EFFECT OF COMMERCIAL ARBUSCULAR MYCORRHIZAL PRODUCTS ON COLONIZATION AND GROWTH OF ORNAMENTAL ANNUAL PLANTS

by

CHRISTINE LOUISE HOFFMAN

(Under the Direction of Timothy J. Smalley)

ABSTRACT

These studies evaluated the effect of commercial arbuscular mycorrhizal (AMF) products on root colonization and growth of ornamental annuals grown in Cecil sandy clay loam.

Adding phosphorus (P) decreased Mycor (Mycor Flower Saver Plus™; Plant Healthcare, Inc.) colonization of *Impatiens wallerana* and *Salvia splendens* without reducing colonization by native AMF.

An April 2001 study indicated Mycor or Healthy Start™, fertilizer in Mycor, did not increase native AMF root colonization of *Impatiens* (32%) and demonstrated that Mycor did not colonize *Impatiens* (0%) in sterilized soil.

In an August 2001 study, soil sterilization did not limit colonization by native AMF in *Impatiens* and *Salvia* grown in a 3 sterile: 1 non-sterile soil. Mycor and endoRoots™ (Roots, Inc.) colonization of *Impatiens* (0%, 0.3%) and *Salvia* (0%, 0%) was negligible.

Using product obtained from the producers, an April 2002 study demonstrated that Mycor can colonize *Impatiens* (22%), but endoRoots colonization was again low (0.2%).

INDEX WORDS: AMF, endoRoots, Mycor, phosphorus, Salvia, Impatiens

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DEDICATION

This work is dedicated to my family. First to my husband, Chris, whose love, faith, grace, teachings and friendship has kept me together, strong and laughing. Secondly to the members of my family, which is very large. It includes the amazing Hoffman clan I was born into; to my parents, Charlie and Edna, whose love of family and learning are the greatest gifts they could have given to my sisters and I. To my sisters, Jo, Kathy, Madeline and Charlotte, who are my friends and life mentors, and their wonderful husbands who understand the need of telephone calls. My family also includes the incredibly loving Sparnicht crew I have had the luck and grace of Spirit to marry into and learn from. With them and my own family, my number of nieces, nephews, aunts, uncles, cousins and grandparents has doubled. And they have added more blessings to our lives. This work and our life here has been supported by them with love, encouragement and the strength of their many shoulders. And thirdly, to those of my family who have become a part of our families. To my "adopted sister" Brina, who shared the path of graduate school and showed me the ropes and gave me much guidance and encouragement. And to my "surrogate parents", the Squires, whose humor and love has made life so much sweeter. These friends and beautiful spirits (and so many others I have not named, but you know who you are) have pulled, carried and held hands with all of us through some of the hardest years of our lives. To all of them, I am incredibly grateful.

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CHAPTER 1

INTRODUCTION

Georgia's drought has continued for 5 years. Climatologists find North Central Georgia, which includes Clarke County, in a moderate drought (Stooksbury and Knox, 2001). Counties throughout the state have been under mandatory water-use restrictions (Dollard, 2001), which make it difficult for homeowners and landscapers to provide water to keep plants alive during Georgia's hot and dry summers. Products advertised to increase drought tolerance, such as those containing hydrogel or mycorrhizal fungi, are available on the market, and may provide a partial solution to this problem.

Scientists studying arbuscular mycorrhizal fungi (AMF) have found that colonized host plants can tolerate (Waterer and Coltman, 1989) and recover from drought better than non-mycorrhizal plants (Sweatt and Davies, 1984). Arbuscular mycorrhizal fungi are a symbiotic obligate that live in the roots of 80% of flowering plants. The host plant provides photosynthates and a safe place for the fungi to live. The fungi, through its extensive external hyphae network, can provide greater access to nutrients (Marschner and Dell, 1994) and water (Hardie, 1985; Busse and Ellis, 1985).

This mycorrhizal benefit has been attributed to external hyphae absorption of water (Hardie, 1985; Ruiz-Lozano and Azcon, 1995); soil aggregation by fungi exudates and hyphae, which allows the soil to hold more water (Fitter, 1985); and increased nutrition and continued leaf conductance (open stomates) even under drought (Duan *et al*,

1996). The degree of drought benefit will depend on the host plant (Hetrick *et al*, 1987) and species of fungi (Ruiz-Lozano and Azcon, 1995).

Commercial AMF products, containing AMF propagules (spores, hyphae and/or colonized roots) to inoculate soil, may provide this drought benefit to ornamental annuals in the Georgia landscape. Colonization by commercial AMF products has been observed in flowering annuals (Koide *et al*, 1999).

Field studies with commercial AMF inocula (Smalley, personal communications) have yielded variable results in AMF colonization and plant growth. This variability could be due to non-viable inocula, improper storage, high phosphorous levels in the soil inhibiting colonization or competition from native AMF. Other components of the commercial AMF products, such as fertilizer, organic matter and biostimulants, may also be affecting the viability of the inocula. The purpose of this study was to investigate the effect of some of these factors on colonization and growth of annual flowers by commercial sources of mycorrhizal inocula.

CHAPTER 2

LITERATURE REVIEW

The Mycorrhizae

Mycorrhizae are a group of fungi that create a symbiotic relationship with plants' roots. Mycorrhizae, which means "fungus-root" in Greek, was defined in 1885 by A. B. Frank, a German pathologist, who first described the fungus-root association he discovered in trees. In the symbiotic relationship, a fungi-root interface is created between the fungi and plant, where an exchange of nutrients and water occurs. Approximately 95% of all land plants have mycorrhizal associations. These relationships are believed to have existed for over 400 million years (Remy *et al*, 1994). Some scientists believe that without these relationships, colonization of land by plants would not have been possible (Pirozunski and Malloch, 1975).

Mycorrhizal symbiosis, like the *Rhizobium*-legume and the *Frankia*-plant symbiosises, follows the endosymbiont theory of chloroplast and mitochondria evolution. This endosymbiont theory states that the hosts' necessity for elements C, H, and O catalyzed the symbiosis of cyanobacteria with eukaryotic algae, Archegoniatae and spermatophytes. This necessity also catalyzed the symbiosis of mitochondria with eukaryotes. The latter relationship provides oxygen for internal use by eukaryotes; and the cyanobacterias provide carbon in the form of CO_2 and hydrogen from H_2O (Werner, 1992).

The four groups of mycorrhizae are the ectomycorrhizae, the ericaceous mycorrhizae, the orchidaceal mycorrhizae and the arbuscular mycorrhizae (AM). The ericaceous mycorrhizal fungi are associated with ericaceous plants, which grow in acidic and highly organic soils. The orchidaceal mycorrhizae are specifically associated with the Orchid family where the association is established as the seed germinates, providing nutrients for the developing embryo, as orchid seeds contain little stored food. This association may or may not end, depending on the orchid species. Orchid evolution is considered to be continuous because of its mycorrhizal associations and is the only host group, which has evolutionarily developed specific root modifications for the association (Rasmussen, 1995).

Ectomycorrhizal fungi partner with tree roots in forests and produce a Hartig net to bring in nutrients. The Hartig net is a network of hyphae found between the root cortical cells and includes a thick hyphal coat on the outside of the now swollen and stubby mycorrhizal feeder roots. Because of their potential economic importance for plantation tree farming, ectomycorrhizal fungi have been heavily researched.

Arbuscular mycorrhizae fungi (AMF) colonize approximately 80% of the plant kingdom. The fungus-plant interface occurs in the root cortex where C and safe habitation is exchanged by the plant for minerals and water provided by the extensive fungal mycelium.

The name endomycorrrhizae is an older term referring to mycorrhizal fungi that exist in the root cortex. This name refers not only to AM fungi, but also to the orchidaceous mycorrhizal fungi and the ericaceous mycorrhizae. This research focused

on the arbuscular mycorrhizal fungi (AMF or AM), also named vesicular-arbuscular mycorrhizal fungi (VAM or VAMF).

Arbuscular Mycorrhizal Fungi

Taxonomy

The arbuscular mycorrhizal fungi are a group of 150 plus species (not including individual isolates) that are still being named, identified, and catalogued (Schultz *et al*, 1999; Morton and Redecker, 2001). This cataloguing increased dramatically in 1990 when Joseph Morton became curator of the largest living AMF collection after N.C. Schenck, creator of the collection, retired. The collection was moved and renamed INVAM (International Collection of Vesicular and Arbuscular Mycorrhizal Fungi) and is housed at West Virginia University, in Morgantown.

On the basis of mainly spore identification (cell wall thickness, coloring, and make-up) (Walker, 1983; Morton, 1988), Morton refined classification of AM to help name the five families and eight genera in this group of fungi (Morton 1988; Morton *et al*, 1993a*or1990; Morton & Redecker, 2001). AMF belong in the phylum Zygomycota and in the order Glomales, in which all members of the order produce arbuscules (therefore the name Arbuscular Mycorrhizal Fungi). Scientists had assumed that all AMF produced vesicles, therefore the earlier name VAMF. Some continue to use the name VAMF since the majority fungi produce vesicles and all produce arbuscules. Taxonomists divide the order Glomales into two suborders: Glomineae and Gigasporineae. Suborder Glomineae is divided into families Acaulosporaceae and Glomaceae, which both produce vesicles. Suborder Gigasporineae has only one family;

Gigasporaceae, which does not produce vesicles. Two new families were created due to DNA sequencing and different morphological characteristics; Archaeosporaceae and Paraglomaceae (Morton and Redecker, 2001).

These families are divided into eight genera: Acaulosporaceae into *Entrophospora* and *Acaulospora*; Glomaceae into *Glomus* and *Sclerocystis*, family Gigasporaceae into generas *Gigaspora* and *Scutellospora*, Archaeosporaceae into a single genera *Archaeospora* and Paraglomaceae into *Paraglomus*. These divisions are based upon structure and development (Morton, 1988) and now DNA sequencing (Morton and Redecker, 2001). Species are identified by mode of spore formation and in subcellular structure of spores. This method of identification is diverse enough to allow recognition and naming of over 150 species (Morton and Bentivenga, 1994). Starting with the work of Gerdemann and Trappe in 1974 (Morton, 1993a; Gerdemann and Trappe, 1974), the taxonomy of these fungi is approximately 25 years old.

AMF Isolates

Variations within these genera can be identified beyond species to isolate. Morton (1993a) defines isolates as all fungal propagules recovered in a single sample; so multiple samples from the same site are considered multiple isolates. Morton clarifies this definition from a methods approach: an isolate is the closest entity to a fungal individual collected from a soil. The individual has originated from a single spore. Its boundaries are definable only when grown in a containerized plant. This is due to ecological pressures, which drive changes through life cycle adaptations. Because of this, isolates from different soil environments (this can include micro-habitats) will have different traits such as infectivity, primary colonization rates, secondary colonization rates, foraging

ability of external hyphae, survivability, and changes in soil or root environments (Morton, 1993b).

Isolates of the same species can and will behave differently. For example, Medeiros *et al* (1994) compared isolates *Glomus intraradices* UT 143 (named I) and *Glomus intraradices* UT 126 (named B) response to pH. In all four treatments (pH rates of 4, 5, 6, & 7), Isolate I was more effective in increasing plant growth irrespective of pH (especially at pH 5) than Isolate B.

International Culture and Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM), CIAT (Centro Internacioal de Agricultura Tropical - Columbian equivalent of INVAM) and BEG (European Bank of Glomales - European version of INVAM) use letter and number systems to identify their isolates. The INVAM letter and number identification explains where the sample was collected, i.e., the isolates I and B mentioned above carry the letters UT for Utah. The number indicates which isolate from Utah it is (Morton *et al*, 1993c). The INVAM isolate identification information will also include soil information if available (e.g., type, pH) and any pertinent facts regarding the sample. When trying to tie taxonomy and mycorrhizal functioning together, one must study organisms from shared habitat (location) versus organisms sharing spore wall structure (taxonomy) (Morton and Bentivenga; 1994).

Mycorrhizal Specificity

AMF are obligate symbionts - they grow and reproduce only by obtaining C and nutrients from a living host (Sylvia *et al*, 1998). This makes growing AMF axenically nearly impossible. These biotropic relationships are complex. The fungi must establish a connection with the host plant in the root cortical cells. In order to grow and reproduce,

all fungi must have the innate ability to colonize the host and to breach the plant's defenses. The efficacy of the fungus is based on its ability to penetrate and colonize plant roots. The effectiveness of each fungus is its ability to enhance plant growth and/or stress tolerance of the host or both (Sylvia *et al*, 1998). This includes the bi-directional exchange of water, nutrients, and hormones so that each fulfills its life cycle to the point of reproduction for survival until the next growing season or host. If successful, this may include secondary infection in the case of the fungi as a result of the primary infection (Agrios, 1997).

Fungal Structure

Arbuscular mycorrhizal fungi reproduce through spores, extramatrical hyphae, vesicles and chlamydospores. Extramatrical hyphae from infected roots or germinated spores move into the soil to colonize potentially receptive root hair or young unsuberized root tips.

Root exudates (i.e., flavonoids) and volatiles (i.e., CO_2) in the rhizosphere can increase germination of spores and attract germ tubes and hyphae toward the roots. Identifying these hundreds of root exudates and volatiles and determining their impact on the rhizosphere is taking longer than expected (Koske and Gemma, 1992). The flavonoid, formononetin, (Siqueira *et al*, 1991) used by Koide *et al* (1999) in a commercial product named Myconate, increased colonization. In the case of non-mycorrhizal plants (i.e., *Brassica napus*), volatile compounds can reduce the rate of germination and hyphal and germination tube growth without reducing the quantity (Tommerup, 1984).

Upon reaching the potential host plant, a specialized structure called an apressorium penetrates the root (called the entry point) by forcing its way between two

epidermal cells. Intraradical hyphae grow to form intercellular and intracellular hyphae, vesicles, arbuscules, and coiled hyphae. Vesicles contain lipids or spores and are formed at hyphal terminal ends or side branches.

Arbuscules are the interfaces between the fungus and root and are formed when a cortical cell wall near the vascular tissue is thinned or lysed by intraradical hyphae entering the cell. The hyphae enter the cell wall without contacting the cell cytosol because the plant produces a thin but encasing membrane around the forming arbuscule (branched and tree-like in shape), which is itself surrounded by a thin fungal chitin membrane. These membranes protect the plant cell but allow the bi-directional exchange of minerals, photosynthates, water and hormones, between the partners. An arbuscular network can survive many months (2-4 months) as long as the relationship is healthy, nutrients are available and arbuscules continue to develop to replace dying ones (Morton, 2000 – INVAM website).

Benefits of Symbiosis

Why does a plant allow "invading" fungi to colonize its roots? Under normal circumstances such an invasion will cause a defensive reaction as seen for pathogenic fungi. The major benefit plants receive from the relationship is a source of P. In soils, P can be very limited due to phosphorus' anionic nature and due to the season the plant is growing (i.e., cool spring soils). Mycorrhizal safflower plants (*Carthamus tinctorius*) were larger than their non-mycorrhizal counterparts when grown in a low P fine loamy clay, and non-mycorrhizal plants needed 100-fold more P to be of equal size to the mycorrhizal plants (Bryla and Duniway, 1997a).

External hyphae absorb and impart other minerals such as Ca, Mg, (Borie and Rubio, 1999; Medeiros *et al*, 1994; Clark *et al*, 1999b), Cu, K, NH_{4+}, SO_{4+}, Zn, (Marschner and Dell, 1994; Clark *et al*, 1999b) B and Fe (Clark and Zeto, 1996). In mycorrhizal plants, Al tolerance of soybean (*Glycine max* L.) increased, (Maddox and Soileau, 1991), shoot concentrations of Mn and Al in switch-grass (*Panicum virgatum*) (Clark *et al*, 1999b) and geranium (*Pelargonium* x *hortorium* L.H. Bailey) (Biermann & Linderman, 1983b) was decreased, and chloride tolerance in soybean increased (Maddox and Soileau, 1991).

In the case of soil pH, mycorrhizal infection provided protection of sorghum (*Sorghum bicolor*) and soybean (*Glycine max* L. Merr. cv Essex) in acidic soils (Medeiros *et al*, 1994; Maddox and Soileau, 1991).

Larger leaf areas and leaf sizes can occur as seen in geraniums (*Pelargonium x hortorium* L.H. Bailey) (Bierman and Linderman, 1983aa), because of greater nutrition. Greater nutrition was also suggested as the cause for larger vascular bundles and more heavily lignified fibers in mycorrhizal tomatoes (*Lycopersicon esculentum*), petunias (*Petunia* x *hybrida*) and corn (*Zea mays*) (Daft and Okusanya, 1973). Increased stoloniferous growth has been observed in strawberries (*Fragaris* x *ananassa* Duch. 'Senga Sengana') (Niemi and Vestberg, 1992; Khanizadeh *et al*, 1995) and vine growth in grapes (*Vitis* 'Kober 5BB') (Biricolti *et al*, 1997).

Mycorrhizal plants acquire reproductive benefits over non-mycorrhizal plants such as earlier and increased flowering in petunias and normal male tasseling in corn (Daft and Okusanya, 1973). Other reproductive benefits include earlier senescence, and increased fruit size in cultivated oats (*Avena sativa* L.) (Koide *et al*, 1988) and seed

weight and production (Koide and Li, 1991; Zambolim and Schenck, 1983) with increased P seed content (Powell, 1981). Even under stress, increased seed weight and reduced fruit abortion occur (Busse and Ellis, 1985). Koide found increased capsule and seed production (Koide *et al*, 1994) as well as superior, stronger offspring (Koide and Lu, 1992; Lewis and Koide, 1990; Shumway and Koide, 1994) in AMF colonized plants.

Other physiological benefits include salt tolerance, CO_2 exchange rate, stomatal conductance and water use efficiency (Ruiz-Lozano *et al*, 1996) as well as drought tolerance. Improved drought tolerance for peppers (*Capsicum annuum* L. 'Emerald Green Giant' and 'Early Bountiful') has been observed (Waterer and Coltman, 1989; Davies *et al* 1992). Leaves of inoculated *Taxus* x *media* var. *densiformis* had greater chlorophyll content (Gemma *et al*, 1998). Life span has been shown to both increase (tomatoes - *Lycopersicion esculentum* Mill; Bryla and Koide, 1990b) and decrease (oats - *Avena sativa*) due to mycorrhizal infection.

Disease protection/resistance/tolerance from soil-borne plant pathogens for the colonized plant (Azcon-Aguiler and Barea, 1996; Caron *et al*, 1986; Cordier *et al*, 1996 Dehne, 1982; Dugassa *et al*, 1996; Giovannetti *et al*, 1991; Newsham *et al*, 1995a) and plants (dianthus – *Dianthus caryophyllus*) living near AMF colonized plants (marigolds – *Tagetes patula*) (St.-Arnaud *et al*, 1997) is another benefit from the symbiosis.

Soil stabilization occurs through fungi hyphae exudates and mycelium, which, create and hold soil aggregates (Tisdall, 1994). These exudates and mycelium also increase soil water holding capacity. This is an external environmental benefit for the mycorrhizal host and neighboring plants.

Problems with Symbiosis

The symbiotic relationship can be deterious to the host plant because the fungi act as a sink for photosynthates (Trimble and Knowles, 1995b). This loss of photosynthates can cause growth depressions and flower and fruit abortions depending on the plant, plant cultivar, fungi, and soil combination (Trimble and Knowles,1995a,b; Buwalda *et al*, 1982; Koide, 1985). Koide *et al* (1999) saw growth depressions (shoot development) in bedding annuals even when P nutrients were applied to reduce root colonization. Koide (1999) believed the growth depression was associated with the non-P fixing nature of peat moss. Other scientists saw similar growth depressions in mycorrhizal annual geraniums grown in peat moss and attributed it to the medium (Biermann and Linderman, 1983a, 1983b). Growth depressions have also been seen in sunflowers (leaf and stem) even under high light and low P (Koide, 1985), in ryegrass (*Lolium perenne*) caused by C reduction due to host-fungi competition (Buwalda *et al*, 1982), in a variety of nursery stock grown in a sandy loam, and in annual VAM-infected Indian mallow (*Abutilon theophrasti*) when grown beside non-mycorrhizal dependent bristle grass (*Setaria lutescens*) due to reduced available soil P (Koide and Li, 1991). In Koide's 1985 experiment he grew sunflowers (*Helianthus annuus*) in autoclaved soil. The growth depressions in leaf areas of mycorrhizal plants' was temporary. The growth depressions in the shoot growth, specifically stem weights, were not temporary and were due to the symbiosis photosynthate sink.

Koide (1988) studied the growth depressions in mycorrhizal sunflowers (*Helianthus annuus*) and non-mycorrhizal mustard (*Brassica napus*) using soil sievings with and without AM. Early leaf measurements of mycorrhizal plants showed growth

depressions before mycorrhizal colonization could have occurred and non-mycorrhizal plants with non-autoclaved soil sievings also had this growth depression in relation to the control. Possible reasons for this depression are pathogens eliminated by autoclaving and re-introduced with the soil sievings and saprophytic micro-organisms that would have been outcompeted by the AM if it had been present. The leaf areas in mycorrhizal plants increased once colonization occurred and mycorrhizal leaves had the largest leaf area.

Glomus macrocarpum is the cause of tobacco stunt disease where root development is inhibited, the plant is stunted, flowering is delayed, and leaf quality reduced (Jones and Hendrix, 1987). Tobacco stunt disease is treated with soil fungicides to eliminate the fungi.

AM Quantification

Root colonization is quantified through evaluating and/or counting: a) hyphae infection b) vesicle/arbuscule c) length of infected root = total root length/root dry matter or d) length of infected root. When evaluating root colonization, roots are cleared, stained and fungal structures are counted using the grid line intersection method (Giovannetti and Mosse, 1980). Arbuscule quantification provides a clearer picture of functioning mycorrhizae as arbuscules have a short life span of 15 days or less (Sylvia *et al*, 1998).

The percentage of colonization and plant dry weight were not always positively correlated, that is, high colonization does not always equate with high plant dry weights (Mosse, 1972; Medeiros *et al*, 1994; Clark *et al*, 1999a). Medeiros *et al* (1994) found that *Glomus etunicatum* had a higher dry matter yield for the whole plant and shoot, though a lower root colonization than another *Glomus sp.*, which had a lower dry matter yield.

Clark *et al* (1999a) found eight AMF (including *Glomus clarum*, *Gomus etunicatum* and *Glomus intratadices*) grown in an acidic soil at pH 4 and pH 5 that enhanced the dry matter of switchgrass (*Panicum virgatum*) when the total colonization was greater than 20%. When these mycorrhizal plants were compared to non-mycorrhizal plants, mycorrhizal plants had as high as 52% increase in dry matter in pH 4 soil, and as high as 26% increases in dry matter at pH 5.

Davis *et al* (1984) saw a change in active AMF from 1982 to 1983 in nine test fields where mint (*Mentha spp.*) was grown. The only noted difference between the two seasons was a change in weather conditions, from dry and hot in 1982 to a record wet/overcast and cool conditions in 1983. *Glomus etunicatum* was one of these fungi that appeared and colonized the mint with the cooler/wetter conditions, while other species disappeared.

Introduced vs. Native AMF

Porter *et al* (1987) described the challenge of introducing AMF inoculum as similar to that which occurred when *Rhizobium trifolii* was introduced to Australian soils and failed because the strain was poorly adapted to the soil. They recognized that not only does growth need to be increased by the fungi, but also the fungi must be able to establish and persist in the soils where introduced. Siqueira *et al* (1990) found that AM fungi from acidic soils sporulated more and had greater effectiveness with brachiaria grass (*Brachiaria decumbens*) when the soil was not limed. Davis *et al* (1983) in discussing the results of their experiment of pH and AMF effects on sweetgum seedlings (*Liquidamber styraciflua*), believe that regardless of the diversity of symbiotic hosts for AM fungi, if AMF is used as an inoculum, the AM species should be used in the soils and

under weather conditions which closest matches those from which it originates to maximize the benefits of the symbiosis. When this is done, the reintroduction of AMF to a disturbed site where AMF have been removed or eliminated may be possible.

Seasonality and Survival

AM fungi can survive within the roots of the host plant. Monitored on a monthly basis, fields at the Rothamsted Farm in England showed AM infection growing slowly in the spring, spore production increasing by mid-summer and infection plateauing by autumn (Hayman, 1982). In woodland perennials, Brundrett and Kendrick (1990) saw that arbuscule production occurred in summer, declined in late summer and early fall. When winter came and the host plant either died or went dormant, only reproductive structures (spores, vesicles, hyphae, chlamydospores) survived.

The mycorrhizal life cycle will depend on the root growth of the plant. *Erythronium americanum*, a spring ephemeral, grows its roots in the late summer to autumn until the ground becomes too cold. Colonization of the newly grown roots occurs in September and October (Brudrett and Kendrick, 1990).

AM fungi can remain in the soil for years and still germinate and infect roots (Tommerup, 1983). The structure and composition of the spore wall is the reason for this long-term viability (Bonfante and Bianciotto, 1995). External mycelia are able to survive freezing stress by contracting the cytoplasm into a smaller segment of its aseptate hyphae only to extend it in the spring. External mycelia can remain connected to the rest of a mycelia system (Addy *et al*, 1994), re-creating or creating a quick connection and continue in the spring with the old or a new host.

Soil

Lime and soil pH

Arbuscular mycorrhizal fungi exist in soils with pHs ranging from a very acidic 2.7 (Daft *et al*, 1975) to an alkaline 9.2 (Bowen *et al*, 1980). AMF are described as being basophilic or acidophilic. For example, *Glomus mosseae* is considered basophilic, and prefer a soil pH of 6.0 (Davis *et al*, 1983) and above, while *Glomus etunicatum* or *Acaulospora laevis* Gerd. & Trappe are acidophilic, prefer a lower pH (4.6; Porter, 1987). Germination and germ tube growth is dependent on medium pH (Bartolome & Schenck, 1990, Green & Graham 1976), but it is not the controlling factor (Siqueira *et al*, 1984). Fungi function best in soil pHs closest to their soil of origin and will function as long as the soil is brought to a pH range nearer its origin (Porter *et al*, 1983; Mosse, 1972). Nurlaeny *et al* (1996) saw by adjusting the pH (4.7, 5.6 and 6.4) of an Oxisol and an Ultisol with CaCO3, mycorrhizal inoculation increased shoot dry weight and total root length of soybeans (*Glycine max*) in Oxisol and increased P concentration in roots and shoots (soybean and maize (*Zea mays* L.)). Mycorrhizal roots in maize actually became coarser than non-mycorrhizal roots. But even with liming, pH modification may or may not work. *Glomus mosseae* had a greater germination in the control soil (sand) than in the lime-modified acidic soil (Siqueira *et al*, 1984). Davis (1983) showed the opposite was true with *G. mosseae* when he colonized sweetgums (*Liquidamber styraciflua*) in an acidic soil that he had modified with lime to a variety of pHs. In this same study, *Glomus fasciculatum* increased plant growth in more acidic soils, both limed and unlimed, and at all pH values (5-8) over non-mycorrhizal plants, but the mycorrhizal root and shoot weights were similar, when at or closer to the provincial pHs of the AMF.

Medeira *et al* (1994) showed that even though root colonization was better for three of the four isolates at pH 7, the dry matter yield was higher at pH 5. Hayman and Tavare (1985) working with nine species of AMF saw that most colonized well from pH 4 to 7, though when they did not increase plant growth.

Very acidic soils can hamper the growth of plants due to the toxicity of the expressed ions in the soils, such as Al, Fe, Mn and H+ or the deficit of available P, Ca, Mg, and K. Extremes in pH can cause mineral stress which can be alleviated by AMF infection and plants may use this as a stress avoidance mechanism under acidic conditions (Marschner, 1991).

The ability of plants and fungi to deal with pH and its influences on mineral availability and toxicity is very specific. AM-positive, aluminum-tolerant barley (*Hordeum vulgare* L.) had a higher AM response than liming response, while Al-sensitive barley responded to lime and had a smaller response to AM. The AM positive, Al-tolerant plants had lower levels of Al in the shoots than non-AM plants (with and without lime), showing that the AM plants were more efficient in decreasing the levels of phytotoxic Al in the shoots. The Al-tolerant mycorrhizal plants had increases in root and shoot dry matter and increased Ca, Mg, and P concentrations and content in the shoots (Borie and Rubio, 1999).

Phosphorus

Phosphorus-deficient plants will have increases in root/shoot weight ratios due to increases in root weights, lengths and fineness. Increasing root surfaces increase P uptake (Marschner, 1995). As pH reaches a range between 6 and 7, the availability of P peaks in the soil as Al-P and Fe-P bonds break. A plant's ability to acquire P is a determining

factor on its dependency on AMF (Koide, 1991; Hetrick *et al*, 1986). AMF can be P tolerant or intolerant. Mosse's (1972) use of P deficient soils documented the connection between phosphorus needs and deficiencies and AMF. Nurlaeny *et al* (1996) saw that P-depletion was greater in the rhizospheric soil around mycorrhizal plants than non-mycorrhizal plants.

Sylvia and Schenck's (1983) research on applied superphosphorus drenches showed an increase in sporulation of P-tolerant AMF isolate from Florida. In the high P-soil (199 mg/kg-1 P soil, pH of 6.5), *Gigaspora margarita, Glomus clarum* and *Glomus mosseae* (from soils with P levels up to 448 mg kg -1P) produced mycorrhizal relationships with their host (bahiagrass; *Paspalum notatum*). All produced significantly more spores when drenched with P (20% P2O5 at 2.5 g/kg-1 of soil) than non-treated mycorrhizal plants. *Glomus etunicatum, Glomus macrocarpum, Gigaspora gigantean* and *Gigaspora heterogama* did not colonize roots in the high P soil. In the moderate P soil (98 mg kg-1 P soil, pH 6.5), all fungi colonized the soil. At 9 weeks, *G. clarum* and *G. etunicatum* had 67% and 39% colonization, respectively. When drenched, *G. etunicatum* sporulation and colonization was reduced.

Davis *et al* (1984) also found *G. clarum* tolerated low and high available P soil levels in three peppermint (*Mentha piperita*) fields growing in a silty clay loam (low P at pH 6.0) and two well fertilized silty loam soils registering a pH of 5.6 with a high P content. In 1982, the peppermint roots had a low level of root colonization (averaged 26.5% over the three fields), with fewer arbuscules and more vesicles (due to age of sampled roots); external hyphae were abundant. This reversed in 1983, when arbuscules became more abundant and external hyphae decreased. Bolan *et al* (1984) found in soil

that is P deficient, adding P not only benefited the plant host, but benefited the mycorrhizal fungi's growth.

Depending on the AMF, plant tissue P content may or may not increase. The dilution factor caused by growth and time of harvest will cause differences in P concentrations. Plants with relatively low tissue concentrations are sometimes considered to have higher P-use efficiency (DMY produced per unit P in tissue) values (Medeiro *et al*, 1994; Clark *et al*, 1999a). Clark *et al* (1999a) also saw that the AMF species influenced plant P-content differently and that the extent of root colonization did not matter. *Glomus etunicatum* plants had the highest P concentrations with only 10% root colonization and *Gigaspora margarita* with 50% colonization the next highest P concentration in pH 4 soil. Dogwood seedlings (*Cornus florida*) inoculated with *Glomus etunicatum* provided a greater survival, shoot dry mass and root fresh mass than seedlings inoculated with *Glomus intraradices* or the control (Sylvia,1986). *G. etunicuatum* did not improve P content in shoots, indicating that dogwoods are P-efficient. Bryla and Koide's (1990b) research showed that P-use efficiency was not significantly correlated with response to infection.

The increase in P causes an increase in plant health and growth. Plant size, increased flowering, reproduction and seed/offspring superiority are all seen as benefits of the increased P that the AM fungi provide (Sanders *et al*, 1998; Koide and Lu, 1996a,b).

Plant Host

Over 200,000 angiosperms are able to form AM symbiotic relationships. Only a few gymnosperms as well as Pteridophytes and Bryophytes, form this relationship with

AMF (mainly gymnosperms form such relationships with ectomycorrhizal fungi (actinomycetes and basidiomycetes).

It is believed that the non-mycorrhizal trait has evolved several times through several plant families and therefore there are many physiological and genetic reasons for this trait (Smith and Read, 1997). The following plant families are non-mycorrhizal: Chenopodiaceae, Amaranthaceae, Portulacaceae, Caryophyllaceae, Polygonaceaeae, Brassicaceae, Sapotaceae, and Proteaceae. These plants have found alternate methods of finding nutrients, such as increased root hairs or root hair lengths, or in the case of proteoid plants, excretion of organic acids or phenolic acids or both, to chemically extract nutrients from the immediate root zone (Marschner, 1995). In some cases, non-mycorrhizal plants planted with mycorrhizal plants received some of the benefits from the AM symbiosis. Non-mycorrhizal *Dianthus* was protected from *Fusarium* when planted with mycorrhizal *Tagetes*. Without the mycorrhizal *Tagetes*, the *Dianthus* survivial rate dropped (St.-Arnaud *et al*, 1997).

Plants and Potential Industrial Mycorrhizal Use

Harley and Harley (1987) created a compendium of British flora known to be mycorrhizal dependent (AM, Ecto-, Orchidaceous and Ericoid), showing the great diversity of mycorrhizal plant hosts, and specifically those AM dependent. This list encompasses annuals, biennials, and perennials (herbaceous perennials, groundcovers, vines, shrubs and trees).

Plants with AM symbionts include a variety of vegetable crops, grains, grasses, ferns, flowering annuals, herbaceous perennials, shrubs, vines and trees. Research has occurred due to the potential benefits already mentioned of the AM infection to plants in

agriculture, horticulture and forestry (Robson *et al*, 1994). Scientists have researched the potential use of AM in the greenhouse and nursery industries for earlier mentioned benefits during production and after transplanting in pot and ground, and now due to the required reduction of P in production wastewaters (Hottovy, 1994). Earlier research on chrysanthemums (*Chrysanthemum morifolium* Ramat cvs.) (Johnson *et al*, 1982a,b; Johnson *et al*, 1984) and poinsettias (*Euphorbia pulcherrima*) (Barrows and Roncadori, 1977) showed that providing extra P accomplished little; there was low colonization and no additional benefits. Two problems arose in the early attempts to use AM in greenhouse production: media and uniformity/quality (personal communication, Roncadori, 1998). The industry use of lighter peat-bark mixes make moving plants easier but depresses plant growth. Koide (1999) believes this may be partly due to poor P holding capacity of such mixes. Biermann and Linderman (1983) found a reduction in colonization when using 100% peat compared to a peat/soil mix. Colonization did increase when vermiculite was added to peat. In media containing soil AM, increased shoot growth of geranium (*Pelargonium* x *hortorum* 'Sprinter Scarlet' L.H. Bailey) occurred. The expected uniformity and size required for a salable crop, which is easily created with well-balanced fertilizers to maximize growth, will depress most AM colonization (Koide *et al*, 1999, Biermann and Linderman, 1983a). Pre-transplanted, AM- inoculated geranium seedlings grown in vermiculite were the same size as non-mycorrhizal control geraniums (Biermann and Linderman, 1983b). Once transplanted into soil however, the mycorrhizal plants were larger with greater shoot weights, leaf areas, leaf dry weights and more flower stems. Crop uniformity was seen. Unfortunately, the work of Johnson *et al* (1982b) work with mycorrhizal chrysanthemum cultivar

'Circus' did not produce a quality plant for commercial use, even though the AM fungi increased height and flower, stem and dry weights.

In the latest research attempting to use AM in the greenhouse, seedlings of six common summer annuals were inoculated with AMF at the plug stage, at transplanting, or at both plug and transplanting stages to determine the best inoculum timing and cost while still using a P fertilizer (Koide *et al*, 1999). Inoculation at seeding and then at transplanting accomplished the greater colonization than at transplant only. Inoculation at the seeding stage depressed growth in some plants. *Coleus*, *Petunias* and *Viola* were the most colonized while *Impatiens*, *Tagetes*, and *Salvia* were the least colonized.

Inoculated marigolds (*Tagetes erecta*) and zinnias (*Zinnia elegans*) showed similar results of earlier flowering, increased flower numbers, increased shoot growth early in development and final fresh weights and increased fresh root weight when compared to uninoculated plants (Aboul-Nasar, 1996).

Gaur *et al* (2000) looked at three annuals exposed to AM inocula or chemical fertilizers in a low P soil. Petunia (*Petunia hybrida*), china aster (*Callistephus chinensis*) and balsam impatiens (*Impatiens balsamina*) were grown in a marginal soil amended with organic matter. Inoculation caused an increase in the numbers of flowers, earlier flowering, greater dry matter and taller plants. Fertilizer cost showed approximately 30% savings when the inoculum was used.

Cultivated and Wild Mycorrhizal Plants

Koide *et al* (1988) and Bryla and Koide (1990a,b) focused on the growth of cultivated versus mycorrhizal-treated wild species. Cultivated mycorrhizal oats (*Avena sativa* L.) had a longer life span and increased number of panicles, length of flowering,

averaged seed weights, and P concentrations than wild mycorrhizal oats (*Avena fatua* L.). The wild oats were not as mycorrhizal dependent as the cultivated varieties. Two cultivars of mycorrhizal tomatoes (*Lycopersicon esculentum* Mill.) in general were also more responsive to the fungi than the wild tomato species. The cultivars had shorter life spans. The responsiveness to mycorrhizal fungi varied among all types, wild or cultivars.

In Sanders and Koide's (1993) research on nutrient acquisition and community structure in wild oats (*Avena fatua* L.), the scientists state that infection may have long term effects on the structure of plant communities due to the higher quality of the seed produced in mycorrhizal plants. This is important for annual plants that live for only one season.

Boerner (1992) looked at the responses of mycorrhizal perennial Panicum (*Panicum virgatum*) and Bromus (*Bromus inermus*), and mycorrhizal annual Panicum (*Panicum capillare*) and Bromus (*Bromus secalinus*) under high and low P conditions. The perennial grasses were more dependent and responsive to mycorrhizae at high and low P than the annual grasses, which were only responsive at low P and less dependent on mycorrhizae. The survey suggests that perennials are more mycorrhizal dependent than annuals. Ecologically, this supports his idea of grasslands successional replacement of annual grasses by perennial grasses as land and plantings mature, and as soil nutrient levels lower.

Root Architecture and Mycorrhizae

Angiosperms (flowering plants) are divided into two groups: monocotyledons and dicotyledons. Dicots are seen as the earliest angiosperms with monocots evolving from early dicots. Baylis (1975) suggests the simpler the root architecture, i.e., a stubbier,

coarser root with no or few root hairs like the Magnoloidal, the more likely the plants are to be mycorrhizal dependent, (Schweiger *et al* 1995). Plants with greater root structures (greater branching with many root hairs like the Graminonidal grasses, rushes and sedges) the less likely the need for the fungi. Evolutionary speaking, the Magnoliidae subclass is thought to be the most primitive angiosperms. Monocots also have an order of primitive members (i.e., *Hyacinthoides non-scripta* (bluebells) of the Liliaceae) and these lesser developed members, like the Magnoliidae, have a greater need to acquire P and mycorrhizae than the rushes, sedges and grasses (i.e., *Vulpia cilita* sp. *ambigua*) (Baylis, 1975).

Hetrick *et al* (1990,1991) found that mycorrhizal dependence on root architecture also depended on the seasonality of the plants. Cool-season grasses had a greater number of finer and longer primary, secondary and tertiary roots, and were the less mycorrhizal-dependent than warm-season grasses with smaller root systems that were more mycorrhizal dependent and more accommodating to AM fungi. Mycorrhizal fungal activity decreased in soil with drops in temperatures (Hayman, 1982), which may be evolutionarily part of the reason mycorrhizal fungi has little influence on cool season grasses.

The differences in plants' nutrient efficiencies are related to the different abilities of roots to acquire nutrients, the plants' use or both (Marschner, 1995). Schweiger *et al* (1995) found that AMF benefit was not well related to root diameter, root length per plant its root/shoot ratio, but was related to extent of root hair development. Fitter and Strickland (1991) found support in their prediction that for plants in low nutrient conditions, root systems should also be more herringbone-like. Herringbone root systems

divert photosynthates from shoot growth to root growth in order to mine nutrients. Dicots became more herringbone-like, but not the grasses. The ability of plants to modify the structure of their roots due to environmental changes is called plasticity. Cool season grasses had larger root systems and smaller root diameters when compared to warm season grasses (Hetrick *et al*, 1988,1991). Phosphorous did not affect the root systems of the cool or warm season plants. Mycorrhizal fungi did affect the warm season grasses by inhibiting their growth but did not change the growth of the cool season grasses. Warm-season grasses are considered to be more plastic, therefore more accommodating to mycorrhizal fungi than the cool season grasses.

Desiccation Avoidance

Scientists studying arbuscular mycorrhizal fungi have found that colonized host plants can tolerate (Waterer and Coltman, 1989) and recover from drought better than non-mycorrhizal plants (Sweatt and Davies, 1984). This mycorrhizal benefit has been attributed to external hyphae absorption of water (Hardie, 1985, Ruiz-Lozano and Azcon, 1995), soil aggregation by fungi exudates and hyphae that allow the soil to hold more water (Fitter, 1985); increased nutrition and continued leaf conductance (open stomates) even under drought (Duan *et al*, 1996).

How mycorrhizal fungi increase plant drought tolerance is not fully understood (Ruiz-Lozano *et al*, 1995a,b), since it does not occur for all plants or all AM fungi (Byrla and Duniway, 1997a).

Fungal properties protecting plants from drought stress include extramatrical mycelium and soil aggregation. Extramatrical mycelia are able to extract water from soil

pores that roots cannot mine (Hardie, 1985; Hardie and Leyton, 1981) and acquire nutrients from the soil even under drought conditions (Al-Karaki and Clark, 1999; Tobar *et al*, 1994). Fitter (1985) and Kothari *et al* (1990) do not believe that hyphae water uptake explains all the water needs of a drought stressed plant. Bethylenfalvay *et al* (1988) found the amount and length of extraradical hyphae was greater in stressed soybeans (*Glycine max* (L.)). Soil aggregation occurs due to the binding qualities of the mycelium and exudates produced by roots and mycelium with the soil (Tisdall, 1994). This binding allows the soil to hold more water. Auge and Stodola (1990) found AM roses (*Rosa hybrida* L.) had greater turgor pressure over a variety of soil water potentials due to increased water into the symplast. The roots had a greater quantity of symplastic solutes because of the higher quantity of symplastic water. Under well-watered conditions, the reverse was true.

Fungal species will determine the degree of drought tolerance imparted. Ruiz-Lozano *et al* (1995a,b) found in lettuce (*Lactuca sativa* L. cv Romana) that drought tolerance differed according to AMF species. AMF species tested demonstrated the following best to worst drought tolerance in lettuce: *Glomus deserticola>Glomus fasciculatum>Glomus mosseae>Glomus etunicatum>Glomus intraradices>Glomus caledonium>Glomus occultum*. Under drought conditions, the difference in plant growth between *Glomus occultum* and *Glomus deserticola* was 821%. *Glomus deserticola* produced tolerance, which created the smallest decrease in growth (Ruiz-Lozano *et al*, 1995a,b). Ruiz-Lozano and Azcon (1995) compared *Glomus deserticola* to *Glomus fasciculatum* in lettuce. While *Glomus deserticola* positively influenced nutrition, leaf

conductance and transpiration under drought, *Glomus fasciculatum* effectively and significantly increased net photosynthesis and water use efficiency.

Drought acclimation

Cyclical drought affects the AMF drought benefit. Drought acclimation (imposed cyclical drought) combined with AMF imparted the greatest drought resistance to pepper (*Capsicum annuum* L.) plants as seen in higher leaf water potential, turgor, water content and fewer wilted plants. When AMF or drought acclimation was applied separately, drought resistance did occur but at a lower frequency (Davies *et al*, 1992). Drought acclimation did increase AMF colonization in AM plants (Hetrick *et al*, 1986) but drought did not affect colonization in all cases (Sylvia *et al*,1993) and depended on AM species (Al-Karaki *et al*, 1998). Well-watered plants and non-acclimated AM plants had lower colonization in rose (*Rosa hybrida* L. cv Ferdy) (Davies, 1996).

Quantifications to determine AMF benefits/effects to plants under drought stress include water use efficiency (Al-Karaki, 1998 ; Ruiz-Lozano *et al*, 1995a,b), plant growth (Simpson and Daft, 1990), leaf and root growth and weights (Waterer and Coltman, 1989), root/shoot ratios (Henderson and Davies, 1990), nutrient acquisitions (Al-Karaki *et al*, 1998; Al-Karaki and Clark, 1999; Subramanian *et al*, 1997), stomatal conductance (Green *et al*, 1998; Fitter, 1988), transpiration rates (Auge, 1989; Davies *et al*, 1996), increase in photosynthesis (Ruiz-Lozano and Azcon, 1995), leaf turgor (Davies *et al*, 1993), osmotic adjustment (Auge, *et al* 1986), hormonal (Duan *et al*, 1996), and osmolyte biosysthesis (Ruiz-Lozano *et al*, 1995a,b), root and leaf hydraulic conductivity/resistance (Koide, 1985; Graham *et al*, 1987), non-hydraulic root:shoot signaling (Auge and Duan, 1991), chlorophyll content, CO_2 assimilation (Ruiz-Lozano *et al*, 1995a,b) and leaf

elasticity. These plant physiological aspects are measured since they can be affected by drought, but may or may not be affected by AMF (Graham *et al*, 1987).

Plant reaction

Plants may or may not be helped by AMF when under drought stress. Hetrick *et al* (1987) found that *G. etunicatum* did not increase the growth of corn (*Zea mays* L.) or sudan grass (*Sorghum vulgare* (Piper) Hitch) but did benefit native big blue stem (*Andropogon gerardii* Vitman) in cyclical drought. *Glomus intraradices* significantly reduced root hydraulic conductiviy in Carrizo citrus seedlings (*Poncirus trifoliate* x *Citrus sinensis*) after a 20-day drought cycle with a re-watering period at mid-point (Graham *et al*,1987). Sylvia *et al* (1993) showed that AMF increased grain yield in maize and water treatment did not matter. Fruit in AM bell peppers (*Capsicum annuum*) were larger in P-deficient soils under drought stress than non-AM bell peppers (Waterer and Coltman, 1989). More advanced floral formation and quicker recovery from drought was seen in AM geraniums (*Pelargonium x hortorum* Bailey cv. Cherry Glow) under water stress compared to non-inoculated plants (Sweatt and Davies, 1984).

Plant cultivars can react differently to AM colonization. Wheat (*Triticum durum*) cultivar CR006, which is drought sensitive, had lower AM colonization than its drought tolerant counterpart (CR057) (Al-Karaki and Clark, 1998; Al-Karaki, 1998).

Root Morphology and Uptake

Root morphology becomes more extensive under drought as top growth decreases and root growth increases. The root:shoot ratio increases with drought as abscisic acid increases (Taiz and Zeiger, 1998). Mycorrhizal roots will also change under normal

moisture conditions. The root growth at apical ends slow and more lateral initials occurs (Read and Smith, 1997), though Kothari *et al* (1990) found less lateral root growth.

Fitter's (1988) experiment with red clover (*Trifolium pratense* L.) and Bryla and Duniway's (1997b) experiment with wheat (*Triticum* aestivum L. cv Anza) and safflower (*Carthamus tinctorius* L. cv S555) suggested that AM plants were not able to acquire more water from the soil than non-AM plants. AM clover roots had less estimated water uptake. Droughted AM-wheat and safflower had no significant affect on root dry mass, root diameter, or root length compared to well-watered AM-wheat that had significantly less root length than well-watered non-AM plant (Bryla and Duniway, 1997a).

Nutrition Effects and Drought

Healthy AMF colonization requires a low P environment. To ensure AM plants and non-AM-control plants are of equivalent size at the onset of a drought experiment or during the experiment, non-AM plants are provided with P (along with other nutrients as are AM-plants) to ensure similar plant weights and root and shoot growth (Bryla and Duniway, 1997a,b). AM lettuce had increased P-uptake relative to control plants under water-stressed conditions (Tobar *et al*, 1994). Equivalent P content assures that plants are not drought tolerant due to nutrition, but are being measured for an AM-plant interaction (Davies *et al*, 1996).

Shoot concentrations of Zn, Cu and Fe were higher for AM plants grown under drought stress than non-stressed plants. AMF species *Glomus monosporum* and *Glomus mosseae* had similar shoot concentrations whether water stressed or not (Al-Karaki *et al*, 1998).

Nitrogen, whether in the form of NH_4^+ or NO_3^-, is taken up by mycorrhizal plants. Under drought stress, N as NO_3^- produced greater biomass in AM (*G. fasciculatum*) lettuce than P-only plants. Phosphorous, N, K and Mg were all taken up, but contents depended on the fungi and the N source under drought conditions (Azcon *et al*, 1996).

Al-Karaki and Clark (1999) showed AM-barley having greater P shoot contents than non-AM plants, whether water-stressed or not. Water stress reduced the AM plants' content of Zn, Cu, and Mn. AMF increased P levels regardless of water stress.

ABA and proline

ABA (Abscissic acid), a phytohormone, and proline, an amino acid, both affect drought stressed plants. ABA is thought to work as a direct signal, which is produced in drying roots and transported to leaves to close stomata. Allen *et al* (1982) and Duan *et al* (1996) both saw drops in ABA levels in AM plants under drought stress. Duan *et al* (1996) showed lower ABA concentration levels in xylem sap and lower concentrations in leaves of cowpeas (*Vigna unguiculata*). Allen *et al* (1982) saw ABA decreases in leaves and unchanged levels in roots in blue gramma grass (*Bouteloua gracilis* (H.B.K.) Lag ex Steud). Stomatal conductivity was higher in AM plants, with lower ABA levels, when compared to non-AM, nutritionally similar, plants during drought stress. This conductivity was not related to the xylem saps' content of calcium, zeatin riboside, xylem pH or the AM infection of the plant. It required greater drought for AM plants to have the same concentration and movement of ABA as non-mycorrhizal plants (Duan *et al*, 1996).

Proline, an osmolyte biosynthate, may regulate drought stress through osmotic adjustment. This makes it a good parameter to measure drought tolerance response. Lettuce (*Lactuca sativa* L. cv. Romana) colonized with *Glomus deserticola* and under

drought had greater levels of proline in the foliage versus well-watered plants (Ruiz-Lozano *et al*, 1995). These plants had the least reduction in shoot growth even when compared to P-fertilized plants. *Glomus mosseae* colonized lettuce, when under drought stress, had increased proline quantities (Azcon *et al*, 1996).

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CHAPTER 3

EVALUATING COMMERCIAL INOCULANTS FOR ARBUSCULAR MYCORRHIZAL COLONIZATION AND GROWTH OF *IMPATIENS WALLERANA* 'SUPER ELFIN WHITE'

Introduction

As the landscape industry is required to reduce both water use (Wade, 1999) and fertilizer runoff into the soil and water (Hottovy, 1994; Hubbard *et al*, 1988), methods to reduce plant drought stress (Omahen, 2001) and to limit fertilizer application amounts must be found. One such alternative is arbuscular mycorrhizal fungi.

Arbuscular mycorrhizal fungi (AMF) create symbiotic relationships through root colonization of approximately 80% of the plant kingdom. In this symbiotic relationship, AMF provide nutrients (Borie and Rubio, 1999; Clark *et al*, 1999b), especially P (Marschner, 1995) and water (Hardie, 1985; Busse and Ellis, 1985) to the plant, as the plants contribute photosynthates to the fungi. Arbuscular mycorrhizal fungi can access other nutrients such as Zn and Cu (Marschner, 1995) while ameliorating other heavy metals (Maddox and Soileau, 1991; Clark *et al*, 1999b; Biermann & Linderman, 1983 a,b).

Other plant benefits of AMF colonization include protection from plant diseases (Azcon-Aguiler and Barea, 1996; Caron *et al*, 1986), salt tolerance (Ruiz-Lozano *et al*, 1996), increased chlorophyll content (Gemma *et al*, 1998), drought tolerance (Waterer and Coltman, 1989; Davies *et al*, 1992), increased shoot growth (Bierman and

Linderman, 1983) and increased flower number (Daft and Okusanya, 1973), and earlier bloom times. The relationship will also increase soil stabilization through soil aggregation (Tisdall, 1994).

Current landscaping methods for new homes often remove the topsoil been and add high amounts of P to the remaining subsoil. This practice can eliminate or decrease native AMF populations in the soil (Mosse,1972). To offset this loss of AMF, commercial AMF inocula have been developed to return the AMF to the soil. These commercial AMF products may contain spores, vesicles, infected root fragments, extramatrical mycelium, and chlamydospores as inocula. In addition to potential inoculants, commercial AMF products may include a combination of fertilizers, peat moss, mineral carriers, biostimulants and clay pellets.

Preliminary experiments in 2001 using commercial AMF inocula yield varied results, including no root colonization in annuals grown in Cecil sandy clay loam typical of a Georgia piedmont landscape (Hoffman, 2003). The purpose of this experiment was to evaluate the ability of two AMF inocula products, Mycor Flower Saver Plus™ and endoRoots™ to colonize *Impatiens wallerana* in sterilized Cecil sandy clay loam.

Materials and Methods

Soil and Soil Preparation: A Cecil sandy clay loam subsoil was collected in March 2002 from the University of Georgia Horticulture Farm in Watkinsville, GA. The soil was sieved through a 20.0 mm screen and air-dried for 1 week. A portion of the soil was reserved as a source of native AMF, and the remaining soil was fumigated with methyl bromide. Ten days after fumigation, a soil sample was planted with *Impatiens*, which after 7 days, showed no signs of stress caused by residual methyl bromide.

Soil Analysis. The soil was analyzed using a Mehlich I extraction for minerals and Adams-Evans buffer for pH. The soil pH_w was 6.1. The P, K and Fe levels were low at 2, 40 and 13.9 ppm, respectively. Calcium (375 ppm), Mg (38 ppm), Zn (.29 ppm), and Mn (11.5 ppm) were all at medium sufficiency levels for *Impatiens*. Aluminum (131.7 ppm) was high (MMI, Athens, GA).

Mycorrhizal Inocula. Two commercial arbuscular mycorrhizal inocula products, endoRoots™ (Roots, Inc., Independence, MO) and Mycor Flower Saver Plus™ , (henceforth, Mycor, Plant Healthcare, Inc., Pittsburgh, PA), were evaluated. Mycor includes the arbuscular mycorrhizal fungi *Entrophorospora columbiana* (Spain & Schenck), *Glomus clarum* (Nicol. & Schenck), *Glomus entunicatum* (Becker & Gerdemann) and *Glomus intraradices* (Schenck & Smith). Product specifications state a minimum of 3,000 spores exist per 0.45 kg (1 lb) of product, with the remaining dry ingredients including proprietary fertilizers (3N-1.8P-2.5K), humic and fulvic acids, sea kelp, amino acids, vitamins, "growth factors," sugars, yucca extract, and beneficial bacteria. The spores chosen because they were able to grow in soils of pH 3 to 8.

endoRoots is also a dry blend of fertilizers (3N-1.3P-3.3K) and arbuscular mycorrhizal fungi with a total of 200,000 spores and propagules contained in each 22.6 kg (50 lb) bag. Arbuscular mycorrhizal fungal species in the formula are *Glomus mosseae* (Nicol. & Gerd.) Gerd. & Trappe, *G. intraradices*, *G. clarum*, *G. monosporus* (Gerdemann & Trappe), *G. deserticola* (Trappe, Bloss & Menge), *G. brasilianum*, *G. aggregatum* (Schenck & Smith), and *Gigaspora margarita* (Becker & Hall). Kelp, humus, vitamins, and amino acids are also included.

Inoculants were acquired in Summer 2001 from local distributors, and more inoculants were acquired directly from the producers in Spring 2002. All inocula were stored according to Plant Healthcare's instructions: cool and dry storage, not exposed to excessive heat or direct sunlight. No instructions were found for the storage of the endoRoots.

Treatments. The experiment consisted of seven treatments with five replications. The treatments were 1) sterile soil only; 2) sterile soil amended with P (1.22 g/3.8 L nursery pot; 0.04 oz/1 gal); 3) sterile soil amended with stored (purchased 2001) endoRoots (1.22 g/3.8 L nursery pot; 0.04 oz/1gal); 4) sterile soil amended with stored (purchased 2001) Mycor (7.27 g/3.8 L nursery pot; 0.26 oz/1 gal); 5) sterile soil amended with new endoRoots (1.22 g/3.8 L nursery pot; 0.04oz/1gal); 6) sterile soil amended with new Mycor (7.27 g/3.8 L nursery pot; 0.26 oz/1 gal) and 7) sterile soil amended with non-sterile soil (0.95 L/3.8 L nursery pot; .03ft^3/1 gal). The applications were 51 spores/pot for Mycor and 10 spores/pot for endoRoots. Quantities were based on manufacturers' recommendations.

All Mycor and endoRoots treatments were mixed into the top 5 to 7.5 cm (2 to 3 in) of soil and around the rootball according to Plant Healthcare's planting instructions. endoRoots' instructions stated to "work in thoroughly."

Experimental Design. Following INVAM recommendations for growing mycorrhizal plants (Morton *et al*, 1993), the greenhouse was pre-treated with a 10% bleach solution to eliminate any viable mycorrhizal spores. All equipment was treated with a bleach wash. All pots were topped with sand treated with methyl bromide and sub-

irrigated to reduce mycorrhizal spore spread. Before the experiment began, 8 plants were tested for existing arbuscular mycorrhizal colonization. No colonization was discovered.

The five replications were placed in a randomized complete block design by species on a shaded (45%) greenhouse bench on April 7, 2002. Plants were watered as needed and fertilized once a week with a 15N-0P-12.5K dark weather-nitrate liquid feed at 150 ppm nitrogen. Greenhouse temperatures averaged from 20 to 30 degrees C (68 F to 86 F). Plants were treated with pesticides as needed for thrips (*Frankliniella occidentalis*), whitefly (*Bemisia sp*.) and aphids (*Aphis sp*. and *Myzus persicae*).

After 11 ½ weeks, growth indices (growth index = plant height + ((plant width[1] + plant width[2]) /2) /2), flowers numbers, and shoot, root and flower weights were determined. A portion of fresh roots were weighed, cleaned, cleared with 10% KOH, stained, and colonization percentages determined as described by Kormanik and McGraw (1980). Colonization was determined with the grid-line intersect method (Giovannetti and Mosse, 1980).

Results and Discussion

No mycorrhizal colonization was observed in the control, p-amended, or stored endoRoots treatments (Table 3-1). Since the soil had been sterilized, no colonization was expected in the control and P treatments. This experiment verified a preliminary study in August 2001 (Hoffman, 2003) when this endoRoots source, purchased in July 2001, did not colonize *Impatiens*. Low colonization (0.2%) was observed in the new endoRoots treatments (Table 3-1), obtained directly from the factory (March 2002) to acquire the freshest sample possible, was also similar to the preliminary studies (Hoffman, 2003).

Root colonization by the Mycor (purchased 2001) also verified the 2001 preliminary study (Hoffman, 2003) when low colonization occurred using the stored Mycor treatment product when it had just been purchased. However, the new Mycor obtained from the factory (March, 2002), showed colonization of 22% (Table 3-1) in contrast to the earlier samples purchased from distributors in which no colonization was observed (Hoffman, 2003). Colonization has been shown not to impact plant dry mass unless the colonization percentages are greater than 20% in switchgrass (*Panicum virgatum*) (Clark *et al* 1999).

These data indicate that the viability of commercial sources of inocula varies. In all cases, the mycorrhizal products were stored as instructed by the manufacturers after purchase. The storage conditions before purchase are unknown.

Because effective AMF colonization was seen in the new Mycor and the non-sterile soil alone, the stability of both products is in question. Storage conditions are an important issue when working with mycorrhizal inocula. Moisture and temperature can affect spore viability (Talukdar and Germida, 1993, Doud and Schenck, 1991, Wagner *et al*, 2001) and dormancy of AMF spores (Tommerup, 1983). Time of storage can reduce viability of infected roots stored in soils (Rives *et al*, 1980). All these factors effecting inocula will vary according to AMF species (Tommerup, 1983, Doud and Schenck, 1991, Gemma and Koske, 1988).

Adding non-sterilized soil to the sterilized soil was included to verify that native AMF propagules were not affected by the methyl bromide sterilization. Colonization was greatest in the non-sterilized soil treatment (Table 3-1) because of the large numbers of

native AMF spores reintroduced into sterilized soil or from the native AMF spores being re-introduced into their own native soils.

The greater growth indices and shoot weights of the Mycor plants compared to the other treatments (Table 3-1) are likely caused by the fertilizers or biostimulants in the Mycor product. No differences occurred between the two Mycor treatments with respect to growth indices and shoot weights, even though the new Mycor plants had greater mycorrhizal colonization. This growth suggests that the fertilizers or biostimulants in the Mycor product had a greater effect in increasing growth than colonization by native AMF.

The greater growth indices and shoot weights of the two Mycor treatments compared to the endoRoots (Table 3-1) may reflect the greater amount of fertilizer in the recommended rates of the two products; 7.27 g/pot of Mycor (3N-1.8P-2.5K) compared to 1.27 g/pot of endoRoots (3N-1.3P-3.3K). The greater growth indices and shoot weight of the P-treated plants compared to the control indicated that the lower amounts of P were limiting growth in the control.

The shoot mass of non-sterilized treatment plants was greater than the P amended and control plants (Table 3-1). Thus, lower P fertility limited growth of the control, but not the non-sterilized treatment, which had been colonized by AMF. This implied that the mycorrhizae of the colonized non-sterilized treatment plants were able to overcome the P deficit (Nurlaeny *et al*, 1996).

The root dry masses of both Mycor treatments were greater than all other treatments because of the greater amount of fertilizer supplied in the Mycor product (Table 3-1). AMF colonization often reduces root growth (Berta *et al*, 1990), but no

difference was noted between the Mycor treatments, which differed in colonization percentages.

The shoot:root ratio of non-sterilized soil plants was greater than those of all other treatments (Table 3-1). The non-sterilized soil plants also had the greatest mycorrhizal colonization rates. AMF colonization increased shoot:root ratios because colonization increases shoot weights due to increase nutrition (Marschner, 1995; Aboul-Nasar, 1996) and often decreases the size of the root systems (Berta *et al* 1990).

The Mycor treatments had the greatest flower mass and flower number because the greater amount of fertilizer in the Mycor. Fertilizer as well as AMF colonization (Aboul-Nasar, 1996; Gaur *et al*, 2000) can increase flowering. The greater flowering of the non-sterilized soil plants compared to the control plants indicated that mycorrhizal colonization increased flowering. However, no difference in flowering occurred between the new and stored Mycor treatments, even though the new Mycor treatments had a greater colonization rate (Gaur *et al*, 2000; Barrows and Roncadori, 1977, Mosse, 1972, Sylvia and Schenck, 1983; Davis *et al*, 1984).

Conclusion

Commercial mycorrhizal inocula, Mycor and endoRoots, varied in their ability to colonize *Impatiens*. Only Mycor obtained directly from the manufacturer had AMF that colonized *Impatiens* at a significant level. Mycor and endoRoots obtained from distributors, and endoRoots obtained from the producer had AMF that failed to colonize *Impatiens* because of poor colonization from no inocula, low inocula viability and/or inocula degradation in production, distributor handling, or proper or improper storage.

This colonization failure could explain variable results observed in earlier field and greenhouse experiments (Hoffman, 2003; Smalley, personal communications).

The increase in growth observed in Mycor and endoRoots treated plants is most likely because of additives (biostimulants, fertilizer) in the product, as the increases in growth occurred in the absence of root colonization.

Table 3:1. Effect of new and stored Mycor Flower Saver Plus™,[z] and endoRoots™,[z] products on *Impatiens wallerana* 'Super Elfin White' in sterilized soil[y].

Treatments	Colonization percentages	Growth indices[x] (cm)	Shoot dry mass (g/plant)	Root dry mass (g/plant)	Shoot: Root (g/plant)	Flower dry mass (g/plant)	Flowers/ plant
Control	0.0 c[w]	12.9 c	1.5 e	0.3 c	7.3 b	0.01 d	0.7 d
Phosphorus	0.0 c	17.5 b	4.6 c	0.9 b	5.3 b	0.19 c	21.4 c
Stored[v] endoRoots	0.0 c	15.6 b	3.2 d	0.5 bc	6.3 b	0.05 cd	6.6 d
New endoRoots	0.2 c	16.4 b	3.4 d	0.6 bc	5.7 b	0.08 cd	9.2 d
Stored Mycor	1.2 c	24.6 a	14.5 a	2.9 a	5.5 b	0.59 a	46.8 ab
New[v] Mycor	22.0 b	22.4 a	14.0 a	2.3 a	6.1 b	0.67 a	49.6 a
Non-sterilized soil	39.6 a	18.0 b	6.7 b	0.6 bc	11.4 a	0.41 b	36.0 b

[z] Mycor Flower Saver Plus™ (Plant Healthcare, Inc., Pittsburgh, PA). AMF inocula. (7.27 g/3.7 L pot; 3N-1.8-2.5K)

endoRoots™ (Roots, Inc., Independence, MO). AMF inocula. (1.22g/3.7 L pot; 3N-1.3-3.3K).

[y] Cecil sandy clay loam subsoil sterilized with methyl bromide.

[x] growth index = height + ((w1+w2)/2) /2.

[w] treatment means with same letters are not significantly different (Duncan groupings; <.05).

[v] Stored products purchased from distributor - July 2001.

New products acquired directly from the producers - March 2002.

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CHAPTER 4

CONCLUSION

These studies indicate variability in the colonizing ability of the inocula contained within Mycor and endoRoots in sterile and non-sterile soils. For Mycor, colonization varied yearly (2000, 2001 and 2002) and product source (distributor vs. producer). For endoRoots, neither time nor source changed the inoculant's inability to colonize. Even under proper storage, the Mycor product did not colonize roots for its full 18-month shelf life, but did colonize when obtained directly from the producer and used immediately. Another study to test this sample's shelf life is needed to clarify its length of shelf life.

In two studies, native AMF-colonized *Impatiens wallerana* and *Salvia splendens* plants exhibited greater growth than non-colonized control plants grown under the same fertility regime. This demonstrated the growth benefit provided by plant-AMF symbiosis. Colonization can occur in landscape annuals and be beneficial to the plants within the 8 weeks that these experiments ran. The most consistent inoculation came from native AMF found in the Cecil sandy clay loam.

Landscapers and homeowners could use the two AMF products, Mycor and endoRoots, because plants in the studies benefited from the fertilizers and biostimulants in the products. Each product produced plants larger than the control.

Because the plants were only tested for colonization and growth, no conclusions from this research can be reached about other potential AMF benefits, such as disease resistance and drought tolerance. Disease resistance and drought studies are impossible to conduct with the commercial products until a reliable inoculation is achieved when using these products.

If commercial inoculants remain so variable and if landscapers and homeowners wish to use AMF to benefit their landscapes, soil can be harvested from uncultivated sites

and added to inoculate the planting bed with native AMF. Landscapers should reduce P applications, since it will reduce AMF populations.

APPENDICES

PRELIMINARY RESEARCH SUMMARIES

Appendix A

Effect of phosphorus and Mycor on mycorrhizal root colonization and growth of *Impatiens wallerana* 'Lipstick' and *Salvia splendens* 'Red Hot Sally'

(May 11, 2000 – July 10 & July 25, 2000)

This study's purpose (Table A) was to determine the ability of a commercial arbuscular mycorrhizal fungi (AMF) inoculum (Mycor Flower Saver Plus™ (Plant Healthcare, Inc., Pittsburgh, PA)) to colonize *Impatiens wallerana* 'Lipstick' and *Salvia splendens* 'Red Hot Sally' in a phosphorus-amended Cecil sandy clay loam soil. Mycor contains four species of arbuscular mycorrhizal fungi spores, Colonize™ (flavanoid product) purported to stimulate spore germination and attract hyphae, fertilizer (3N-1.8P-2.5K), biostimulants and beneficial bacteria.

Soil treatments were 1) low phosphorus (no P added) and no inoculum, 2) low P and Mycor (7.27 g/3.7L pot), 3) high P (1.21 g/3.7L pot) and no Mycor, and 4) high P and Mycor. The high P treatment consisted of P added according to the rate recommended after a soil test.

For *Impatiens*, the largest mycorrhizal root colonization occurred in low-P + Mycor plants. Similarly, Mycor increased colonization only in low-P treatments of *Salvia*. The addition of P did not effect colonization of native AMF population for either plant species as no difference existed between the no P - Mycor and high P - Mycor treatments. These data indicated that Mycor increases colonization only at low phosphorus levels.

The growth indices did not differ between treatments for *Impatiens* or *Salvia*. In *Impatiens*, shoot dry weights of the no P+Mycor treatment plants were less than those of the high P+Mycor treatment plants. Two explanations are possible: 1) carbohydrates were diverted from shoot growth to support AMF growth in AMF colonized roots or 2) addition of P increased shoot growth. *Salvia* shoot weights did not differ.

The high P+Mycor treatment plants had the greatest root dry weight in *Impatiens*, which can be attributed to the additional ingredients in Mycor. Root dry weights of *Salvia* treatments did not differ. Flower dry mass and flowers/plant were similar among treatments for *Impatiens* and *Salvia*.

Subsequent experiments are needed to determine the cause of the increase in colonization of the no P+Mycor treatments. This increase can be attributed to augmented colonization by the AMF inoculants in the Mycor. Future experiments will be conducted in sterilized soil to prevent colonization by native AMF populations.

Table A. Effect of phosphorus and Mycor[z] on mycorrhizal root colonization and growth of *Impatiens wallerana* 'Lipstick'[y] and *Salvia splendens* 'Red Hot Sally'[y].

	Treatments				Shoot	Root[x]	Flower	
Taxa	Mycor (g/pot)	Phosphorus	Colonization Percentage[x]	Growth index[w]	dry mass (g/plant)	dry mass (g/plant)	dry mass (g/plant)	Flowers/ plant
Impatiens	0.00	Low[v]	34.0 b[u]	29.2 a	11.0 ab	1.80 b	0.53 a	43.8 a
	7.27	Low	52.0 a	26.6 a	10.1 b	1.54 b	0.47 a	40.4 a
	0.00	High[t]	36.0 b	27.7 a	11.6 ab	1.70 b	0.35 a	25.8 a
	7.27	High	38.0 b	28.0 a	12.6 a	2.53 a	0.35 a	27.0 a
Salvia	0.00	Low	38.0 b	24.5 a	4.2 a	1.63 a	2.09 a	n.a.
	7.27	Low	51.0 a	25.7 a	4.0 a	1.66 a	2.09 a	n.a.
	0.00	High	41.0 ab	24.7 a	4.3 a	1.60 a	1.90 a	n.a.
	7.27	High	48.0 ab	25.6 a	4.3 a	1.55 a	1.84 ab	n.a.

[z] Mycor Flower Saver Plus™ (Plant Healthcare, Inc., Pittsburgh, PA) contains arbuscular mycorrhizal fungi inoculum.

[y] Planted May 11, 2000. *Salvia* harvested July 10, 2000. *Impatiens* harvested July 25, 2000.

[x] Colonization percentage determined through grid-line intersect method . Colonization and root samples: n = 20; all other samples n = 32.

[w] Growth index = (height + ((w1+w2)/2)) /2.

[v] Soil P content.

[u] Treatment means w/ same letters are not significantly different (Duncan groupings; <.05).

[t] Added superphosphate - 1.21 g/3.7 L pot. Level as recommended by soil test.

Appendix B

Arbuscular mycorrhizal fungal colonization of *Impatiens wallerana* 'Super Elfin Red' by Mycor Flower Saver Plus™ and Healthy Start™ in non-sterilized and sterilized soils. (Oct. 31, 2000-Jan. 5, 2001)

The purpose of this experiment (Table B) was to document colonization of *Impatiens wallerana* 'Super Elfin Red' by native arbuscular mycorrhizal fungi (AMF) and introduced commercial AMF inoculum (Mycor Flower Saver Plus™ (Plant Healthcare, Inc., Pittsburgh, PA)) in non-sterilized and sterilized Cecil sandy clay loam soil. The effect of Healthy Start™ (Plant Healthcare, Inc., Pittsburgh, PA) on colonization was also determined. Healthy Start™ (3N-1.76P-2.5K) is the fertilizer found in Mycor Flower Saver Plus™.

The treatments were 1) Control – non-sterilized soil + Healthy Start™ (6.06 g/3.7L pot); 2) Non-sterilized soil + Mycor VAM Flower Saver Plus™ (7.27 g/3.7L pot); 3) Steam sterilized soil + Healthy Start™ (6.06 g/3.7L pot); 4) Steam sterilized soil + Mycor Flower Saver Plus™ (7.27 g/3.7L pot).

At project's end, it was realized that the soil was not sterilized as earthworms were discovered in the soil and AMF colonization in all treatments. In order to prevent future problems in the next studies, contamination prevention methods suggested by International Culture and Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) were implemented, and the soil was sterilized with methyl bromide.

Table B. Arbuscular mycorrhizal fungal colonization of *Impatiens wallerana*'Super Elfin Red' by Mycor Flower Saver Plus[TMz] and Healthy Start [TMy] in non-sterilized and sterilized soil[xw].

	Colonization Percentages[v]			
	Treatments			
Reps:	Healthy Start Non-sterilized soil	Mycor (AMF) Non-sterilized soil	Healthy Start Steamed Soil	Mycor (AMF) Steamed Soil
1	5	29	9	24
2	3	30	8	8
3	86	14	54	17
4	8	18	15	52
5	2	3	24	0
Means:	20.8	18.8	22	20.2

[z] Mycor Flower Saver Plus[TM] (Plant Healthcare, Inc., Pittsburgh, PA) is a commercial arbuscular mycorrhizal fungi inoculum (7.27 g/3.7 L pot) (3N-1.8P-2.5K).

[y] Healthy Start[TM] (Plant Healthcare, Inc., Pittsburgh, PA) is the fertilizer contained in Mycor (6.06g/3.7 L pot) (3N-1.8P-2.5K).

[x] Cecil sandy clay loam subsoil.

[w] Planted on October 31, 2000 and harvested January 5, 2001.

[v] Colonization percentages determined by grid-line intersect method.

Appendix C:

Arbuscular mycorrhizal fungus colonization of *Impatiens wallerana* 'Super Elfin Red' by Mycor Flower Saver Plus™ and Healthy Start™ interaction in sterilized and non-sterilized soils.

(April 3, 2001 – June 3, 2001)

In order to test for colonization (Table C) by arbuscular mycorrhizal fungi (AMF) inocula in commercial products, soil must be sterile to eliminate confounding colonization by the native AMF population. In this study, *Impatiens wallerana* 'Super Elfin Red' were planted in Cecil sandy clay loam that was sterilized with methyl bromide. Before planting, the soil was tested for methyl bromide residues. *Impatiens* showed no physical reactions of residual methyl bromide. International Culture and Collection of Arbuscular and Vesicular-Arbuscular Mycorrhizal Fungi (INVAM) production standards were incorporated into the experiment to eliminate cross-contamination of AMF between treatments.

Treatments included 1) sterilized soil only, 2) Healthy Start™ (6.06 g/3.7L pot) + sterilized soil, 3) Mycor Flower Saver Plus™ (7.27 g/3.7L pot) + sterilized soil, 4) Healthy Start™ (6.06 g/3.7L pot) + non-sterilized soil, 5) Mycor Flower Saver Plus™ (7.27 g/3.7L pot) + non-sterilized soil, 6) non-sterilized soil. Both products were purchased new for this experiment.

Colonization was not observed in the sterilized soil treatments. Healthy Start™ (3N-1.8P-2.5K) (Plant Healthcare, Inc., Pittsburgh, PA), the fertilizer found in the AMF commercial inoculum, Mycor Flower Saver Plus™ (3N-1.8P-2.5K) (Plant Healthcare,

Inc., Pittsburgh, PA), did not increase native AMF colonization when compared to the control in non-sterile soil. The newly purchased inoculum, Mycor Flower Saver Plus™, did not increase colonization when compared to the control in either the sterilized or non-sterilized soil.

Possible reasons for the lack of colonization in sterilized soil are no viable inoculum existed in the Mycor or sterile soil could not support AMF. These results suggested future research needed to look at 1) newly purchased inocula 2) other commercial inocula and 3) and the ability of AMF to colonize annuals in methyl bromide sterilized soil.

Table C. Arbuscular mycorrhizal fungi colonization of *Impatiens wallerana* 'Super Elfin Red' by by Mycor Flower Saver Plus[TMz] and Healthy Start[TMy] in sterilized and non-sterilized soil[xw].

	Colonization Percentages[v]					
	Treatments					
	Sterilized Soil			Non-Sterilized Soil		
Reps:	Control	Healthy Start	Mycor	Control	Healthy Start	Mycor
1	0	0	0	37	33	18
2	0	0	0	55	37	30
3	0	0	0	30	20	26
4	0	0	0	17	23	49
5	0	0	0	22	40	35
Means:	0 b[u]	0 b	0 b	32.2 a	30.6 a	31.6 a

[z] Mycor Flower Saver Plus[TM] (Plant Healthcare, Inc.,Pittsburgh, PA) is a commercial arbuscular mycorrhizal fungi inoculum (7.27 g/3.7L pot; 3N-1.8P-2.5K).

[y] Healthy Start[TM] (Plant Healthcare, Inc., Pittsburgh, PA) is the fertilizer contained in Mycor. (6.06 g/3.7 L pot; 3N-1.8P-2.5K).

[x] Cecil sandy clay loam subsoil was sterilized with methyl bromide.

[w] Planted April 3, 2001 and harvested June 3, 2001.

[v] Colonization percentages determined through grid-line intersect method.

[u] Treatment means with same letters are not significantly different (<0.05) (Duncan groupings)..

Appendix D

Effects of Mycor Flower Saver Plus™ and endoRoots™ on colonization and growth of *Impatiens wallerana* and *Salvia splendens* in sterilized soil and non-sterilized soil.

(August 19, 2001 – Oct 19, 2001)

A previous study indicated no colonization of annual plants by a commercial arbuscular mycorrhizal fungi (AMF) inoculum (Mycor Flower Saver Plus™ (Plant Healthcare, Inc., Pittsburgh, PA)). The purpose of this study (Table D) was to compare the ability of a native AMF population and the abilities of two commercial AMF inocula, Mycor Flower Saver Plus™ (Plant HealthCare, Inc., Pittsburgh, PA) and endoRoots™ (Roots, Inc., Independence, MO), to colonize two annual plant species in methyl bromide sterilized and non-sterilized Cecil sandy clay loam subsoil.

The treatments were 1) sterile soil only; 2) endoRoots™ (3N-1.32P-3.3K) (1.22g/3.7L pot) + sterile soil; 3) Mycor (3N-1.8-2.5K)(7.27 g/3.7 L pot) + sterile soil; 4) non-sterile soil + sterile soil. Treatment 4 allowed for testing the response of native AM fungi to residual methyl bromide or other possible limitations to AMF colonization in the sterilized soil. The products were purchased from local distributors and handled according to producers' instructions to assure inocula health and numbers.

Little to no colonization occurred in sterilized soil in either commercial AMF inocula or control for either species. Colonization in the non-sterile soil treatment indicated that methyl bromide sterilization did not limit colonization of native AMF species.

For *Impatiens*, Mycor treatment shoot weights were greater than those of endoRoots, non-sterile soil treatments, and the sterilized soil. Non-sterile soil treatment plants had smaller root dry weights than the other treatments. Smaller root dry weights often occur in mycorrhizal plants as root functions are partially assumed by fungal mycelia. Because of greater nutrients accessed by the mycelia, shoot weights of mycorrhizal plants increased. Both endoRoots™ and Mycor™ contain fertilizers, (3N-1.3P-3.3K and 3N-1.8P-2.5K, respectively). The higher Mycor shoot weights of *Impatiens* can be attributed to the higher fertility supplied by the Mycor product.

In the *Salvia*, shoot and root weights were greatest in the Mycor treatment plants. All other weights were similar to each other. Again, because no root colonization occurred, higher shoot and root weights can be attributed to the fertilizers in Mycor.

Table D. Effects of Mycor Flower Saver Plus™[z] and endoRoot™[y] on colonization and growth of *Impatiens wallerana and Salvia splendens* in sterilized and non-sterilized soil[xw].

	Impatiens wallerana 'Super Elfin Red'			*Salvia splendens* 'Vista Red'		
Treatments	Colonization percentages[v]	Shoot dry mass (g/plant)	Root dry mass (g/plant)	Colonization percentages	Shoot dry mass (g/plant)	Root dry mass (g/plant)
Sterilized soil	0 b[u]	3.6 c	0.59 a	0 b	1.26 b	0.14 b
endoRoots (AMF) Sterilized soil	0 b	5.0 b	0.64 a	0 b	1.80 b	0.17 b
Mycor (AMF) Sterilized soil	0.3 b	7.5 a	0.65 a	0 b	7.43 a	1.05 a
Non-sterilized soil Sterilized soil	8.2 a	5.2 b	0.28 b	11.2 a	1.34 b	0.08 b

[z] Mycor Flower Saver Plus™ (Plant Healthcare, Inc., Pittsburgh, PA) (7.27 g/3.7 L pot; 3N-1.8P-2.5K).

[y] endoRoots™ (Roots, Inc., Independence, MO) (1.22g/3.7 L pot; 3N-1.3P-3.3K).

[x] Cecil sandy clay loam subsoil sterilized with methyl bromide.

[w] Planted on August 19, 2001 and harvested October 19, 2001.

[v] Colonization percentages determined by grid-line intersect method.

[u] Treatment means with same letters are not significantly different (<0.05)(Duncan groupings).