INFORMATION TO USERS

This manuscript has been reproduced from the microfilm master. UMI films the text directly from the original or copy submitted. Thus, some thesis and dissertation copies are in typewriter face, while others may be from any type of computer printer.

The quality of this reproduction is dependent upon the quality of the copy submitted. Broken or indistinct print, colored or poor quality illustrations and photographs, print bleedthrough, substandard margins, and improper alignment can adversely affect reproduction.

In the unlikely event that the author did not send UMI a complete manuscript and there are missing pages, these will be noted. Also, if unauthorized copyright material had to be removed, a note will indicate the deletion.

Oversize materials (e.g., maps, drawings, charts) are reproduced by sectioning the original, beginning at the upper left-hand corner and continuing from left to right in equal sections with small overlaps. Each original is also photographed in one exposure and is included in reduced form at the back of the book.

Photographs included in the original manuscript have been reproduced xerographically in this copy. Higher quality 6" x 9" black and white photographic prints are available for any photographs or illustrations appearing in this copy for an additional charge. Contact UMI directly to order.

UMI

A Bell & Howell Information Company
300 North Zeeb Road, Ann Arbor MI 48106-1346 USA
313/761-4700 800/521-0600

EVALUATION OF THE EFFICIENCY OF DIFFERENT ARBUSCULAR MYCORRHIZAL FUNGI ON CORN (*Zea mays* L.) AND PEPPER (*Capsicum frutescens* L.) UNDER GREENHOUSE AND FIELD CONDITIONS

by

Luis Barnola

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science

Department of Plant Science
Macdonald Campus of McGill University
Sainte-Anne-de-Bellevue, Québec, Canada

May, 1997

Short title:

EVALUATION OF THE EFFICIENCY OF MYCORRHIZAE ON CORN AND PEPPER

M.Sc. Plant Science

ABSTRACT

Luis Barnola

EVALUATION OF THE EFFICIENCY OF DIFFERENT ARBUSCULAR MYCORRHIZAL FUNGI ON CORN (*Zea mays* L.) AND PEPPER (*Capsicum frutescens* L.) UNDER GREENHOUSE AND FIELD CONDITIONS

The objective of this research was the selection of the most efficient arbuscular mycorrhizal (AM) fungus on pepper (*Capsicum frutescens* L. cv. North star) and sweet corn (*Zea mays* L. cv. Bicolor) growing under controlled and field conditions. The inoculum treatments consisted of 9 different AM inocula and an autoclaved mix of roots and sand used as a control. Plants were inoculated and planted in a pasteurized growth medium (greenhouse) and non-fumigated soil in 4 different field locations for each crop. *Glomus intraradices* (GinA) and both the same strain (GinA) and a mix of *Sclerocystis rubiformis* and *G. fasciculatum* (Sru+) developed the highest AM colonization in sweet corn and pepper, respectively, under controlled conditions. However, no significant increases in growth were found compared with non-mycorrhizal plants. Only a mix of *G. microaggregatum*, *G. mosseae* and *G. fasciculatum* (Gmi+) produced a greater shoot dry mass compared with the control treatment in sweet corn under controlled conditions. None of the mycorrhizal strains used in the field experiments increased the growth of sweet corn or pepper compared with non-inoculated plants under field conditions.

M.Sc. Phytotechnie

RESUME

Luis Bamola

ÉFFICACITÉ DE DIFFÉRENTS CHAMPIGNONS ENDOMYCORHIZIENS SUR LE MAÏS (*Zea mays* L.) ET LE POIVRON (*Capsicum frutescens* L.) DANS DES CONDITIONS DE SERRE ET DE CHAMP

Cette recherche avait pour objectif la sélection de souches de champignons endomycorhiziens le plus efficace sur le poivron (*Capsicum frutescens* L. cv North star) et le maïs (*Zea mays* L. cv Bicolor), poussant dans des conditions contrôlées et dans des champs. Les traitements consistait en 9 différents souches de champignons et un mélange d'inoculum et sable stérilisé à l'autoclave utilisé comme temoin. Les plantes étaient inoculées et plantées dans un milieu de croissance pasteurisé à la vapeur (serre) et un sol non-fumigé dans 4 sols agricoles différents pour chaque espèce végétal. *Glomus intraradices* (GinA) et la combinaison de la même variété (GinA) avec un mélange de *Sclerocystis rubiformis* and *G. fasciculatum* (Sru+) ont développé la colonisation endomycorhizien la plus élevée sur le maïs sucré et le poivron, respectivement, dans des conditions contrôlées. Toutefois, aucune augmentation de croissance significative n'a été constatée par comparaison avec les plantes temoines. Seul le mélange de *G. microagreggatum*, *G. mosseae* et *G. fasciculatum* (Gmi+) a produit une masse de pousses sèches plus grande par comparaison avec le temoin sur le maïs sucré, dans des conditions contrôlées. Aucune des différentes variétés de champignons endomycorhiziens utilisées dans les expériences dans les champs n'a augmenté la croissance du maïs sucré et du poivron par comparaison avec les plants non-inoculées dans des conditions de champ.

ACKNOWLEDGMENTS

The author wishes to thank Dr. Chantal Hamel, who kindly helped me throughout this thesis, giving me support, encouragement and her friendship. Merci beaucoup!

I am thankful to Dr. Donald Smith, who was always helpful and supervised all my work with patience and interest.

I am grateful to Dr. Yolande Dalpé for her time and help in spore identification and who kindly supplied the mycorrhizal inoculum. I also thank Dr. Marc St-Arnaud for his helpful comments.

I am indebted to Stewart Liebovitch, who not only helped me in the field but also cooperated with me in numerous other matters. I thank all the personnel of the Macdonald Campus greenhouse facilities and especially, Denis, for his technical assistance and support. To Carlos Costa my gratitude for his help and encouragement.

I am particularly indebted to my friends Moraima, Carla, and Elidmar who unconditionally helped me with the field work and gave me support throughout this work.

I deeply appreciate the financial support of the *Consejo Nacional de Investigaciones Científicas y Tecnológicas de Venezuela* which provided me with a scholarship to fulfill my academic program at McGill University. This research was also supported by a grant of the Conseil des Recherches en Pêche et en Agro-alimentaire du Québec (CORPAQ) held by D.L. Smith.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

The impact of conventional agriculture on the environment is assuming immense proportions. The intensive use of agro-chemicals is causing both on- and off farm effects which pollute rivers and lakes. Soil erosion, lack of organic matter, and a reduced stability due to a loss of soil physical aggregation are also accelerated under high-input agriculture. As a consequence, the soil—mistakenly considered as an inexhaustible resource—requires increasing amounts of energy to keep a profitable economic margin for the producer, but at a very high environmental cost.

A sustainable future for agriculture is under continuous debate, transcending issues of economics, ecological principles, stability of rural communities, and technology (Wilson and Morren, 1990). From the agro-ecological perspective, the study and development of biologically based systems of farming represents a large challenge for modern agriculture. More closed chemical and biological cycles, as well as reduced impacts on soil structure are features that should be encouraged. The biotic component of the soil plays an important role in alternative models of agricultural resource management, but only a comprehensive approach will be appropriate to interpret the unpredictable behaviour of such a complex system as the "living soil".

Mycorrhizae are essential in terrestrial ecosystems. Many aspects of plant physiology are intrinsically linked to the occurrence of mycorrhizal fungi, which in turn connect the plant with other plants and the surrounding soil. The flux of matter and energy mediated by this symbiosis and its effects in natural ecosystems and cultivated land have not been fully elucidated. A delicate balance underlies this symbiosis: The overall increase in the performance of the host plant due to multiple factors mediated by the mycorrhizal fungi are counterbalanced with the investment of carbon in fungal structures, which in turn supports a more diverse microflora and also enhances soil stability. This equilibrium is disrupted by many practices associated with high-input agriculture.

Very little is known about the conditions under which arbuscular mycorrhizal (AM) fungi elicit particular responses in host plants. Competitive interactions between introduced AM fungal

strains and indigenous AM fungi account for this effect. A highly variable environment and the occurrence of more aggressive AM fungi in the field are likely to interfere with the potential positive effects of an inoculated AM fungal strain on a target host, when compared with the plant response under controlled conditions, evaluated in the absence of competing micro-organisms and other micorrhizal fungi. The selection of effective and competitive AM fungal strains becomes a crucial step for mycorrhizal research, in order to allow the establishment of effective management strategies that maintain beneficial AM populations in cultivated land.

The aim of this research is to evaluate different AM fungal strains isolated from both agricultural fields and non-disturbed habitats that have the potential to enhance growth and yield of two important crops of Eastern Canada, sweet corn (*Zea mays* L.) and pepper (*Capsicum frutescens* L.). The following activities were pursued in order to carry out the experiments:

- ***Inoculum production.*** Field collected and herbarium samples were used to produce AM inoculum in pot cultures.
- ***Screening for effectiveness of AM fungal isolates under greenhouse conditions.*** Different AM fungal isolates were used to inoculate sweet corn and pepper growing in pasteurized soils in pot experiments under controlled environment conditions. The extent of mycorrhizal infection, plant growth, and phosphorus tissue contents were used to evaluate the effectiveness of the AM isolates by comparison with non-mycorrhizal control treatments.
- ***Screening for competitive ability of these AM fungal isolates under field conditions.*** The same AM fungal isolates were used to inoculate both crops in non fumigated soils with different crop histories and at various locations. The extent of mycorrhizal infection, plant growth, and crop yield were used to estimate mycorrhizal effectiveness and competitive abilities of the considered strains by comparison with autoclaved non-mycorrhizal inoculum applied to the field soils.

Chapter 1

GENERAL ASPECTS OF ARBUSCULAR MYCORRHIZAE

A mycorrhiza is a mutualistic symbiosis between plants and fungi and is localized in a root or a root-like structure in which energy moves primarily from plant to fungus and inorganic resources move from fungus to plant (Allen, 1991). Mycorrhizae are considered the most important symbiosis on earth and the AM the primitive condition for plants (Allen, 1996). Colonization of land by plants was facilitated by this mutualistic association as shown by the fossil record (Pirozynski and Dalpé, 1989; Pirozynski and Malloch, 1975) and molecular evidence indicates its appearance over 300 million years ago (Simon et al. 1993). AM are ubiquitous and most of terrestrial plant species form AM symbioses.

The importance of AM is crucial in determining plant community structure. This symbiosis enhances competitiveness of colonized plants which in turn obtain more soil resources than surrounding non-mycorrhizal neighbours (Allen, 1996). By the same token, the effective application of AM in the field largely depends on a better understanding of the complex interactions between the biotic and abiotic components of the soil surrounding mycorrhizal roots (Klironomos and Kendrick, 1993). At the same time, it becomes necessary to elucidate the range of conditions by which AM elicit specific responses (Allen, 1996).

1.1.1 TAXONOMY

The order Glomales has been designated to include only fungi capable or presumed to be capable of forming AM with plants; whether or not this is a valid character to define an order is questionable (Walker, 1992). There is also an obvious problem of definition since a mycorrhiza is defined on a mutualistic functional basis which can not be proved in all cases (Allen, 1996). Arbuscules are the most distinguishable characteristic of this association but also important is the external matrix of fungal mycelium which grows into the surrounding soil. Various architectural features of these hyphae suggest some spatial functionality among them (Allen, 1996).

Morphological attributes of fungal spores and/or sporocarps have been normally used to characterize 3 different families among the Glomales (Walker, 1992):

a) The Gigasporaceae includes the genus *Gigaspora* and *Scutellospora* which characteristically do not produce vesicles. The genus *Gigaspora* was thought to form zygospores but it was later considered to form azygospores. It is now more convenient to designate these structures simply as spores until their identity can be fully elucidated.
b) The Acaulosporaceae is formed by two different genera, *Acaulospora* and *Entrophospora*. The former characteristically forms spores laterally on the proximal part of a sporiferous saccule.
c) The Glomaceae includes two genera: *Sclerocystis* and *Glomus*. The former only contains a single species that deserves further reconsideration within this group. *Glomus* is a complex and probably a polyphyletic group composed of species, some of which at least require extensive review (Walker, 1992).

More than 150 AM fungal species have been described but only few of the old descriptions of the Glomales have been re-described to match new concepts and terminology regarding AM morphology. As a consequence it becomes extremely difficult to identify some of these species (Walker, 1992). However, the use of more advanced techniques like the polymerase chain reaction (PCR), which amplifies small amounts of DNA, could represent powerful tools for the development of mycorrhizal taxonomy, particularly for AM (Kloronomos and Kendrick, 1993; Piché et al., 1994). The PCR technique has already been useful for showing the phylogenetic relationships among twelve species of the main taxonomic groups of endomycorrhizal fungi (Simon et al., 1993).

1.1.2 SOME GENETIC ASPECTS OF ARBUSCULAR MYCORRHIZAE

The Glomales are thought to be asexual organisms and for this reason the interbreeding approach for species definition is not valid (Walker, 1992). Each fungal spore contains thousands of nuclei and it is not clear at all if these are a single genotype; as a consequence, the term clone can not be used even for pot cultures originated from single spores (Walker, 1992).

Although AM fungi cannot fully develop in the absence of a plant host they are actually capable of DNA replication and the mycelium of a germinating spore exhibits migration of nuclei and nuclear division (Bonfante and Perotto, 1995).

Different sequences of ribosomal genes occurring in different nuclei but in the same spore, as described by the heterocaryote hypothesis, illustrate the point that these spores might better be considered populations than genetic individuals (Sanders et al., 1996). When hyphae encounter each other in the soil they can fuse, potentially bringing new nuclear types together (Allen, 1991). Whether there is an actual exchange of nuclei between different AM fungi or not is still unknown, but other genetic mechanisms, like molecular drive or gene turnover would also explain the high genetic diversity and thus the high plasticity exhibited by the Glomales (Sanders et al., 1996). A higher genetic variability among and within populations of morphologically identical spores collected from the field compared with the genetic consistency among spores obtained from pot cultured material developed from isolated spores, suggests that there is some mechanism that affects natural populations and promotes high genetic diversity (Sanders et al., 1996).

1.1.3 EARLY STAGES OF MYCORRHIZAL COLONIZATION

Appressoria formation is the first morphological sign of a recognition process which takes place between AM fungi and the root of host plants. Symbiosis-specific plant genes seem to play an important role in the early infection stages; these are also related with a more general spectra of mechanisms in plant mediated-microbe interactions, as suggested by using *myc* and *nod* mutants (Bonfante and Perotto, 1995; Gianinazzi-Pearson et al., 1996). Host plants show little or no reaction at the cellular level when the appressorium connects the invading fungus with the root (Gianinazzi-Pearson et al., 1996). This clearly contrasts with the enhanced metabolism of host cells surrounding the branching hyphae during arbuscle formation, which can not be related to any specific defence reactions (Gianinazzi-Pearson et al., 1996).

In the presence of the host, some root exudates (principally phenolic compounds) function as biochemical signals that activate fungal genes related to the morphological integration and functional compatibility between both symbiotic partners (Bonfante and Perotto, 1995;

Gianinazzi, 1991). Some reports have indicated that certain flavonoid compounds (especially quercetin) are especially important for enhancing mycorrhizal establishment in *in vitro* systems (Bécard and Piché, 1989; 1992; Chabot et al., 1992b; Tsai and Phillips, 1991) but flavonoids are not essential plant molecular signals since root-organ cultures form mycorrhizae in their absence (Bécard et al., 1995). The penetration of the AM fungi into the root seems to be largely due to mechanical force as shown by the low rate production of root-cell wall hydrolitic enzymes (Bonfante and Perotto, 1995). However, control of fungal establishment and long term regulation of arbuscule development are determined by an interplay between host rejection and continual re-invasion by the fungus and thus, the system is never in equilibrium (Allen, 1991; Gianinazzi, 1991). After infection, changes take place in the root cells including an increase in nuclei size (Bonfante and Perotto, 1995).

1.1.4 THE MYCORRHIZAL INTERFACE

The newly formed and highly complex interface zone is characteristic of the symbiotic status (Bonfante and Perotto, 1995). The plant membrane extends from the peripheral plasmamembrane, around the fungal haustorium, while the fungal partner remains in the apoplast outside the plant protoplast (Smith et al., 1994). Arbuscules are formed from intercellular hyphae which proliferate longitudinally (Smith et al., 1994) and one of these can be defined as a highly complex and specialized apoplastic compartment. These structures allow for nutritional exchange between both partners (Bonfante-Fasolo, 1992). It was observed that the macromolecular chitin organization of the fungus changes dramatically from fibrillar to amorphous when forming the arbuscular cell wall (Arines et al., 1993).

Bi-directional transfer of nutrients takes place in functional mycorrhizae where carbohydrates move from plant to fungus and mineral nutrients move from fungus to plant. The extent to which this process occurs at the same interfaces is uncertain (Smith et al., 1994). It has been proposed that arbuscules and intercellular hyphae can function as separated interfaces: P would theoretically be released by arbuscule branches and be taken up by the highly energized periarbuscular host membrane. Carbohydrates would be released from the cortical cells into the intercellular spaces and then be taken up by intercellular hyphae (Smith et al., 1994).

1.1.5 NUTRIENT UPTAKE IN MYCORRHIZAL SYMBIOSES

Nutrients that are available to plants can be directly absorbed by them and this process depends basically on their movement to the absorbing roots, the potential nutrient supply of the soil, and the active absorption of these nutrients by cortical root cells (Chapin, 1980). The flux of nutrients into the depletion zone surrounding the absorbing root depends on diffusion and/or mass flux. Nutrients move through diffusion across a concentration gradient perpendicular to the root surface and from areas of higher concentration in soil solution into the depletion zone surrounding the roots (Bolan, 1991). Mass flux is a convective process that occurs during plant transpiration, in which nutrients dissolved in soil water move into depletion zones (Bolan, 1991).

Diffusion is frequently the limiting factor in nutrient absorption by root plants. Some low-mobility nutrients, such as PO_4^+, NH_4^+, K^+ and to a lesser extent NO_3^-, have diffusion rates much lower than the absorption rates required for optimal plant growth, resulting in a depletion zone of these nutrients around plant roots (Chapin, 1980; Hayman, 1983; Koide, 1991; Smith and Gianinazzi-Pearson, 1988).

It is well known that the larger volume of soil explored by the extramatrical hyphae, compared with the volume of soil explored by roots of a non-mycorrhizal plant, enhances the uptake of phosphorus as well as other poorly mobile nutrients in soil. Nutrient uptake efficiency at a given infection level it is likely to be affected by the occurrence and physiology of arbuscules as well as the physiological status of the external hyphae (Marshner and Dell, 1994).

1.1.5.1 *Phosphorus uptake*

It has been suggested that the increase in phosphorus uptake by mycorrhizal plants is related to the following mechanisms: Development of an extensive network of extramatrical hyphae in soil which extends far away from the depletion zone facilitating nutrient movement across depleted areas (Miller, 1987; Smith and Gianinazzi-Pearson, 1988), a notable increase in the root absorption surface mediated by the external mycelium, which explores small soil pores where root hairs do not have access (Bolan, 1991; Smith and Gianinazzi-Pearson, 1988), and a greater affinity for soil P by fungal hyphae (Bolan, 1991; Smith and Gianinazzi-Pearson, 1988).

Although experimental evidence shows that mycorrhizal plants obtain labelled phosphorus from the same source as nonmycorrhizal plants do (Bolan, 1991), a significant increase of acid phosphatase activity by mycorrhizal hyphae suggests an AM-mediated release of P from organic complexes in the soil (Kucey et al., 1989; Marshner and Dell, 1994). However, there is no conclusive proof of the AM-mediated effect on the increased P uptake from non-soluble sources since it is practically impossible to discriminate this form of uptake from other rhizosphere activities (Smith and Gianinazzi-Pearson, 1988).

1.1.5.2 *Other nutrients*

The increase in phosphorus uptake by mycorrhizal plants induces an overall improvement in plant performance and thus, a notable increase in the foliar concentration of some nutrients such as copper, zinc, nickel, chlorides and sulphates (Koide, 1991). However, mycorrhizal plants can increase the direct uptake of the macronutrient K, minor nutrients such as Ca and $SO_4^=$, and the micronutrients Cu and Zn, which are transported to the roots through the external hyphae, but at lower rates than P (Marshner and Dell, 1994).

Nitrogen is taken up as NH_4^+ by mycorrhizal plants, which explains why these plants are dominant in some ecosystems where P does not limit plant production (Allen, 1996). This ion moves very slowly in soil and it is transported through mycorrhizal hyphae as confirmed under both controlled conditions (Ames et al., 1983) and field experiments (Barea et al., 1987)

1.1.6 PHYTOHORMONE BALANCE

It is suggested that one of the factors that can regulate the intraradical colonization of the fungus is the level of phytohormones, which in turn can modify the functioning of the plant (Allen, 1996). Significant increases in cytokinin, abscisic acid and gibberellin-like substances have been detected in mycorrhizal plants, but whether or not this is an effect of the improved nutrient status of the host is not clear (Smith and Gianinazzi-Pearson, 1988). However, the overall control of the mycorrhizal root architecture (Hetrick, 1991), stomatal control and photosynthesis,

and water relations (Allen, 1996) are some of the plant processes that would be mediated by phytohormone balance.

1.1.7 DROUGHT RESISTANCE

Arbuscular mycorrhizae enhance the movement of water through fungal hyphae even during times of increasing drought, when roots shrink and the soil root interface is filled with air pockets (Allen, 1996). Some other plant features related to increased drought resistance mediated by the AM symbiosis are the change in leaf elasticity, an improved leaf water turgor potential, the maintenance of stomatal opening and high transpiration rates, and increased rooting length and depth (Sylvia and Williams, 1992). Nelson (1987) also noted that increased levels of tissue P also increase drought resistance due to changes in membrane permeability.

1.1.8 THE MYCORRHYZOSPHERE EFFECT

The mycorrhyzosphere is the soil surrounding the mycorrhizal roots. The soil mycoflora is modified by an altered amount and quality of root exudates as well as the presence of extramatrical hyphae (Garbaye, 1991; Linderman, 1992, 1993). The importance of the transport of carbon to microbial communities through the external hyphae, outside the influence of the rhizosphere, is now widely accepted (Bethlenfalvay and Schüep, 1994; Hamel, 1996). Selective changes in the soil mycoflora are likely to affect the further development of hyphae in soil and the absorption of metabolites produced by these microbial associates, but the implicit mechanisms are virtually unknown (Linderman, 1992).

Synergistic effects between AM fungi and several microbial groups that can influence plant growth and health have been experimentally demonstrated. Some of these groups of micro-organisms, reviewed by Linderman (1992), include both nodule producing and free-living N_2 fixing bacteria, phosphate solubilizing bacteria (PSB), and plant growth promoting rhizobacteria (PGPR).

1.1.9 PLANT DISEASE CONTROL

The scientific literature contains plenty of examples supporting the hypothesis that the mycorrhizal symbiosis leads to a suppression of root diseases caused by pathogenic fungi and nematodes (Linderman, 1992). However, some of these results are controversial and many different mechanisms have been proposed to explain how AM fungi mediate such a response, which varies with the fungal and plant species as well as cultural and environmental conditions (Varma, 1995). Increased production of phytoalexin compounds has been suggested to explain the enhanced disease tolerance mechanisms in mycorrhizal plants (Varma, 1995). Some other examples of biocontrol mediated by AM fungi and their proposed mechanisms of action have been reviewed elsewhere (Garbaye, 1991; Ingham and Molina, 1991; Jalali and Jalali, 1991; Linderman, 1992).

1.1.10 SOIL STRUCTURE

The relevance of AM fungi in soil aggregation has became an important focus of sustainable agriculture research. It has been suggested that the extracellular polysaccharides produced by mycorrhizal hyphae attaches to soil microaggregates and binds them into stable macroaggregates (Tisdall, 1994). The exact mechanisms involved in this process are not known but the extraction of water from small soil pores by AM hyphae is thought to stabilize soil aggregates (Schreiner and Bethlenfalvay, 1995).

The hierarchical theory proposes that floccules are composed of primary clay particles, held together along with inorganic oxides and organic polymers, by electrostatic bonds (Schreiner and Bethlenfalvay, 1995). These floccules encrust small particles of organic matter, forming small aggregates, and then combine with small grains of sand to form microaggregates (Schreiner and Bethlenfalvay, 1995). The physical entanglement permits the external hyphae to enmesh microaggregates thereby creating larger aggregated units within the rhizosphere (Miller and Jastrow, 1992a). The hierarchical theory may not apply for all soils due to different sized microaggregate fractions and also the fact that organic matter is not the only binding material for all soils (Tisdall, 1994).

1.1.11 PHOTOSYNTHETIC RATES AND CARBON USE

It is well documented that photosynthetic rates are higher in mycorrhizal than non-mycorrhizal plants. It has been postulated that this effect is caused by the increased below-ground sink demand for C in mycorrhizal plants. Accumulation of sucrose-6-P in leaf mesophyll cells is the limiting step in photosynthesis as it binds up Pi in inactive pools. An increase in the C sink of mycorrhizal roots allows a mobilization of starch pools stored in leaves whereas ADP:ATP ratios decrease, resulting in higher photosynthetic rates (Fitter, 1991; Harris and Paul, 1987; Ingham and Molina, 1991).

Plant carbohydrates that enter into the fungus are converted into lipid globules that can be carried along concentration gradients by protoplasmic streaming. These lipids are accumulated in vesicles and spores and can help maintain a carbohydrate concentration gradient towards the fungus, facilitating the movement of reduced C from the plant (Harris and Paul, 1987).

It has been suggested that the carbon cost to maintain the mycorrhizal symbiosis is equivalent to the amount required to maintain the root system (Fitter, 1991). Fungal biomass may represent between 5 to 10 % of the total root biomass but maintenance costs of the former are at least ten times higher per unit weight than those of root biomass (Fitter, 1991). Despite of the fact that respiration of the fungus may represent 30 to 40 % of the total photosynthate consumption by roots (Harris and Paul, 1987), the carbon cost required to construct fungal hyphae is lower per unit length than that required to construct rootlets (Fitter, 1991).

The carbon:phosphorus ratio has an important effect on mycorrhiza-mediated plant responses. In very poor soils that only support sub-optimal growth rates and have low P availability, the mycorrhizal symbiosis may be restricted by a limited carbon transport to fungal structures as well as the inadequate level of P, allowing other soil micro-organisms to invade the roots of plants growing under these conditions (Koide, 1991; Van Duin et al., 1991). On the other hand, a drain of C to mycorrhizal roots in soils with high fertility and moisture, without the benefit of an increased P status of the plant due to the symbiosis, might induce a depression of growth (Allen, 1996; Harris and Paul, 1987; Hendrix et al., 1992; Koide, 1991). This effect may induce what has been called the change from mutualism to parasitism (Hayman, 1987; Van Duin

et al., 1991) but it has been suggested that C expenditure may be regulated by the host plant (Graham and Eissenstat, 1994).

PRACTICAL USES OF AM IN AGRICULTURE

The conventional approach to evaluate the effect of AM in agriculture has emphasized the potential increase in plant growth and yield derived from mycotrophy. As a consequence, the soil has never had the same priority as the productivity of plant shoots (Bethlenfalvay and Schüep, 1994). The extent to which the natural environment has been modified to achieve a high agricultural productivity has resulted in a degradation of natural resources, loss of topsoil, and the contamination of surface and groundwater with fertilizers and pesticides that have promoted a lack of profitability for farmers (Hesterman and Thorburn, 1994; Oberle, 1994). Soils must be viewed not as a profitable resource but a complex, living, fragile system that must be protected to ensure long-term productivity (Reganold et al., 1990).

For all these reasons, a number of authors have commented on the potential of the AM symbiosis to enhance crop productivity and soil conservation. This is especially important for sustainable agriculture research, the principal goal of which is to minimize soil erosion while maintaining soil fertility for ecological stability and long term agricultural productivity (see Allen et al., 1992; Bethlenfalvay and Schüep, 1994; Douds et al., 1993; Jeffries and Barea, 1994; Jeffries and Dodd, 1991; Miller and Jastrow, 1992a; Sieverding, 1991).

Equally important is the regulatory effect of mycorrhizae at a lower level, promoting linkages between the biotic and geochemical components of ecosystems which in turn are a determining factor in their large scale behaviour (O'Neill et al., 1991). Moreover, a sustainable agricultural system should resemble more mature ecosystems characterized by a rapid cycling of nutrients within the biotic sub-system (Jeffries and Barea, 1994). In conclusion, the ability of AM to permeate the soil, picking up nutrients and directing them quickly to the host plant (Allen et al., 1992) and the gain of C by the soil mediated by AM, which in turn enhances microbial activity and increases soil organic matter content and soil stability, must play a critical role in a conservationist set of agricultural practices to be implemented in order to ensure a more balanced and stable life-supporting soil matrix (Bethlenfalvay and Schüep, 1994).

Very little is known about the complexity of plant-soil-fungus interactions in agricultural conditions (Hamel, 1996). Therefore, it will be difficult to implement long term strategies to use mycorrhizae in productive agroecosystems until we achieve a better understanding of AM functioning and their long term effects.

1.2.1 AGRONOMIC STANDARDS FOR MYCORRHIZAL RESEARCH

The proper identification and assessment of AM isolates under field conditions is one of the main goals for mycorrhizal research. The isolation of AM spores from the field is not a good indicator of fungal diversity since sporulation normally occurs after a threshold colonization level and often field collected spores are difficult to identify (Morton and Bentivega, 1994). The presence of non-sporulating fungi, morphologically identical but different spore types, differences between the collected number of spores and the actual ecological importance of the considered species, and the fact that spore counting only reflects past events in the symbiosis lead to erroneous conclusions and normally underestimate natural diversity (Sanders et al., 1996).

Early molecular methods used allozyme patterns allowing the detection of single AM species in roots. All new techniques, based on the polymerase chain reaction (PCR), are promising but require further refinement to develop more reliable molecular markers (Sanders et al., 1996). Diagnosis tests designed for AM quantification are now being developed and include, among others, PCR amplification of AM chitin synthase genes (Hamel, 1996) and the co-amplification of PCR standards with root-derived AM fungal templates using known primers (Sanders et al., 1996).

A conventional method to assess the degree of reliability of a plant upon the mycorrhizal symbiosis is the relative field mycorrhizal dependency (RFMD), which was defined by Gerdemann (Plenchette et al., 1983). It expresses the difference between the dry weight of mycorrhizal and non-mycorrhizal plants as a percentage of the dry weight of the mycorrhizal plant at a known available P soil level (Plenchette et al., 1983). The morphology of the roots was related to mycorrhizal dependency and P absorption. Baylis (1975) described the magnolioid and graminoid roots and the positive relationship between the coarseness of the root and its mycorrhizal dependency. Thus, magnolioid roots without well developed root hairs are more

dependent upon mycorrhizae for P uptake than graminoid roots, with abundant root hairs (Baylis, 1975).

The ability of AM fungal strains to rapidly colonize a host depends largely on the formation of appressoria or infection points (Giovannetti and Gianinazzi-Pearson, 1994). The soil mycorrhizal potential, on the other hand, takes into consideration the number of AM propagules and their ability to develop the symbiosis under the prevalent soil conditions (Plenchette and Strullu, 1995). It is estimated with a bioassay using a mycorrhizal test plant growing in a logarithmic series of dilution of the considered soil, although this method is quite labour intensive to perform on a regular basis (Plenchette and Strullu, 1995).

The parameters to be chosen for the evaluation of mycorrhizal effectiveness depend on what is expected from the symbiosis. They can be yield, a better growth or survival of the host plant, improved tolerance to biotic or abiotic stresses, improved nutrient transport, etc. (Gianinazzi et al., 1989; Giovannetti and Gianinazzi-Pearson, 1994). Other authors have argued that soil aggregate stability should be considered for the evaluation of effective AM isolates (Bethlenfalvay and Schüep, 1994; Miller et al., 1994; Schreiner and Bethlenfalvay, 1995). Thus, the improvement of soil structure which reduces erosion in succeeding years and a decrease in P runoff are benefits that cannot be calculated into the increased value of the crop but undoubtedly have to be considered for the effectiveness concept (Miller et al., 1994).

1.2.2 INTRASPECIFIC VARIATION OF ARBUSCULAR MYCORRHIZAE

Differences in host response to morphologically identical isolates of AM fungal species have been reported by several authors (i.e. Bethlenfalvay et al., 1989; Stahl and Christensen, 1991; Stahl et al., 1990; Sylvia et al., 1993). It was suggested that phenotypic plasticity, although present to varying degrees in populations of AM endophytes, was not enough to explain the widespread distribution of species such as *Glomus mosseae* (Stahl and Christensen, 1991).

The use of molecular techniques has confirmed the high genetic variability among morphologically identical spores. The internal transcribed spacers (ITS) of ten indistinguishable spores of *Glomus* sp. were genetically different (Sanders et al., 1995) and some AM isolates of *Glomus mosseae*, originating from the same culture but kept in different laboratories for several

years, have revealed genetic differences when their fingerprints were compared by the restriction fragment length polymorphism (RAPD)-PCR method (Wyss and Bonfante, 1993). Consequently, it has become evident that developing a reliable method to properly identify AM isolates is of prime importance.

For practical reasons, if the objective of the research is to identify fungal isolates of the same species (edaphotypes), which are effective in promoting plant growth in diverse host-soil systems, it is necessary to assess the edaphic and floristic characteristics of the native site and the goals of the prospective site of utilization (Bethlenfalvay et al., 1989).

1.2.3 SPECIFICITY OF ARBUSCULAR MYCORRHIZAE

Most of the experimental evidence obtained under controlled conditions show that AM fungi are generalists and display little specificity in their relationships with host plants. However, ecological specificity and functional compatibility determines that the host plant may select for a particular combination between symbionts (Allen et al., 1995; Bagyaraj, 1992; Molina et al., 1992; Sanders and Fitter, 1992). As a consequence, AM fungi can be distributed within a community, across any landscape in a shifting mosaic and associated with particular plant species but whether these patches represent a single mycelium or multiple genotypes dispersed throughout the hyphal network is not known (Allen, 1996).

The coexistence of different AM fungi in the same root has recently been corroborated using molecular tracers. Clapp et al. (1995) developed the selective enrichment of amplified DNA (SEAD) methodology to confirm the simultaneous presence of three different genera of AM fungi (*Acaulospora, Glomus, and Scutellospora*) in a single root of bluebell (*Hyacinthoides non-scripta*). The coexistence of different AM fungi in the same host may exert competitive interactions among them and cause masking effects of some fungus over positive interactions of the coexisting isolates with host plants (Sanders et al., 1996).

Hepper et al. (1988) found that species of *Glomus* can compete with each other and one fungus totally exclude another from the root in a short period of time. It has been suggested that the fastest AM fungus colonizing the host plant would have a competitive advantage over other AM fungi (Abbott and Robson, 1984; Giovannetti and Gianinazzi-Pearson, 1994; Hepper et al.,

1988). On the other hand, AM fungi are rather promiscuous and attach to any compatible root, forming bridges between plants that may transport resources between different organisms. Thus, the controversy between the relative importance of competition and sharing of resources in mycorrhizal communities has never been elucidated (Allen, 1996).

1.2.4 OCCURRENCE OF AM IN CROPS

AM mediated enhanced growth has been observed in numerous crops after inoculation in low P soils and it is possible to rank the mycorrhizal dependency of such crops at a known P level (Hayman, 1987; Jeffries and Dodd, 1991; Plenchette et al., 1983). Some tropical crops (i.e. *Manihot esculenta, Pueraria phaseoloides, Sorghoum* sp.) have shown enormous yield increases due to inoculation (Jeffries and Dodd, 1991). Legumes are particularly responsive to mycorrhizal inoculation, with the exception of *Lupinus*, a non-mycorrhizal genus (Hayman, 1987). Among others, some plantation crops like coffe (*Coffea arabica*), some woody species like *Citrus* spp., apple (*Malus* spp.), sweetgum (*Liquidambar styraciflua*), yellow poplar (*Cucumbertree magnolia*), and some vegetables like *Allium* spp., peppers (*Capsicum* spp.), asparagus (*Asparagus officinalis*), carrot (*Daucus carota*), and cucumber (*Cucumis sativus*) have also shown increased growth after AM inoculation under field conditions (Hayman, 1987; Jeffries and Dodd, 1991).

A different plant response has been observed under high P fertility. Mycorrhizal-induced growth depression has been observed in different species of *Citrus*, ryegrass (*Lolium* sp.), ferns, forage legumes, apple, tobacco (*Nicotiana tabacum*) and *Allium* spp. growing in high P soils (Graham and Eissenstat, 1994). In other cases, a positive effect of inoculation under high fertility conditions is only obtained when the soil is fumigated before seeding, which can be explained by a reduction on the incidence of highly competitive native AM fungi (Hamel, 1996). Diverse agronomic practices and the selection of efficient AM isolates are likely to affect the practical application of the AM symbiosis in agriculture (Hamel, 1996).

Low-input agricultural systems seem to increase mycorrhizal colonization and sporulation compared with conventional agronomic systems (Douds et al., 1993) and select for different groups of AM fungi (Douds et al., 1995). A low-input agricultural system resembles more a natural ecosystem and it includes diverse sustainable practices like reduced soil tillage, increased

plant diversity, reduced chemical inputs, etc. (Hamel, 1996). An increased number of weeds in the conversion from high to low-input systems was likely to promote higher root density and higher colonized root length, and thus an increase in AM fungal spore populations in the low-input area (Kurle and Pfleger, 1994).

1.2.5 ARBUSCULAR MYCORRHIZAE AND CULTURAL STRESS

Some agricultural practices have profound effects on quantitative and qualitative mycorrhizal dynamics. The following briefly outlines the cultural stresses imposed in agricultural systems and their effects on VAM fungal populations. An integral understanding of this information and the development of reliable techniques to properly identify and quantify AM fungi will improve the management of mycorrhizae as components of sustainable agriculture.

1.2.5.1 *Fallowing and crop rotation*

The long fallow disorder is the reduced growth of some crops planted after a long period of fallow (Johnson and Pfleger, 1992). The mycorrhizal inoculum decline after a long period of fallow can be the main cause of plant growth reduction (Bethlenfalvay, 1992; Johnson and Pfleger, 1992). The accumulation of dead biomass in a fallow system could promote the occurrence of some Glomales with saprophytic capabilities which could also account for the growth reduction effect (Hamel, 1996). The inclusion of non-host plants also reduces mycorrhizal inoculum densities but growth depression is normally greater when the land is kept fallow (Johnson and Pfleger, 1992). Fallowing and the inclusion of non-mycorrhizal plants can be good management strategies that could promote more diverse AM populations. This is particularly true after the monoculture of cassava (*Manihot esculenta*), that promotes the occurrence of non-effective AM isolates (Sieverding, 1989, 1991), or where the native AM mycoflora is highly competitive (Hamel, 1996).

Crop rotation is known to optimize yield but the mechanisms responsible for this effect are not fully elucidated (Bethlenfalvay, 1992). It has been suggested that the most vigorous and sporulating AM isolates, selected by monoculture systems, are not necessarily the best symbionts

because these fungi could be more opportunistic than mutualistic (Johnson et al., 1992). A highly diverse low-input system, consisting of at least 5 different crop species, resulted in a more diverse AM population compared with a conventional management with only two crops (Douds et al., 1993). In conclusion, crop rotation could be useful in the management of AM fungal communities as it favours the most beneficial fungal species (Johnson et al., 1992). Choosing for the appropriate crop sequence which favours the mycorrhizal symbiosis should be taken into account when designing the most efficient cropping system (Hamel, 1996; Hendrix et al., 1995).

1.2.5.2 *Soil fertility*

The importance of AM in the uptake and transport of nutrients has been already mentioned, but an efficient recycling of nutrients mediated by mycorrhizae plays a particularly important role in sustainable agriculture. Provided that nutrient transfer occurs through hyphal bridges under adverse conditions, the absorption and re-distribution of C and nutrients from senescing and dying roots is greatly improved, allowing plants to cope with such stress by minimizing losses from the system (Jeffries and Barea, 1994).

After excessive fertilization (considered to be a very widespread form of soil degradation), agricultural soils are likely to develop reduced levels of mycorrhizal symbiosis (Hamel, 1996). It is well documented that P-deficient roots show an increased rate of exudation of soluble sugars, aminoacids, and carboxylic acids compared with well fed roots (Schwab et al., 1991). A high P status in the plant host, in response to fertilization, is thought to decrease the amount of carbohydrate allocated to root exudates. As a consequence, the proportion of AM fungal strains that most rapidly acquire the available C (less beneficial strains) may be selected over less aggressive but mutualist strains (Johnson, 1993).

The mycorrhizal symbiosis affects plant fitness over a long time scale; even if the fungus may reduce plant growth during a short period of time, the association can still remain mutualistic if the symbiosis enhances plant fitness over the long run (Allen, 1991; Allen et al., 1992). For example, the quality of soils increases due to the production of an extensive external mycelium even if this is at the expense of the host plant growth (Bethlenfalvay and Barea, 1994; Hamel,

1996). This effect, in turn, represents an important factor affecting crop yield over the long term (Bethlenfalvay and Schüep, 1994).

In some cases high rates of fertilization do not reduce mycorrhizal activity, especially if it is estimated from root colonization, which is not a good indicator of mycorrhizal effectiveness (Miller et al., 1995). Mycorrhizal dependency of the involved plant species would also explain the high levels of mycorrhizal colonization in some fertile soils (Hamel, 1996).

The following variables are useful to predict mycorrhizal responses to P-fertilization. In naturally infertile soils, mycorrhizal colonization is reduced after fertilization whereas in naturally fertile soils there is little or not effect (Johnson and Pfleger, 1992). On the other hand, small additions of fertilizer in extremely poor soils can stimulate mycorrhizal colonization and sporulation but larger additions of fertilizer suppress this (Johnson and Pfleger, 1992). There is also evidence that balanced fertilization (N-P-K) stimulates mycorrhizal colonization (Johnson and Pfleger, 1992). Amending the soil with fresh organic matter in appropriate amounts could protect mycorrhizal plants against Al toxicity in acid soils (Soedarjo and Habte, 1993).

1.2.5.3 *Soil disturbance and tillage*

Soil disturbance can affect the distribution and abundance of arbuscular mycorrhizae by changing the chemical, physical or biological characteristics of soils or by changing the botanical composition of a site (Abbott and Robson, 1991). The soil stable aggregates provide physical protection, especially in arable systems, in which the nutrient reserve is afforded by inputs from crop residues, organic amendments or fertilizers (Miller and Jastrow, 1992a). As a consequence, the contribution of the mycorrhizal association in creating both vegetation and soil heterogeneity in disturbed landscapes is of vital importance in restoration processes, in order to avoid a rapid loss of organic matter and associated nutrients from soil through both mineralization and erosion (Miller and Jastrow, 1992a, 1992b).

Soil erosion reduces yield and contributes to river and lake pollution. It can severely decrease both the extent of AM development and the number of propagules; as a consequence, eroded soils often have very low fertility levels (Abbott and Robson, 1991; Sylvia and Williams, 1992). Compacted soil also reduces plant growth, as well as mycorrhizal colonization, due to a decrease

in root growth, soil water content and soil aeration (Sylvia and Williams, 1992). Land restoration, helped by AM, can be categorized under different organizational levels that include the study of soil aggregate formation due to extraradical hyphae, the influence of AM on the physiology of the whole plant, the composition of succession series, and AM population dynamics (Miller and Jastrow, 1992b). The management of indigenous populations and inoculation with native mycroflora have succeeded restoring degraded land (i.e. Lumini et al., 1994).

Cultivation accelerates mineralization of the soil organic matter which induces a loss of water-stable soil aggregates (Schreiner and Bethlenfalvay, 1995). Conventional tillage consists on mouldboard plow plus two or three passes with secondary tillage equipment, which causes the physical disruption of the AM hyphal network in the soil (Miller et al., 1995). Alternatively, maize plants grown on undisturbed soils have shown an increase in P absorption capacity during early growth (Miller et al., 1995). It was suggested that the persistence of the mycelium (resting hyphae) from the previous crop maintains its infectivity after the winter and is also functionally capable of absorbing P and transporting it into new attached roots (Miller et al., 1995). Tillage could induce a strong selection pressure for more vigorous AM species (Hamel, 1996) or for those that produce infective propagules most rapidly (Johnson and Pfleger, 1992). The production of intraradical vesicles may be an important feature of these opportunist strains (Johnson and Pfleger, 1992).

Tillage affects the vertical distribution of AM spores (Smith, 1978) and AM hyphae (Kabir *et al.*, unpublished results). Tillage also influences biotic factors, especially the soil fauna, which are vectors of VAM propagules (Johnson and Pfleger, 1992). Rabatin and Stinner (1989) found a significantly higher proportion of invertebrates containing AM spores in no-till systems compared with conventional farming. These results promote a promising and almost unexplored area of research concerning AM and sustainable agriculture practices.

1.2.5.4 *Biocides*

Biocides may directly or indirectly affect AM fungi modifying either the complex host-soil biota or the host plant (Bethlenfalvay, 1992). Mycorrhizal effects caused by pesticides may induce a confusing cause and effect interpretation which could be partly explained because

pesticides react differently under different edapho-climatic conditions (Sieverding, 1991). Harmful effects of some biocides on AM fungal populations could be reduced by testing them in both controlled and field conditions prior to their marketing (Hamel, 1996).

Soil fumigants are used to reduce populations of pathogens and pests but their use normally eradicates AM fungi as well as other beneficial organisms (Johnson and Pfleger, 1992; Sieverding, 1991). AM fungi are more sensitive to methyl-bromide than most soil micro-organisms, with its potency depending on soil type, moisture level, temperature and method of application (Johnson and Pfleger, 1992). Soil solarization has little or no detrimental effect on AM fungi and is mostly used in tropical areas (Johnson and Pfleger, 1992).

The effect of fungicides on AM fungi is quite variable and depends on the specific fungicide, its mode of action and its interaction with the fungus in each soil type (Schreiner and Bethlenfalvay, 1996). Benzimidazole fungicides (Benomyl) and substituted aromatic hydrocarbons, used at recommended rates, are consistently detrimental to AM fungi while two anti-oomycete fungicides frequently stimulate AM fungi (Johnson and Pfleger, 1992). A reduction in the population of antagonists to AM fungi and an increased allocation of carbohydrates into root exudates due to the fungicide are two alternative hypothesis suggested to explain this beneficial effect on AM fungi (Johnson and Pfleger, 1992).

Many herbicides do not directly induce an adverse effect on AM fungi (Johnson and Pfleger, 1992). Since herbicides are used to control weed populations, AM host diversity is reduced after their application which results in a reduced support for native mycorrhizal fungi (Hamel, 1996). Managing weed populations, especially where crop rotation is not applicable, would increase soil fertility due to an increased diversity of AM fungi, favouring the occurrence of slow-growing but beneficial fungal strains (Hamel, 1996). A synergistic effect on weed injury was observed when the herbicide bentazon was applied to a mycorrhizal weed-crop association under controlled conditions (Bethlenfalvay et al., 1996). The presence of AM hyphal bridges and a functional sink driven transferring of nutrients from weeds into crop plants was suggested to explain this effect but a direct hyphal transfer was not explicitly demonstrated (Bethlenfalvay et al., 1996).

1.2.5.5 *Crop breeding*

Conventional breeding programs that select for high-yielding crop varieties, by choosing genotypes that respond well to the recommended fertilization regimes, may have selected cultivars that are unresponsive to mycorrhizae (Hamel, 1996; Johnson and Pfleger, 1992) or that show growth depression at high P (Graham and Eissenstat, 1994; Hetrick et al., 1993). Hetrick et al. (1995) have found concrete evidence of the heritability of mycorrhizal dependence by locating the genes responsible for the reduced responsiveness to mycorrhizae in new varieties of wheat (*Triticum aestivum*).

An alternative approach of plant breeding would be selection of genotypes under stress conditions and then evaluating the F1 generation under normal production conditions, has been proposed to indirectly select for appropriate AM hosts (Hamel, 1996). This could require a return to older varieties of crop plants that may be able to strike an optimum balance between mycorrhizal responsiveness and a balanced fertilizer requirement (Jeffries and Barea, 1994). There is also a growing concern about the potential impact of pathogen-resistant transgenic cultivars over non target organisms like AM fungi that could induce a notable decrease in their susceptibility to mycorrhizal infection (Gianinazzi-Pearson et al., 1996; Jeffries and Barea, 1995).

1.2.6 MANAGEMENT OF ARBUSCULAR MYCORRHIZAL FUNGI

AM management refers to the manipulation of AM populations to enhance the beneficial effects of the symbiosis (Sieverding, 1991). The selection of appropriate agronomic practices that enhance AM fungi is the only economically feasible way to increase farm productivity in large areas of the world (Sieverding, 1991) and especially in P-deficient soils supporting a large proportion of highly mycorrhizae dependent plants (i.e. legumes) (Abbott et al., 1995). The indigenous AM population is an indispensable prerequisite for predicting the potential benefit that can be achieved (Abbott et al., 1995).

In order to design a truly sustainable agricultural system that enhances the AM symbiosis, a holistic approach has to take into account the effect of native AM fungi, agrochemical inputs, crop rotation, soil disturbance, crop varieties, and the selection of appropriate fungal isolates to

be used as inoculants when necessary. Selective management of AM fungi can account for a reduction in less beneficial strains, shifting to the relative dominance of more efficient AM fungi (Abbott et al., 1994; Sieverding, 1991) and even favouring the establishment of AM fungi inoculated directly into the soil (Dodd and Thomson, 1994).

1.2.7 SELECTION OF ARBUSCULAR MYCORRHIZAL INOCULANTS

As mentioned above, conventional agricultural practices can select for non-efficient AM fungi. The main goal of the agricultural system to be designed will then determine the exact meaning of "effective strain". Mycorrhizal inoculation shows its greatest potential in areas where indigenous mycorrhizal populations have been reduced by human influence or natural disturbance, such as mine sites, badly eroded soils, reforestation areas (Dodd and Thomson, 1994), fumigated soils or after a long fallow (Hamel, 1996). Equally important is the potential benefit of AM inoculation on micropropagated plants (Varma and Schüep, 1995) and its effect on shortening acclimatization periods (Gianinazzi et al., 1995).

Soil characterization, isolation of AM fungi and screening procedures to effectively select and evaluate AM fungi from natural soils have been proposed by Dodd and Thomson (1994).

1.2.7.1 *Site characterization*

This implies the description of the edaphic and climatic conditions of the prospective site in order to define the range of environmental stresses that are likely to affect the inoculated plants. The use of indigenous fungi isolated from the same or a similar area is encouraged, to increase the chance of compatibility between symbionts (Dodd and Thomson, 1994).

1.2.7.2 *Collection and isolation of fungi*

An adequate description of the native AM fungi should be developed from soil samples taken from the rhizosphere of the representative vegetation at various developmental stages (Dodd and Thomson, 1994). There is an increased effort to keep available AM fungal germ plasm listed

both as on-line hypertext information (INVAM, http://invam.caf.wvu.edu/, Morton et al., 1993) and off-line, in expert systems (BEG, http://kiwi.ukc.ac.uk/biolab/beg/index.html/, Dodd and Rosendahl, 1996) through international collections of AM fungi.

1.2.7.3 *Screening of fungi.*

Dodd and Thomson (1994) suggested three steps to properly screen for appropriate AM fungi. Isolates should first be evaluated in sterile soil with saturating amounts of inoculum to avoid differences in growth responses due to unequal distribution of inoculum. A further test should be conducted in natural soil but under controlled conditions, in order to compare the competitive ability with other AM fungi and soil micro-organisms. Finally, field tests should be carried out to evaluate the competitive ability of the selected fungal strains in a natural setting and to verify their capacity to persist and spread on roots over the long run. A poor competitor, like *Glomus intraradices*, is likely to persist in the field for only one season after being introduced, although this response is highly dependent on the native AM fungi already present in the bulk soil (Harinikumar and Bagyaraj, 1996).

The conventional criteria for selecting good AM strains include improved P-absorption and transfer to the plant, increased plant growth, fast root colonization after inoculation, and formation of a large number of infective propagules (Abbott et al.,1992). Some other important features include the ability to perform well on a wide range of plant hosts, the rapid development of the extramatrical hyphae (Dodd and Thomson, 1994; Hamel, 1996), and the production of soil binding agents which may account for synergistic effects on soil stability (Schreiner and Bethlenfalvay, 1994).

AM symbioses are complex webs of interactions where neither the AM fungi, the soil, nor the host plant can be considered as isolated factors in determining the ideal conditions for the effective use of AM mycorrhizae in agriculture. Selection for plant-fungus combinations and their evaluation under targeted conditions is probably a preferable strategy to increase the number and quality of AM fungi in agricultural soils (Hamel, 1996).

1.2.8 PRODUCTION OF AM INOCULA

AM fungi are obligate biotrophs and must grow on living roots. Pot culture inoculum production is the standard procedure to obtain fresh material for AM inoculation (Gianinazzi et al., 1989; Severding, 1991). Root organ culture has also proven to be a reliable source of sterile material with the advantage of being a controllable and reproducible system (Piché et al., 1994). Initially, roots are genetically transformed with the Ri plasmid of *Agrobacterium rhizogenes*, then they are inoculated with surface-sterilized AM spores (Bécard and Piché, 1992). Relatively large numbers of spores and extraradical hyphae of *Gigaspora margarita* (Diop et al., 1992) and *Glomus intraradices* (Chabot et al., 1992a, St-Arnaud et al., 1996) were produced using this culture system.

The use of AM spores as a source of inoculum in the field is questionable because spores require several days to germinate and also show dormancy (Sieverding, 1991). Furthermore, the spore inoculum might not be able to compete with indigenous AM and other micro-organisms due to slow spread of the initial infection (Sieverding, 1991). Miller et al. (1995) also suggest that the source of infection in disturbed soils is through hyphae rather than spores. Multiplying AM fungi in pot cultures, on the other hand, has the advantage of permanently monitoring AM cultures by controlling the overall performance of the host plants while they grow (Gianinazzi et al., 1989).

The production of mixed AM inocula containing more than one isolate is a good alternative because it may account for differential responses over a broader range of environmental conditions, increasing the probability of a successful inoculation (Dodd and Thomson, 1994, Schreiner and Bethlenfalvay, 1995). Multiagent inoculants containing different micro-organisms (i.e. phosphate solubilizing bacteria and fungi, symbiotic and free-living nitrogen-fixing rhizobacteria, biocontrol agents, and disease-suppressive fungi) promise to carry a broader spectrum of potential benefits over a broader range of uses (Wood and Cummings, 1992).

1.2.9 POT CULTURES

Roots or soil can be used to inoculate roots of trap plants seeded in a sterilized substratum or soil in order to obtain crude field pot culture isolates (Menge, 1984). A main disadvantage of

pot-cultured AM inoculum is the concomitant risk of pathogen stimulation by adding fresh organic material into the soil, which can be an attractive source of nutrients for native micro-organisms (Siverding, 1991). The large volume of AM-infected roots required for field inoculation would represent a constraint for its large-scale use (Sieverding, 1991).

1.2.9.1 *Selection of host plants*

The success of a good pot-cultured inoculum production largely depends on the host plant and the environmental conditions of the greenhouse or growth chamber (Sieverding, 1991). These plants should be a good host, have a fast growth rate and have no pathogens in common with the final host (Menge, 1984). Sorghum and sudangrass are widely used in temperate areas but the former have a stronger root system and its seedlings are much less subject to transplant shock than sudangrass (Morton et al., 1993).

1.2.9.2 *Growth media*

The growth medium is also an important aspect of AM pot culture inoculum production. The standard growth medium is a coarse-textured low nutrient sandy soil with a high cation exchange capacity to reduce P availability (Menge, 1984) but Morton et al. (1993) recommended a sand-soil mix which is more versatile with increased aeration, water holding and cation exchange capacities. Fertilization should be kept to a minimum and be applied only when plants show signs of phosphorus or nitrogen deficiencies (Morton et al., 1993).

1.2.9.3 *Other factors*

Watering conditions are likely to affect pot-cultured AM inoculum production and it is preferable to under-water infected plants than to over-water them (Menge, 1984). Light intensity and photoperiod must be maximized, especially during the winter months, in order to produce good mycorrhizal colonization and spore development in pot cultures (Menge, 1984). High temperatures also increase AM colonization and spore production while pruning reduces

mycorrhizal activity and should be avoided (Menge, 1984). Finally, it is desirable to produce AM inoculum in large containers because host plant size and root biomass can be influenced by the amount of available soil and the pot volume (Menge, 1984).

1.2.9.4 *Quality control of AM pot-cultured inocula production*

Quality control in pot culture AM inoculum production is important in order to obtain pathogen-free material. This inoculum must also be able to tolerate variable conditions during and after its preparation (Dodd and Thomson, 1994). The following basic rules were proposed by Menge (1984) to produce clean, pathogen-free AM inoculum under greenhouse conditions:

1. Use sterile pots and growth media.
2. Start the culture with clean inoculum obtained from surface-disinfected spores on aseptically grown plants in petri plates or test tubes.
3. Test for non-target organisms and discard questionable cultures.
4. Alternate selected host plant species and always avoid using inoculum from one host species to inoculate the same plant species.
5. Practice sanitary greenhouse procedures (Menge, 1984).

The infectivity of the inoculum must be tested before its use. The simplest method is to count the number of AM spores present in the substrate (Feldmann and Idczak, 1992). The most probable number (MPN) method is a useful tool to quantify the number of infective propagules in a given substrate. This bioassay consists of the cultivation of a host plant in dilution series of the soil or the inoculum carrier (An et al., 1990). The choice of the host plant, the length of the incubation period, and the homogeneous distribution of propagules in the successive dilutions exert a dramatic influence on the results of this bioassay (Feldmann and Idczak, 1992).

The occurrence of common pests (Chytridiacous fungi, Phytium-like fungus, nematodes, flies, mites, spring tails, some insects, amoeba, and bacteria) affect the overall performance of the host plant and eventually affects directly the AM fungi and it is better to keep the plants free of them (Menge, 1984). Keeping the cultures for a longer period of time (more than 4-5 months), excessive or sloppy handling of plants, and water splashing during irrigation of the pots have

been identified as the main source of cross-contamination of AM cultures kept in the INVAM collection (Morton et al., 1993).

1.2.10 ON-FARM AM INOCULUM PRODUCTION STRATEGIES

The following steps were suggested by Sieverding (1991) for a low cost on-farm inoculum production system, suitable to small farmers and nurserymen:

1. Prepare 25 m^2 of an infertile land which must be cleared by turning the soil over and chopping it with a hoe to break up lumps.
2. Disinfect the soil with granular dazomet (Basamid at 50-60 g $\times$ m^{-2}). The soil should be watered, covered with a sheet of plastic and sealed for 10-14 days. After soil sterilization a small amount of inorganic fertilizer is recommended.
3. After the complete evaporation of the soil fumigant (10 days after the soil is uncovered) apply 5-10 g of mycorrhizal inoculum of an already selected AM isolate, pouring it in small holes. Planting distance depends on the choice of host plant; *Brachiaria decumbens* is suggested for tropical soils.
4. Water when necessary and clip flowers to prevent seed contamination of the inoculum.
5. After 4-6 months the host plant is harvested at the ground level. The soil substrate up to a 20 cm depth (including roots) is used as biofertilizer (Sieverding, 1991).

MATERIALS AND METHODS

2.1 POT INOCULUM PRODUCTION

Eight different AM fungal isolates and a commercial inoculum source (Table 1) were cultured with suitable host plants in order to produce a mix of roots, spores, and sand to be used as a source of inoculum for both greenhouse and subsequent field experiments. Leek (*Allium porrum*) infected roots of pure cultures of *Glomus intraradices* Schenck & Smith (GinN), *G. aggregatum* Schenck & Smith emend. Koske (GagN), and *G. etunicatum* Becker & Gerdemann (GetN) were provided by Yolande Dalpé from the National Herbarium (Ottawa, Canada) and were originally collected from native soils (NAT). Different Quebec agricultural sampled soils (AGR) were collected by Chantal Hamel and used as raw inoculum for the pot culture production. Only spores of *G. fasciculatum* (Thaxter) Gerd. & Trappe emend. Walker & Koske (GfaA) and *G. mosseae* (Nicol. & Gerd.) Gerdeman & Trappe (GmoA) were respectively found and identified by Y. Dalpé in two different soil samples. In order to evaluate the effect of different AM species in the same treatment, *G. aggregatum* Schenck & Smith emend. Koske (Gag+), *G. microaggregatum* Koske, Gemma & Olexia (Gmi+), and *Sclerocistis rubiformis* (Sru+) consisted of a mix of different fungal strains (Table 1).

A first set of pot cultures was started with surface sterilized (1 min in 70 % ethanol and 10 min in 30 % H_2O_2) sorghum seeds (*Sorghum vulgare*) sown on clean 15 cm plastic pots using autoclaved sand (126°C twice for 20 min). Later on, a similar set of pots was started using surface sterilized (1 min in 70% ethanol and 4 min in 30 % H_2O_2) marigold seeds (*Tagetes erecta* L.) to overcome the severe damage caused by several pests on sorghum seedlings. Another set of pots was arranged in 1996 using colonized pepper roots, obtained from the 1995 greenhouse experiment, to inoculate surface sterilized (1 min in 70 % ethanol and 10 min in 30 % H_2O_2) sorghum seeds. This was done in order to check the infectivity and purity of the AM isolates.

The inoculum, which consisted of 1 g of colonized roots with sand and soil, from each one of the above mentioned AM strains, was placed 5 cm below the seeds. Pots were carefully watered. A low P modified Hoagland's nutrient solution (Hamel, 1991) was added weekly. Each week plants were carefully washed with Safer soap (Safer Ltd., Scaraborough, ON MLB 2K4) to avoid the development of thrips, white flies and other common greenhouse pests. Plants were grown for 4 months and then allowed to dry prior to harvest. A mix of sand and chopped roots from the pots of this work was used as source of inoculum for subsequent experiments.

Table 1. List of AM strains obtained from: natural (NAT) and agricultural (AGR) soils.

Code	AM species	Source
GagN	*G. aggregatum*	NAT #2279 (Y. Dalpé)
GinN	*G. intraradices*	NAT #3089 (Y. Dalpé)
GetN	*G. etunicatum*	NAT (Y. Dalpé)
GfaA	*G. fasciculatum*	AGR (C. Hamel)
GmoA	*G. mosseae*	AGR (C. Hamel)
Gag+	*G. aggregatum* mixed with *G. geosporum*	AGR (C. Hamel)
Gmi+	*G. microaggregatum* mixed with *G. mosseae* and *G. fasciculatum*	AGR (C. Hamel)
Sru+	*S. rubiformis* mixed with *G. fasciculatum*	AGR (C. Hamel)
GinA	*G. intraradices*	AGR (Premier Tech)

2.2 GREENHOUSE EXPERIMENTS

Both sweet corn (*Zea mays* L. cv. Bicolor) and pepper (*Capsicum frutescens* L. cv. North Star) were each inoculated with one of the 9 AM strains (Table 1). A non-mycorrhizal control, which received autoclaved (126°C for 20 min) marigold roots was also included. The growth medium for the 1995 experiment consisted of a mix of 4 parts pasteurized soil (30 min at 72°C)

(Thompson, 1990), and 1 part autoclaved sand (126°C twice for 20 min). This substratum contained, on average, 16.1 mg of P and 45.3 mg of K per kg of growth medium (Mehlich 3). The initial pH (water) was 6.0 and the organic matter content (Walkley-Black) was 20.31 %. The growth medium for the 1996 experiment consisted of a pasteurized sandy soil (30 min at 72°C) which contained, on average, 152 mg of P and 108.9 mg of K per kg of sample (Mechlich 3). This soil was sampled from an agricultural field which explains its higher nutrient content compared with the 1995 growth medium. The initial pH (water) was 6.2. Average temperature was 25°C and a 15 hour-photoperiod was supplemented with 400 Watt high pressure sodium lamps. These experiments were carried at the Macdonald Campus of McGill university greenhouse facilities during 1995 and 1996.

For each crop species, the experiment consisted on a randomized complete block design with 5 and 2 blocks in the 1995 and 1996 experiments, respectively. Blocks were arranged on two separate benches in the greenhouse. Each block had 1 and 2 replicates of each treatment for the 1996 and 1995 experiments, respectively. In 1995, plants were inoculated with colonized roots and sand from the marigold pot cultures for each one of the 8 mycorrizal strains plus the commercial inoculum of *G. intraradices* (Premier Peat Ltd., C.P. 2600, G5R 4C9 Canada), which was originally cultured from an agricultural field isolate (AGR). In 1996, plants were inoculated with colonized roots obtained from each one of the treatments from the 1995 greenhouse pepper experiment, including a non-mycorrhizal control treatment. Pots were re-randomized weekly within each block. Sweet corn was sown on May 05 and June 14, and harvested on August 11 and September 11 for the 1995 and 1996 experiments, respectively. Pepper seedlings were transplanted into pots on July 17 and July 08, and harvested on October 23 and September 26 for the 1995 and 1996 experiments, respectively. The first set of sweet corn plants of 1995 (7 weeks after sowing) was harvested and roots were used to supplement the colonized marigold roots used as the inoculum source for subsequent experiments.

2.2.1 *Plant material*

Corn seeds were surface sterilized (1 min in 70 % ethanol and 10 min in 30 % H_2O_2) and 5 seeds were sown in 10 L plastic pots. One gram of the inoculum was placed 5 cm below the

seeds that were later thinned to one plant per pot. Surface sterilized pepper seeds (1 min in 70 % ethanol and 10 min in 30 % H_2O_2) were sown several days before transplanting in plastic trays in a very low P peat moss growth medium (SB-mix, Premier Peat Ltd.). The inoculum source was uniformly spread beneath the pepper seeds. Uniform seedlings were later transplanted into 15 cm clean plastic pots and also used in the field experiment. In order to equalize microbial populations in all the non-mycorrhizal control treatments, 100 ml of a solution of filtered (37 µm mesh) inoculum washing was added to each one of the pots. All plants were carefully watered and nutrients were added weekly or when plants showed deficiency symptoms using a low P modified Hoagland's solution (Hamel, 1991).

2.2.2 *Sampling methodology*

The height of the plants was measured as the distance from cotyledons to terminal buds, and the distance from the ground to the terminal extreme of the longest fully extended leaf, for pepper and sweet corn, respectively. Plant height was recorded weekly until the plants were harvested, 11 weeks after sowing (sweet corn) and the 13 weeks after transplanting (pepper). At the final harvest, fruits (if present) and the remaining aboveground material were separated for each pot, dried (65 °C for 48 hours) and weighed. Shoot P levels in wet digested material (Thomas *et al.*, 1967) obtained from mature leaf blades with petioles and ear leaf blades of pepper and sweet corn, respectively, were measured at the final harvest by colorimetry (Murphy and Riley, 1962). In 1995, the first set of pepper plants (5 weeks after transplanting) was harvested for AM colonization assessment. A sub-sample of each root system was taken at each harvest, washed free of soil, and kept in 50 % ethanol until processing. These roots were later cleared at 126 °C for 15 min. in 10 % KOH and stained with acid fushin (Brundrett, 1994). Percentage of root mycorrhizal colonization was determined by the grid intersect method observing 100 root intersections under a dissecting microscope (× 50) (Giovanetti and Mosse, 1980). The relative mycorrhizal dependency (RMD) is the difference between the dry mass of inoculated plants and the dry mass of non-mycorrhizal plants expressed as a percentage of the dry mass of the mycorrhizal plant and was calculated as described by Plenchette *et al.* (1983).

2.2.3 *Statistical analysis*

For each crop species, analysis of variance was conducted to test the effects of different AM isolates. Means were compared using the least significant difference multiple comparisons test ($LSD_{0.05}$) at a 95 % confidence level. Prior to statistical analysis, normality assumption was tested. Percentage root mycorrhizal colonization data were arc sine-transformed, which prevents the variance from being a function of the mean (Gomez and Gomez, 1984). For each crop species, 1995 and 1996 data were pooled to test for differences between years. Due to differences in the number of blocks, homogeneity of variances was carefully checked prior to analyses and a logarithmic transformation was applied whenever necessary (Gomez and Gomez, 1984). Orthogonal contrasts were performed by partitioning the sum of squares according to a selected pair of group comparisons (Winer *et al.*, 1991). This was done in order to test for differences in plant growth between particular treatments. The repeated measures statistical analysis was used to evaluate height data as a time series (Crowder and Hand, 1990). Analyses were performed with SAS (SAS, 1988).

2.3 FIELD EXPERIMENTS

Both sweet corn and pepper were inoculated in non-fumigated soils with the 9 different AM fungal isolates listed in Table 1 and a non-mycorrhizal treatment containing a mix of autoclaved (126°C for 20 min) marigold and sweet corn roots. Experimental fields were located in 4 different sites at the Horticultural Research Center and the Emile Lods Agricultural Research Center, both of McGill University (Ste-Anne-de-Bellevue, Québec). All sites were < 5 Km from each other. Figure 1 shows the monthly average precipitation (1995) and the normal precipitation estimated for the 1961-1990 period and measured at the Montréal International airport (Dorval), located at less than 20 km from the field experiments.

2.3.1 *Experimental design*

For each crop species, the experimental design was a 4 by 10 factorial experiment arranged in a randomized complete block design with 4 blocks and 1 replicate per block. Four different sites for each crop species were selected depending on the previous crop and various soil chemical characteristics (Table 2).

Table 2. Soil variables and cropping history of the experimental study sites.

Crop	Site #	pH[a]	P (mg kg^{-1})[b]	K (mg kg^{-1})	Organic matter (%)[c]	Cropping history
Corn	1	6.7	21.7	72.8	4.7	Corn
Corn	2	7.1	177.7	162.7	3.3	Corn
Corn	3	7.4	187.9	235.3	5.7	Fallow
Corn	4	7.0	384.8	235.7	3.0	Lupin
Pepper	5	6.9	424.1	263.8	3.2	Raspberry and buckwheat
Pepper	6	7.0	384.8	235.7	3.0	Lupin
Pepper	7	7.2	195.5	224.1	5.5	Corn
Pepper	8	7.5	167.6	215.0	3.1	Fallow

[a]Soil pH (water)
[b]Soil available phosphorus (Mehlich 3)
[c]Soil organic matter (Walkey-Black)

2.3.1.1 ***Sweet Corn.*** Site 1 was a Chicot fine sandy loam soil with a pH (water) of 6.7 and a 4.7 % organic matter content (Walkey-Black); corn was the previous crop. The soil contained 21.7 mg of P and 72.8 mg of K (Mehlich 3) per kg of soil. Sites 2 and 3 were both a Chateauguay clay loam. Corn was the previous crop for site 2, which contained 177.7 mg of P and 162.7 mg of K (Mehlich 3) per kg of soil. Soil pH (water) was 7.1 and organic matter content was 3.3 % (Walkey-Black). Site 3 was fallow the year before and the soil contained 187.9 mg of P and 235.3 mg of K (Mehlich 3) per kg of soil. Soil pH (water) was 7.4 and organic matter content was 5.68 % (Walkey-Black). Site 4 was a St. Bernard sandy loam under organic management (no agrochemicals applied) and planted with lupine the year before. The soil contained 384.8 mg of P

and 235.7 mg of K (Mehlich 3) per kg of soil. Soil pH (water) was 7.0 and organic matter was 3 % (Walkey-Black).

Fertilization rates for sweet corn were those recommended by the Conseil des Productions Végétales du Québec (Anonymous, 1994) and were adjusted for each site. Site 1 received 210 kg ha^{-1} and 95 kg ha^{-1}, site 2 received 30 kg ha^{-1} and 65 kg ha^{-1}, and site 3 received 30 kg ha^{-1} and 40 kg ha^{-1} of P_2O_5 and K_2O, respectively. The applied N rate was 120 kg ha^{-1} for these three sites. Only N (blood meal) was applied in site 4 at the recommended rate of 120 kg ha^{-1}.

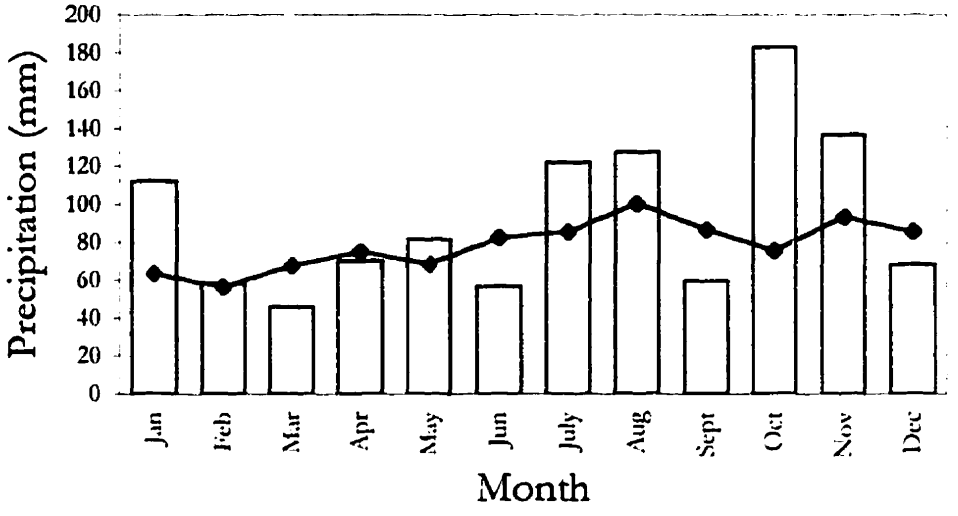


Fig. 1. Monthly (♦) precipitation in 1995 and the 30-year average precipitation (1961-1990) at the Montréal International airport (Dorval).

The experimental field at each site was 20 m × 22.5 m in total. Each plot was 4 m × 2.25 m and consisted on 2 non-inoculated border rows and 1 inoculated row in the center with an inter-row distance of 0.75 m and an inter-plant distance of 0.20 cm. Weeds at sites 1, 2, and 3 were controlled with Round-up (Glyphosate isopropylammonium), a non-selective postemergence herbicide (Monsanto, Mississaugua, ON, Canada). Inoculated rows were planted manually and 1 g of inoculum was placed 5 cm under each seed. Non-inoculated rows were planted mechanically. Supplementary weed control, between rows, was performed by hoeing and rototilling.

2.3.1.2 *Pepper.* Site 5 was on a St. Bernard clay loam soil planted with raspberry and buckwheat the year before. Soil pH (water) was 6.9 and organic matter content (Walkey-Black) 3.2 %. The soil contained 424.1 mg of P and 263.8 mg of K (Mehlich 3) per kg of soil. Site 6 was on a St. Bernard sandy loam under organic management (no agrochemicals applied) and planted with lupine the year before. The soil contained 384.8 mg of P and 235.7 mg of K (Mehlich 3) per kg of soil. Soil pH (water) was 7.0 and organic matter content was 3.0 % (Walkey-Black). Sites 7 and 8 were both on a Chateauguay clay loam. Site 7 was planted with corn the year before and the soil contained 195.5 mg of P and 224.1 mg of K (Mehlich 3) per kg of soil. Soil pH (water) was 7.2 and organic matter content was 5.5 % (Walkey-Black). Site 8 was fallow the year before and the soil contained 167.6 mg of P and 215 mg of K (Mehlich 3) per kg of soil. Soil pH (water) was 7.5 and organic matter content was 3.1 % (Walkey-Black).

Fertilization rates for pepper followed those recommended by the Conseil des Productions Végétales du Québec (Anonymous, 1994) and were adjusted for each site. Site 5 received 80 kg ha^{-1} of N-P-K 19-19-19 in the early spring. Site 6 was amended with blood meal at a rate of 70 kg ha^{-1}. Sites 7 and 8 received 40 kg ha^{-1}, 80 kg ha^{-1}, and 70 kg ha^{-1} of P_2O_5, K_2O, and N, respectively.

The experimental field in each site was 20 m × 8 m in total. Each plot was 2 m × 1.6 m and consisted on 2 inoculated rows with an inter-row distance of 0.45 m and the inter-plant distance was 0.45 m containing 7 plants per plot. The fertilizer was broadcast on the soil surface and the land was rototilled prior to laying a black embossed plastic mulch (Plastitech, St-Remi, Québec). A drip irrigation line was installed beneath the mulch and uniform pre-inoculated pepper seedlings were transplanted into the field. The irrigation regime was, in general, 6 hours every 4 days but changed somewhat with the amount of rainfall. Weed control between rows was performed by combination of hand hoeing and rototilling.

2.3.2 *Sampling methodology*

2.3.2.1 *Sweet corn.* Sweet corn plants were grown for 11 weeks in 1995. Site 4 was discarded since no seed germinated, probably due to the presence of a pathogenic fungi in this soil. At site 1, a first root harvest was conducted 6 weeks after sowing and 9 weeks after sowing at sites 2 and

3. Ten random samples were taken on inoculated rows with a fine soil core. The second and final root harvest was done by excavating 5 plants and taking samples from the root systems. Roots were separated, washed free of soil, and kept in 50 % ethanol until processing. A sub-sample was taken and roots were cleared at 126 °C for 15 min. in 10 % KOH and stained with acid fushin (Brundrett, 1994). Percentage of root mycorrhizal colonization was determined by the grid intersect method observing 100 root intersections under a dissecting microscope (× 50) (Giovanetti and Mosse, 1980). Plant height was measured as the distance from the ground to the uppermost exposed ligule. This was recorded weekly until the tasseling stage by measuring 5 plants randomly chosen from each plot. Ear leaf blades were randomly sampled at the tasseling stage (5th week for site 1 and 10th week for sites 2 and 3), dried at 65 °C for 48 hours, and milled for colorimetric shoot P determination (Murphy and Riley, 1962) in wet digested material (Thomas *et al.*, 1967). Corn ears and the remaining aboveground material from 5 consecutive plants, randomly sampled in each plot, were separated, dried (65 °C for 48 hours) and weighed. Sweet corn yield (kg plant^{-1}) and shoot dry mass per plant (g) were measured at the final harvest, 11 weeks after sowing. A mixed sample of soil from each non-mycorrhizal control treatment was taken by site at the end of the growth season in order to identify the AM species present in the bulk soil (native fungi).

2.3.2.2 ***Pepper.*** Pepper plants were grown in the field for 12 weeks in 1995. A first root harvest was carried out 6 weeks after transplanting by taking 10 random samples on the inoculated row with a fine soil core. The second and final root harvest was done by excavating 5 plants and taking samples from their root systems. These roots were washed free of soil and kept in 50 % ethanol until processing. Percentage of root mycorrhizal colonization was determined by the grid intersect method, observing 100 root intersections under a dissecting microscope (× 50) (Giovanetti and Mosse, 1980) after clearing and staining with acid fushin (Brundrett, 1994). Pepper height was measured as the distance from cotyledons to terminal buds and was determined weekly in 5 random plants per plot until the plants reached a stable size. Mature leaf blades were randomly collected at the 15th week, dried at 65 °C for 48 hours and milled for colorimetric P determination (Murphy and Riley, 1962) in wet digested material (Thomas *et al.*, 1967). Fruits and the remaining aboveground material from the whole plot were separated, dried

(65 °C for 48 hours) and weighed. Pepper yield (kg plant^{-1}) and shoot dry mass per plant (g) were measured at the final harvest, 19 weeks after sowing. A mixed sample of soil from each non-mycorrhizal control treatment was taken by site at the end of the growth season in order to identify the AM species present in the bulk soil (native fungi).

2.3.3 *Statistical analysis*

For each crop species, analysis of variance was used to test the effects of different AM isolates by site. The number of plants per plot was recorded for each site and crop for use as a possible covariate in the analysis of yield and shoot dry mass. Means were compared using the least significant difference multiple comparisons test ($LSD_{0.05}$) at a 95 % confidence level. Prior to statistical analysis, the normality assumption was tested. Percentage of root mycorrhizal colonization was arc sine-transformed transformed, which prevents the variance from being a function of the mean (Gomez and Gomez, 1984). For each crop, results from different sites were pooled in order to estimate the range of adaptability of AM strains. Homogeneity of variances was carefully checked using the chi-square (Bartlett's) test prior to analyses and the logarithmic transformation was applied whenever it was necessary (Gomez and Gomez, 1984). The repeated measures statistical analysis was used to evaluate height data as a time series (Crowder and Hand, 1990). Analyses were performed with SAS (SAS, 1988).

RESULTS AND DISCUSSION

3.1 POT INOCULUM PRODUCTION

The *G. etunicatum* (GetN) isolate obtained from a natural soil developed the largest percentage of mycorrhizal colonization in marigold plants (Table 3). Very few spores were detected in the marigold pot cultures which prevented checking the purity of AM isolates. A very low percentage of mycorrhizal colonization on pepper seedlings (Table 3) probably reflects the lag phase, which consists of a slow initial establishment of the infection when the growing root encounters an appropriate mycorrhizal propagule in the soil (Mosse et al., 1981). A mix of *G. aggregatum* and *G. geosporum* developed the largest percentage of mycorrhizal colonization in sorghum plants inoculated with colonized pepper roots during the 1995 greenhouse experiment (Table 3). Number of AM infective propagules were not counted for any of the pot cultures included in this study.

3.2 GREENHOUSE EXPERIMENTS

3.2.1 *Sweet corn*

The inoculum source explained a significant amount of the variance in AM colonization for the 1995 ($P < 0.001$) and 1996 ($P < 0.05$) sweet corn experiments (Table 4). Plants inoculated with the commercial inoculum of *G. intraradices* (GinA), formerly isolated from an agricultural soil, developed the largest AM colonization in both years (Fig. 2a, b). *G. mosseae* (GmoA) and the *G. intraradices* strain isolated from a natural soil (GinN) also had a high proportion of AM colonization although less than the commercial *G. intraradices* inoculum (Fig. 2a, b). Interestingly, the proportion of colonized roots in plants inoculated with the *G. intraradices* strain from a natural

soil (GinN) was significantly lower than the corresponding values in plants inoculated with the other strain of the same species (GinA) for the 1995 experiment. (Fig. 2a).

Table 3. Percentage mycorrhizal colonization of marigold, pepper seedlings, and sorghum roots inoculated with 9 different AM strains under controlled conditions.

		Mycorrhizal colonization (%)		
Code	Inoculum source	Marigold[a]	Pepper[b]	Sorghum[c]
GagN	*G. aggregatum*	54	1	19
GinN	*G. intraradices*	68	2	39
GetN	*G. etunicatum*	74	9	35
GfaA	*G. fasciculatum*	44	1	23
GmoA	*G. mosseae*	28	0	46
Gag+	*G. aggregatum* mixed with *G. geosporum*	19	0	57
Gmi+	*G. microaggregatum* mixed with *G. mosseae* and *G. fasciculatum*	65	3	32
Sru+	*S. rubiformis* mixed with *G. fasciculatum*	42	0	33
GinA	*G. intraradices*	n.a.	0	58

[a]Marigold roots (1995) inoculated with the original source of inoculum
[b] 7th week old inoculated pepper seedlings (1995) transplanted into the field
[c]Sorghum roots (1996) inoculated with colonized pepper roots obtained from the 1995 greenhouse experiment

At harvest in the 1995 experiment, the inoculum source accounted for a significant amount of the variance in height ($P < 0.01$) and the $\log_e$ transformed shoot dry mass ($P < 0.001$) (Table 4). In the same year, heights of plants inoculated with *G. intraradices* (GinA) were the largest, although not significantly higher than non-mycorrhizal plants (Fig. 3a). A lack of growth response in sweet corn height to AM inoculation was also found by Olsen *et al.* (1996) in plants inoculated with a mix of *G. mosseae* and *G. aggregatum* at different P rates. On the other hand, in this experiment, the height of sweet corn plants inoculated with *G. intraradices* (GinN) and *G. aggregatum* (GagN) was significantly smaller than the height of non-mycorrhizal control plants and those inoculated with *G. intraradices* (GinA) (Fig. 3a).

Table 4. *F* ratios from the MANOVA approach of the time repeated measures (TRM) on height and the ANOVA test for inoculum effects on sweet corn shoot dry mass, shoot P, height at harvest, relative mycorrhizal dependency (RMD), and AM colonization for the 1995 and 1996 experiments under controlled conditions.

	Source of variance		
	Main effects		Interactions
Response variable	Inoculum	Time	Time*inoculum
Sweet corn 1996			
AM colonization (final harv.)	4.74*		
Shoot dry mass	2.37		
Shoot P (final harv.)	8.42**		
Height at harvest	0.68		
Height (TRM) until final harv.[a]	0.96	1185.07***	1.00
Rel. myc. Dependency (RMD)	2.31		
Sweet corn 1995			
AM colonization (final harv.)	10.23***		
Shoot dry mass	6.76***		
Height at harvest	3.70**		
Height (TRM) until final harv.[a]	2.72***	2646.30***	2.742***
Rel. myc. Dependency (RMD)	6.83***		

*,**,***, indicate that *F* ratios were significant at $P \leq .05$, .01, and .001, respectively

[a]*F* equivalent value from the Wilks' Lambda statistics of the MANOVA approach of the time repeated measures

In 1995, the shoot dry mass of plants inoculated with a mix of *G. microaggregatum*, *G. mosseae*, and *G. fasciculatum* (Gmi+) was the largest (Fig. 3b). The same trend, but not significantly different, was observed in 1996 when plants inoculated with the same mix of AM species (Gmi+) weighed more than the other treatments (data not shown). The occurrence of several species and hence, a more diverse temporal pattern of colonization in roots could be related to the phosphorus requirements of sweet corn, accounting for the observed increase in shoot dry mass in plants inoculated with Gmi+. The use of mixed AM inocula under field conditions may also enhance the persistence of the introduced strains and this is especially important in revegetation and rehabilitation studies (Dodd and Thomson, 1994).

There was a depression of shoot dry mass for plants inoculated with *G. intraradices* (GinN) and *G. aggregatum* (GagN) in the same year compared with non-mycorrhizal control plants (Fig. 3b). Reductions of corn shoot dry mass has been reported previously, especially at high and

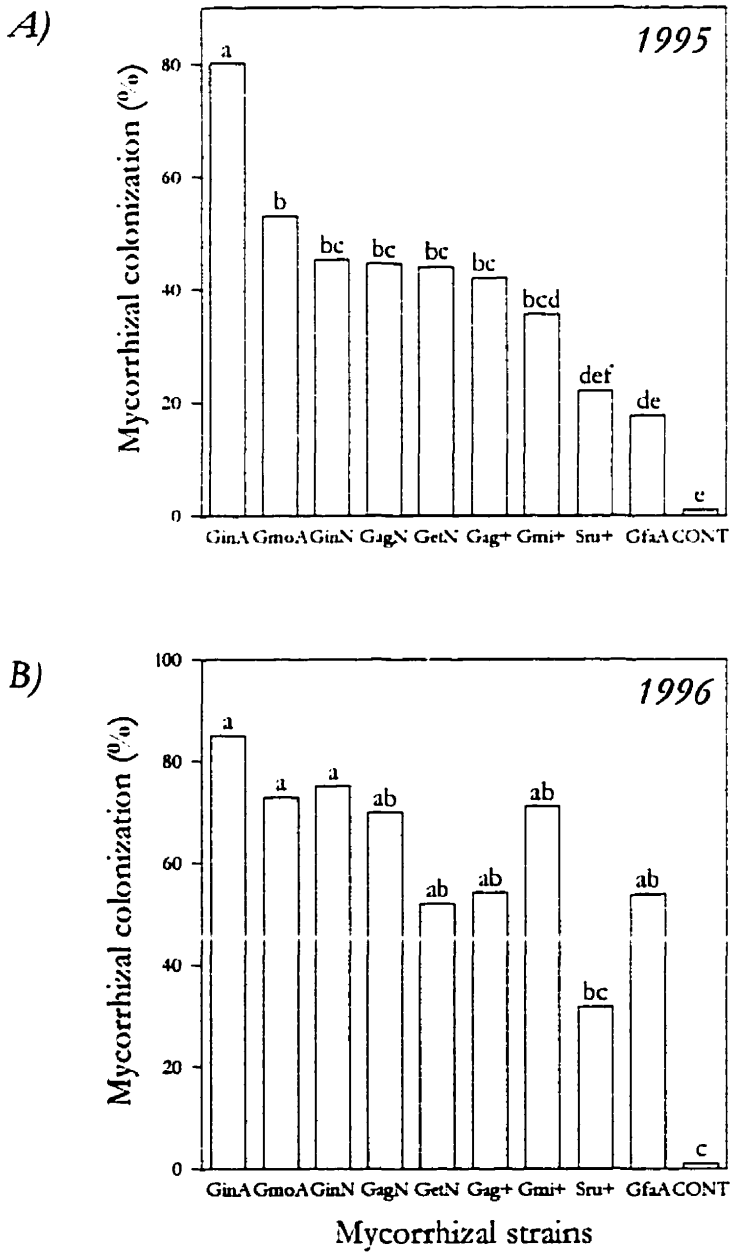


Fig. 2. Mycorrhizal colonization in sweet corn plants inoculated with 9 different mycorrhizal strains and a non-mycorrhizal control treatment under controlled conditions in: (a) 1995 and (b) 1996. Data points represent average values of 5 and 2 plants, for (a) and (b), respectively. Bars with different letters differ at a .05 level.

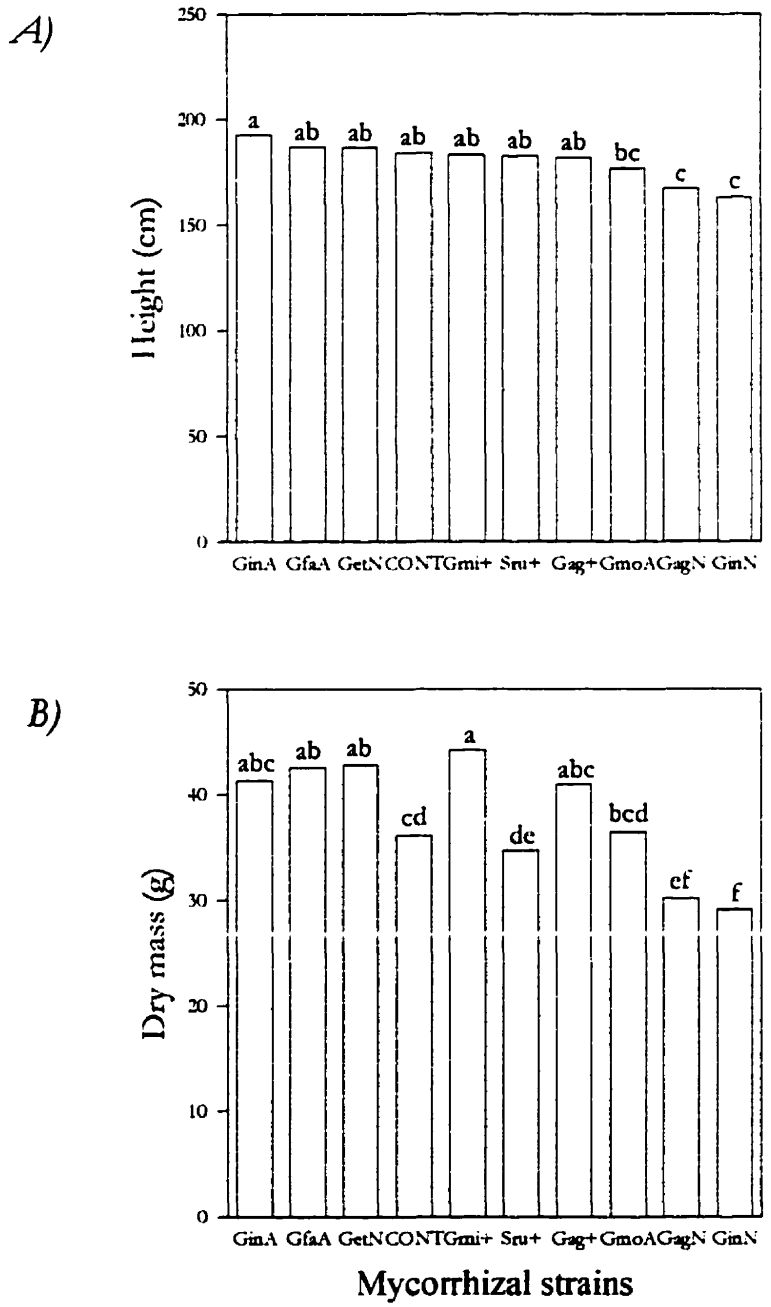


Fig. 3. Responses of sweet corn plants to inoculum treatments at harvest for the 1995 experiment under controlled conditions: (a) Height, (b) shoot dry mass. Data points represent average values of 5 plants. Bars with different letters differ at a .05 level.

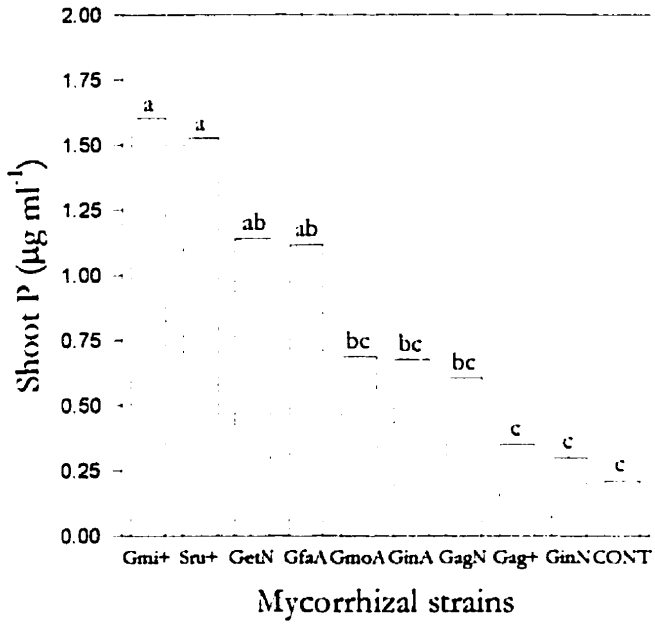


Fig. 4. Shoot P content at harvest in sweet corn plants inoculated with 9 different mycorrhizal strains and a non-mycorrhizal control treatment in 1996 under controlled conditions. Data points represent average values of 2 plants. Bars with different letters differ at a .05 level.

moderate P levels (Hetrick *et al.*, 1984, Olsen *et al.*, 1996). Additionally, shoot dry mass for plants in this experiment, inoculated with *G. intraradices* (GinA) in the same year, was significantly greater than the corresponding values of plants inoculated with the other strain of the same species (GinN) (Fig. 3b).

The inoculum source accounted for a significant amount of the variance in shoot P ($P < 0.01$) in the 1996 sweet corn experiment (Table 4). In 1995, the incidence of several pests caused severe damage on corn leaf tissue at harvest which prevented shoot P measurement. In 1996, the orthogonal contrast for shoot P between the effect of all the mycorrhizal strains and the non-mycorrhizal control treatment was significant ($P < 0.05$). Plants inoculated with a mix of *G. microaggregatum*, *G. mosseae*, and *G. fasciculatum* (Gmi+) had the largest amount of shoot P levels (Fig. 4). As mentioned above, height and shoot dry mass were the highest, but not significantly different, in sweet corn plants inoculated with the same mix of AM species (Gmi+) in 1996 (data

not shown). Olsen et al. (1996) also found that a higher shoot P for sweet corn, inoculated with a mix of *G. mosseae* and *G. etunicatum*, was not associated with a significant increase of shoot dry mass, even at a low P rate. It is interesting to note that inoculation in this experiment with both strains of *G. intraradices* only caused a slight increase in shoot P, which was not significantly different from non-mycorrhizal plants (Fig. 4). At the same time, AM colonization was the highest in plants inoculated with both strains in 1996 (Fig. 2b).

The inoculum source accounted for a significant amount of the variance for sweet corn relative to mycorrhizal dependency for the 1995 experiment ($P < 0.001$) (Table 4). A mixed inoculum *of G. microaggregatum*, *G. mosseae*, and *G. fasciculatum* (Gmi+) resulted in the greatest increase in shoot dry mass compared with the non-mycorrhizal control in 1995. As a consequence, this inoculum source (Gmi+) showed the highest RMD on inoculated plants in 1995 (Table 5). Negative RMD values corresponded to a lower shoot dry mass of inoculated plants than the corresponding value for non-mycorrhizal plants. Thus, this was indicative of a detrimental effect on sweet corn growth due to the effect of the respective mycorrhizal strains. A significant difference in the RMD of sweet corn, between plants growing at the same P level and inoculated with different fungal strains, indicated that different AM species, and even geographical isolates, exerted a differential response on the degree to which sweet corn depended upon the mycorrhizal association, measured under these experimental conditions.

The temporal repeated measures on height for the 1996 experiment showed no effect of the inoculum source on the growth rate of sweet corn plants (Table 4). In 1995, the inoculum source accounted for a significant amount of the variance in plant height ($P < 0.001$) and the interaction between time and inoculum source ($P < 0.001$) (Table 4). Thus, the effect of different AM strains on height varied with time and non-mycorrhizal plants were the shortest until the 7th week (Fig. 5 a,b). All the treatments displayed a sigmoidal response curve and 7 weeks after transplanting, the growth rate of control plants increased significantly compared with plants inoculated with *G. intraradices* (GinN) and *G. aggregatum* (GagN) (Fig. 5b). This could indicate a drain of C to fungal structures and a reduction in growth of plants inoculated with these strains, especially during sweet corn reproductive stages.

Significant differences were found between sweet corn plants inoculated with both strains of *G. intraradices* (GinA and GinN). Orthogonal contrasts showed that in 1995, plants inoculated

Table 5. Relative mycorrhizal dependency (RMD) of sweet corn and pepper inoculated with 9 different mycorrhizal strains, under controlled conditions in 1995 and 1996.

		Sweet corn		Pepper	
Code	Inoculum source	1995[ab]	1996[c]	1995[b]	1996[c]
Gmi+	*G. micronaggregatum* mixed with *G. mosseae* and *G. fasciculatum*	17.44a	−4.24	−17.89	−25.20
GetN	*G. etunicatum*	15.11ab	−84.17	−17.76	−13.31
GfaA	*G. fasciculatum*	14.47ab	−4.64	−4.72	−20.02
GinA	*G. intraradices*	12.29ab	−57.43	−23.58	−41.94
Gag+	*G. aggregatum* mixed with *G. geosporum*	11.23abc	−21.65	−17.83	−18.90
GmoA	*G. mosseae*	−1.03bc	1.26	−17.31	−47.54
Sru+	*S. rubiformis* mixed with *G. fasciculatum*	−6.08cd	−24.78	−23.12	−5.68
GagN	*G. aggregatum*	−20.69d	−19.16	−16.67	−20.92
GinN	*G. intraradices*	−25.26d	−63.04	−15.81	−11.40

[a]Means followed by different letters differ at .05 level
[b]Data represent average values of 5 plants
[c]Data represent average values of 2 plants

with the isolate formerly collected from an agricultural soil (GinA), developed a greater AM colonization ($P < 0.001$), were taller ($P < 0.001$), weighted more ($P < 0.001$), and shown a higher RMD ($P < 0.001$) compared with plants inoculated with the isolate from a natural soil (GinN). The same trend, although not significantly different, was observed in 1996 for AM colonization, shoot dry mass, shoot P, and RMD. These differences could be due either to changes in the amount and quality of the used inoculum or indicate an inherently different mutualistic capacity of the compared strains. Fertilizing low nutrient soils is thought to favour the proliferation of inferior AM mutualists (Johnson, 1993). There is also evidence that AM isolates obtained from low P soils are more beneficial for plant growth than isolates obtained in richer soils (i.e. Henkel *et al.*, 1989, Louis and Lim, 1987). In this experiment, a more beneficial effect of GinA compared

with GinN, is difficult to explain since the exact location and soil characteristics where the strains were obtained are unknown.

The commercial inoculum of *G. intraradices* (GinA) used in the 1995 experiment was a preparation which consisted on a peat moss-based material containing spores and colonized roots. On the other hand, the same treatment (GinA) used in 1996 consisted of colonized pepper roots and sand obtained from the preceding year's greenhouse experiment. The commercial inoculum (GinA) may have had a higher number of infective propagules compared with the other strain (GinN) in 1995, which consisted of colonized marigold roots. This, in turn, could explain why GinA caused the highest mycorrhizal colonization in sweet corn and a better plant response to inoculation than GinN. The advantages of using carriers other than the bulk soil for infective mycorrhizal structures (roots, hyphae, spores), have encouraged the development of alternative inoculum production systems. Moreover, the use of peat and expanded clay as carriers increases the viability and infectivity of AM propagules for a very long time (Dehne and Backhaus, 1986).

When pooling the data from the 1995 and 1996 experiments, the year effect accounted for a significant amount of the variance in plant height at harvest ($P < 0.001$), shoot dry mass ($P < 0.001$), AM colonization ($P < 0.01$), and RMD ($P < 0.01$). A significantly higher overall shoot dry mass, plant height, and mycorrhizal colonization were measured in 1996 (Table 6). Nutrient levels of the growth medium in 1996 (152 and 108 mg kg^{-1} for P and K, respectively) were higher than those measured in 1995 (16.1 and 45.3 mg kg^{-1} for P and K, respectively) which can explain the better growth response of sweet corn. The overal RMD, on the other hand, was significantly higher in 1995 (Table 6) when the growth medium contained a much lower P level. This could be indicative of a better plant growth response to inoculation in a lower P level. Moreover, a higher overall AM colonization in 1996 did not represent a more beneficial effect on sweet corn growth response in colonized plants since the relative increase in shoot dry mass due to inoculation, compared with non-inoculated plants (RMD), was higher in 1995 at a lower mycorrhizal colonization (Table 6).

Table 6. Sweet corn and pepper overall response to inoculation for the 1995 and 1996 experiments under controlled conditions. Values with different letters, in the same row and within each crop species differ at the .05 level.

	Sweet corn		Pepper	
Response variable	1995[a]	1996[b]	1995[a]	1996[b]
Height at harvest (cm)	180.05b	209.49a	35.09	38.26
Shoot dry mass (g)	37.63b	91.80a	11.88a	7.66b
Fruit dry mass (g)			6.86a	3.30b
AM colonization (%)	43.76b	64.12a	51.83	46.54
Shoot P ($\mu g\ ml^{-1}$)			0.90b	3.72a
RMD (%)	1.94a	−30.87b	−17.19	−22.77

[a]Data represent average values of 45 plants
[b]Data represent average values of 18 plants

3.2.2 *Pepper*

The inoculum source accounted for a significant amount of the variance in AM colonization for 1996 ($P < 0.001$) and both harvests during the 1995 pepper experiment ($P < 0.001$) (Table 7). In 1995, the percentage of colonized roots was higher at the second harvest (13 weeks after transplanting) than values measured at the first harvest (5th week) (Fig. 6a,b). This can be explained by the lag phase, when roots become colonized at a slow rate, depending on the amount of mycorrhizal inoculum present in the soil (Mosse et al., 1981). In the same year, *G. intraradices* (GinA) developed the largest mycorrhizal colonization on roots at the first harvest (Fig. 6a). At the same time, both the same strain (GinA) and the mix of *S. rubiformis* and *G. fasciculatum* (Sru+) induced the greatest AM colonization at the second harvest (Fig. 6b). In 1996, the same mix of *S. rubiformis* and *G. fasciculatum* (Sru+) developed the largest mycorrhizal colonization on roots of inoculated plants (Fig. 7).

The *G. fasciculatum* (GfaA) treatment developed a remarkably low level of mycorrhizal colonization. The orthogonal contrast of AM colonization in plants inoculated with this strain (GfaA), compared with the effect of the rest of the fungal isolates, was significant in 1996

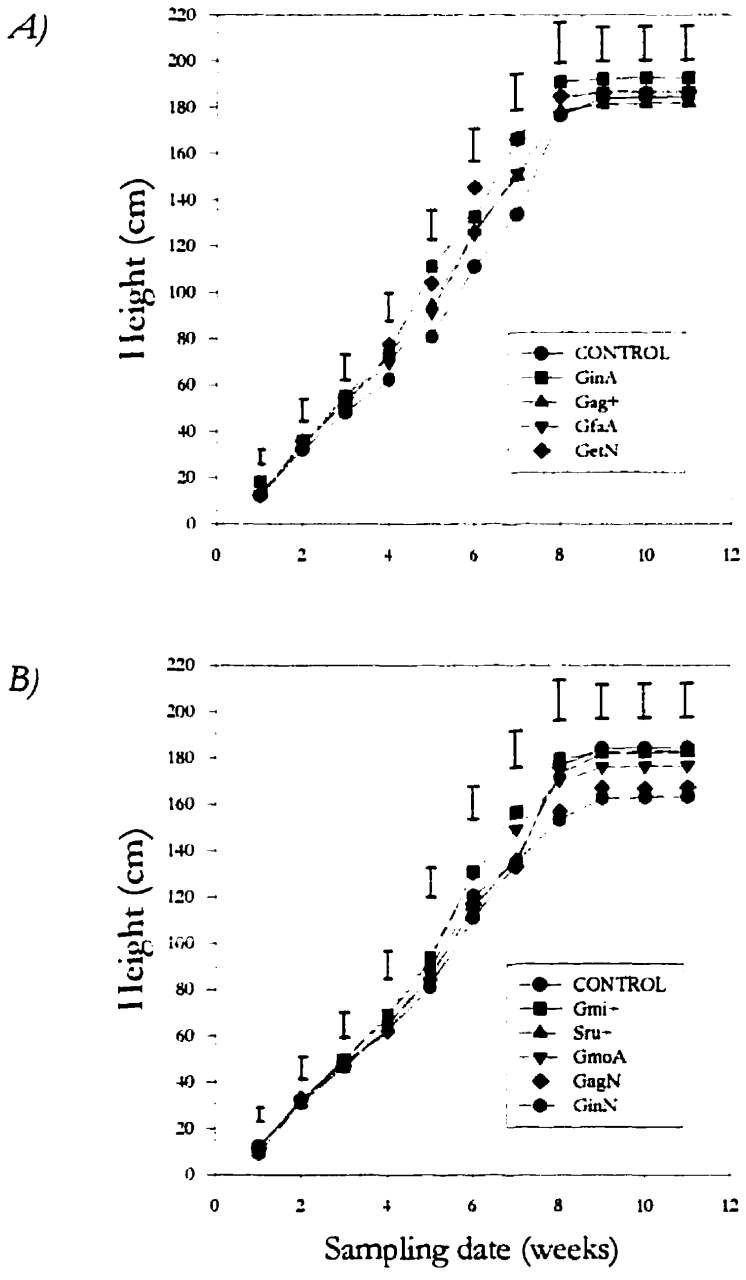


Fig. 5. Heights of sweet corn plants inoculated with 9 different mycorrhizal strains and a non-mycorrhizal control treatment during the 1995 greenhouse experiment: (a) *G. intraradices* AGR (GinA), a mix of *G. aggregatum* (Gag+), *G. fasciculatum* (GfaA), *G. etunicatum* (GetN), and (b) a mix of *G. microaggregatum* (Gmi+), a mix of *S. rubiformis* (Sru+), *G. mosseae* (GmoA), *G. aggregatum* (GagN), and *G. intraradices* NAT (GinN). Data points represent average values of 5 plants. Vertical bars represent the L.S.D. at a .05 level.

Table 7. *F* ratios from the MANOVA approach of the time repeated measures (TRM) on height and the ANOVA test for inoculum effects on pepper shoot dry mass, height at harvest, fruit dry mass, shoot P, relative mycorrhizal dependency (RMD), and AM colonization for the 1995 and 1996 experiments under controlled conditions.

	Source of variance		
	Main effects		Interactions
Response variable	Inoculum	Time	Time*inoculum
Pepper 1996			
AM colonization (final harv.)	26.11***		
Shoot dry mass	0.74		
Fruit dry mass	0.77		
Shoot P (final harv.)	2.68		
Height at harvest	1.19		
Height (TRM) until final harv. [a]	0.68	936.46*	1.17
RMD	0.86		
Pepper 1995			
AM colonization (5th week)	6.00***		
AM colonization (final harv.)	35.24***		
Shoot dry mass	2.01		
Fruit dry mass	1.52		
Shoot P (final harv.)	0.94		
Height at final harvest	1.01		
Height (TRM) until 5th week [a]	2.13***	655.09***	1.50*
Height (TRM) until final harv. [a]	1.31	681.50***	1.04
RMD	1.55		

*,**,***, indicate that *F* ratios were significant at $P \leq .05, .01$, and .001, respectively

[a] *F* equivalent value from the Wilks' Lambda statistics of the MANOVA approach of the time repeated measures

($P < 0.001$) and both harvests in 1995 ($P < 0.05$ and $P < 0.001$ for the 1st and 2nd harvests, respectively). Furthermore, the L.S.D. test showed no significant differences in AM colonization between plants inoculated with *G. fasciculatum* (GfaA) and the non-mycorrhizal control treatment at the 1st and 2nd harvests in 1995 (Fig. 6a and 6b, respectively).

In 1995, plants inoculated with *G. fasciculatum* (GfaA) were the tallest but differences among means were not significantly different (data not shown). Variances in plant height and both shoot and fruit dry mass were not related to the inoculum source in the same year (Table 7). However, orthogonal contrasts revealed that non mycorrhizal plants had greater fruit and shoot dry mass than inoculated plants ($P < 0.01$) (data not shown). In both years, variance in shoot P

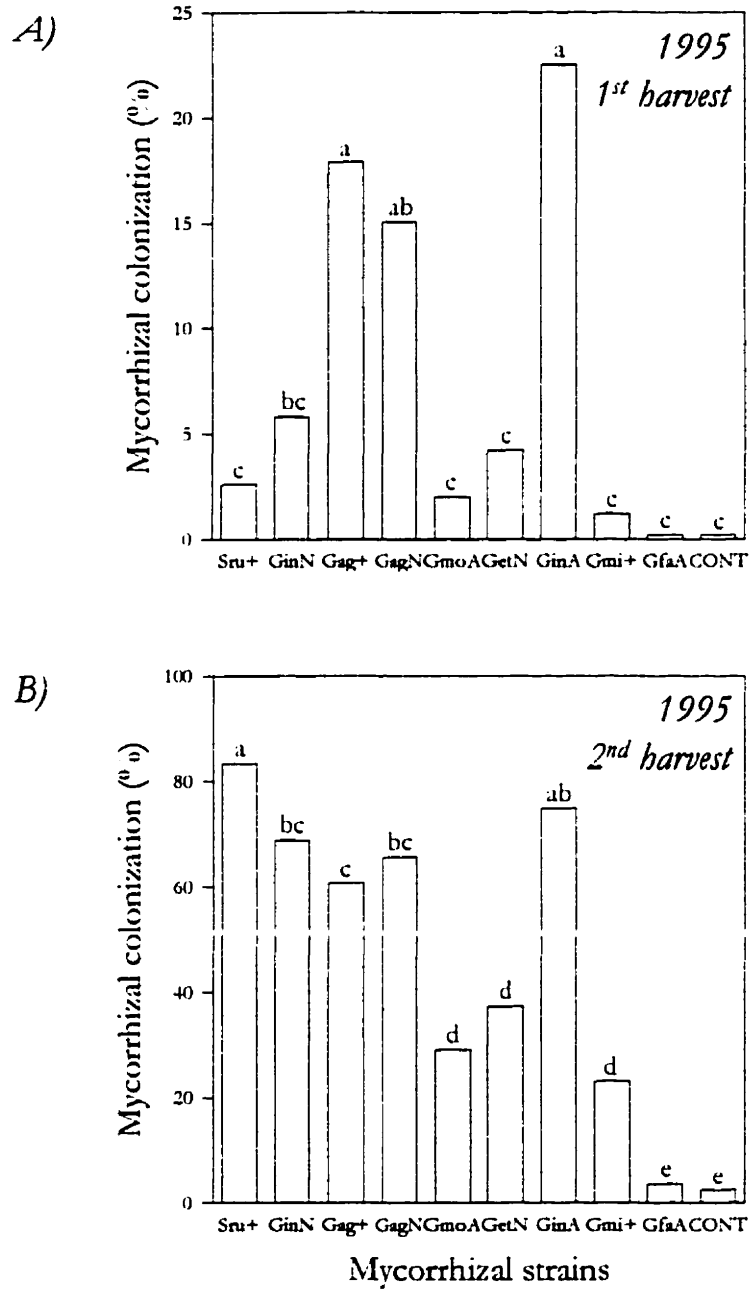


Fig. 6. Mycorrhizal colonization in pepper inoculated with 9 different mycorrhizal strains and a non-mycorrhizal control treatment during 1995 under controlled conditions at: (a) First harvest and (b) second harvest (5 and 13 weeks after transplanting, respectively). Data points represent average values of 5 plants. Bars with different letters differ at a .05 level.

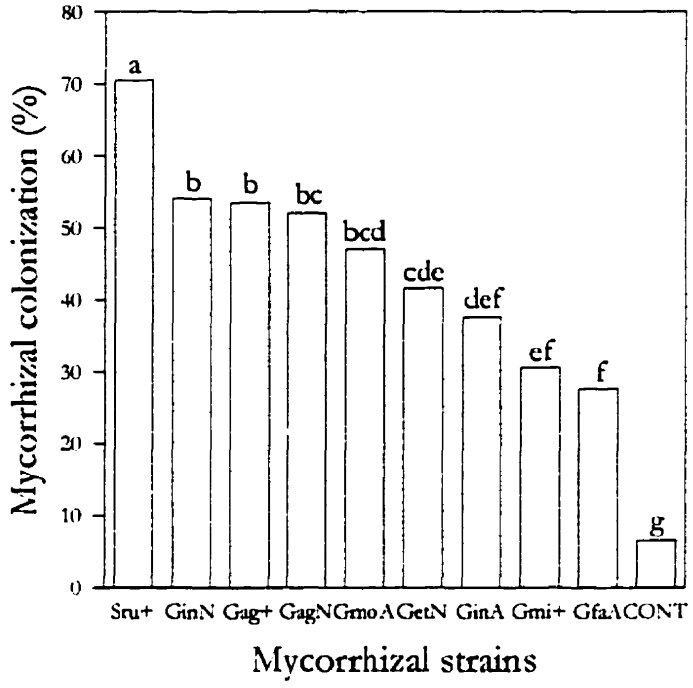


Fig. 7. Mycorrhizal colonization in pepper plants inoculated with 9 different mycorrhizal strains and a non-mycorrhizal control treatment under controlled conditions in 1996 (13 weeks after transplanting). Data points represent average values of 2 plants. Bars with different letters differ at a .05 level.

was not related to the inoculum source (Table 7). However, non-mycorrhizal plants had the lowest, although not significantly different, shoot P levels in 1995 (data not shown). In 1996, the orthogonal contrast of shoot P between the non-mycorrhizal and the rest of the treatments was significant ($P < 0.05$) and control plants had the lowest shoot P level (Fig. 8).

Several authors have reported positive growth responses for *Capsicum* at low P rates when inoculated with different AM strains under greenhouse conditions (Olsen et al., 1996, Waterer and Coltman, 1989). However, inoculation with *G. desertícola* at three P levels did not have any significant effect on pepper growth with a low AM colonization (10 to 20%) (Davies and Linderman, 1991). Also important is the variable response of plant growth and the extent of AM colonization, which depended on the inoculum source (Dodd et al., 1983, Haas and Krikun, 1985) and density of mycorrhizal inoculum (Haas and Krikun, 1985).

The relative mycorrhizal dependency of inoculated plants were all negative values (Table 5) and the inoculum source did not account for a significant amount of the variance of the pepper RMD in both years (Table 7). A decrease in shoot dry mass of inoculated plants, compared with non-mycorrhizal plants, indicates that all the evaluated AM strains were detrimental for the growth of pepper under these experimental conditions. Olsen et al. (1996) obtained a decrease in the RMD of pepper plants inoculated with a mix of *G. mosseae* and *G. etunicatum*, which was related to an increased rate of P application. Negative, but non-significantly different, values of pepper RMD were also reported by the same authors at higher P levels (over 92.7 mg kg^{-1}) (Olsen et al., 1996).

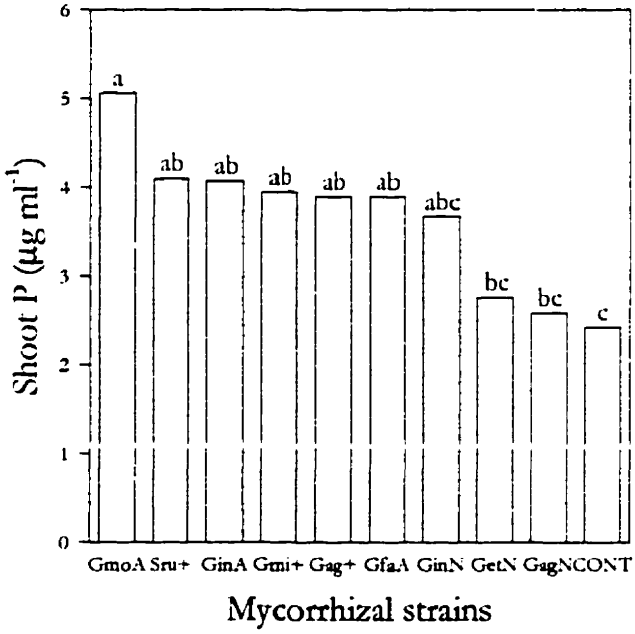


Fig. 8. Shoot P content of pepper plants to inoculum treatments at harvest for the 1996 experiment under controlled conditions. Data points represent average values of 2 plants. Bars with different letters differ at a .05 level.

Inoculum source accounted for a significant amount of the variance in plant height measured over time ($P < 0.001$) and the interaction between the time factor and the inoculum source until 5 weeks after transplanting, in 1995 ($P < 0.05$) (Table 7). Conversely, the temporal repeated

measures for the second harvest of 1995 (13 weeks after transplanting), and the 1996 experiment, showed no effect of inoculum source on the growth rate of sweet corn plants (Table 7). All the treatments displayed a sigmoidal response curve and height of plants inoculated with different AM strains varied with time in 1995 (Fig. 9 a,b). Non-mycorrhizal plants were the tallest during the first 5 weeks after transplanting but, after this time became smaller than for the rest of the treatments. Plants inoculated with *G. fasciculatum* (GfaA) noticeably increased their size 7 weeks after transplanting, becoming the tallest by the end of the second harvest (Fig. 9 a).

As was noted before, plants inoculated with *G. fasciculatum* (GfaA) developed a very low level of mycorrhizal colonization, not significantly different than non-mycorrhizal plants. The lack of responsiveness of pepper to inoculation with this strain in 1995 may have resulted in a less detrimental effect on plant growth, compared with the effect of the other mycorrhizal strains. In this year, orthogonal contrasts between the effect of this strain (GfaA) and all other treatments, were significant for pepper height ($P < 0.05$), shoot dry mass ($P < 0.05$), and RMD ($P < 0.05$) (data not shown). The factors that determined the low receptivity of pepper to *G. fasciculatum* are unknown, but the effect of this strain on plant growth was more extreme than that of any other strain.

When pooling the data from both experiments, the year effect accounted for a significant amount of the variance in the overall shoot dry mass ($P < 0.001$), fruit dry mass ($P < 0.001$), and shoot P ($P < 0.01$). In 1995, inoculated plants had a greater shoot and fruit dry mass, but at a lower shoot P level than plants grown in 1996 (Table 6). Non significant differences in the overall RMD and AM colonization between years, and the fact that plants grew better in the low P growth medium in the 1995 greenhouse experiment, may indicate that an unknown non P-related factor was an advantage for pepper growth in 1995.

3.3 FIELD EXPERIMENTS

3.3.1 *Sweet corn*

In each of the 3 sweet corn field site experiments the inoculum source accounted for a significant amount of the variance in the measured variables (Table 8). At the same time, there was no clear trend indicating that inoculation with any of these AM strains enhanced plant growth more than the native AM populations under field conditions. Since no significant differences were observed for AM colonization, P shoot content analysis was not performed.

Soil was not fumigated before seeding since it was of interest to compare the performance of the tested AM strains when competing with native mycorrhizal fungi. Hamel et al. (1991a) found no difference in yield of corn in any of the inoculation treatments with either *G. versiforme*, *G. intraradices*, or *G. vesiculiferum* in non-fumigated soils located near the experimental field sites of this study. Conversely, yield of inoculated corn, planted in soils fumigated with methyl bromide, was greater than non-inoculated plants but differences among the used AM species were not significantly different (Hamel et al., 1991a). These findings support the hypothesis that the lack of responsiveness of sweet corn to inoculation in non-fumigated soil, is due to competition between the AM indigenous community and the introduced strains. An alternative explanation for the lack of response of inoculated sweet corn under field conditions in this experiment is an inadequate amount of inoculum, such that plant roots were not provided with enough AM propagules.

When pooling the data, the site effect explained a significant amount of the variance for overall mycorrhizal colonization at both the first ($P < 0.01$) and the second harvest ($P < 0.001$), shoot dry mass ($P < 0.001$), corn yield ($P < 0.001$), and height at the 7th week ($P < 0.001$). Inoculated corn plants in site 1 developed the greatest AM colonization at both harvests (Fig. 10) which could be related with a greater overall shoot dry mass (Fig. 11a), yield (Fig. 11b), and height at the 7th week (Fig. 11c) of inoculated plants in the same site.

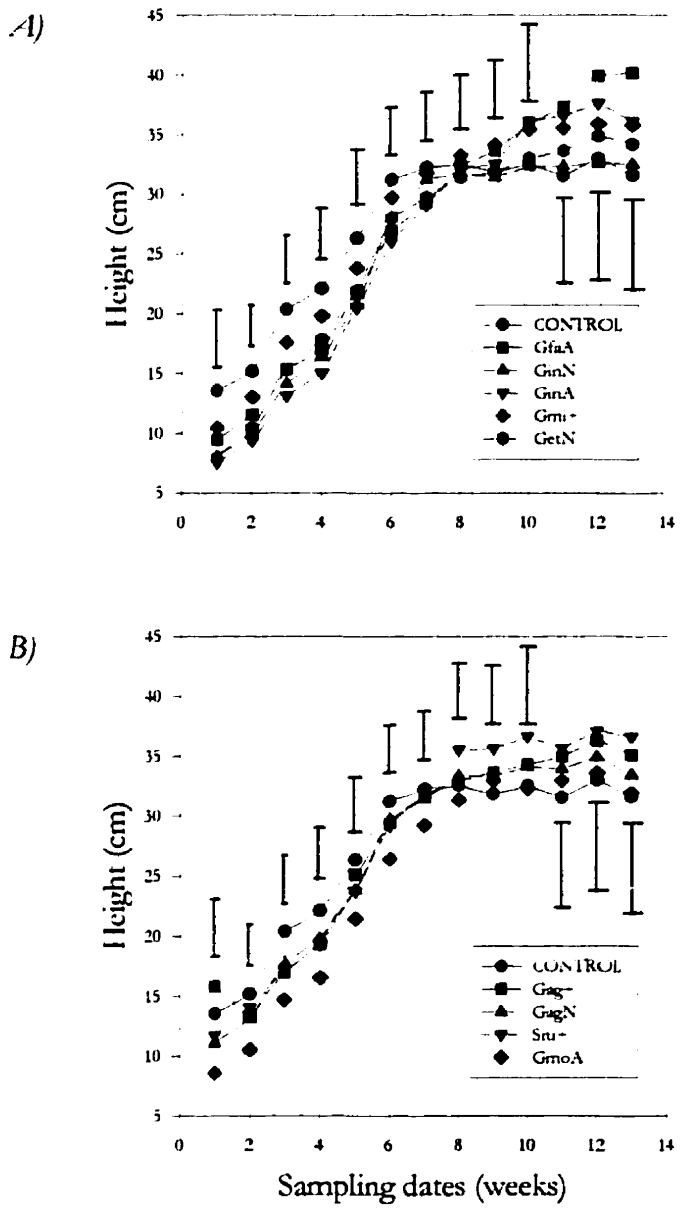


Fig. 9. Heights of pepper plants inoculated with 9 different mycorrhizal strains and a non-mycorrhizal control treatment during the 1995 greenhouse experiment: (a) *G. fasciculatum* (GfaA), *G. intraradices* NAT (GinN), *G. intraradices* AGR (GinA), a mix of *G. microaggregatum* (Gmi+), and *G. etunicatum* (GetN), (b) a mix of *G. aggregatum* (Gag+), *G. aggregatum* (GagN), a mix of *S. rubiformis* (Sru+), and *G. mosseae* (GmoA). Data points represent average values of 5 plants. Vertical bars represent the L.S.D. at a .05 level.

Table 8. *F* ratios from the MANOVA approach of the time repeated measures on height and the ANOVA test for inoculum effects on sweet corn shoot dry mass, height (7th week), yield, and AM colonization in 3 different sites under field conditions.

	Source of variance			
	Main effects			Interactions
Response variable	Cov[b]	Inoculum	Time	Time*inoculum
Site 1				
AM colonization (6th week)	0.15	1.38		
AM colonization (final harv.)	0.43	0.90		
Shoot dry mass	6.73*	0.47		
Yield	4.19*	1.15		
Height (7th week)	1.30	1.15		
Height (TRM) until 9th week[a]		1.04	1581.4***	1.1556
Site 2				
AM colonization (9th week)	0.81	0.75		
AM colonization (final harv.)	1.94	1.92		
Shoot dry mass	2.57	1.19		
Yield	3.55	0.58		
Height (7th week)	4.69*	1.70		
Height (TRM) until 9th week[a]		0.85	572.4***	0.8627
Site 3				
AM colonization (9th week)	0.02	1.02		
AM colonization (final harv.)	0.37	1.25		
Shoot dry mass	5.78*	1.70		
Yield	11.15**	0.78		
Height (7th week)	0.00	1.05		
Height (TRM) until 9th week[a]		0.78	834.1***	0.7816

*,**,***, indicate that *F* ratios were significant at $P \leq .05$, .01, and .001, respectively

[a]*F* equivalent value from the Wilks' Lambda statistics of the MANOVA approach of the time repeated measures

[b]Covariate: number of plants per plot

Soil available P at site 1 was low (21.7 mg kg^{-1}). In contrast, available P at site 2 (177.7 mg kg^{-1}) and 3 (187.9 mg kg^{-1}) was very high which could account for a reduced responsiveness of corn to AM inoculation in sites 2 and 3. The accumulation of P due to excessive fertilization may reduce the usefulness of mycorrhizal inoculation under field conditions (Hamel, 1996) and no significant differences, or even detrimental effects, have been observed in inoculated corn grown under controlled conditions (Hetrick et al., 1984, Olsen et al., 1996). Continuos cropping and fertilizing of originally low nutrient soils would also favour the proliferation of inferior AM symbionts (Johnson, 1993, Johnson et al., 1991a). Sites 1 and 2 were planted with corn the year

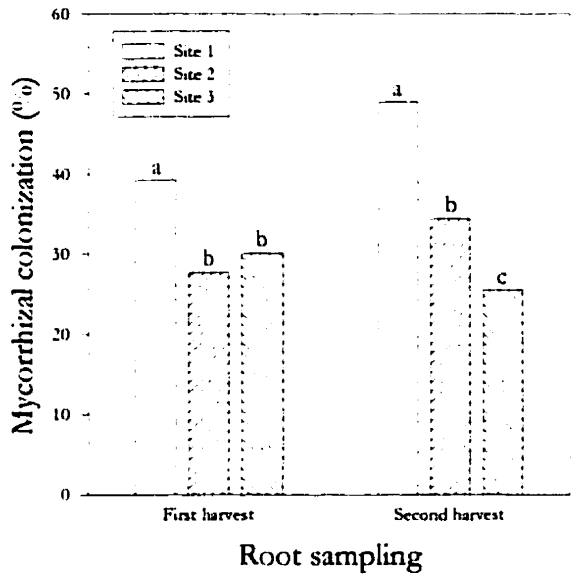


Fig. 10. Overall mycorrhizal colonization in sweet corn plants from 3 different field sites in 1995. Data points represent average values of 40 plants. Bars with different letters within each root sampling differ at a .05 level.

before, and site 3 was fallow (Table 2). However, the crop history of all these 3 sites is corn, which was produced under conventional management for several years. As a consequence, a more difficult establishment of beneficial AM strains in these soils can be expected; fertilization may have selected for highly competitive and less mutualist AM fungi (Hamel, 1996).

Very dry conditions for 11 consecutive days, immediately after planting the inoculated seeds at site 1, caused a delay in seed germination at sites 2 and 3, which were planted the day after planting site 1. Root formation may have been affected by this dry period. As a consequence, differences in plant growth could be related with the physiological constraints caused by this stress during the very early stages of sweet corn development.

A preliminary survey of the AM species present at the field experimental sites shows that Glomales predominated in all of them (Table 9). *Glomus mosseae* and *Sclerocystis rubiformis* were common to all of the 3 field sites where sweet corn was planted (Table 9). As mentioned above, sites 1 and 2 were planted with corn the year before and site 3 was fallow (Table 2).

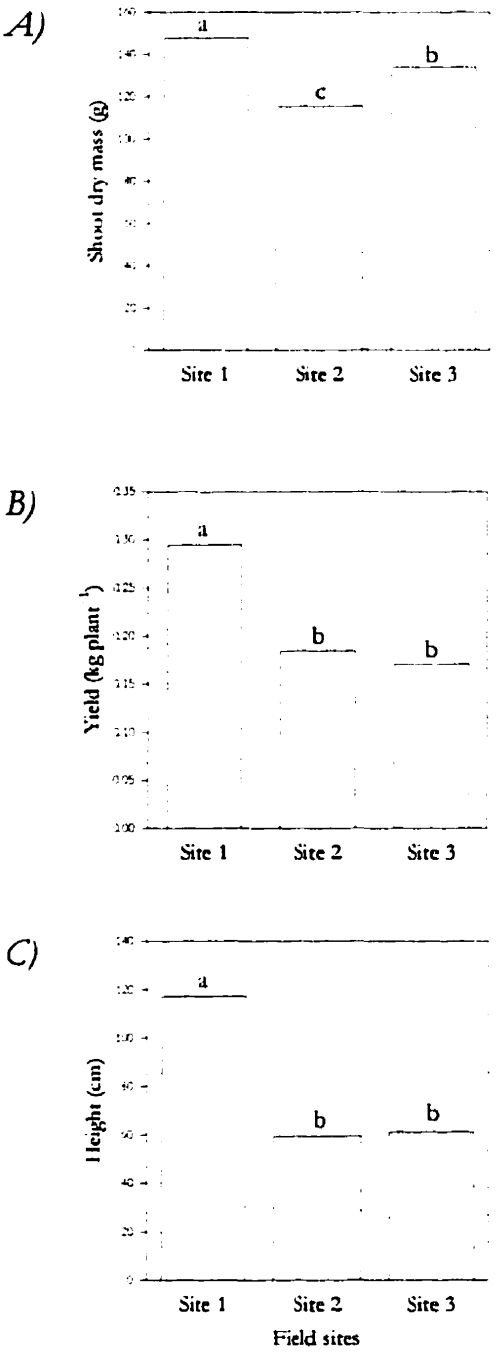


Fig. 11. Overall plant responses to mycorrhizal colonization in sweet corn from 3 different field sites in 1995: (a) Shoot dry mass, (b) yield, and (c) height at the 7th week. Data points represent average values of 40 plants. Bars with different letters differ at a.05 level.

However, all 3 of these sites had been continuously planted with corn for several years. Other studies have revealed that the number of spores of *G. mosseae* and *G. occultum* in soils was associated with history of corn production (Johnson et al., 1991b). This is indicative of the important effect that cropping history and edaphic factors have in controlling populations of AM mycorrhizal fungi (Johnson et al., 1991b).

3.3.2 *Pepper*

The inoculum source accounted for a significant amount of the variance in AM colonization at the first harvest of both sites 5 and 7 ($P < 0.05$ and $P < 0.001$, respectively) (Table 10). However, the cortex of pepper roots was partially lost due to a longer KOH clearing period, which was done in order to obtain a good staining of these roots. As a consequence, counting of fungal structures in these roots was not a reliable estimation of the actual AM colonization (data not shown).

Table 9. List of indigenous AM species separated from the non-inoculated plots in sweet corn and pepper field experiments.

Site	Crop	Identified species:
1	Sweet corn	*G. mosseae*, *G. caledonium*, *G. albidum*, *S. rubiformis*
2	Sweet corn	*G. fasciculatum*, *G. albidum*, *G. mosseae*, *G. aggregatum*, *S. rubiformis*, *Glomus* sp.
3	Sweet corn	*S. rubiformis*, *G. mosseae*, *Acaulospora* sp.,
5	Pepper	*G. mosseae*, *S. rubiformis*
6	Pepper	*G. mosseae*, *S. rubiformis*, *G. constrictum*
7	Pepper	*G. mosseae*, *Glomus* sp., *S. rubiformis*
8	Pepper	*G. albidum*, *G. fasciculatum*, *S. rubiformis*, *G. constrictum*, *Acaulospora* sp., *Glomus* sp.

Table 10. *F* ratios from the MANOVA approach of the time repeated measures (TRM) on height and the ANOVA test for inoculum effects on pepper shoot dry mass, yield, and AM colonization in 4 different sites under field conditions.

	Source of variance			
	Main effects			Interactions
Response variable	Cov[b]	Inoculum	Time	Time*inoculum
Site 5				
AM colonization (6th week)	0.00	2.42*		
AM colonization (final harv.)	0.13	0.61		
Shoot dry mass	2.27	1.41		
Yield	1.81	1.05		
Height (TRM) until 8th week[a]		1.57*	341.7***	1.88
Site 6				
AM colonization (6th week)	0.54	0.84		
AM colonization (final harv.)	0.08	1.00		
Shoot dry mass	0.44	0.84		
Yield	0.03	0.49		
Height (TRM) until 8th week[a]		0.95	428.1***	0.95
Site 7				
AM colonization (6th week)	0.01	5.24***		
AM colonization (final harv.)	0.88	1.09		
Shoot dry mass	0.52	2.28*		
Yield	0.36	2.01		
Height (TRM) until 8h week[a]		1.92**	387.3***	1.79*
Site 8				
AM colonization (6th week)	0.78	1.63		
AM colonization (final harv.)	0.20	0.96		
Shoot dry mass	0.09	1.52		
Yield	2.50	3.48**		
Height (TRM) until 8h week[a]		1.87**	303.9***	1.81*

*,**,***, indicate that *F* ratios were significant at P ≤ .05, .01, and .001, respectively

[a]*F* equivalent value from the Wilks' Lambda statistics of the MANOVA approach of the time repeated measures

[b]Covariate: number of plants per plot

The inoculum source accounted for a significant amount of the variance in plant height over time in sites 5 ($P < 0.05$), 7 ($P < 0.01$), and 8 ($P < 0.01$) (Table 10). The interaction between time and inoculum source for plant height over time was also significant for sites 7 ($P < 0.05$) and 8 ($P < 0.05$) (Table 10). For all these sites, plant growth of all treatments displayed a sigmoidal response curve. At site 5, heights of control plants and those inoculated with a mix of *G.*

aggregatum and *G. geosporum* (Gag+) was higher than the other treatments (Fig. 12a, b). At the same time, only plants inoculated with both strains of *G. intraradices* (GinA and GinN) were significantly smaller than control plants and those inoculated with Gag+, during the considered period of time (Fig. 12b). For sites 7 and 8, there was not a clear trend for the effect of inoculum source, since it varied in time. However, plants inoculated with a mix of *G. aggregatum* and *G. geosporum* (Gag+) tended to grow taller than plants for the rest of the treatments at site 7 (Fig. 13a) and 8 (Fig. 14a). Interestingly, plants at site 8 inoculated with a mix of *S. rubiformis* and *G. fasciculatum* (Sru+) increased their size 6 weeks after transplanting and grew taller than plants receiving other treatments (Fig. 14b).

The inoculum source accounted for a significant amount of the variance in shoot dry mass at site 7 ($P < 0.05$) and yield at site 8 ($P < 0.05$) (Table 10). For site 7, plants inoculated with *G. intraradices* (GinN) had a greater shoot dry mass than the rest of the plants, although this was not significantly different from the control plants (Fig. 15a). For site 8, plants inoculated with *G. aggregatum* (GagN) and *G. intraradices* (GinN) produced a greater yield than the rest of the plants, although not significantly more than the non-inoculated control treatment (Fig. 15b). On the other hand, plants inoculated at the same site with *G. intraradices* (GinA) and *G. mosseae* (GmoA) produced a significantly smaller yield than the control plants (Fig. 15b).

Sclerocystis rubiformis was present at all sites where pepper was planted (Table 9). Interestingly, the indigenous community of AM species was more diverse at site 8, which also had been fallow the year before. It is not possible to determine if introduced AM inoculum competed favourably with the native mycorrhizal fungi in this site, but each of the treatments induced a significant increase in plant growth when compared with the control plots.

When pooling the data, the site effect explained a significant amount of the variance in the overall shoot dry mass ($P < 0.001$), pepper yield ($P < 0.001$), and height at the 6th week ($P < 0.001$). As was mentioned before, AM colonization values were not a reliable estimation of the actual extent of the symbiosis in pepper roots and variance was not homogeneous (data not shown). The effect of the inoculum source also varied by site in pepper height 6 weeks after transplanting ($P < 0.001$) and shoot dry mass ($P < 0.05$). These significant interactions could indicate, assuming that differences are solely due to the mycorrhizal inoculum, that even similar

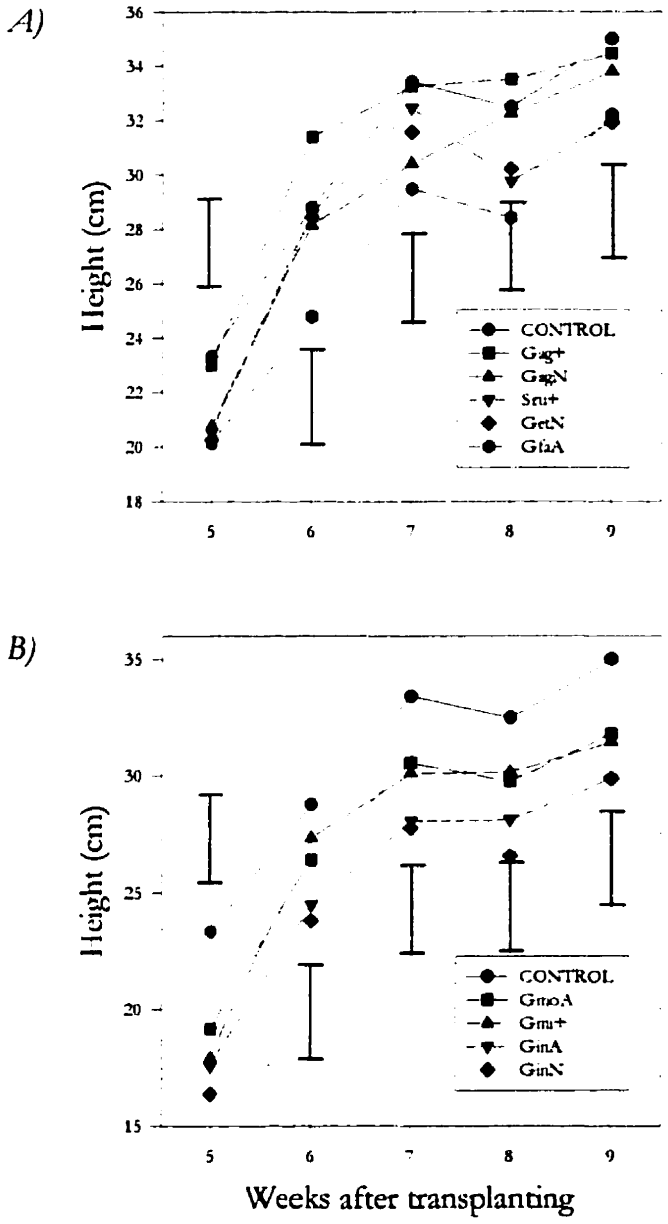


Fig. 12. Heights of pepper plants inoculated with 9 different mycorrhizal strains and a non-mycorrhizal control treatment at field site 5 between the 5th and 9th week (1995): (a) a mix of *G. aggregatum* (Gag+), *G. aggregatum* (GagN), a mix of *S. rubiformis* (Sru+), *G. etunicatum* (GetN), and *G. fasciculatum* (GfaA), (b) *G. mosseae* (GmoA), a mix of *G. microaggregatum* (Gmi+), *G. intraradices* AGR (GinA), and *G. intraradices* NAT (GinN). Data points represent average values of 20 plants. Vertical bars represent the L.S.D. at a .05 level.

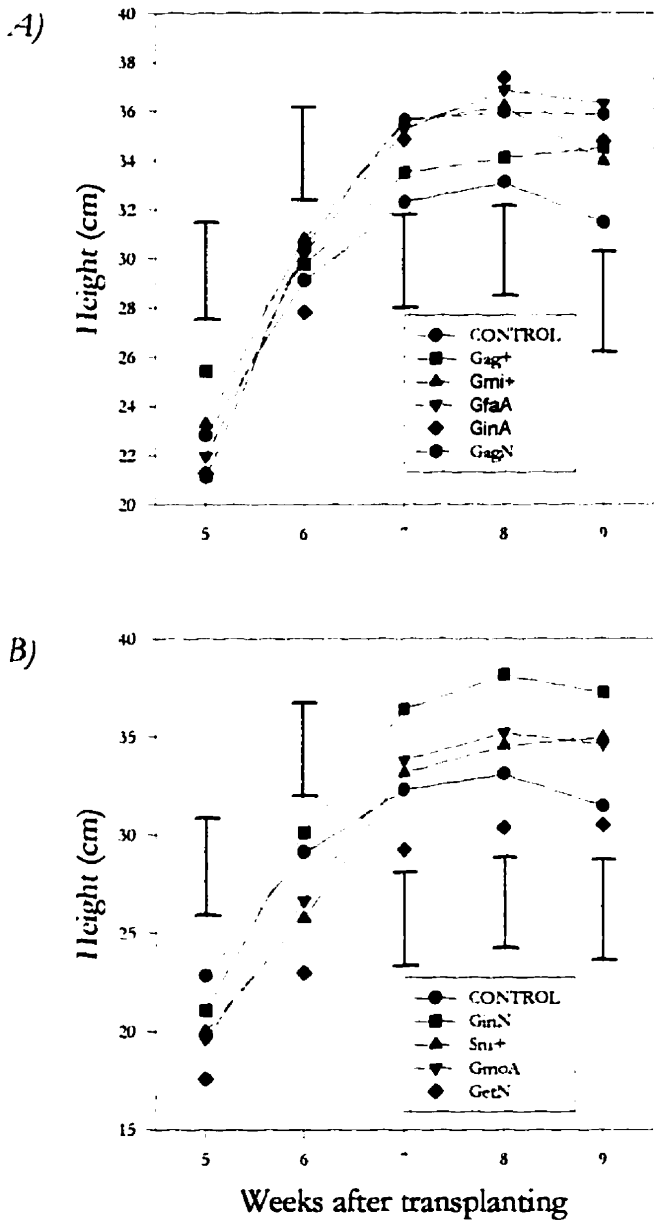


Fig. 13. Heights of pepper plants inoculated with 9 different mycorrhizal strains and a non-mycorrhizal control treatment at field site 7 between the 5th and 9th week (1995): (a) a mix of *G. aggregatum* (Gag+), a mix of *G. microaggregatum* (Gmi+), *G. fasciculatum* (GfaA), *G. intraradices* AGR (GinA), and *G. aggregatum* (GagN), (b) *G. intraradices* NAT (GinN), a mix of *S. rubiformis* (Sru+), *G. mosseae* (GmoA), and *G. etunicatum* (GetN). Data points represent average values of 20 plants. Vertical bars represent the L.S.D. at a .05 level.

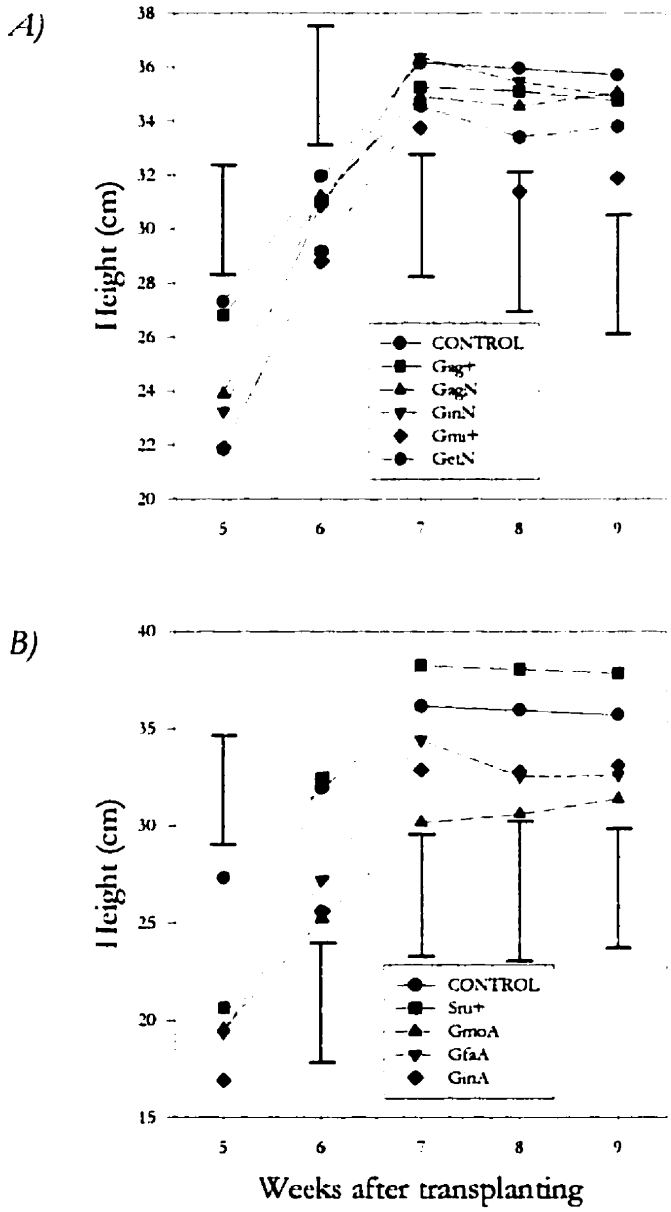


Fig. 14. Heights of pepper plants inoculated with 9 different mycorrhizal strains and a non-mycorrhizal control treatment at field site 8 between the 5th and 9th week (1995): (a) a mix of *G. aggregatum* (Gag+), *G. aggregatum* (GagN), *G. intraradices* NAT (GinN), a mix of *G. microaggregatum* (Gmi+), and *G. etunicatum* (GetN), (b) a mix of *S. rubiformis* (Sru+), *G. mosseae* (GmoA), *G. fasciculatum* (GfaA), and *G. intraradices* AGR (GinA). Data points represent average values of 20 plants. Vertical bars represent the L.S.D. at a .05 level.

types of soil can exert a selective response of pepper to AM inoculation. Differences in fertilization regimes and agricultural practices can also select for particular mycorrhizal strains that are less beneficial for crop growth and compete with the more beneficial isolates used as inoculum (Hamel, 1996; Johnson, 1993).

Site 5 had the highest levels of available soil P (424.1 mg kg^{-1}) and K (263.8 mg kg^{-1}) among the experimental sites. However, shoot dry mass (Fig. 16a), pepper height (Fig. 16b), and plant yield in this site (Fig. 16c) were the lowest among the compared sites. It was not possible to establish a relationship between the occurrence of AM and pepper growth since root colonization were not a reliable estimation of the symbiosis in the field. Pepper shoot dry mass at site 6, on the other hand, was the greatest among the compared sites (Fig. 16a). This site was planted with lupine the year before, a crop which has proved to be non-responsive to mycorrhizal inoculation (Hayman, 1987). A preliminary survey of indigenous AM species present at the field sites shows that *G. mosseae*, *S. rubiformis* and *G. constrictum* were present at site 6. Whether or not the inclusion of a non-mycorrhizal plant (*Lupinus*) determined a relative increase in the overall plant response to mycorrhizal inoculation is not known, but it may have accounted for this effect. Fallow, or the inclusion of a non-mycorrhizal crop, can be a useful practice to improve the effectiveness of introduced AM strains since this causes a reduction in the background level of very competitive indigenous AM fungi in soil (Hamel, 1996; Sieverding, 1991).

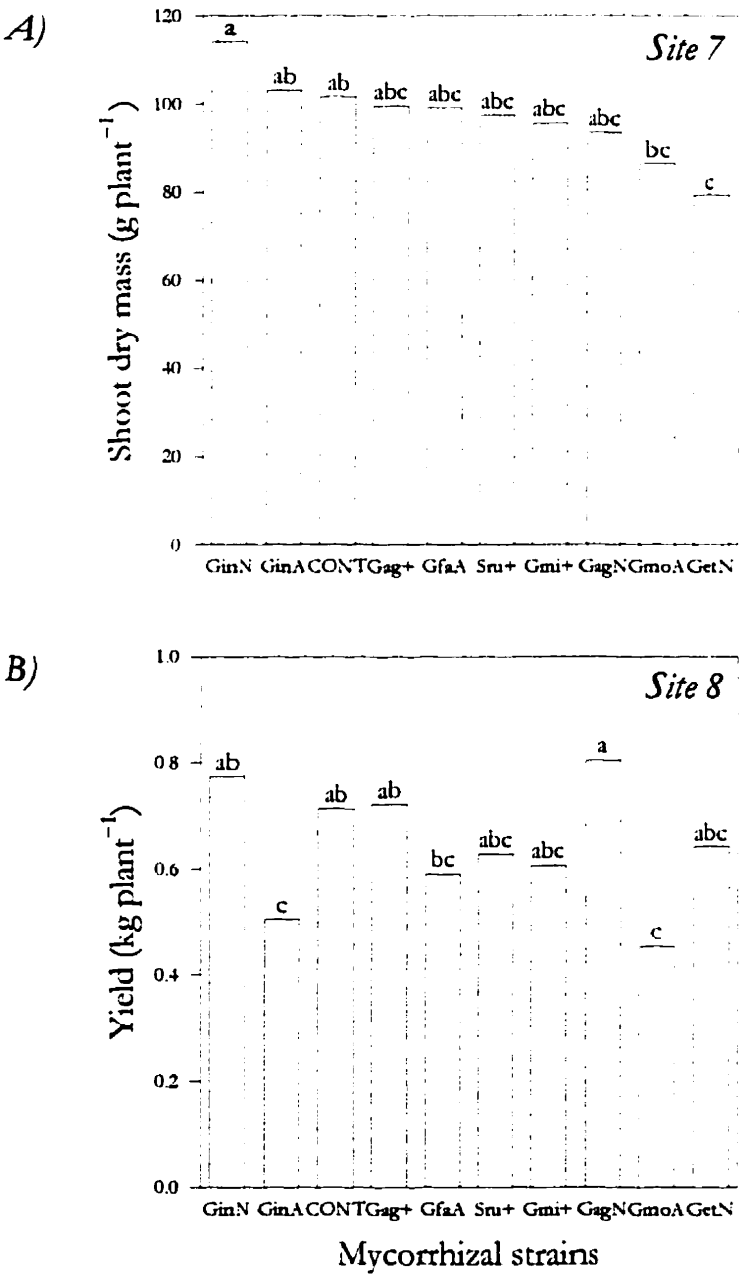


Fig. 15. Responses of pepper plants to inoculum treatments for: (a) Shoot dry mass in field 7 and (b) yield in field 8, for the 1995 experiment. Data points represent average values of 28 plants. Bars with different letters differ at a .05 level.

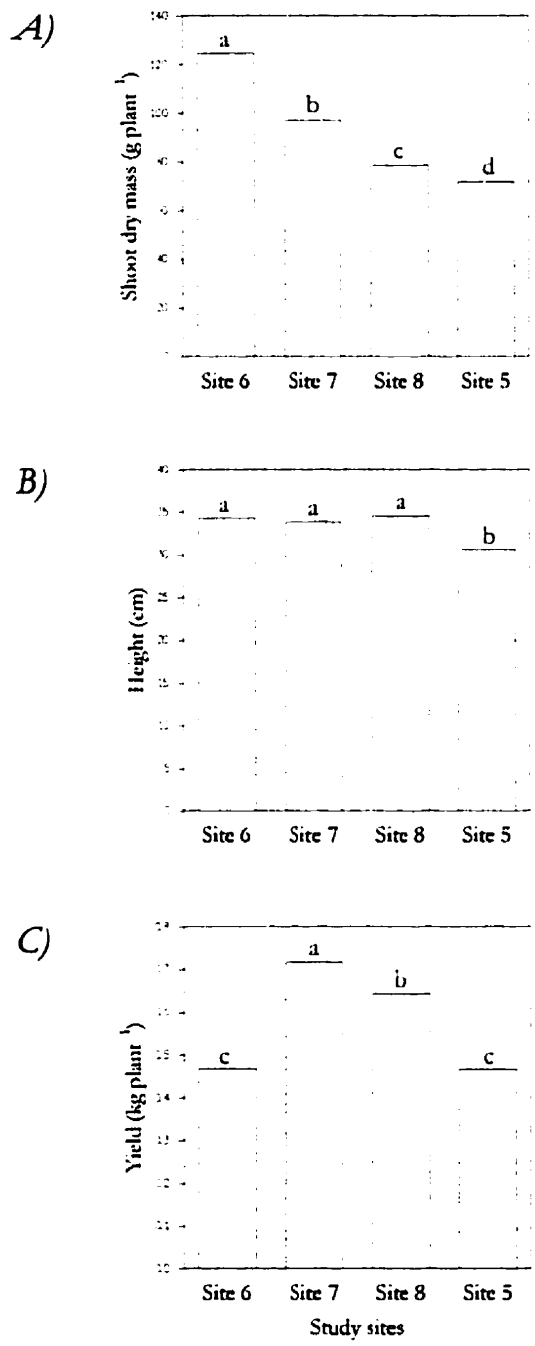


Fig. 16. Overall plant responses to mycorrhizal colonization in pepper from 4 different field sites in 1995: (a) Shoot dry mass and (b) plant height at the 6th week, and (c) yield. Data points represent average values of 280 plants. Bars with different letters differ at a .05 level.

GENERAL CONCLUSIONS

The effect of the considered AM strains varied according to the plant species used in this experiment. A strain of *Glomus aggregatum* isolated from a natural soil (GagN), a mix of *G. aggregatum* and *G. geosporum* (Gag+) collected from an agricultural soil, a commercial inoculum of *G. intraradices* formerly obtained from an agricultural soil (GinA), and this same strain (GinA) altogether with a mix of *Sclerocystis rubiformis* and *G. fasciculatum* (Sru+), both collected in an agricultural soil, produced the greatest levels of AM colonization in marigold, sorghum, sweet corn, and pepper, respectively. These results were obtained for plants growing under controlled environmental conditions, in the absence of competing indigenous mycorrhizal fungi.

A high proportion of colonized roots due to the effect of a particular treatment was not necessarily related to a significant growth increase due to the same mycorrhizal strain. Sweet corn plants inoculated with *G. intraradices* (GinA) developed the highest AM colonization under controlled environmental conditions. However, no significant increases in shoot dry mass were found in these plants compared with non-mycorrhizal sweet corn. In addition, both strains of *G. intraradices* (GinA and GinN) had low levels of shoot P in 1996 which may indicate the less beneficial effect of this AM species for sweet corn growth under experimental conditions. On the other hand, a mix of *G. microaggregatum*, *G. mosseae* and *G. fasciculatum* (Gmi+) resulted in a greater dry mass than the control treatment, but at a lower mycorrhizal colonization. At the same time, sweet corn inoculated with the same inoculum source (Gmi+) had the highest shoot P and relative mycorrhizal dependency among the treatments tested in 1996 and 1995, respectively.

A less clear response of sweet corn to inoculation with different AM strains was found under field conditions. In fact, none of the inoculum treatments used in this experiment resulted in a significant increase in plant growth, compared with non-inoculated plants in non-fumigated plots. This lack of growth response by sweet corn to inoculation may have been caused by direct competition between the introduced strains and the indigenous population of AM fungi. A reduced number of infective AM propagules contained in the source of inoculum may also have

accounted for the low responsiveness of sweet corn to inoculation under field conditions. Moreover, the occurrence of very dry conditions caused a delay in seed germination and may have induced a severe stress during the very early stages of sweet corn development. It is unknown if changes in root development due to environmental stress can cause a differential response on plant response to mycorrhizal inoculation.

The available P levels may affect sweet corn response to inoculation. Overall root colonization was the highest in the 1996 experiment under controlled conditions, and in site 1 under field conditions. The available P level was higher in the 1996 experiment under controlled conditions and an overall plant growth response to inoculation was observed in this year. This effect can be attributed to a better P nutrition of these plants growing in a richer growth medium. However, the overall relative mycorrhizal dependency of inoculated sweet corn in 1996 was significantly lower than for plants in 1995. Moreover, the overall RMD of sweet corn in 1996 was negative which indicates that AM inoculation was detrimental to plant growth. The overall AM colonization in inoculated plants at site 1, which had the lowest available P level among the compared sites, was the highest. Overall sweet corn growth was the greatest at site 1, which may indicate an improved response to AM inoculation in a less fertile soil. However, there was no difference among the mycorrhizal inocula for plant growth at this site.

None of the mycorrhizal inocula used in this experiment produced a beneficial effect on pepper growth under controlled conditions. Growth of non-mycorrhizal pepper plants in both years was greater than inoculated plants. As a consequence, the relative mycorrhizal dependency of pepper to AM inoculation for the different fungal isolates was negative for all treatments in both years. However, *G. intraradices* (GinA) and a mix of *Sclerocystis rubiformis* and *G. fasciculatum* (Sru+) developed the highest AM colonization on pepper roots growing under controlled environment conditions, but this effect was only related to an increase in shoot P of pepper inoculated with these treatments (Sru+ and GinA) compared with the corresponding values in non-mycorrhizal control plants. Interestingly, the effect of *G. fasciculatum* (GfaA) inoculation on AM colonization of inoculated pepper plants was almost negligible. The factor that determined a low response of pepper to this fungal strain is unknown but the effect of this treatment on plant growth was less detrimental than the other mycorrhizal strains.

None of the mycorrhizal strains used in the field experiments increased growth of inoculated pepper compared with non-inoculated plants. However, the relative response of pepper plants to inoculum treatments varied by site and the reasons for these differences remain unknown. The differential effect of inoculation with different AM isolates becomes more difficult to explain due to the lack of a reliable estimation of AM colonization of pepper roots from the field experiments. Pepper seedlings were pre-inoculated which may have resulted in a shorter acclimatization period after transplanting. Whether or not this effect represents an advantage for the introduced AM inocula when competing with the indigenous mycorrhizal fungi, remains unknown.

A final checking of the mycorrhizal species present in the different treatments used in these experiments shows discrepancies between the original AM strains and observed species in each treatment (Table 11). These spores were separated from pot cultures where sorghum seeds were inoculated in 1996 with colonized pepper roots and sand (which remained from the 1995 experiment). It is unclear if some of the identified species are dead material remaining in the pot culture after pasteurization of the original sandy soil (i.e. *G. mosseae* and *S. rubiformis*). The extent and exact moment of cross-contamination occurrence, as well as the disappearance of some AM species (i.e. *G. intraradices*) in the final checklist (Table 11) remains unexplained.

In order to overcome these difficulties in future studies, it is recommended to carefully check the pasteurization procedure which may have been insufficient to kill the background levels of AM fungi. In addition to this, it is also advisable to have more available room in the greenhouse in order to avoid cross-contamination among treatments. A considerable amount of AM inoculum should be produced in separate benches and well in advance of starting this type of experiment. Thus, both the risk of cross-contamination and the use of remaining material from preceding experiments will be avoided.

This preliminary survey of the effectiveness of different mycorrhizal isolates under field conditions has shown no differences in growth among inoculated and non-inoculated plants growing in non-fumigated soils. The cost of large-scale fumigation is prohibitive for farmers and the detrimental long-scale consequences of such a strategy on plant productivity and the environment are unpredictable. As a consequence, it is advisable to further evaluate the effectiveness of different mycorrhizal partners in the presence of a competitive indigenous

Table 11. Final checklist of AM species observed in inoculum treatments.

Code	Original AM strains	[a]AM species (final checking)
GagN	*Glomus aggregatum*	*Sclerocystis rubiformis*
		Glomus fasciculatum
		Glomus macrocarpum
GinN	*Glomus intraradices*	*Glomus mosseae*
		Glomus fasciculatum
		Glomus aggregatum
		Sclerocystis rubiformis
GetN	*Glomus etunicatum*	*Glomus aggregatum*
		Sclerocystis rubiformis
		Glomus mosseae
GfaA	*Glomus fasciculatum*	*Glomus fasciculatum*
		Glomus mosseae
		Sclerocystis rubiformis
GmoA	*Glomus mosseae*	*Glomus mosseae*
		Glomus aggregatum
		Glomus sp.
		Glomus albidum
		Sclerocystis rubiformis
Gag+	*Glomus aggregatum*	*Glomus albidum*
	Glomus geosporum	*Glomus fasciculatum*
		Glomus macrocarpum
		Sclerocystis rubiformis
Gmi+	*Glomus microaggregatum*	*Glomus fasciculatum*
	Glomus mosseae	*Sclerocystis rubiformis*
	Glomus fasciculatum	*Glomus aggregatum*
Sru+	*Sclerocystis rubiformis*	*Sclerocysistis rubiformis*
	Glomus fasciculatum	*Glomus aggregatum*
GinA	*Glomus intraradices*	*Glomus aggregatum*
		Glomus mossea
		Sclerocystis rubiformis

[a]Spores were separated from the sorghum pot culture (1996) and identified by Yolande Dalpé

mycorrhizal community. It is also advisable to keep in mind that AM species differentiation is still a matter of intense debate. Molecular techniques are now being used to elucidate the actual degree of affinity among morphologically similar spores.

The following are some suggested future directions to follow in this work:

- Selected AM fungi should be evaluated at different P rates under field conditions.
- Determine the effect of agricultural management practices on plant responses to indigenous AM communities and introduced strains. Native mycorrhizae should be evaluated prior to inoculation.
- It is advisable to implement other methodologies than AM colonization to assess mycorrhizal activity and hence, have a better indicator of the effectiveness of introduced AM strains or native mycorrhizal populations.
- Extend the study of AM effectiveness beyond the scope of plant growth or cash-crop benefits into the long term effects on soil stability due to mycorrhizal activity.

BIBLIOGRAPHY

Abbott, L.K., and A.D. Robson. 1984. Colonization of the root system of subterranean clover by three species of vesicular-arbuscular mycorrhizal fungi. New Phytol. 110:275-281.

Abbott, L.K., and A.D. Robson. 1991. Factors influencing the occurrence of vesicular-arbuscular mycorrhizas. Agric. Ecosystems Environ. 35:121-150.

Abbott, L.K., A.D. Robson, and C. Gazey. 1992. Selection of inoculant vesicular-arbuscular mycorrhizal fungi. Meth. Microb. 24:1-21.

Abbott, L.K., A.D. Robson, and M.A. Scheltema. 1995. Managing soils to enhance mycorrhizal benefits in Mediterranean agriculture. Crit. Rev. Biotech. 15:213-228.

Allen, E.B., M.F. Allen, D.J. Helm, J.M. Trappe, R. Molina, and E. Rincon. 1995. Patterns and regulation of mycorrhizal plant and fungal diversity. Plant and Soil 170:47-62.

Allen, M.F. 1991. The Ecology of Mycorrhizae. Cambridge University Press, Cambridge.

Allen, M.F. 1996. The ecology of arbuscular mycorrhizas: a look back into the 20th century and a peek into the 21st. Mycol. Res. 100:769-782.

Allen, M.F., S.D. Clouse, B.S. Weinbaum, S.L. Jeakins, C.F. Friese, and E. Allen. 1992. Mycorrhizae and the integration of scales: From molecules to ecosystems. p. 488-515. *In* M.F. Allen (ed.) Mycorrhizal Functioning, an Integrative Plant-fungal Process. Chapman and Hall, New York.

Ames, R.N., D.P. Reid, L.K. Porter, and D. Cambardella. 1983. Hyphal uptake and transport of nitrogen from two ^{15}N labelled sources by *Glomus mosseae*, a vesicular-arbuscular mycorrhizal fungus. New Phytol. 95:381-396.

An, Z.Q., J.W. Hendrix, D.E. Hershman, and G.T. Henson. 1990. Evaluation of the "most probable number" (MPN) and wet sieving methods for determining soil-borne populations of endogonaceous mycorrhizal fungi. Mycologia 82:576-581.

Anonymous 1994. Grilles de Référence en Fertilisation. Conseil des Productions Végétales du Québec, Canada.

Arines, J., J.M. Palma, and A. Vilariño. 1993. Comparison of protein patterns in non-mycorrhizal and vesicular-arbuscular mycorrhizal roots of red clover. New Phytol. 123:763-768.

Bagyarah, D.J. 1992. Vesicular-arbuscular mycorrhiza: Application in Agriculture. Meth. Microb. 24:359-373.

Barea, J.M., C. Azcón-Aguilar, and R. Azcón. 1987. Vesicular-arbuscular mycorrhizae improve both symbiotic N_2 fixation and N uptake from soil with ^{15}N technique under field conditions. New Phytol. 106:717-725.

Baylis, G.T. 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. p. 373-389. *In* Endomycorrhizas, Proceedings of a symposium held at the University of Leeds, Academic Press.

Bécard, G., and Y. Piché. 1992. Establishment of vesicular-arbuscular mycorrhiza in root organ culture: review and proposed methodology. Meth. Microb. 24:89-108.

Bécard, G., L.P. Taylor, D.D. Douds, P.E. Pfeffer, and L.W. Doner. 1995. Flavonoids are not necessary plant signals compound in arbuscular mycorrhizal symbiosis. Mol. Plant-Microbe Inter. 8:252-258.

Bethlenfalvay, G.J. 1992. Mycorrhizae and crop productivity. p. 1-27. *In* G.J. Bethlenfalvay and R.G. Linderman (ed.) Mycorrhizae in Sustainable Agriculture. American Society of Agronomy, Crop Science Society of America, ASA Spec. Pub. No. 54, Madison, WI.

Bethlenfalvay, G.J., and J.M. Barea. 1994. Mycorrhizae in sustainable agriculture. I. Effects on seed yield and soil aggregation. Amer. J. Alternative Agric. 9:157-161.

Bethlenfalvay, G.J., and H. Schüep. 1994. Arbuscular mycorrhizas and agrosystem stability. p. 117-146. *In* S. Gianinazzi and H. Schüep (ed.) Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems. Birkhäuser Verlag Basel, Switzerland.

Bethlenfalvay, G.J., R.L. Franson, M.S. Brown, and K.L. Mihara. 1989. The *Glycine-Glomus-Bradyrhizobium* symbiosis. IX. Nutritional, morphological and physiological responses of nodulated soybean to geographic isolates of the mycorrhizal fungus *Glomus mosseae*. Physiol. Plant. 76:226-232.

Bethlenfalvay, G.J., R.P. Schreiner, K.L. Mihara, and H. McDaniel. 1996. Mycorrhizae, biocides, and biocontrol. 2. Mycorrhizal fungi enhance weed control and crop growth in a soybean-cocklebur association treated with the herbicide bentazon. Appl. Soil Ecol. 3:205-214.

Bolan, N.S. 1991. A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plants. Plant and Soil 134:189-207.

Bonfante-Fasolo, P. 1992. Plant-fungal interface in VA mycorrhizas: A structural point of view. p. 340-347. *In* D.J. Read et al. (ed.) Mycorrhizas in Ecosystems. University Press, Cambridge.

Bonfante, P., and S. Perotto. 1995. Strategies of arbuscular mycorrhizal fungi when infecting host plants. New Phytol. 130:3-21.

Brundrett, M.C. 1994. Clearing and staining mycorrhizal roots. p. 42-46. *In* M. Brundrett et al. (ed.) Practical Methods in Mycorrhizal Research. Mycologue Publications.

Chabot, S., G. Bécard, and Y. Piché. 1992a. Life cycle of *Glomus intraradix* in root organ culture. Mycologia 84:315-321.

Chabot, S., R. Bel-Rhlid, R. Chênevert, and Y. Piché. 1992b. Hyphal growth promotion in vitro of the VA mycorrhizal fungus *Gigaspora margarita* Becker & Hall, by the activity of structurally specific compounds under CO_2-enriched conditions. New Phytol. 122:461-467.

Chapin, F.S. 1980. The mineral nutrition of wild plants. Ann. Rev. Ecol. Syst. 11:233-260.

Clapp, J.P., J.P.W. Young, J.W. Merryweather, and A.H. Fitter. 1995. Diversity of fungal symbionts in arbuscular mycorrhizas from a natural community. New Phytol. 130:259-265.

Crowder, M.J., and D.J. Hand. 1990. Analysis of Repeated Measures. Chapman and Hall, London.

Davies, F.T., and R.G. Linderman. 1991. Short term effects of phosphorus and VA-mycorrhizal fungi on nutrition, growth and development of *Capsicum annuum* L. Scientia Horticulturae 45:333-338.

Dehne, H.W., and G.F. Backhaus. 1986. The use of vesicular-arbuscular myco-rrhizal fungi in plant production. I. Inoculum production. Z. Pflanzaenkr. Pflanzaenschutz 93:415-424.

Diop, T.A., G. Bécard, Y. Piché. 1992. Long-term *in vitro* culture of an endomycorrhizal fungus, *Gigaspora margarita*, on Ri T-DNA-trasformed roots of carrot. Symbiosis 12:249-259.

Dodd, J.C., Krikun, J., and J. Haas. 1983. Relative effectiveness of indigenous populations of vesicular mycorrhizal fungi from four sites in the Negev. Israel J. Bot. 32:10-21.

Dodd, J.C., and S. Rosendahl. 1996. The BEG expert system—a multimedia identification system for arbuscular mycorrhizal fungi. Mycorrhiza 6:275-278.

Dodd, J.C., and B.D. Thomson. 1994. The screening of inoculant arbuscular-mycorrhizal and ectomycorrhizal fungi. Plant and Soil 159:149-158.

Douds, D.D., L. Galvez, R.R. Janke, and P. Wagoner. 1995. Effect of tillage and farming system upon populations and distribution of vesicular-arbuscular mycorrhizal fungi. Agric. Ecosystems Environ.. 52:111-118.

Douds, D.D., R.R. Janke, and S.E. Peters. 1993. VAM fungus spore populations and colonization of roots of maize and soybean under conventional and low-input sustainable agriculture. Agric. Ecosystems Environ. 43:325-335.

Feldmann, F., and E. Idczak. 1992. Inoculum production of vesicular-arbuscular mycorrhizal fungi for use in tropical nurseries. Meth. Microb. 24:339-357.

Fitter, A.H. 1991. Costs & benefits of mycorrhizas: implications for functioning under natural conditions. Experientia 47:350-355.

Garbaye, J. 1991. Biological interactions in the mycorrhizosphere. Experientia 47:370-375.

Gianinazzi, S. 1991. Vesicular-arbuscular (endo-) mycorrhizas: cellular, biochemical and genetic aspects. Agric. Ecosystems Environ. 35:105-119.

Gianinazzi, S., A. Trouvelot, and V. Gianinazzi-Pearson. 1989. Conceptual approaches for the rational use of VA endomycorrhizae in Agriculture: posibilities and limitations. Agric. Ecosystems Environ. 29:153-161.

Gianinazzi, S., A. Trouvelot, P. Lovato, D. Van Tuinen, P. Franken, and V. Gianinazzi-Pearson. 1995. Arbuscular mycorrhizal fungi in plant production of temperate agroecosystems. Crit. Rev. Biotech. 15:305-311.

Gianinazzi-Pearson, V., E. Dumas-Gaudot, A. Gollotte, A. Tahiri-Alaoui, and S. Gianinazzi, S. 1996. Cellular and molecular defence-related root responses to invasion by arbuscular mycorrhizal fungi. New Phytol. 133:45-57.

Giovannetti, M., and V. Gianinazzi-Pearson V. 1994. Biodiversity in arbuscular mycorrhizal fungi. Mycol. Res. 98:705-715.

Giovanetti, M., and B. Mosse. 1980. An evaluation of techniques for measuring vesicular-arbuscular infection of roots. New Phytol. 84:489-500.

Gomez, K.A., and A.A. Gomez. 1984. Statistical Procedures for Agricultural Research. John Wiley & Sons, Singapore.

Graham, J.H., and D.M. Eissenstat. 1994. Host genotype and the formation and function of VA mycorrhizae. Plant and Soil 159:179-185.

Hamel, C., C. Nesser, U. Barrantes-Cartín, and D.L. Smith. 1991a. Endomycorrhizal fungal species mediate ^{15}N transfer from soybean to maize in non-fumigated soil. Plant and Soil 138:41-47.

Hamel, C. 1991b. Mycorrhizal effects on ^{15}N transfer from legumes to grass intercrops, plant growth and interspecific competition. Ph.D. thesis. Macdonald College of McGill University. Québec, Canada.

Hamel, C. 1996. Prospects and problems pertaining to the management of arbuscular mycorrhizae in agriculture. Agric. Ecosystems. Environ. 60:197-210.

Harinikumar, K.M., and D.J. Bagyaraj. 1996. Persistence of introduced *Glomus intraradices* in the field as influenced by repeated inoculation and cropping systems. Biol. Fert. Soils. 21:184-188.

Harris, D.S., and E. Paul. 1987. Carbon requirements of vesicular-arbuscular mycorrhizae. p. 93-105. *In* G.R. Safir (ed.) Ecophysiology of VA Mycorrhizal Plants. CRC Press, Boca Raton.

Haas, J.H., and J. Krikun. 1985. Efficacy of endomycorrhizal-fungus isolates and inoculum quantities required for growth response. New. Phytol. 100:613-621.

Hayman, D.S. 1983. The physiology of the vesicular-arbuscular endomycorrhizal symbiosis. Can. J. Bot. 61:944-963.

Hayman, D.S. 1987. VA mycorrhizas in field crop systems. p. 171-192. *In* G.R. Safir (ed.) Ecophysiology of VA Mycorrhizal Plants. CRC Press, Boca Raton.

Hendrix, J.W., B.Z. Guo, and Z.Q. An. 1995. Divergence of mycorrhizal fungal communities in crop production systems. Plant and Soil 170:131-140.

Hendrix, J.W., K.J. Jones, and W.C. Nesmith. 1992. Control of pathogenic mycorrhizal fungi in maintenance of soil productivity by crop rotation. J. Prod. Agric. 5:383-386.

Henkel, T.W., W.K. Smith, and M. Christensen. 1989. Infectivity and effectivity of indigenous vesicular-arbuscular mycorrhizal fungi from contigous soils in southwestern Wyoming, USA. New Phytol. 112:205-214.

Hepper. C.M., C. Azcon-Aguilar, S. Rosendahl, and R. Sen. 1988. Competition between three species of *Glomus* used as spatially separated introduced and indigenous mycorrhizal inocula for leek (*Allium porrum* L.). New Phytol. 110:207-215.

Hesterman, O.B., and T.L. Thorbum. 1994. A comprehensive approach to sustainable agriculture: W.K. Kellog's integrated farming systems initiative. J. Prod. Agric. 7:132-134.

Hetrick, B.A. 1991. Mycorrhizas and root architecture. Experientia 47:355-362.

Hetrick, B.A., J.A. Hetrick, and J. Bloom. 1984. Interaction of mycorrhizal infection, phosphorus level, and moisture stress in growth of field corn. Can. J. Bot. 62:2267-2271.

Hetrick, B.A., G.W. Wilson, and T.S. Cox. 1993. Mycorrhizal dependence of modern wheat cultivars and ancestors: a synthesis. Can. J. Bot. 71:512-518.

Hetrick, B.A., G.W. Wilson, B.S. Gill, and T.S. Cox. 1995. Chromosome location of mycorrhizal responsive genes in wheat. Can. J. Bot. 73:891-897.

Ingham, E.R., and R. Molina. 1991. Interactions among mycorrhizal fungi, rhizosphere organisms and plants. p. 169-197. *In* P. Barbosa et al. (ed.) Microbial Mediation of Plant-herbivore Interactions. John Willey & sons.

Jalali, B.L. and I. Jalali. 1991. Mycorrhizae in plant disease control. p. 131-154. *In* D.K. Arora et al. (ed.) Handbook of Applied Mycology, Vol. 1: Soil and plants. Marcel Dekker Inc., New York.

Jeffries, P., and J.M. Barea. 1994. Biogeochemical cycling and arbuscular mycorrhizas in the sustainability of plant-soil systems. p. 101-115. *In* S. Gianinazzi and H. Schüep (ed.) Impact of Arbuscular Mycorrhizas on Sustainable Agriculture and Natural Ecosystems. Birkhäuser Verlag Basel, Switzerland.

Jeffries, P., and J.C. Dodd. 1991. The use of mycorrhizal inoculants in forestry and agriculture. p. 155-185. *In* D.K. Arora et al. (ed.) Handbook of Applied Mycology, Vol 1: Soil and plants. Marcel Dekker Inc.

Johnson, N.C. 1993. Can fertilization of soil select less mutualistic mycorrhizae? Ecol. Appl. 31:749-757.

Johnson, N.C., and F.L. Pfleger. 1992. Vesicular-arbuscular mycorrhizae and cultural stresses. p. 71-79. *In* G.J. Bethlenfalvay and R.G. Linderman (ed.) Mycorrhizae in Sustainable Agriculture. American Society of Agronomy, Crop Science Society of America, ASA Spec. Publ. No. 54, Madison, WI.

Johnson, N.C., P.J. Coopeland, R.K. Crookston, and F.L. Pfleger. 1992. Mycorrhizae: possible explanation for yield decline with continous corn and soybean. Agron. J. 84:387-390.

Johnson, N.C., P.J. Copeland, R.K. Crookston, and F.L. Pfleger. 1991a. Mycorrhizae: possible explanation for yield decline with continous corn and soybean. Agron. J. 84:387-390.

Johnson, N.C., F.L. Pfleger, R. K. Crookston, S.R. Simmons, and P.J. Copeland. 1991b. Vesicular-arbuscular mycorrhizas respond to corn and soybean cropping history. New Phytol. 117:657-663.

Klironomos, J.N., and W.B. Kendrick. 1993. Research on mycorrhizas: trends in the past 40 years as expressed in the 'Mycolit' database. New Phytol. 125:595-600.

Koide, R.T. 1991. Nutrient supply, nutrient demand and plant response to mycorrhizal infection. New Phytol. 117:365-386.

Kucey, R.M., H.H. Janzen, and M.E. Leggett. 1989. Microbially mediated increases in plant-available phosphorus. Adv. Agric. 42:199-228.

Kurley, J.E., and F.L. Pfleger. 1994. Arbuscular mycorrhizal fungus spore populations respond to conversions between low-input and conventional management practices in a corn-soybean rotation. Agron. J. 86:467-475.

Linderman, R.G. 1992. Vesicular-arbuscular mycorrhizae and soil microbial interactions. p.45-70. *In* G.J. Bethlenfalvay and R.G. Linderman (ed.) Mycorrhizae in Sustainable Agriculture. American Society of Agronomy, Crop Science Society of America, ASA Spec. Publ. No. 54, Madison, WI.

Linderman, R.G. 1993. Effects of microbial interactions in the mycorrhizosphere on plant growth and health. p. 138-152. *In* R. Ferrera Cerrato and R. Quintero (ed.) Agroecología, Sostenibilidad y Educación. Centro de Edafología, Colegio de Postgraduados, México.

Louis, I., and G. Lim. 1987. Response of *Glycine max* to two tropical isolates of *Glomus clarum*. p. 49. In D.M. Sylvia *et al.* (ed.) Mycorrhizae in the next decade: practical applications and research priorities. 7th North American Conference on Mycorrhizae, University of Florida, Gainesville, USA.

Lumini, E., M. Bosco, G. Puppi, R. Isopi, M. Fratttegiani, E. Buresti, and F. Favilli. 1994. Field performance of *Alnus cordata* Loisel (Italian alder) inoculated with *Frankia* and VA-mycorrhizal strains in mine-spoil afforestation plots. Soil Biol. Biochem. 26:659-661.

Marshner, H., and B. Dell. Nutrient uptake in mycorrhizal symbiosis. Plant and Soil. 59:89-102.

Menge, J.A. 1984. Inoculum production. p. 187-203. *In* C.L. Powell and D.J. Bagyaraj (ed.) VA Mycorrhizae. CRC Press, Boca Raton.

Miller, R.M. 1987. The ecology of vesicular-arbuscular mycorrhizae in grass and shrublands. p. 135-170. *In* G.R. Safir (ed.) Ecophysiology of VA Mycorrhizal Plants. CRC Press, Boca Raton.

Miller, R.M., and J.D. Jastrow. 1992a. The role of mycorrhizal fungi in soil conservation. p. 29-44. *In* G.J. Bethlenfalvay and R.G. Linderman (ed.) Mycorrhizae in Sustainable Agriculture. American Society of Agronomy, Crop Science Society of America, ASA Spec. Publ. No. 54, Madison, WI.

Miller, R.M. and Jastrow, J.D. 1992b. The application of VA mycorrhizae to ecosystem restauration and reclamation. p. 438-467. *In* M.F. Allen (ed.) Mycorrhizal Functioning, an Integrative Plant-fungal Process. Chapman & Hall, New York.

Miller, R.M., T. McGonigle, and H.D. Addy. 1994. An economic approach to evaluate the role of mycorrhizas in managed ecosystems. Plant and Soil. 159:27-35.

Miller, R.M., T. McGonigle, and H.D. Addy. 1995. Functional ecology of vesicular arbuscular mycorrhizas as influenced by phosphate fertilization and tillage in an agricultural ecosystem. Crit. Rev. Biotech. 15:241-255.

Molina, R., H. Massicotte, and J.M. Trappe. 1992. Specificity phenomena in mycorrhizal symbiosis: community-ecological consequences and practical implications. p. 357-423. *In* M.F. Allen (ed.) Mycorrhizal Functioning, an Integrative Plant-fungal Process. Chapman and Hall, New York.

Morton, J.B., and S.P. Bentivenga. 1994. Levels of diversity in endomycorrhizal fungi (Glomales, Zygomycetes) and their role in defining taxonomic and non-taxonomic groups. Plant and Soil. 159:47- 59.

Morton, J.B., S.P. Bentivenga, and W.W. Wheeler. 1993. Germ plasm in the International collection of arbuscular and vesicular-arbuscular mycorrhizal fungi (INVAM) and procedures for culture development, documentation and storage. Mycotaxon 48:491-528.

Mosse, B., D.P. Stribley, and F. Le Tacon. 1981. Ecology of mycorrhizae and mycorrhizal fungi. Adv. Microb. Ecol. 5:137-210.

Murphy, J., and J.P. Riley. 1962. A modified single solution method for the determination of phosphate in natural water. Anal. Chem. Acta. 27:31-36.

Nelson, C.E. 1987. The water relations of vesicular-arbuscular mycorrhizal systems. p. 71-91. *In* G.R. Safir (ed.) Ecophysiology of VA Mycorrhizal Plants. CRC Press, Boca Raton.

Oberle, S. 1994. Farming systems options for U.S. agriculture: an agroecological perspective. J. Prod. Agric. 7:119-123.

O'Neill, E.G., R.V. O'Neill, and R.J. Norby. 1991. Hierarchy theory as a guide to mycorrhizal research on large-scale problems. Environ. Poll. 73:271-284.

Olsen, J.K., J.T. Schaefer, M.N. Hunter, D.G. Edwards, V.J. Galea, and L.M. Muller. 1996. Response of capsicum (*Capsicum annuum* L.), sweet corn (*Zea mays* L.), and tomato (*Lycopersicon esculentum* Mill.) to inoculation with vesicular-arbuscular mycorrhizae. Aust. J. Agric. Res. 47:651-671.

Piché, Y., L. Simon, and A. Séguin. 1994. Genetic manipulation in vesicular-arbuscular mycorrhizal fungi. Plant and Soil 159:171-178.

Pirozynski, K.A., and Y. Dalpé. 1989. Geological history of the Glomaceae with particular reference to mycorrhizal symbiosis. Symbiosis 7:1-36.

Pirozynski, K.A., and D.W. Malloch. 1975. The origin of land plants: a matter of mycotropism. Biosystems 6:153-164.

Plenchette, C., and D.G. Strullu. 1995. Mycorrhizes et agriculture intégrée: standards expérimentaux. p. 125-144. *In* J.A. Fortin et al. (ed.) La Symbiose Mycorhizienne, État des Connaissances. Éditions Orbis, Québec.

Plenchette, C., J.A. Fortin, and V. Furlan. 1983. Growth responses of several species to mycorrhizae in a soil of moderate P fertility. I. Mycorrhizal dependency under field conditions. Plant and Soil 70:199-209.

Rabatin, S.C., and B.R. Stinner. 1989. The significance of vesicular-arbuscular mycorrhizal fungi-soil macroinvertebrate interactions in agroecosystems. Agric. Ecosystems. Environ. 27:195-204.

Reganold, J.P., R.I. Papendick, and J.F. Parr. 1990. Sustainable agriculture. Sci. Amer. 262:112-120.

Sanders, I.R., and A.H. Fitter. 1992. Evidence for differential responses between host-fungus combinations of vesicular-arbuscular mycorrhizae from a grassland. Mycol. Res. 96:415-419.

Sanders, I.R., J.P. Clapp, and A. Wiemken. 1996. The genetic diversity of arbuscular mycorrhizal fungi in natural ecosystems—a key to understanding the ecology and functioning of the mycorrhizal symbiosis. New Phytol. 133:123-134.

SAS Institute Inc. 1988. SAS/STAT User's Guide, release 6.03, Cary, NC.

Schreiner, R.P., and G.J. Bethlenfalvay. 1995. Mycorrhizal interactions in sustainable agriculture. Crit. Rev. Biotech. 15:271-185.

Schreiner, R.P., and G.J. Bethlenfalvay. 1996. Mycorrhizae, biocides, and biocontrol. 4. Response of a mixed culture of arbuscular mycorrhizal fungi and host plant to three fungicides. Biol. Fert. Soils. 23:189-195.

Schwab, S.M., J.A. Menge, and P.B. Tinker. 1991. Regulation of nutrient transfer between host and fungus in vesicular-arbuscular mycorrhizas. New Phytol. 117:387-398.

Sieverding, E. 1989. Ecology of VAM fungi in tropical agrosystems. Agric. Ecosystems Environ. 29:369-390.

Sieverding, E. 1991. Vesicular-arbuscular Mycorrhiza Management in Tropical Agrosystems. Deutshe Gesellschaft für Technische Zusammen arbeit, GmbH., Eschborn.

Simon, L., J. Bousquet, R.C. Lévesque, and M. Lalonde. 1993. Origin and diversification of endomycorrhizal fungi and coincidence with vascular land plants. Nature 363:67-69.

Smith, S.E., and V. Gianinazzi-Pearson. 1988. Physiological interactions between symbionts in vesicular-arbuscular mycorrhizal plants. Ann. Rev. Plant Phys. Plant Mol. Biol. 39:221-244.

Smith, S.E., V. Gianinazzi-Pearson, R. Koide, and J.W. Cairney. 1994. Nutrient transport in mycorrhizas: structure, physiology and consequences for efficiency of the symbiosis. Plant and Soil. 159:103-113.

Smith, T.F. 1978. A note on the effect of soil tillage on the frequency and vertical distribution of spores of vesicular-arbuscular endophytes. Aust. J. Soil Res. 16:359-361.

Soedarjo, M., and M. Habte. 1993. Vesicular-arbuscular mycorrhizal effecti-veness in an acid soil amended with fresh organic matter. Plant and Soil 149:297-203.

St-Arnaud, M., C. Hamel, B. Vimard, M. Caron, and J.A. Fortin. 1996. Enhanced hyphal growth and spore production of the arbuscular mycorrhizal fungus *Glomus intraradices* in an *in vitro* system in the absence of host roots. Mycol. Res. 100:328-332.

Stahl, P.D., and M. Christensen. 1991. Population variation in the mycorrhizal fungus *Glomus mosseae*: breadth of environmental tolerance. Mycol. Res. 95:300-307.

Stahl, P.D., M. Christensen, and S.E. Williams. 1990. Population variation in the mycorrhizal fungus *Glomus mosseae*: uniform garden experiments. Mycol. Res. 94:1070-1076.

Sylvia, D.M., A.G. Jarstfer, and M. Vosátka. 1993. Comparisons of vesicular-arbuscular mycorrhizal species and inocula formulations in a commercial nursery on diverse Florida beaches. Biol. Fert. Soils 16:139-144.

Sylvia, D.M., and S.E. Williams. 1992. Vesicular-arbuscular mycorrhizae and environmental stress. p. 101-124. *In* G.J. Bethlenfalvay and R.G. Linderman (ed.) Mycorrhizae in Sustainable Agriculture. American Society of Agronomy, Crop Science Society of America, ASA Spec. Pub. No. 54, Madison, WI.

Tisdall, J.M. 1994. Possible role of soil microorganisms in aggregation in soils. Plant and Soil 159:115-121.

Thomas, R.L., R.W. Sheard, and J.R. Moyer. 1967. Comparison of conventional and automated procedures for nitrogen, phosphorus and potassium analysis of plant material using a single digestion. Agron. J. 59:240-243.

Thompson, J.P. 1990. Soil sterilization methods to show VA-mycorrhizae aid P and Zn nutrition in vertisoils. Soil Biol. Biochem. 22:229-240.

Tsai, S.M., and D.A. Phillips. 1991. Flavonoids released naturally from alfalfa promote development of symbiotic *Glomus* spores *in vitro*. Appl. Environ. Microb. 57:1485-1488.

Van Duin, W.E., W.A. Griffioen, and J.H. Ietswaart. 1991. Occurrence and function of mycorrhizae in environmentally stressed soils. p. 114-123. *In* J. Rozema and J.A. Verkleij (ed.) Ecological Responses to Environmental Stress. Kluwer Acad. Publ., Netherlands.

Varma, A. 1995. Arbuscular mycorrhizal fungi: the state of art. Crit. Rev. Biotech. 15:179-199.

Varma, A., and H. Schüep. 1995. Mycorrhization of the commercially important micropropagated plants. Crit. Rev. Biotech. 15:313-328.

Walker, C. 1992. Systematics and taxonomy of the arbuscular endomycorrhizal fungi (Glomales)—a possible way forward. Agronomie 12:887-897.

Waterer, D.R., and R.R. Coltman. 1989. Response of mycorrhizal bell peppers to inoculation timing, phosphorus, and water stress. HortScience 24:688-690.

Wilson, K., and G.E. Morren Jr. 1990. Systems Approaches in Agiculture and Resource Management. MacMillan Pub., New York.

Wood, T., and B. Cummings. 1992. Biotechnology and the future of VAM commercialization. p. 468-487. *In* M. Allen (ed.) Mycorrhizal Functioning, an Integrative Plant-fungal Process. Chapman & Hall, New York.

Winer, B.J., D.R. Brown, and K.M. Michels. 1991. Statistical Principles in Experimental Design. McGraw Hill, New York.

Wyss, P., and P. Bonfante. 1993. Amplification of genomic DNA of arbuscular-mycorrhizal (AM) fungi by PCR using short arbitrary primers. Mycol. Res. 97:1351-1357.

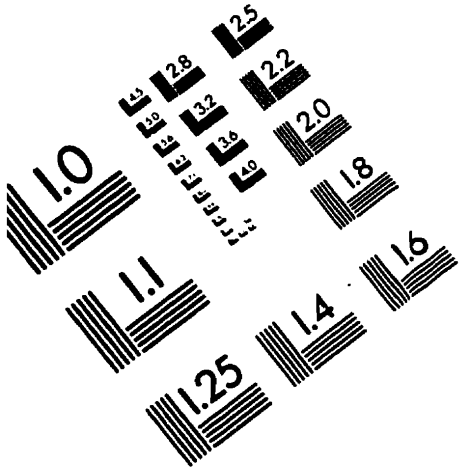
APPENDIX I

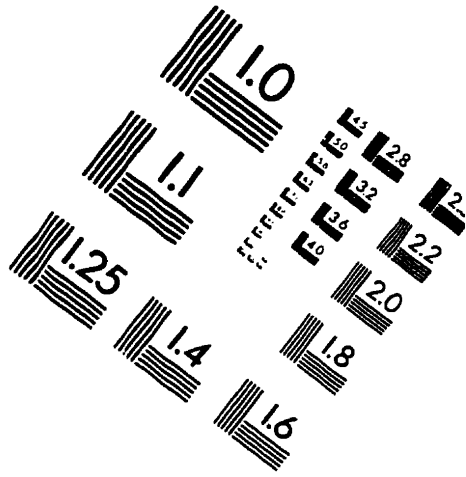
Modified Hoagland's nutrient solution.

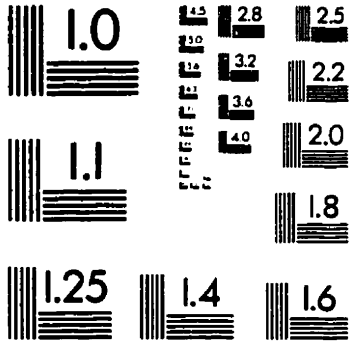
Compound	Concentration
K_2SO_4	1.00 mM
$MgSO_4$	0.25 mM
KH_2PO_4	0.04 mM
$CaCl_2$	1.00 mM
KNO_3	8.30 mM
Fe (Chelate 9 %)	444.0 $\mu g\ l^{-1}$
H_3BO_3	150.0 $\mu g\ l^{-1}$
$MnCl_2.4H_2O$	100.0 $\mu g\ l^{-1}$
$ZnSO_4$	12.5 $\mu g\ l^{-1}$
$CuSO_4.2H_2O$	12.5 $\mu g\ l^{-1}$
$Na_2MoO_4.2H_2O$	5.0 $\mu g\ l^{-1}$

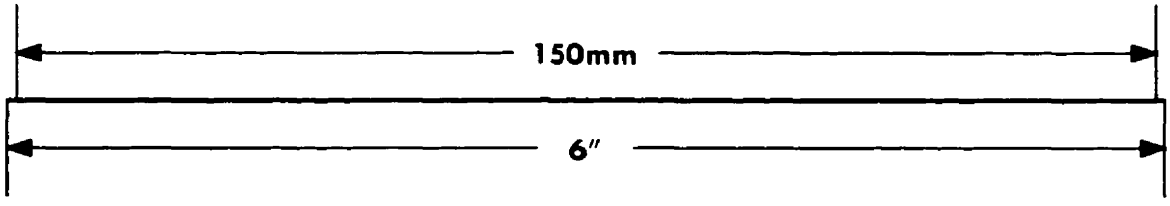
Source: Hamel (1991b)

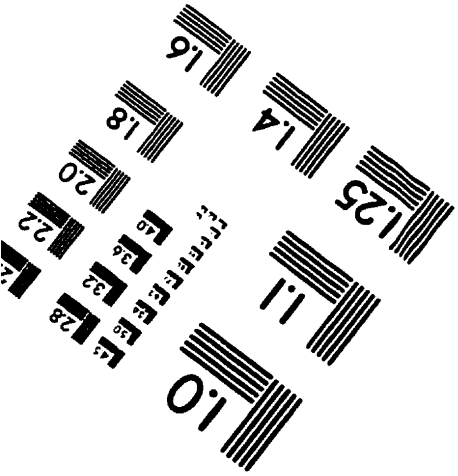
IMAGE EVALUATION
TEST TARGET (QA-3)

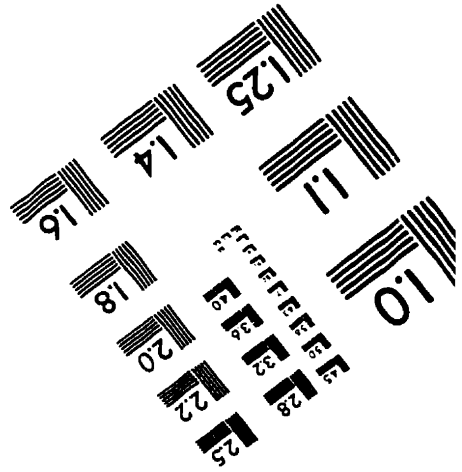












APPLIED IMAGE, Inc
1653 East Main Street
Rochester, NY 14609 USA
Phone: 716/482-0300
Fax: 716/288-5989