POPULATION AND IDENTIFICATION OF MYCORRHIZAL FUNGI IN ST. AUGUSTINEGRASS IN FLORIDA AND THEIR EFFECT ON SOILBORNE PATHOGENS

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

2006

DEDICATION

This dissertation is dedicated to my family in the memory of my father, Malcome Elmore.

ACKNOWLEDGMENTS

I would like to thank my parents, Malcome and Donna Elmore, for their loving support and my sister, Emilee. I would also like to acknowledge a very special person, LaVette Burnette, for all of the patience and caring attention she has shown me for many years. I would also like to thank Dr. James Kimbrough and his wife, Jane, for their support, both emotionally and spiritually. I would also like to thank Drs. Jim Graham and Kevin Kenworthy for agreeing to serve on my graduate committee and for their willingness to offer advice on my studies. I also owe Dr. Vertigo Moody a big "thank you" for motivating me to finish my Ph.D. as well as for his technical support in writing.

Additionally, I would like to say a big "thank you" to Dr. Gerald Benny both for serving on my committee and for his attention in the lab. Dr. Benny is always ready to help with research, or simply listen to my ramblings about research and politics which I appreciate greatly. I would like to extend a personal "thank you" to the Department of Plant Pathology staff, Gail Harris, Lauretta Rahmes, and Donna Perry. These ladies always have a smile ready and a helping hand for students. I would also like to thank Eldon Philman and Herman Brown for their assistance in experimental studies at the greenhouse complex. They seem to always have a good solution or answer to any problem or question. Finally, I would like to extend my sincerest appreciation to the Department of Plant Pathology, namely Dr. Gail Wisler, at the University of Florida and to the Institute of Food and Agricultural Sciences for financial and technical support in

this endeavor. I would not have been able to fulfill my dreams without the help and support from all of these people.

TABLE OF CONTENTS

LIST OF TABLES

Table page

LIST OF FIGURES

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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August, 2006

Chair: James W. Kimbrough
Major Department: Plant Pathology

Arbuscular mycorrhizal fungi (AMF) are obligate symbionts of more than 90% of all land plants. Mycorrhizae are documented in many crops as positive associations with roots of plants that help reduce disease severity soilborne pathogens and increase nutrient and water uptake while lowering plant stress and ultimately management costs. However, there is no information concerning the effects of AMF colonization in St. Augustinegrass.

In Florida, St. Augustinegrass sod production contributes hundreds of millions of dollars to the economy annually while supplying a product to homeowners and commercial entities with great aesthetic value. The use of AMF in St. Augustinegrass sod production has many potential benefits to the sod industry and the environment including lowered management costs, pesticide use and pollution. In these studies, a survey of St. Augustinegrass sod farms in north central Florida revealed a moderate level of AMF colonization as well as a diverse population of AMF species. Direct and indirect

pathogen challenges with the ubiquitous AMF, *Glomus intraradices*, in St. Augustinegrass plants suggested a limited role for AMF in lowering disease severity in two of the more devastating diseases of St. Augustinegrass in Florida, brown patch and take-all root rot.

While no positive correlation was observed between AMF colonized St. Augustinegrass plants and the soilborne pathogens *Rhizoctonia solani* or *Gaeumannomyces graminis* var. *graminis*, effective assays for mycorrhizal St. Augustinegrass evaluations were developed and foundation information concerning the association between St. Augustinegrass and AMF provided valuable data, which may help in the development of future AMF evaluations in St. Augustinegrass field trials and with other AMF species. These results were the first to suggest an association between AMF and St. Augustinegrass, and to evaluate their potential effects on disease severity.

CHAPTER 1
GENERAL INTRODUCTION

"Mykorrhizen" was a term first applied by the German forest pathologist, A.B. Frank, who described structures in plant roots as "*fungus-roots*" (1885). Harley (1989) described them as a mutualistic symbiosis in which a fungus and host exist as one. Despite minuscule differences in description, mycorrhizas are recognized by scientists as economically important in most agricultural crops. In fact, the mutually beneficial relationships are actually three-way associations in which the soil, plant root, and fungus interact to produce symbiotic effects.

In 1879, de Bary defined symbiosis as "the living together of differently named organisms," which included both parasitic and beneficial relationships. Later, Raymer (1927), commenting on the nature of symbionts, acknowledged such partnerships, but did not provide functional information concerning the fungi involved. However, after many years of advanced research throughout the 1960's and 70's, the meaning of the relationship was refined to refer to naturally beneficial relationships exclusively. Most likely, organisms co-existing became symbiotic as a result of selection pressures exerted over the course of time (Remy et al., 1994). In fact, it is possible that the movement of plants from water to land could not have occurred without mycorrhizal associations (Nicolson, 1975; Pirozynski and Malloch, 1975). It is now recognized that mycorrhizas are the norm and not the exception within the Kingdom *Planta*. With ancient lineages stretching across evolutionary history, *Bryophytes*, *Angiosperms*, *Pteridophytes*, and some *Gymnosperms* all possess these associations

(Fitter, 1991), while members of the *Brassicaceae* seem to evade infection by any type of mycorrhizal fungi (Gerdemann, 1968), even in close proximity to mycorrhizal plants. Involved in mycorrhizal symbiosis are members of the fungal taxa *Ascomycotina*, *Basidiomycotina*, *Zygomycotina*, *Deuteromycotina, and Glomeromycota* (Schüssler et al., 2001; Srivastava et al., 1996). Infrequently found living as saprobes, most of these fungi are widespread across various soil types with strong biotrophic host dependence (Smith and Read, 1997).

Mycorrhizal Types and Phylogeny

Types of mycorrhizae are divided based on their fungal associations, extent of root penetration, presence or lack of an external mantle and/or sheath, as well as the intra- and intercellular structures produced inside of the host root (Srivastava et al., 1996). Presently, seven types of mycorrhizae are recognized by taxonomists (Bagyaraj, 1991). The types of mycorrhizae include: *Ectomycorrhizae*, *Ectendomycorrhizae*, *Arbutoid*, *Monotropoid*, *Ericoid*, *Orchidoid*, and *Endomycorrhizae* or the vesicular-arbuscular mycorrhizae (Bagyaraj, 1991). *Endomycorrhizae*, also known as vesicular-arbuscular mycorrhizae or VAM, were taxonomically placed within the Order *Glomales* of the Phylum *Zygomycota* based on morphological features of asexual spores resembling sexual reproductive structures of the *Zygomycota*. Six genera are recognized within the Glomales: *Glomus*, *Sclerocystis*, *Gigaspora*, *Scutellospora*, *Acaulospora*, and *Entrophospora* (Morton and Benny, 1990). In 2001, Schussler et al., using information provided by small subunit rRNA gene sequences, proposed a new Phylum, to separate arbuscular mycorrhizal fungi from other fungal groups in a monophyletic clade. Schussler et al. (2001) suggested that they be removed from the *Zygomycota* and placed into a newly erected Phylum *Glomeromycota*. Small subunit rRNA gene sequencing also

placed *Geosiphon pyriformis*, an endocytobiotic fungus, which is a distant relative of the arbuscular mycorrhizal fungi, within this new Phylum (Schussler et al., 2001). Within the same article, Schussler et al. (2001) also suggested that the *Glomus* genus be emended to include the termination *–eraceae*, with the family named *Glomeraceae* and the higher taxon names reflecting this change with *Glomerales*. Furthermore, Schussler et al. (2001) suggested three new orders, mostly diverged from the Ascomycetes and Basidiomycetes, be recognized as well. These are the *Archaeosporales*, *Diversisporales*, and the *Paraglomerales*. Based on a combination of molecular, ecological, and morphological characteristics, these fungi can now be separated from other fungal groups. The use of molecular techniques such as small subunit rRNA sequencing has led to the recent introduction of other species within the genus *Glomus*. Walker et al. (2004) and Rani et al. (2004) also used this technology to add *Glomus hyderabadensis* from India, and a new genus *Gerdemannia*, to the growing list of arbuscular mycorrhizal fungi collected and speciated around the world. Based on their distinct molecular differences from the Zygomycota and placement into a new phylum, Goto and Maia (2005) recently suggested that spores of the arbuscular mycorrhizal fungi be referred to as glomerospores. Indeed, these spores are not chlamydospores, conidia, or azygospores, so differentiation based on molecularly distinct features is pertinent.

Forming vesicles and arbuscules within cortical root cells, fungi of the *Glomeromycota* produce aseptate hyphae without the presence of a sheath or mantle. *Gigaspora* and *Scutellospora* produce arbuscules only within roots and vesicles only within the soil, and, therefore, the vesicular-arbuscular mycorrhizal term has been emended to simply read as arbuscular mycorrhizae. The name was amended simply

because arbuscules are the most basic and one of the few commonalities between the members of the group (Morton and Benny, 1990). Taylor et al. (1995) proposed that *Glomites* be included as a new fossil genus of Glomales, and two years later, Wu and Lin (1997) added another genus, *Jimtrappea*. However, these two genera are not widely accepted. Currently, there are about 150 recognized species described within the *Glomales,* of which only a few have been carefully studied and recognized as endo-mycorrhizal (Morton and Bentivenga, 1994; Morton and Benny, 1990; Morton et al., 1992; Pirozynski and Dalpe, 1989; and Stuessy, 1992). Glomeromycota are not known to produce sexual reproductive spores and, therefore, are characterized and classified by their resting structures. These structures vary in wall characteristics, size, shape, and color (Morton et al., 1992; Morton and Bentivenga, 1994; and Morton and Benny, 1990).

Arbuscular Mycorrhiza Physiology

The most widespread of the mycorrhizae, both geographically and among species, the arbuscular mycorrhizae occur frequently in the top 15-30 cm of cultivated soil (Bagyaraj, 1991). Arbuscular mycorrhizae-forming fungi colonize and form associations with most agriculturally and horticulturally important plant species, from fruit and forest trees to shrubs and grasses. Unlike other mycorrhizae, these associations do not typically lead to noticeable external morphological changes in plant roots, and they cannot be observed easily without staining procedures (Phillips and Hayman, 1970). In most cases, plants which have formed associations with other types of mycorrhizal fungi, such as basidiomycetes and ascomycetes, do not form relationships with arbuscular mycorrhizae. From the standpoint of the fungus, host specificity exists while the opposite view would be held about the host due to the wide host range of most of the arbuscular mycorrhizal fungi (Gerdemann, 1955). Their limited capacity to be grown from spores, vesicles, or

hyphae from root residue has led to special methodologies in order to maintain strains and for taxonomic evaluation. Typically, single spore types are cultivated in "pot cultures" on plant roots so that characteristics of spores, their mode of colonization, and effects on plant growth can be studied (Smith and Read, 1997).

Arbuscular Morphology

Named by Gallaud (1905) for the structures formed inside cortical root cells, arbuscules are similar to branched haustoria, which form early on in the association between plant root and the repeatingly branched fungal hyphae. Baylis (1975) and St. John (1980) suggested that the form of the root system is a defining factor in the extent to how plants react, nutritionally, and in growth to mycorrhizal colonization. Evolving across phylogenetic lines many times, it appears that dicotyledons have a large incidence of associations with fungal species which form mycorrhizal associations, with very few being non-mycorrhizal in nature (Trappe, 1987). In comparison, the lines of monocotyledons studied by Cronquist (1981) are heavily mycorrhizal, with arbuscular mycorrhizas predominating except in the *Orchidaceae,* which have mycorrhizas formed by Basidiomycetes. In plants forming primarily magnolioid type roots, with wide diameters up to 1.5 mm, slow growth habits, and little root-hair development, mycorrhizas are usually well accepted and form greatly receptive relationships. On the other hand, roots that are primarily fine and rapidly growing with long root-hairs lack the same responsiveness (Baylis, 1975; St. John, 1980). Mycorrhizal relationships were first described by the type of colonization patterns, referred to as either *Arum*- or *Paris*-type (Gallaud, 1904). In fact, there appears to be a continuum between the two forms, with intermediate types along the way.

The *Arum*-type, which was considered the most common association, develops primarily within cultivated crops and consists of intercellular hyphae and arbuscules. In contrast, the *Paris*-type of symbiosis – involving intercellular hyphae, arbusculate coils, and hyphal coils, typically develops within forest trees and herbs (Dickson, 2004). In surveys of mycorrhizal plants and trees from both natural and cultivated environments, it appears that most plant families are dominated by only one symbiotic type (Smith and Smith, 1997). There are, however, a few plant families that appear to possess intermediate forms of the colonization types, including the *Poaceae* (Smith and Smith, 1997). In an extensive survey of various plant families and mycorrhizal fungi, eight distinct classes of colonization types were found along a continuum ranging from the *Paris*- to *Arum*-type (Dickson, 2004). Most researchers agree that one fungus can form either type of arbuscular colonization with most of the specificity in structure dependent upon the host plant (Barrett, 1958; Gerdemann, 1965). Brundett and Kendrick (1988) commented on the presence of intercellular spaces within the host root cortex as being the main factor influencing arbuscular type. Conversely, in tomato, Cavagnaro et al. (2001) suggested that the colonization type was dependent on both the host and fungus involved.

Mycorrhizal Colonization

In mycorrhizal colonization, the host plasmalemma is invaginated with the encroaching arbuscules. These are physiologically active sites for nutrient translocation, for 4-6 days, within the roots (Bracker and Littlefield, 1973; Brundett et al., 1984). Arbuscules are important sites for P exchange for plants under deficient conditions (Simth and Read, 1997). The vesicles, which are small and usually dark, globular or spherical structures, form later in the association and arise from swelling of terminal and intercalary hyphal cells. Vesicles act as storage sites for lipids (Srivastava et al., 1997).

Transversing long distances of soil beyond nutrient depletion zones and reaching areas untouched by growth limited root hairs, the external hyphae absorb nutrients such as P and make it available to plants, rendering these plants more equipped to survive nutrient competitions (Nicolson, 1967). Once the fungal hyphae and plant roots become closely associated in space, a functionally and structurally complex symbiotic relationship is formed between the compatible organisms.

Formed only on unsuberized root tissue, certain areas of the root are more readily colonized even though mycorrhizae can develop on any portion of young root tissue (Brundett and Kendrick, 1990). Based on mathematical and geometrical models, root tissue directly behind the meristematic area is considerably more susceptible to penetration and colonization when compared to other root segments (Garriock et al., 1989; Bonfante-Fasolo et al., 1990). This area of discrete colonization was described earlier as the mycorrhizal infection zone by Marks and Foster (1973), who considered the area to be "non-static," thus growing with the root. Furthermore, Brundett and Kendrick (1990) found that the fungus penetrates and colonizes root cells with little or no suberin deposition, which has been shown to occur just prior to or after fungal penetration. Usually, epidermal and outermost cortical cell colonization is minimal with the intercellular hyphae formed in the inner cortex and the majority of the colonization is deep within the cortex where arbuscules are formed (Srivastava et al., 1997).

With the aid of cellulolytic and pectinolytic enzymes produced by the fungus, direct penetration of the outermost cell wall is the preferred mode of hyphal entry (Jarvis et al., 1988). Physiochemical aspects of the epidermal cell wall seem to be the primary reasons for preferential site penetration (Jarvis et al., 1988). After cell to cell contact

between fungus and host, the external mycelia swell to form defined appresoria (20-40 µm in length). Within these appresoria, infection hyphae are formed and penetrate host cell walls (Garriock et al., 1989). Once penetration has occurred via mechanical and enzymatic interactions, the host's plasmalemma appears to extend around the fungus (Bracker and Littlefield, 1973). Arbuscule formation takes between 4-5 days after which extramatrical hyphae occurs promoting new penetration sites (Brundett et al., 1984). Arbuscules are major contributors to the transfer of nutrients, in particular sugars, between the plant to fungus and inorganic materials, mainly P, from the fungus to the plant (Smith and Gianinazzi-Pearson, 1988).

Mycorrhizal Rhizosphere Interactions

A necessary component of plant life, the macro element P, occurs as part of DNA and RNA nuclei and as part of plant membranes as phospholipids (Griffiths and Caldwell, 1992; Smith and Read, 1997). Present in high amounts within active meristematic regions as part of nuclear proteins and as part of ADP, ATP, NADP, and NAD, P is partly responsible for oxidation-reduction reactions such as respiration, nitrogen and fat metabolism, and photosynthesis, which are necessary for life (Beever and Burns, 1980; Munns and Mosse, 1980). Symptoms of deficiency often include purple or red leaf pigmentation, dead and/or necrotic leaves, petioles, and fruits, premature leaf drop, stunting, and poor vascular tissue development (Srivastava et al., 1997). An important aspect of arbuscular mycorrhizal associations is the increase in P uptake by the plant.

The importance of arbuscular mycorrhizal fungi for P absorption was first suggested by Baylis (1959) and then Gerdemann (1964). Later, Baylis (1967), Daft and Nicolson (1966), Holevas (1966), and Murdoch et al. (1967) provided advanced

information showing the close association between mycorrhizas and P nutrition of the host. Interestingly, Mosse (1973) once remarked that more than one quarter of mycorrhizal text is devoted to P research. In fact, Sanders and Tinker (1973) stated that "the value of these mycorrhizas for the phosphate nutrition of plants in deficient environments may rival that of *Rhizobium* in nitrogen." Obviously, such a strong statement must be supported by an abundance of research. As mycorrhizal research progressed during the last three decades, P research remained an important topic. For instance, in 1986, Gianinazzi-Pearson and Gianinazzi studied the kinetic associations between P concentration in soil solutions and its effect on root and shoot tissues, while Young et al. (1986) evaluated the effect of arbuscular mycorrhizal fungi inoculation on soybean yield and P utilization in tropical soils. Later, Koide (1991) determined that it is the variation among plant species in phenological, morphological, and physiological traits that influence P demand and supply which are directly connected to potential response of mycorrhizal associations. Once absorbed, P is allocated for plant functions or stored for later use (Cox and Sanders, 1974). Since P deficiency is caused by both P availability and plant demand, mycorrhizal associations can have various effects based on the plant species (Koide, 1991).

In low P soils, mycorrhizal plants have an advantage over non-mycorrhizal plants with root to shoot ratios lowered and shoot fresh weight to dry weight ratios higher in mycorrhizal plants (Tinker, 1978). The plant's growth rate is influenced by interactions in mycorrhizal colonization such as nutritional, and non-nutritional, physiological effects, such as pH, temperature, microbial turnover, phosphatase activity, soil and plant moisture, and/or iron (Fe) or aluminum (Al) chelate concentration (Nye and Tinker,

1977; Rusell, 1973). In P deficient soils, studies have shown that plant species with few root hairs are strongly mycorrhizal, providing evidence that root anatomy has a strong correlation to mycorrhizal colonization (Crush, 1974; Baylis, 1975).

Smith and Read (1997) wrote "the focus (of current research) is on P uptake, as well as on the uptake of other nutrients for which there is now unequivocal evidence of mycorrhizal involvement." Furthermore, they noted that "there is excellent evidence to demonstrate that external hyphae of VA mycorrhizal fungi absorb non-mobile nutrients (P, Zn, Cu) from soil and translocate them rapidly to the plants, thus overcoming problems of depletion in the rhizosphere which arise as a consequence of uptake by roots." Throughout the 1960's, reviews of the occurrence of arbuscular mycorrhizal colonized plants and anatomy were the norm in mycorrhizal research (Smith and Read, 1997). There had been little mention of mineral nutrition until Mosse (1957) released details of an experiment with apple seedlings which provided evidence for increased amounts of potassium (K), iron (Fe), and (copper) Cu in mycorrhizal plant tissue versus noninoculated control plants. Other researchers such as Gerdemann (1964) established that P tissue concentrations were also higher in mycorrhizal plants, although the mechanisms were not yet clearly understood. Mosse (1973) reported a shift in mycorrhizal research from pot experiments to study the anatomy of arbuscular mycorrhizal fungi to that of plant growth and P uptake. Now, the mechanisms underlying the mycorrhizal effect on P uptake are coming to light including extraradical hyphae growing into soil not already colonized by roots; hyphae that are more effective than roots, due to size and spatial distribution, in competing with free-living microorganisms or mineralized or solubilized P; the kinetics of P uptake into hyphae may differ from

roots; and that mycorrhizal roots can use sources of P in soil that are not plant available (Smith and Read, 1997).

Hyphal pathways between plants may offer links for soil-derived nutrient transfer, as is the case with plant-derived carbon (C), which can have important roles in the inter plant and species competition in the environment (Smith and Read, 1997). Enzymes are not the only substances produced by arbuscular mycorrhizal fungi. An Iron-containing glycoproteinaceous substance called glomalin, produced by these fungi, is deposited in soils (Rilling et al., 2003). Glomalin is considered to be linked to soil Carbon storage due to its effect on soil aggregation (Rilling et al., 2003). Consistently correlated with soil aggregate water stability, glomalin is involved in C and N content as well as being useful as a potential land-use change indicator (Rilling et al., 2003). After many years of taxonomic research with proteins and soil stability, micronutrient uptake research has increased following studies by Mosse (1957), Daft et al. (1975), and Gildon and Tinker (1983) where uptake of Cu and zinc (Zn) were observed in apples and maize when inoculated with arbuscular mycorrhizal fungi. The uptake of other micronutrients is not well documented, however, Marschner and Dell (1994) observed that the uptake of manganese (Mn) is usually reduced by mycorrhizal associations. Occasionally, instances of increased K concentrations in plant tissues have been reported, which is to be expected given the immobility of the K ion within the soil matrix (Srivastava et al., 1997). Conversely, with increased P uptake as well as other nutrients in mycorrhizal plants comes the risk of accumulating toxic elemental levels. With improved P nutrition and plant growth, the uptake of heavy metals per plant is greatly increased as demonstrated

by El-Kherbawy et al. (1989) on alfalfa inoculated with arbuscular mycorrhizae in various soil pH levels with and without rhizobia.

Effects of Abiotic Factors on Mycorrhiza

Many climatic and physiochemical or abiotic features of the soil influence arbuscular mycorrhizal establishment, growth and benefit. For instance, light, which is not directly required by mycorrhizas in some cases, is essential for the host to thrive and translocate photosynthates to the root, which in turn provides a home for mycorrhizal fungi. In other cases, arbuscular mycorrhizal fungi are stimulated by light to increase root colonization and spore production as well as plant response to mycorrhizal colonization (Furlan and Fortin, 1973; Hayman, 1974).

The rate of photosynthesis and translocation of its products are heavily influenced by air temperature (Furlan and Fortin, 1973; Hayman, 1974). By increasing air temperature to 26^{o} C an increase in plant growth is typical (Hayman, 1974). Soil temperatures also influence mycorrhizal development at all stages: spore germination, hyphal penetration, and proliferation within cortical root cells (Schenck and Schroder, 1974; Smith and Be, 1979). Optimal temperatures vary for spore germination between species and other stages in development. The ability of the arbuscular mycorrhizal spores to survive following host death or harvest is also dependent on soil temperature, though also affected by soil texture (Bowen, 1980).

Soil pH is an additional determinant factor in mycorrhizal growth and development. The efficiency of the mycorrhizae is directly determined by its ability to adapt to soil pH. Soil pH affects both spore germination and hyphal development (Angle and Heckman, 1986; Green et al., 1976). The interaction of soil pH and mycorrhizal development is difficult with soil type, plant and fungal species and P forms involved.

Typically, mycorrhizas are able to colonize and grow well in soils of pH 5.6 to 7.0, but not in soils of pH 3.3 to 4.4, as reported by Hayman and Mosse (1971).

Generally, mycorrhizas are not found within aquatic conditions, due to a reduction in colonization, however, some aquatic plants are commonly mycorrhizal, such as *Lobelia dortmanna* L. and *Eichhornia crassipes* [Martius] Solms (Read et al., 1976). Conversely, most plants found within drought are typically mycorrhizal, which aids in their survival in harsh conditions (Sondergaard et al., 1977). Arbuscular mycorrhizal colonization of roots affects many mechanisms in plant water determination. Root hydraulic conductivity, leaf gas exchange and expansion, phytohormone regulation, and leaf conductants are all affected by interactions with arbuscular mycorrhizas (Gogala, 1991; Hardie and Leyton, 1981; Koide, 1985; Nelson, 1987; Auge et al., 1986). Fungal mycelium is involved in the transport of water especially at low soil potentials, which has made arbuscular mycorrhizae colonization and development a hot research topic in arid and tropical landscapes (Faber et al., 1991).

Mycorrhizal roots and organic matter content play important roles in arbuscular mycorrhizal survival and development as well. Organic root debris may act as a reserve for soil inocula (Warner and Mosse, 1980), while in arid areas contact between susceptible plant roots and colonized root residue is considered by Rivas et al. (1990) to be the most important means for mycorrhizal dissemination when little water is available for spore transport. Soil structure, pH, water, and nutrient availability are all affected by organic matter content, thus influencing mycorrhizal associations (Khan, 1974; Daniels and Trappe, 1980; Johnston, 1949). For instance, Johnston (1949) suggested that organic materials such as manures can enhance tropical soil mycorrhizas in cotton stands. And,

Sheikh et al. (1975) reported that spore population and organic matter content were positively correlated in soils with 1-2% organic matter, but low in soils with 0.5% organic matter or less. Organic matter and root residue are important ecologically as part of the three-way soil, plant and fungal mycorrhizal relationship.

Effects of Seasonality on Mycorrhiza

Seasonality is another abiotic contributor to arbuscular mycorrhizal colonization. Seasonality has been shown to affect spore production as a function of host and climate (Hetrick, 1984), while seasonal patterns can be correlated with P availability and soil water potential in combination with host growth stages, other biotic and abiotic factors, and management practices such as fertilization (Cade-Menun et al., 1991; Yocums, 1985). Hayman (1975) demonstrated that fertilizers such as P and Nitrogen (N) could potentially reduce spore number and fungal colonization with N having a more detrimental effect than P. Despite the possibility for soil chemical treatment injury, arbuscular mycorrhizae can be found in fertile soils, which Hayman et al. (1976) contributed to other factors such as host species, soil type, and management practices influencing fungal survival and development.

As previously mentioned, management practices such as pesticide applications, in particular, fungicides, may inhibit the effect of arbuscular mycorrhizal fungal sporulation and colonization (Nemec and O'Bannon, 1979; El-Giahmi et al., 1976). Rhodes and Larsen (1979) examined arbuscular mycorrhizae of turfgrasses in field and greenhouse conditions. The researchers discovered that when fungicides were applied to bentgrass, infection averaged 9 to 17%, however, in non-treated field plots, the roots were infected at a rate of 40-60 percent. The same observation was reported in the greenhouse evaluations, with one fungicide, PCNB, totally eliminating mycorrhizae (Rhodes and

Larsen, 1979). Conversely, DBCP, a nematicide, has actually been reported by Bird et al. (1974) to enhance arbuscular mycorrhizal development.

It is imperative to mention that mycorrhizal interactions lie along a continuum from mutualistic to parasitic based on the cost to benefit ratio colonization. Obviously, mycorrhizal associations can be mutualistic, but they can also be parasitic, commensal, amensal, and even neutral in nature (Johnson et al., 1997). Where, along this continuum the association will fall, depends on a complex hierarchy mediated by biotic and abiotic factors within the rhizosphere and ecosystem being affected. No doubt, this range of mycorrhizal associations is greatly affected by time and space. The complexity of mycorrhizal investigations is ultimately confounded by the fact that the plant and fungal perspective on costs to benefits differs greatly from situation to situation (Johnson et al., 1997).

With this in mind, Ryan and Graham (2002) presented the point-of-view that arbuscular mycorrhizal fungi do not play such a vital role in production agricultural systems, in relation to nutrition and growth, simply because the high cost of energy from the plant to support the fungal invader outweighs the benefits of that association. This outcome is not beneficial in terms of crop production and may, in fact, be detrimental. Nonetheless, those production systems not considered to be within a natural or traditional cultivated production system, such as sod, still need much attention where mycorrhizal symbiosis is concerned before a definitive yes or no can be applied to functional use of mycorrhizal fungi. Conversely, in 1997, Srivastava et al. concluded that “there is little doubt that vesicular arbuscular mycorrhizae fungi will emerge as a potential tool for improving crop plants in the years to come.” These opinions, in conjunction with the

increased concern for environmental quality and sustainable technologies warrants an examination of more specific research reports in agricultural crops. In this review, the concentration is on turfgrass research.

Mycorrhizas in Grasses

There has been a considerable amount of research on mycorrhizal fungi associated with grasses (Hetrick et al., 1988, 1991; Trappe, 1981; Bethlenfalvay et al., 1984). Though much of the work conducted on grasses was begun in the 1970's, Nicolson (1955) examined mycotrophic nature in grasses and later (Nicolson, 1956) with mycorrhizae in both grasses and cereals. These first studies in grasses and cereals were mainly concentrated on the ecological aspects of mycorrhizal infection. In fact, it was not until Nicolson (1956) showed diagrammatically that external hypha penetrate the root hairs or epidermal cells and spread throughout the cortex of grasses. Additionally, Nicolson noted that arbuscules form later in the inner cortical layers, which was valuable information in the study of grasses and their mycorrhizal partners.

In experiments on fescue (*Festuca ovina* L.), cocksfoot (*Dactylis glomerata* L.), sand fescue (*Festuca rubra* var. *arenaria* L.), and marram grass (*Ammophila arenaria* L.: Link), Nicolson (1956) found that mycorrhizal infection was prevalent throughout a wide range of different habitats and soil types, although the incidence of infection varied greatly between habitats and communities. With a lull in ecological studies throughout the 1960's, environmental issues surpassed many of the more basic research topics. In 1979, Rhodes and Larsen examined the effects of fungicides on mycorrhizal development in cool-season turfgrasses. Again, Rhodes and Larsen (1981) conducted a similar study, where the effects of fungicides on bentgrasses and the mycorrhizal fungus, *Glomus fasciculatus*, were explored. Arbuscular mycorrhizas of 'Penncross' creeping bentgrass

(*Agrostis palustris* Huds.) were studied in greenhouse experiments to evaluate popular fungicides, such as, chloroneb and maneb, which did not affect mycorrhizal development. However, foliar applications of PCNB, chlorothanil, bayleton, anilazine, benomyl, and chloroneb at various weeks after inoculation with *Glomus fasciculatus* resulted in significantly reduced mycorrhizal colonization, thus limiting their beneficial effects.

Later, studies of mycorrhizas in turfgrasses seemed to swing back toward ecological studies with the introduction of seasonal and edaphic variation of arbuscular mycorrhizal infection (Rabatin, 1979). In a population survey, Rabatin (1979) sampled for *Glomus tenuis* infection in *Panicum virgatum* L., *Poa compressa* L., *Poa pratensis* L., *Poa palustris* L., *Phleum pratense* L., and *Festuca etalior* L., all cool-season meadow grasses. Rabatin (1979) determined that the greatest percentage of root infection by this fungus occurred in grass roots from dry, P deficient fields. Moreover, the percent of infection was lowest in the cool, wet months of the spring. Thus, Rabatin (1979) concluded that mycorrhizal infection tends to be greater in drier, P deficient soils versus wet or flooded conditions.

Bagyaraj et al. (1980) concluded that a study of the spread of mycorrhizas from the site of infection along the root to deeper soil layers was necessary to provide important information for plant inoculations. This was done in grasses since the roots grow out of the inoculated sites quickly. Researchers collected root samples from various depths and found that roots at 3 - 4 and 8 - 9 cm were mycorrhizal at 45 days after inoculation. However, when roots were collected from deeper layers, the roots were only mycorrhizal after 75 days. The research lead Bagyaraj et al. (1980) to conclude that mycorrhizal infection of warm-season grasses such as Sudangrass (*Sorghum bicolor* L.:

Moench), was spread to deeper layers by mycelial growth through the root, which was helpful information when researching inoculation methodologies important in such experiments as population surveys where pot cultures are a necessary to speciate the fungi collected. In an attempt to determine the distribution and occurrence of mycorrhizal fungi in Florida's agricultural crops, Schenck and Smith (1981) examined bahiagrass (*Paspalum notatum* Flügge) and digitgrass (*Digitaria decumbens* Stent) among 30 *Cucurbitaceae*, *Leguminosae*, *Solanaceae*, and *Vitaceae* crops. In a population survey, the authors found that mycorrhizal fungi in *Glomus* occurred most frequently in Florida, with species of *Gigaspora* found regularly in central and south Florida and *Entrophospora* collected only once (Schenck and Smith, 1981). Furthermore, *Acaulospora* was found in the highest frequency in the grasses evaluated. In this instance, there was no correlation among species or genera occurrence and the available soil P or soil pH.

In another study, endomycorrhizas and bacterial populations were examined in three cool-season grasses. *Agrostis tenuis* Sibth., *Deschampsia flexuosa* L.: Trin., and *Festuca ovina* L., were collected and examined by Lawley et al., (1982) for mycorrhizal associations. In this case, the researchers noticed that mycorrhizal abundance was lowest when *Agrostis* species were partnered with other plants and highest when partnered with *Festuca*.

Finally, Sylvia and Burks (1988) began working with grasses other than those only found in cool-season climates. Beach erosion in coastal areas became a major economic concern in the late 1980's; beach grasses such as sea oats (*Uniola paniculata* L.) were often utilized to restore southeastern beaches to slow loss of sand. It was

unclear whether or not these grasses relied on arbuscular mycorrhizal associations for survival in the harsh climate. Sylvia and Burks (1988) found that isolates of *Glomus deserticola* and *G. etunicatum* significantly increased the dry mass, height, and P content of the sea oats, while other isolates had little or no effect.

In the search for a better host for inoculum production, compared to the traditional bahiagrass, Sreenivasa and Bagyaraj (1988) evaluated seven grasses for their ability to quickly produce large masses of mycorrhizal spores for inoculations. Grasses such as guinea grass (*Panicum maximum* Jacq.) and rhodes grass (*Chloris gayana* Kunth) were studied and all were found to be mycorrhizal. However, the highest root colonization was observed in the rhodes grass, as well as the highest production of spores and infective propagules. Studies on other warm-season grasses such as St. Augustinegrass (*Stenotaphrum secundatum* [Walt.] Kunze), Centipedegrass (*Eremochloa ophiuroides* [Munro] Hack.), or even bermudagrass (*Cynodon dactylis* L.: Pers.) have not been identified.

In studies of the difference in responses of C_3 and C_4 grasses to P fertility and mycorrhizal symbiosis, Hetrick et al. (1990) showed that warm-season grasses such as big bluestem (*Andropogon geradii* Vitm.) and indian grass (*Sorghastrum nutans* L.: Nash), responded positively to mycorrhizae or P fertilization, or mycorrhization in cool-season grasses, such as perennial ryegrass (*Lolium perenne* L.). In warm-season grasses, there was a positive relationship between root colonization and dry weight, with an inverse relationship between mycorrhizal root colonization and P fertilization. The evaluation provided evidence that the C_3 and C_4 grasses display profoundly different nutrient acquisition strategies (Hetrick et al., 1990b).

The effect of mycorrhizal symbiosis on regrowth of rhizomes of big bluestem was assessed as a function of clipping tolerance (Hetrick et al., 1990a). Mycorrhizal clipped plants were larger than nonmycorrhizal clipped plants, but the effect diminished with successive clippings as did mycorrhizal root colonization. This information on clipping tolerance indicates that mycorrhizal turfgrasses respond similarly when clipped or mowed under constant turf management.

Hetrick et al. (1991) compared the root architecture of five warm and five cool-season grasses in an attempt to evaluate whether mycorrhizal symbiosis confers a greater tolerance to drought, soilborne disease, vigor, and yield through direct or indirect improved nutritional status of the host plant. The cool-season grasses had significantly more primary and secondary roots than the warm-season grasses and the diameter of those roots was smaller than that of the warm-season grasses. The mycorrhizas did not affect the number or diameter of cool-season grass roots, however, the warm-season grasses did respond to mycorrhizal inoculation. Additionally, the root length was significantly increased in the warm-season grasses with mycorrhizal infection when compared to the cool-season grasses. Through the aid of topological analysis of root architecture, mycorrhizal symbiosis was shown to inhibit root branching in warm-season grasses, but had no effect on cool-season grass rooting (Hetrick et al., 1991). The researchers concluded that mycorrhizal-dependent warm-season grasses have unique root architecture, allowing energy to be conserved for root development, while the less dependent cool-season grasses do not exhibit the same benefits of mycorrhizal infection.

In studies designed to determine the dependence of warm-season grasses on arbuscular mycorrhizae and relationships between mycorrhizae and P availability and

plant density, Brejda et al. (1993) and Hetrick et al. (1994) evaluated sand bluestem (*Andropogon geradii* var. *paucipilus* Nash), switchgrass (*Panicum virgatum* L.), and Canada wild rye (*Elymus canadensis* L.).

The popular cool-season grasses, creeping bentgrass (*Agrostis stolonifera* L.) and Kentucky bluegrass (*Poa pratensis* L.) were evaluated in relation to the impact of arbuscular mycorrhizae and P status on plant growth (Charest et al., 1997). The authors revealed that as mycorrhizal infection increased in the grasses, root colonization increased to more than 40% with lowered P fertilization. This information could be particularity helpful in warm-season grasses where P may have a major impact in soils, such as those found throughout Florida. The researchers of this study concluded that arbuscular mycorrhizal symbiosis could be considered as a potential fertilizer reduction agent (Charest et al., 1997).

More recently, mycorrhizal symbiosis and fertilizer relationships have dominated arbuscular mycorrhizal research; however, the majority of this work has concerned cool and warm-season prairie grasses. The emphasis of molecular technologies has resulted in less applied types of research being performed with grasses and mycorrhizas. Using terminal restriction fragment length polymorphism (T-RFLP), Vandenkoornhuyse et al. (2003) assessed the diversity of arbuscular mycorrhizal fungi in various cool-season grasses, which co-occurred in the same research plots. Based on a clone library, the level of diversity was consistent with past studies; showing that mycorrhizae fungal host-plant preference exists, even between grass species.

Obviously, there is limited information on warm-season turfgrasses when compared to the warm-season prairie and cool-season meadow grasses. In the Southeast,

warm-season turfgrasses are highly valued for their drought resistance, aesthetic importance and generally low maintenance on some home lawns, golf courses, soccer, and football fields. Species such as bermuda, St. Augustinegrass, seashore paspalum (*Paspalum vaginatum* Swartz), zoysia (*Zoysia* sp.) bahia, and centipede are used in landscapes throughout Florida. St. Augustinegrass is dominant residential species in Florida (Trenholm, 2004). Haydu et al., (2002) estimated that 36% of the total lawn acreage in Florida, or 1.5 million acres, was comprised of St. Augustinegrass in 1996. Valued for its shade tolerance, ability to adapt to various soils, and color, St. Augustinegrass cultivars such as 'Floratam', 'bitterblue', 'Raleigh', and 'Floratine' became popular with home owners. Chinch bug resistant 'Floratam' quickly became the number one cultivar upon its release in the 1970's. St. Augustinegrass is a desirable species home lawn, however problems with disease susceptibility can be devastating. Two examples are brown patch (*Rhizoctonia solani* Kühn) and take-all root rot (*Gaeumannomyces graminis* (Sacc.) Arx & D. Olivier var. *graminis*).

To date, research evaluating the potential benefit of mycorrhizae in St. Augustinegrass has been neglected such as reduced fertilizer use and production cost. The method of production of St. Augustinegrass may result in limited benefits of mycorrhizal research. St. Augustinegrass is produced vegetatively as sod throughout the southeast. Once or twice a year, the sod is harvested leaving "ribbons" or strips of grass behind. These ribbons are responsible for re-growth, through stolons, of the sod field. Harvesting cycles would make lengthy mycorrhizal studies difficult. An extensive survey of this plant system in relation to the arbuscular mycorrhizal fungi is warranted.

The overall objective of this research is to investigate the impact of mycorrhizal fungi on warm-season turfgrasses in Florida. A survey of the population and identification of arbuscular mycorrhizal fungi associated with St. Augustinegrass roots in Florida sod is provided in Chapter II. In Chapter III, a survey of root pathogens is explored in relation to arbuscular mycorrhizal colonization in sod production fields. Chapter IV includes studies designed to determine whether or not arbuscular mycorrhizal fungi affect root disease caused by pathogenic isolates of *R. solani* and *G. graminis* var. *graminis*, and if potential affects are direct fungal interactions or indirect systemically acquired mechanisms of resistance. In Chapter V, a general summary and conclusions concerning arbuscular mycorrhizal fungi in St. Augustinegrass in Florida are provided.

CHAPTER 2
POPULATION AND IDENTIFICATION OF ARBUSCULAR MYCORRHIZAL FUNGI IN ST. AUGUSTINEGRASS

There is no information regarding arbuscular mycorrhizal fungi (AMF) in the popular warm-season St. Augustinegrass (*Stenotaphrum secundatum*). In Florida, St. Augustinegrass sod is a valuable commodity in home lawns and commercial landscapes. 'Floratam' the most common and widely adaptable cultivar is extensively used across the state. It is also the primary cultivar grown in Florida for sod. In north central Florida, sod production is increasing and growers are eager to increase production and lower pesticide and fertilizer inputs. No information exists about mycorrhizas in this species. The information is potentially useful in sod management to reduce disease severity, chemical usage, and other production costs. In most cases, AMF populations are decreased by agricultural practices are associated with conventional farming. St. Augustinegrass sod production is unique in that it is not a traditional or natural plant system. Currently, no information is available to growers to make informed decision about inoculation with these fungi. The feasibility of inoculation studies for nutrient acquisition, pesticide, and disease management can be performed using mycorrhizal fungi more efficiently in the future once St. Augustinegrass is determined to be mycorrhizal.

Of current interest to mycorrhizal researchers is the ecology of mycorrhizal populations and their benefit to both organic and more conventional cropping systems. Information from less natural and conventional systems like St. Augustinegrass

sod is timely and could shed light on a little known ty cropping method. Mycorrhizal systems and those interactions within it are complex and require extensive evaluation, especially in crops not yet known to possess such associations. This evaluation may supply valuable answers about mycorrhizal ecology. The objective of this study is to determine if AMF colonize St. Augustinegrass, to what extent, and to identify the colonizing fungi.

Materials and Methods

Sampling.|| 'Floratam' St. Augustinegrass plant roots and associated soil were collected monthly from three sod farms in three counties (Marion, Bradford, and Union) in north central Florida from December 2004 through December 2005 with the exception of July. Each of the sod farms had been cropped with 'Floratam' St. Augustinegrass for 12 years or more (Fig. 2-1 A-C).

Ten subsamples of soil were taken from three (3 m^2) plots per sod farm with a 1.27 cm diameter soil probe to a depth of approximately 15 cm as suggested by Brundrett et al. (1995). Root samples from each plot were extracted with a small hand trowel. Subsamples of roots and soil from each plot were pooled, resulting in three separate composite plot samples per location. Root samples were placed into plastic ziplock bags separate from soil samples and stored at room temperature for approximately 1 d prior to spore extraction and root manipulation for mycorrhizal evaluation. Approximately 200 g of field soil from each plot were combined with 200 g of a low P, low organic matter soil mined from the UF/IFAS Plant Science Research and Education Unit in Citra, Florida. This soil was then potted into 10 cm clay pots sown with sorghum-sudangrass hybrid seed (*Sorghum bicolor* [L.] Moench x *Sorghum sudanense*) cv. Summergrazer III. Low P

soil was used in pot cultures to enhance sporulation of potentially cryptic species in order to facilitate their recovery and identification (Fig. 2-2).

The cultures were incubated for 60 d at 20-25 C with 12 h artificial light (day/night). The seed was surface-sterilized using a 10% sodium hypochlorite and deionized water solution for 30 sec and rinsed for 1 min with sterile deionized water prior to planting. The pot cultures received a Peter's 20-0-20 (Spectrum Group, St. Louis, MO) nutrient solution, devoid of P, every two weeks. Approximately 90 d later, single spores from the field soil pot cultures were selected from spore extracts (Fig. 2-3). This process was accomplished by wet sieving, decanting (Gerdemann and Nicolson, 1963), and 40% sucrose (v/v) centrifugation (Jenkins, 1964). These spores were used to inoculate sterile, low P soil (Citra, Florida) and sorghum-sudangrass hybrid seed for spore production and subsequent identification of the sporulating AMF as suggested by Gerdemann and Trappe (1974). The soil was sterilized twice for 90 min at 121 C at 15 psi for two consecutive days. Samples of field soil were also submitted to the IFAS Extension Soil Testing Laboratory in Gainesville, Florida on a tri-monthly basis for soil nutrient composition and pH testing. Soil pH, from all three fields, ranged from 5.6 to 7.0 during the 12 month sampling period. Phosphorous levels ranged from 5 to 119 ppm.

Root preparation. || Young, healthy-appearing fibrous roots were rinsed in tap water and separated with a scalpel from the plant crown and/or seminal roots. Selected roots were cut into 1-2 cm long segments and cleared of cell and wall components in 10% KOH (w/v) under pressure in an autoclave for approximately 20 min (Brundrett et al., 1996). The root segments were cooled, then rinsed in tap water, and placed into hot 0.05% trypan blue with glycerol overnight to stain mycorrhizal structures (Bevege, 1968;

Phillips and Hayman, 1970; Kormanik and McGraw, 1982). Excess stain was rinsed from the root segments with tap water and then mounted in water on glass slides to view vesicles and arbuscules. Slight pressure applied to the cover slip, with occasional heating over an alcohol burner, aided in flattening the root segments adequately for microscopic evaluation of mycorrhizal structures in root cells.

One hundred root segments were evaluated per sample for intensity of colonization and to identify any variations in arbuscular morphology which might exist. Mycorrhizal structures on glass slides were viewed with a Nikon Optiphot compound microscope at 200, 400, and 1000x magnifications, and photographs were taken with a Nikon CoolPix 990 digital camera. In order to judge the amount of mycorrhizal root colonization, the grid line intersect method was used to estimate the total root length colonized by AMF (Newman, 1966; Tennant, 1975; Giovannetti and Mosse, 1980).

Spore extractions. | | Mycorrhizal spores were extracted by wet sieving and decanting by mixing 100 g of air-dried sample soil with 300 ml of tap water, blending at low speed in a commercial Waring blender for 1 min, and then allowed to settle for 1 min. The supernatant was then passed through a series of Tyler 250, 125, and 38 µm mesh sieves (Daniels and Skipper, 1982). The remaining fraction was rinsed with tap water to remove sediment and any organic materials left behind. The fraction was decanted into 50 ml centrifuge tubes containing a 40% sucrose/deionized water solution (w/v) (Jenkins, 1964). The tubes were centrifuged for 3 min at 2,000 rpm in a Dynac III centrifuge. The supernatant, containing the spores, was decanted off the top of the tube into a 38 µm mesh sieve and rinsed to remove the sucrose. The extracted spores were collected in a 9 cm Petri dish with tap water rinse and viewed with a Zeiss dissecting scope.

Mycorrhizal spore densities were enumerated by using an ocular field method described in the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi for high spore densities (Morton, 2005).

Intact and parasite free spores were selected using a Gilson 20 µl pipetman. These spores were used to inoculate 10 cm diameter clay pots containing the low P, sterile soil (as described above) and planted with surface-sterilized sorghum-sudangrass hybrid seed. The monocultures were kept at 20-25 C for approximately 60 d. At that point, any spores that had been produced as a result of the inoculations were extracted as previously mentioned, and used to inoculate another crop of sorghum-sudangrass in sterilized, low P soil. The second generation of monocultures were then maintained for 60-90 d and processed for spore extraction and mycorrhizal identification.

Arbuscular mycorrhizal fungi identification.|| Identification of the mycorrhizal fungi associated with St. Augustinegrass was accomplished by selecting healthy, single spores with a 20 µl Pipetman and mounting in either sterile, deionized water or (1:1 v/v) PVLG (polyvinyl alcohol-lactic acid) + Melzer's reagent (Khalil et al., 1992). The spores were then viewed at 200, 400, and 1000x using a Nikon compound microscope and identified. Using arbuscular mycorrhizal descriptions by Schenck and Pérez (1988), a tentative determination to genus was made based on the average measurement of 20 similar spores per pot. The species was determined based on taxonomic descriptions from the INVAM Species Guide (Schenck and Pérez, 1988). Identifying characteristics of the monocultured spores, such as spore wall number and width, hyphal appendages, the presence or absence of germ shields, approximate overall spore diameter and color in reagents, were used as described by Schenck and Pérez (1988).

Statistical analysis. || Spore density and percent colonization data were analyzed using the General Linear Model procedure (SAS Institute, Version 9.0, 2004) (Appendix F-1). The survey was performed using a random model in a randomized complete block design with multiple samplings at multiple locations. The percent root colonization data were transformed with the arcsine square root transformation prior to an analysis of variance due to distribution of propagules within soil being highly variable resulting in a non-normal frequency of distribution points (St. John and Hunt, 1983; Friese and Koske, 1991). Spore density data were transformed to their natural log prior to analysis of variance to prevent violation of the assumption of normal distribution. Significant interactions were separated using Tukey's Studentized Range Distribution test. Correlations between percent colonization or spore density data, with soil nutrient composition, and percent colonization to spore density were done in SAS using Pearson product-moment correlation coefficients. Regression analyses also were performed with the regression procedure in SAS.

Results

Root Evaluation. || Roots, collected from sod fields evaluated in this survey revealed the first evidence of an interaction between AMF and St. Augustinegrass. In stained roots mounted on glass slides, AMF structures such as internal vesicles, intra and extraradical hypha, and an assortment of arbuscular types were observed. Bulbous appressoria (Fig. 2-4) were noted at inoculation points along the length of the root, giving rise to carbohydrate storage vesicles of various shapes within cortical root cells (Figs. 2-5, 2-6). Copious amounts of intra and extraradical hypha were observed within and along the outer surface of root tissue (Fig. 2-7). Most notably, a variety of arbuscular types were observed within the cortical root cells. Arbuscules, or haustoria-like structures,

have been categorized into two morphological types (Gallaud, 1904); *Arum*- and *Paris*-types. These intercellular mycorrhizal structures are the presumed active fungal sites of nutrient translocation between host and fungus (Bracker and Littlefield, 1973; Brundett et al., 1984).

In this study, field grown plant roots were found to contain both the *Arum*- and *Paris*- type of arbuscules along with a variety of intermediate *Arum*- morphologies. Intermediate forms of the *Arum*- type found in cortical root cells of St. Augustinegrass sod plants ranged from a typical "feathery" form (Fig. 2-8) extending from intracellular hypha to a "dense-compact" form between cells of conjoined intercellular hyphae (Fig. 2-9). A "grainy" form (Fig. 2-10) was also found in cortical root cells on several occasions. This could be a collapsing arbuscule instead of an intermediate arbuscular form. The *Paris*-type arbuscule found in St. Augustinegrass plant roots shows a typical arbusculate coil (Fig. 2-11) in the root cell, while intermediate forms were not observed. An unusual structure was found along intercellular hyphae that resembled a hyphal mat with a mantle-like appearance often found in conjunction with certain types of ectomycorrhizas (Fig. 2-12). This may be a new arbuscular form found in the *Poaceae*. This structure was only observed once in St. Augustinegrass plants harvested in April 2005 at the Fort McCoy location.

Spore density evaluation. | | Further evidence supporting an interaction between AMF and St. Augustinegrass was observed outside the root within the rhizosphere. AMF spores clinging to epidermal tissue on roots were frequently observed in field samples and in pot cultures using field soil from each farm location and sorghum-sudangrass as the trap plant. The three sod farms sampled in this survey have been cropped solely in

'Floratam' St. Augustinegrass sod for more than 12 years. Weeds are heavily controlled with herbicides at each location. The AMF spores recovered from field soil are entirely dependent upon the St. Augustinegrass plants because they are obligate heterotrophs. The limited availability of other plant species at each location, and the availability of numerous spore types for pot culturing and subsequent AMF identification, provides adequate evidence of AMF colonizing St. Augustinegrass plants in North Central Florida soils.

Additional mycorrhizal structures such as auxiliary cells were frequently observed in slide mounts of spores from both pot cultures and field soil (Fig. 2-13). Selected single spores that appeared non-parasitized and viable, were chosen under light microscopy for culturing in sterile, low P soil in order to obtain consistent spore structures compatible with identification procedures. Spores, retrieved from pot cultures were used as sieved soil sub-cultures to produce another generation of spores capable of being readily identified from their morphological structures according to Schenck and Pérez (1988). Table 2-1 lists the species of AMF positively identified from sub-cultures of soil from each location over a year-long period.

Species of *Glomus* were the most commonly encountered AMF in north central Florida soils at each location. At the Lake Butler location, *Glomus* species included: *G. etunicatum* Becker & Gerdemann (Fig. 2-14), *G. intraradices* Schenck & Smith (Fig. 2-15, 2-16), *G. reticulatum* Bhattacharjee & Mukerji (Fig. 2-17, 2-18), and *G. aggregatum* Schenck & Smith (Fig. 2-19). *Glomus* species isolated at the Fort McCoy location included: *G. ambisporum* Smith & Schenck (Fig. 2-20), *G. formosanum* Wu & Chen

(Fig. 2-21), *G. macrocarpum* Tulasne & Tulasne (Fig. 2-22), *G. gerdemannii* Rose, Daniels & Trappe (Fig. 2-23), *G. intraradices*, and *G. etunicatum*. *Acaulospora spinosa* Walker & Trappe (Fig. 2-24) and an unidentified species of *Scutellospora* were isolated at Lake Butler. Additional AMF genera were found at Fort McCoy including: *Entrophospora infrequens* [Hall] Ames & Schneider (Fig. 2-25), *A. denticulata* Sieverding & Toro (Fig. 2-26), *A. lacunosa* Morton (Fig. 2-27), and *Scutellospora minuta* [Ferr. & Herr.] Walker & Sanders (Fig. 2-28). The Starke location was unusual in species diversity with only 3 species isolated: *Glomus etunicatum*, *G. intraradices*, and *Scutellospora minuta*. One unique spore type was found at the Fort McCoy location, but could not be grown in a pot culture successfully. The unidentified spore type was observed on two occasions during the late spring of 2005 in very small numbers and appeared to be either a species of *Acaulospora* or *Entrophospora* based on morphology. Without a sufficient number of cultivated spores for microscopic evaluation, positive identification of the species was not possible.

Sieving field soil from each location not only yielded spores for pot culturing, but also enabled a numerical count of spore density, which is a good indicator of the infectivity of the AMF in the soil and their level of activity in the rhizosphere. The total spore density at the three locations ranged from 78 to 2,132 spores per 100 g of dry soil (non-transformed data). Spore density but did not vary among or within sod farm locations ($P < 0.0001$), indicating that variations in soil factors did not significantly affect AMF spore production between locations from December 2004 through December 2005 (Table 2-2). Spore production did vary significantly ($P < 0.0001$) between monthly sampling, which suggested a possible seasonal influence on spore production. Greater

spore density totals occurred in soils collected during the warmer summer and fall months, as compared to, lowered spore production occurring in the cooler months of winter and spring. Total spore density in December 2004 was significantly lower when compared to December 2005. This might be explained by increased rainfall, prior to the sampling period, in north central Florida during the 2004 hurricane season.

With spore densities varying between dates, analysis of variance for these points showed a significant date by location interaction ($P < 0.05$) indicating that seasonal effects and unknown variations in site-related effects might measurably influence the total spore density. In this survey, rainfall and soil moisture where positively correlated to spore density (Table 2-3).

Based on the regression equations, a quadratic response was generated in total spore density to soil moisture at each location. Spore density at the Starke location increased at soil moisture levels between 0 and 2 cm, but declined until soil moisture levels reached 6 cm where another increase was observed (Fig. 2-29). Above 9 cm a decrease in spore density occurred ($r=0.73$). The same general response to soil moisture was noted at the Fort McCoy location except where soil moisture declined to approximately 8 cm ($r=0.61$) (Fig. 2-30). At the Lake Butler location, spore density increased slightly until soil moisture levels reached 7 to 8 cm when a slight decline in spore density was observed ($r=0.68$) (Fig. 2-31). This lends credibility to the theory that excessive rainfall during the hurricane season of 2004 lowered spore production in December of that year.

A quadratic response was also produced in total spore density to temperature at each location. Spore density at the Starke location ($r= 0.60$) (Fig. 2-32) decreased from

15 C until the temperature reached 20 C. Between 20 C and 28-29 C a gradual increase in spore density was observed until the temperature reached 30 C. At that point there was another gradual decrease in spore density, which seemed to level off near 35 C. At the Fort McCoy location (r=0.84) a gradual increase in spore density was observed until the temperature was approximately 28-29 C, then a decline was noted (Fig. 2-33). At the Lake Butler location (r=0.59) a slight increase in spore density occurred across all temperature ranges (Fig. 2-34). Based on these data, it appears that soil temperatures above 28-30 C have a detrimental effect on the AMF. In addition, this temperature range might also damage host root tissue.

Percent colonization evaluation. || Percent root length colonized by AMF yielded no significant difference among or within location differences, but there was a significant date interaction ($P < 0.0001$). Colonization was generally highest in the cooler months of winter and spring, with lower colonization occurring in the warmer summer and fall months except in December 2005, when colonization was the lowest. The amount of root length colonized ranged from 13 to 39% across the sampling dates (non-transformed data). No correlation was found between temperature and soil moisture in relation to percent root length colonized (Table 2-4).

Discussion

Dickson (2004) suggested an *Arum-Paris* continuum of mycorrhizal symbioses in a survey of 12 colonized plant families, with arbuscule formation dependent on the fungus as well as the host plant. Most mycorrhizal angiosperms were once thought to only produce the *Arum*-type of arbuscule, which consists of both intercellular hyphae and arbuscules, while most angiosperms and bryophytes were thought to only produce the *Paris*-type with intercellular hyphae and arbuscular coils (Dickson, 2004). The majority

of scientific research has been conducted on flowering plants versus trees and bryophytes causing these fallacies to be argued as fact until Smith and Smith (1997) produced a comprehensive list of plant families that included their arbuscule types. The list showed that the *Paris*-type is in fact most common among all plant families and that, "intermediate" or transitional arbuscular morphotypes were observed in some plant species. One genus (*Ranunculus*) forms both types within the same plant (Smith and Smith, 1997).

Experiments on maize (*Zea mays*) and the tuliptree (*Liriodendron tulipfera*), among many others, revealed that AMF can form either type of arbusculate structure based on the host plant (Barrett, 1958; Gerdemann, 1965). In a field experiment using tomatoes (*Lycopersicon esculentum*) and other annual crops, investigators found that arbuscule morphology is actually dependent on intercellular spaces in cortical root cells (Brundrett and Kendrick, 1988; Cavagnaro et al., 2001). Intermediate forms of the *Arum* and *Paris*-type arbuscules are common in certain plant families such as those described in three cultivars of flax (*Linum usitatissimum*), which Dickson et al. (2003) referred to as arbuscules "in pairs in adjacent longitudinally arranged cortical cells arising from a single, radial intercellular hyphae."

On rare occasions, both arbuscule types (*Arum* and *Paris*) occur in the same plant species, which Smith and Smith (1997) noted in the family *Poaceae*. The *Paris*- and *Arum*- types were found in millet, ryegrass, and wheat. In addition, a series of intermediate forms between the two main types of arbuscules were also observed. The same can be said for St. Augustinegrass plants in relation to AMF colonization. In field studies, environmental effects may interact to influence fungal and plant response to the

mycorrhizal interaction. Sylvia et al. (1993) suggested that even in the presence of high amounts of soil P, water stress and pesticide applications can have extensive effects on mycorrhizal response. Rabatin (1979) noted that soil moisture may have the greatest effect on the degree of infection of *Glomus* species in field situations. Furthermore, the stages of plant development (Saif and Khan, 1975) as well as temperature (Giovannetti, 1985; Schenck and Kinloch, 1980; Smith & Smith, 1997; Sylvia, 1986) all play a major role in mycorrhizal activity.

In this survey, AM fungi preferred warmer months for spore production and cooler months for colonization of St. Augustinegrass plants. In the north central region of Florida, St. Augustinegrass does not usually go completely dormant in cooler temperatures, and there is usually some plant activity during the winter months especially in the roots where AMF colonization occurs. This increase in colonization during cooler temperatures may be an effort to preserve valuable carbon and energy reserves for future spore production. Subsequent proliferation in the warmer months, while the plant host is most active, would provide more carbohydrates from a symbiotic interaction (Johnson et al., 1997). It is also possible that AMF are actually acting as a parasite in the winter months when colonization is highest while the plant is less active.

During less than optimal winter growing conditions, the St. Augustinegrass plant is less able to defend itself against infection and colonization due to lowered metabolic activity. Johnson et al. (1997) suggested a mycorrhizal continuum ranging from mutualistic to parasitic in some managed habitats where humans unknowingly altered the association through management regimes. Another possibility is environmentally

induced parasitism due to morphological, phenological, and physiological differences in the symbionts which may influence the mycorrhizal association (Johnson et al., 1997).

Conversely, in natural habitats, mycorrhizal associations have evolved over many years to encourage fitness in the plant and the fungus making the interaction continually mutual (Johnson et al., 1997). St. Augustinegrass sod systems are not traditional cropping systems needing continual management inputs from man, nor are they a natural, non-impacted habitat. St. Augustinegrass sod could be referred to as a non-conventional cropping system due to minimal inputs after harvesting where ribbons of grass are left behind for re-growth. Cloned host plants are in constant supply in sod fields providing the AMF with a dependable host, but when the plant is semi-dormant throughout the winter months the fungi may actually pose a threat to the health of the plant because net costs in carbon might then exceed net benefits in some situations. For example, during instances of lowered metabolic activity in the winter, plants lower photosynthetic ability and subsequent output and will not benefit from the added benefits of a mutual interaction. Acquisition of nutrients and water is less important during these times, but St. Augustinegrass may be harmed by the loss of stored carbon to AMF. Throughout the year, there are potential times when the interaction between plant and AMF is such that the symbiosis might actually be neutral in nature (Johnson et al., 1997).

An attempt was made in this survey to correlate spore density to the percent root length colonized, but no correlation was found. Some researchers have reported a correlation between the two variables (Giovannetti, 1985; Miller et al., 1979) while others have observed no such relationship (Giovannetti and Nicolson, 1983; Hayman and Stovold, 1979). This is most likely due to the vast variations observed in soils, plant

species and their developmental stage, and fungal specificity. Many mycorrhizal studies suggest a significant interaction with soil P where spore production or colonization is lowered by increasing levels of P. Correlations between soil chemical characteristics such as P content to spore density and percent root colonization have been reported in grasses (Brejda et al., 1993). Others suggest that mycorrhizal ecology plays less of a role. P content in south Florida soils had no effect on AMF in tropical forage legume pastures (Medina-Gonzalez et al., 1988), nor did potassium or pH in studies of cultivated soils (Abbott and Robson, 1977; Hayman, 1978).

In this survey, soil samples from each location were evaluated during the months of January, April, August, and November 2005 in an attempt to correlate soil Mg, Ca, K, P, soil pH, and organic matter percentage to spore density and/or percent root length colonized, but a correlation was not observed (Table 5). One theory to explain the lack of correlation between AMF and P content, in this case, might be explained by asexual organisms, without the cost of sexual reproduction and consequently no genetic variability, and having scores of mutations that accumulate over a long period of time (Helgason and Fitter, 2005). The *Glomeromycota* possess ancient asexual lineages (Gandolfi et al., 2003). This apparent genetic isolation would presumably cause mutations to allow for some adaptations such as P tolerance. In AMF the coenocytic mycelium is multinucleate providing a set of mutations within the DNA of all nuclei (Helgason and Fitter, 2005). Reductions in fitness due to a lack of genetic variability due to asexual reproduction may never be noticed in AMF because mutated, non-functional genes from one nuclear lineage might be subjugated by functional alleles on another nucleus (Helgason and Fitter, 2005).

Arbuscular mycorrhizal fungi in these sod fields are secluded, thus reducing genetic variability, so it is possible that the ancient fungi are capable of evolving and adapting through mutations to tolerate large amounts of added nutrients like P. P is widely used in large amounts in St. Augustinegrass to promote root growth and health for winter survival and spring green-up. Through years of isolation in sod fields and large applications of P on a frequent basis, these fungi might have evolved a mechanism through spontaneous mutation to tolerate elevated P levels. This is speculation, but the lack of spore density and percent colonization variable correlation to P levels could be due to genetic mutation in the fungi within these fields leading to a significant adaptation and evolutionary event.

Overall root colonization and spore density were low to moderate, which suggests that the AMF populating St. Augustinegrass sod production soils are moderately active. This situation might lend itself to field inoculation where AMF could potentially provide a level of root disease protection, which might lower pesticide use and cost. It could also lead to increased and more efficient P acquisition and use when combined with more conducive management strategies. On the other hand, inoculation with AMF might be ineffective in situations where genetic isolation combined with perennial cropping and moderate to heavy fertilizer inputs are unavoidable for proper management.

Figure 2-1 A-C. ‘Floratam’ St. Augustinegrass sod farms located at (A) Fort McCoy (Marion County), (B) Lake Butler (Union County), and (C) Starke (Bradford County) in north central Florida.

Fig. 2-2. Sorghum-sudangrass pot cultures containing 50% (w/w) field soil combined with 50% sterile, low P soil.

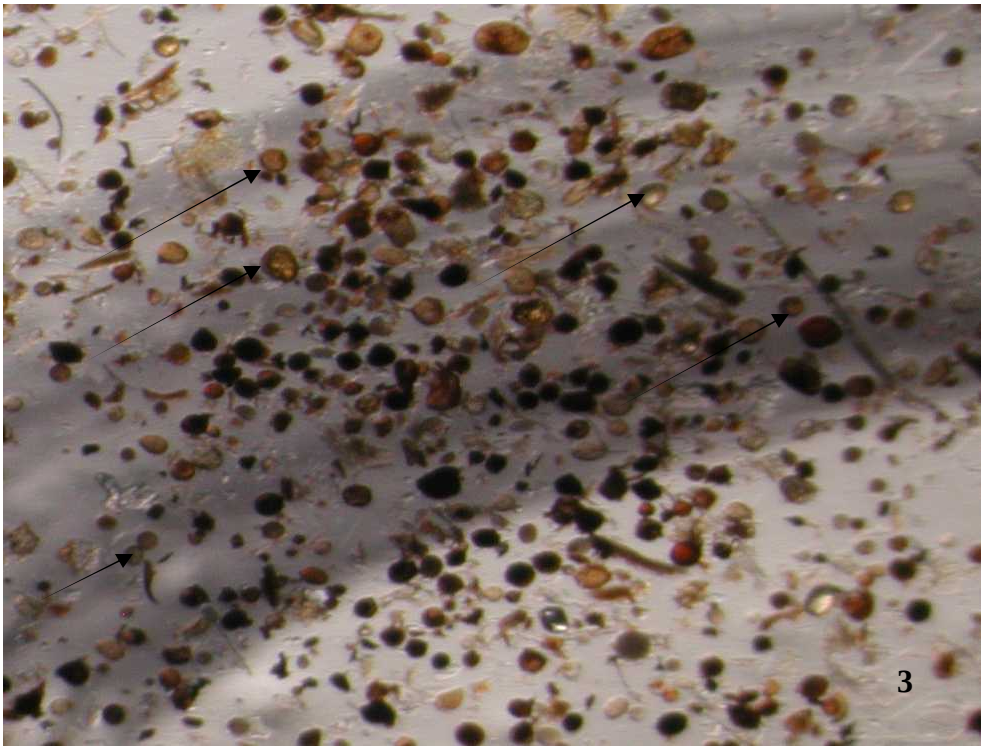
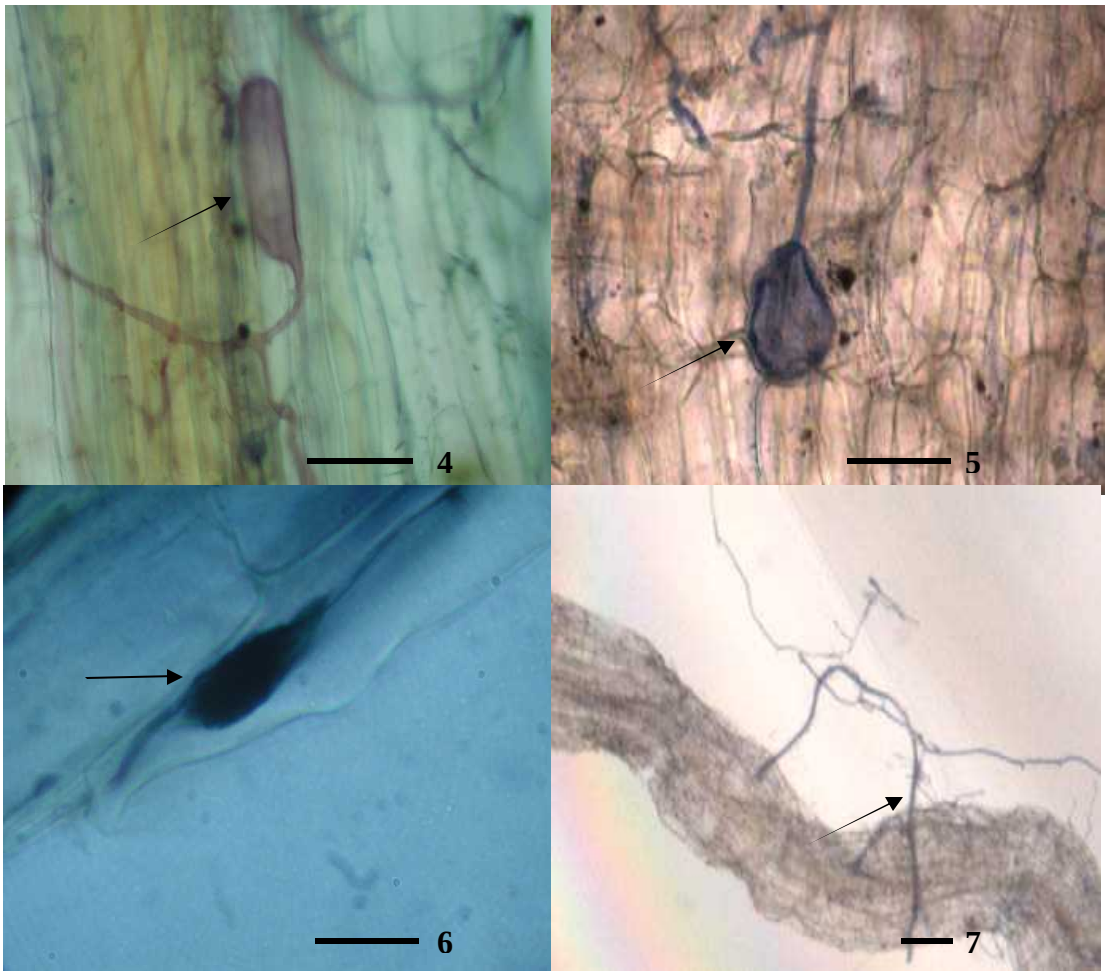
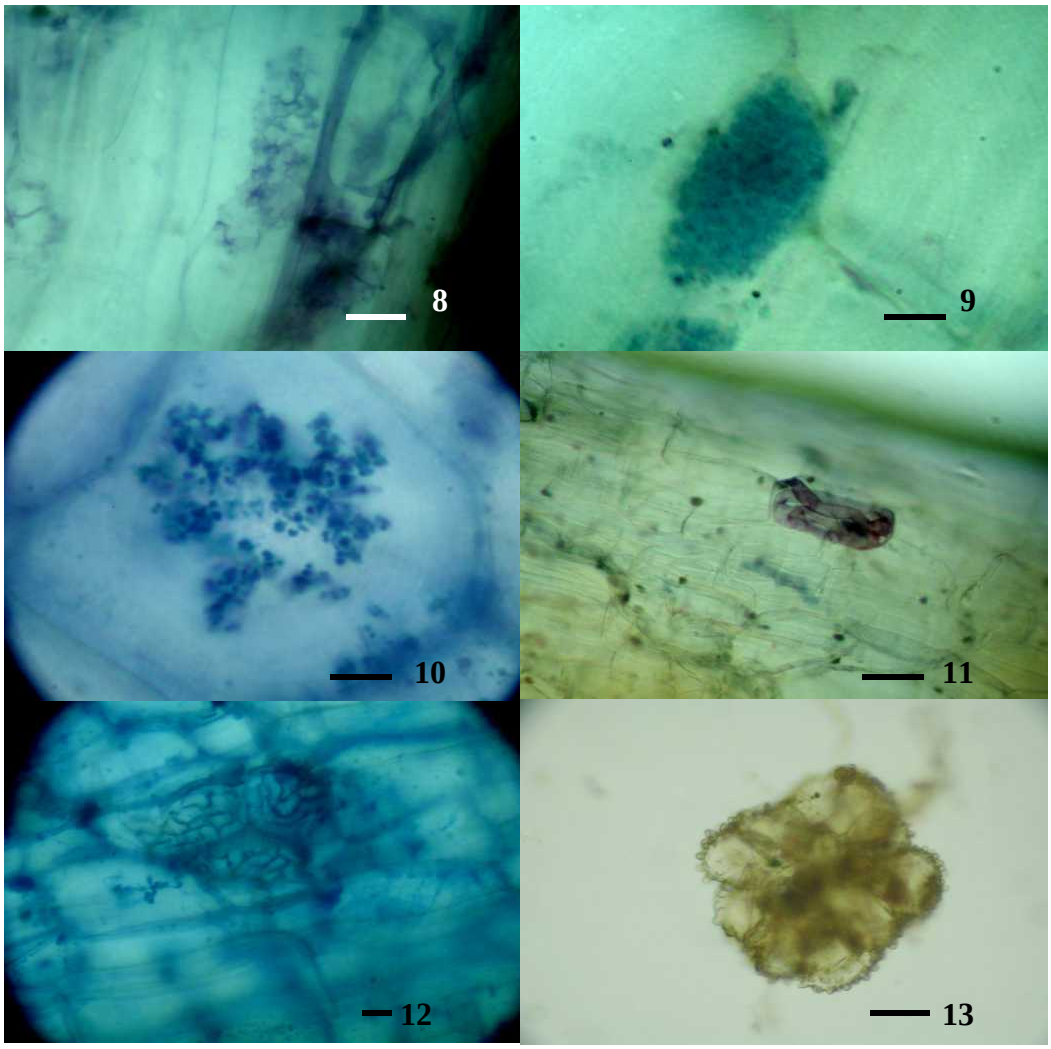


Fig. 2-3. Spore extract from field soil following the wet sieving procedure.



Figs. 2-4 – 2-7. Stained arbuscular mycorrhizal structures observed within 'Floratam' St. Augustinegrass.

Fig. 2-4. Bulbous appressoria found originating from extraradical hypha. Bar = 40 µm.
Fig. 2-5. Circular type of AMF vesicle stained with trypan blue. Bar = 40 µm.
Fig. 2-6. Oblong type of AMF vesicle stained with trypan blue. Bar = 40 µm.
Fig. 2-7. Extraradical hyphae observed with light microscopy infecting and colonizing roots. Bar = 20 µm.



Figs. 2-8 – 2-11. Stained arbuscular morphology types found within 'Floratam' St. Augustinegrass.

Fig. 2-8. Feathery form of the *Arum*-type arbuscule morphology, stained with trypan blue, within cortical root cells. Bar = 40 µm.

Fig. 2-9. Dense and compacted *Arum*-type arbuscule morphology stained with trypan blue. Bar = 40 µm.

Fig. 2-10. Grainy or collapsing *Arum*-type arbuscule morphology stained with trypan blue. Bar = 40 µm.

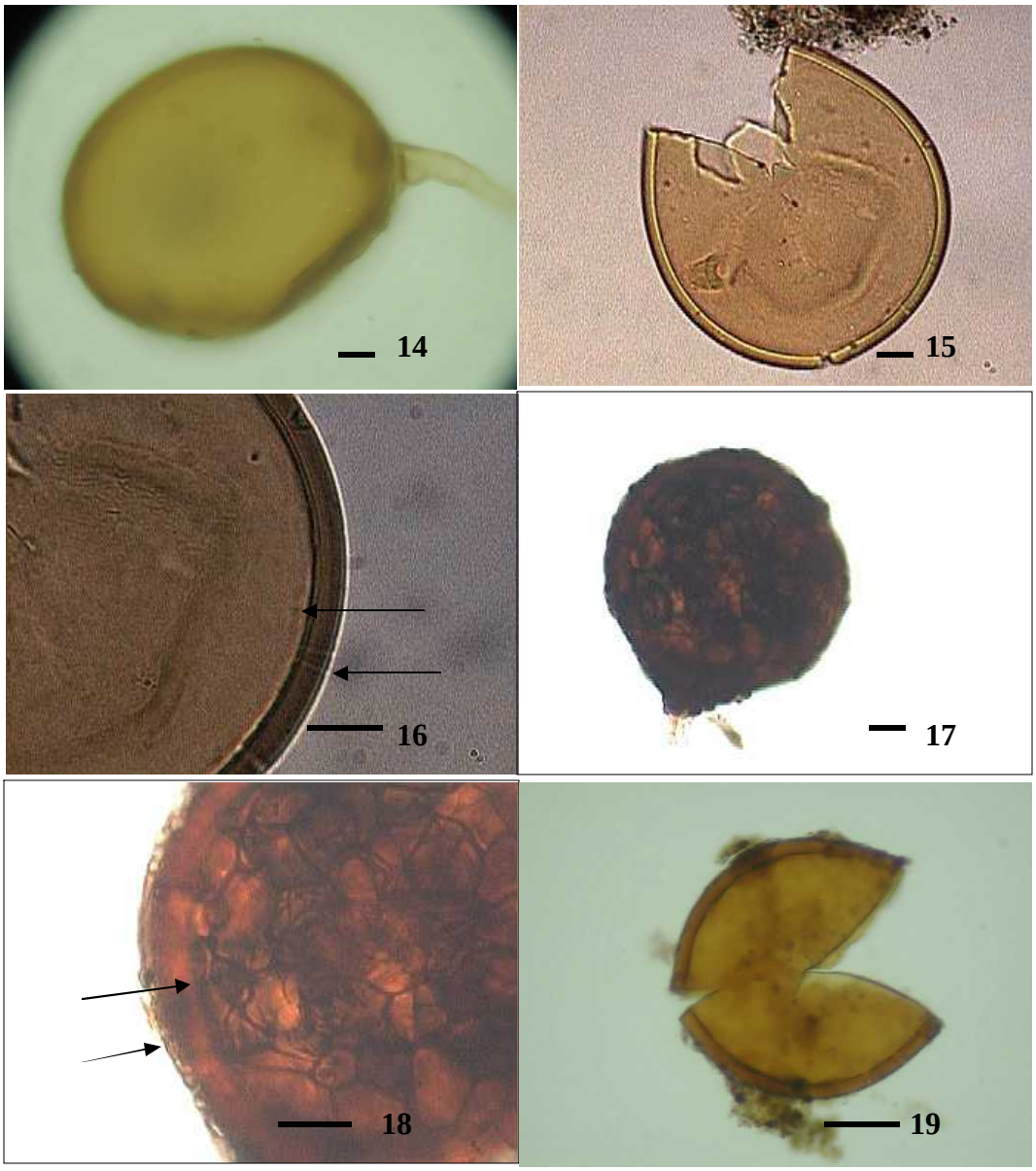
Fig. 2-11. *Paris*-type coiled arbuscule, stained with trypan blue, within cortical root cells. Bar = 40 µm.

Fig. 2-12. Net-like AMF structure observed in roots across adjacent cortical root cells. Bar = 20 µm.

Fig. 2-13. Auxiliary cells of an AMF observed in spore extracts from field soil. Bar = 40 µm.

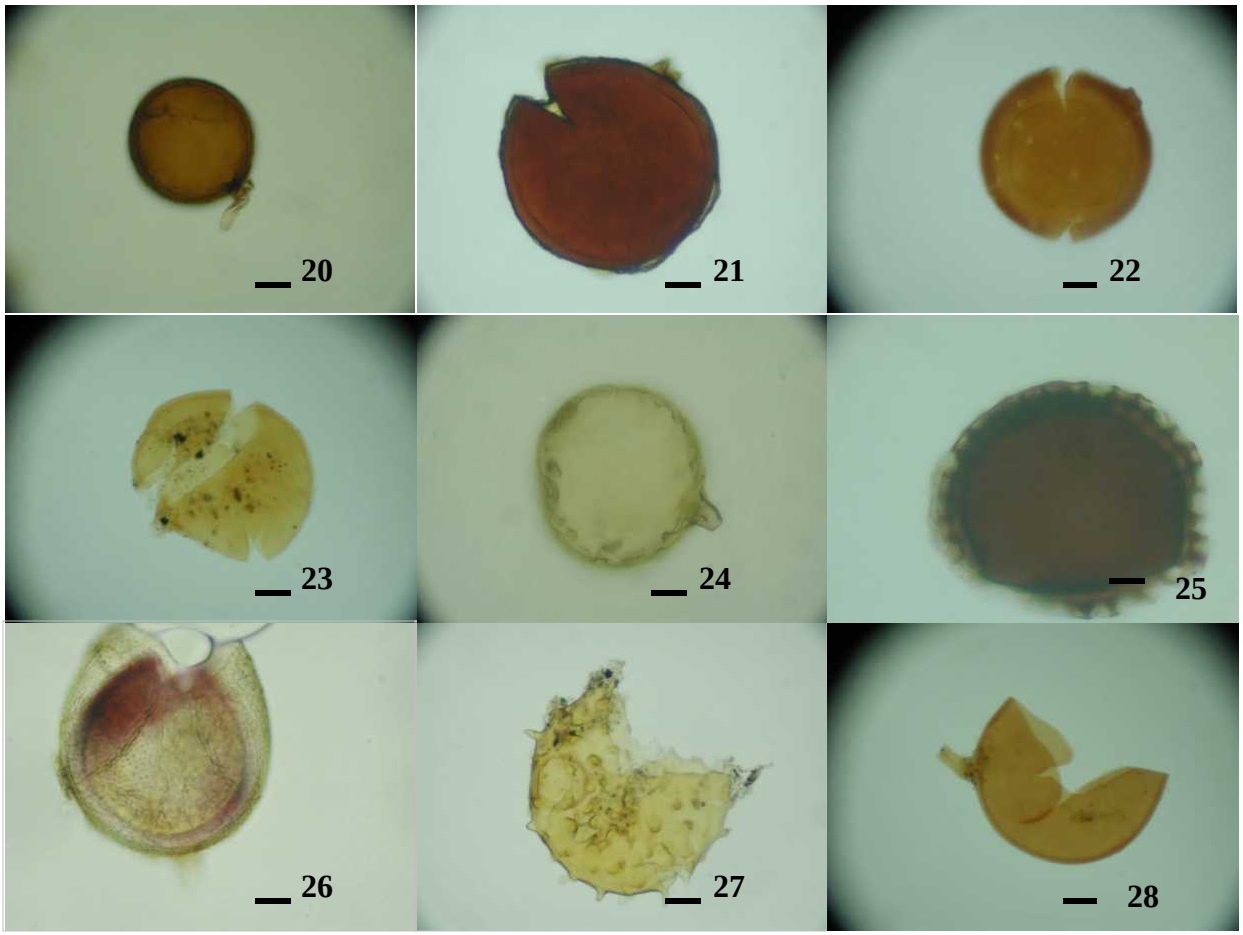
Table 2-1. Species of AMF positively identified at each sod farm location from pot cultures of sorghum-sudangrass within a combination of field and sterile, low P soil.

Location	AMF Species
Lake Butler	*Acaulospora spinosa*
Lake Butler	*Glomus etunicatum*
Lake Butler	*G. intraradices*
Lake Butler	*G. reticulatum*
Lake Butler	*G. aggregatum*
Lake Butler	*Scutellospora* sp.
Fort McCoy	*A. denticulata*
Fort McCoy	*A. lacunose*
Fort McCoy	*Entrophospora infrequens*
Fort McCoy	*G. ambisporum*
Fort McCoy	*G. etunicatum*
Fort McCoy	*G. formosanum*
Fort McCoy	*G. gerdemanii*
Fort McCoy	*G. intraradices*
Fort McCoy	*G. macrocarpum*
Fort McCoy	*Scutellospora minuta*
Starke	*G. etunicatum*
Starke	*G. intraradices*
Starke	*S. minuta*



Figs. 2-14 – 2-19. Arbuscular mycorrhizal fungal spores identified at the Lake Butler sod farm location.

Fig. 2-14. A spore of *Glomus etunicatum* stained in Melzer's reagent. Bar = 20 µm.
Fig. 2-15. A spore of *G. intraradices* in deionized water. Bar = 20 µm.
Fig. 2-16. Spore wall morphology of *G. intraradices* spore stained in Melzer's reagent (arrows point to cell wall layers). Bar = 40 µm.
Fig. 2-17. A spore of *G. reticulatum* in deionized water. Bar = 20 µm.
Fig. 2-18. Spore wall morphology of *G. reticulatum* in deionized water (arrows point to cell wall layers). Bar = 40 µm.
Fig. 2-19. A broken spore of *G. aggregatum* in Melzer's reagent. Bar = 20 µm.



Figs. 2-20 – 2-28. Arbuscular mycorrhizal fungal spores identified at the Fort McCoy sod farm location.

Fig. 2-20. A spore of *Glomus ambisporum* stained in Melzer's reagent. Bar = 20 µm.
Fig. 2-21. A spore of *G. formosanum* stained in Melzer's reagent. Bar = 20 µm..
Fig. 2-22. A spore of *G. macrocarpum* stained in Melzer's reagent. Bar = 20 µm.
Fig. 2-23. A spore of *G. gerdemannii* stained in Melzer's reagent. Bar = 20 µm.
Fig. 2-24. A spore of *Acaulospora spinosa* stained in Melzer's reagent. Bar = 20 µm.
Fig. 2-25. A spore of *Entrophospora infrequens* stained in Melzer's reagent. Bar = 20 µm.
Fig. 2-26. A spore of *A. denticulata* stained in Melzer's reagent. Bar = 20 µm.
Fig. 2-27. A spore of *A. lacunosa* stained in Melzer's reagent. Bar = 20 µm.
Fig. 2-28. A spore of *Scutellospora minuta* stained in Melzer's reagent. Bar = 20 µm.

Table 2-2. Evaluation of analysis of variance data for spore density data from each sod farm location by date.

Date	Location		Total Spore Density (spores/100g air-dried soil)
Dec. '04	Fort McCoy		5.06†
	Lake Butler		4.82
	Starke		5.13
		mean	5.00 d‡
Jan '05	Fort McCoy		5.42
	Lake Butler		5.17
	Starke		5.54
		mean	5.38 cd
Feb '05	Fort McCoy		5.80
	Lake Butler		5.78
	Starke		5.56
		mean	5.71 bcd
March '05	Fort McCoy		5.53
	Lake Butler		6.33
	Starke		5.30
		mean	5.72 bcd
April '05	Fort McCoy		6.48
	Lake Butler		6.84
	Starke		6.32
		mean	6.55 a
May '05	Fort McCoy		6.72
	Lake Butler		6.54
	Starke		6.94
		mean	6.73 a
June '05	Fort McCoy		6.90
	Lake Butler		6.78
	Starke		6.29
		mean	6.66 a
Aug '05	Fort McCoy		5.90
	Lake Butler		7.01
	Starke		5.88
		mean	6.26 ab
Sept '05	Fort McCoy		6.36
	Lake Butler		6.13
	Starke		5.66
		mean	6.05 abc

Oct '05	Fort McCoy		6.65
	Lake Butler		6.08
	Starke		5.70
		mean	6.14 abc
Nov '05	Fort McCoy		6.22
	Lake Butler		6.54
	Starke		6.76
		mean	6.51 a
Dec '05	Fort McCoy		5.81
	Lake Butler		5.80
	Starke		6.36
		mean	5.99 abc

†Each value is the average of three sample plots/location (10 sub-samples/plot).
‡Means followed by the same letter are not significantly different according to Tukey's (HSD) Studentized Range Test (P = 0.0001).

Table 2-3. Pearson correlation coefficients (r) for AMF spore density and soil moisture and temperature.

	Sporeden†	Rainfall†	Soiltemp†
Percolon†	-0.007	-0.14	0.02
Sporeden		0.45***	0.48***
Rainfall			0.61***

*** Significant at P = 0.0001, respectively.
† Percolon = percent root length colonized; Sporeden = spore density; Rainfall = amount of rainfall in month preceding sampling date; Soiltemp = soil temperature for sampling date.

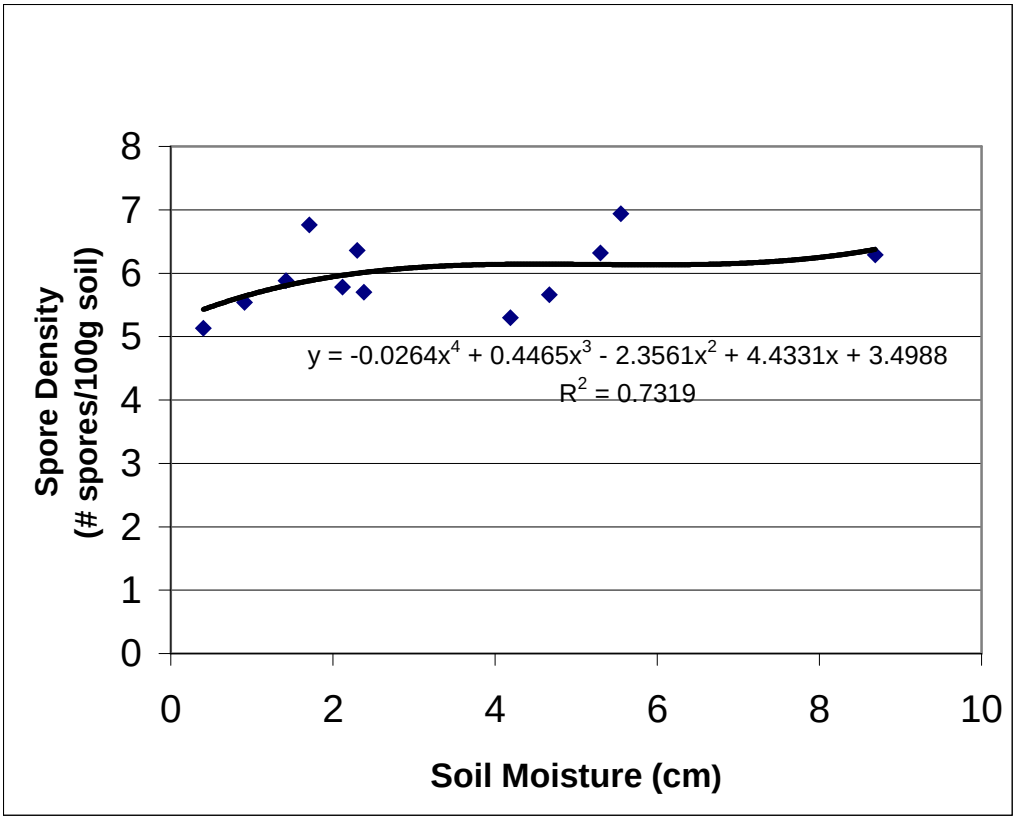


Fig. 2-29. Spore density with increasing soil moisture levels over a 12-month period at the Starke sod farm location.

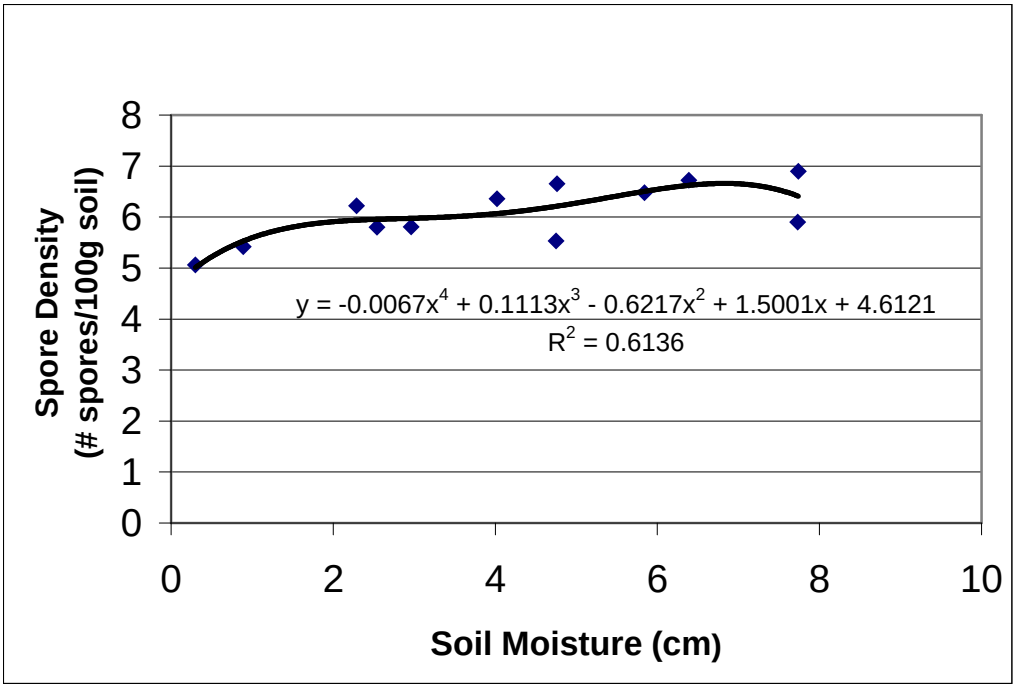


Fig. 2-30. Spore density with increasing soil moisture levels over a 12-month period at the Fort McCoy sod farm location.

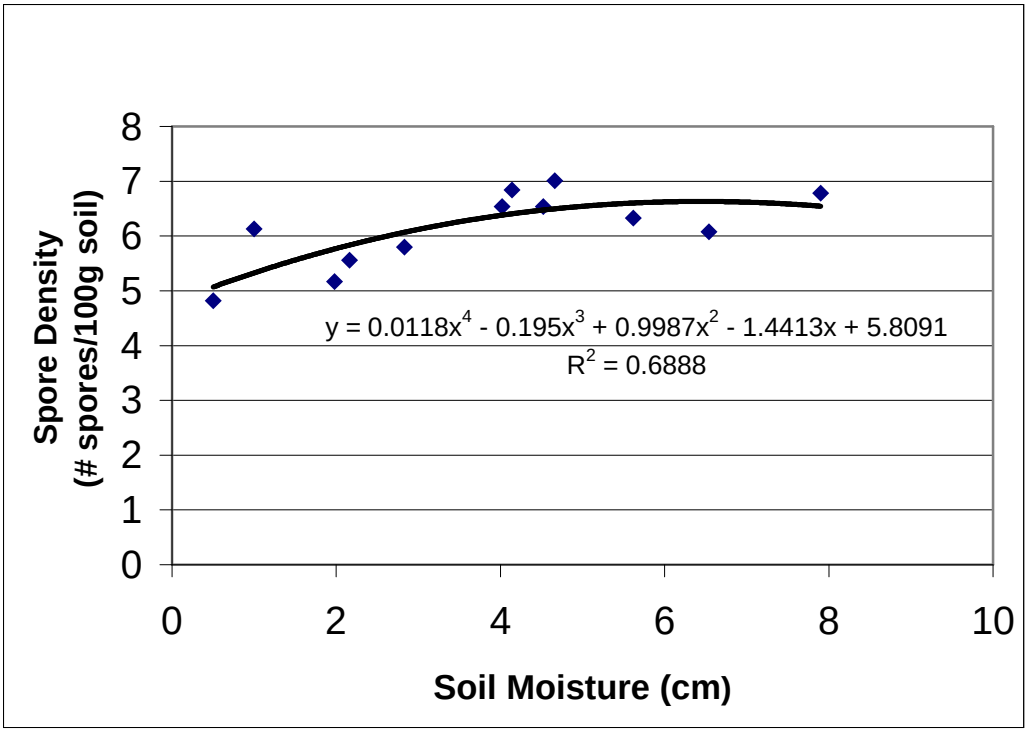


Fig. 2-31. Spore density with increasing soil moisture levels over a 12-month period at the Lake Butler sod farm location.

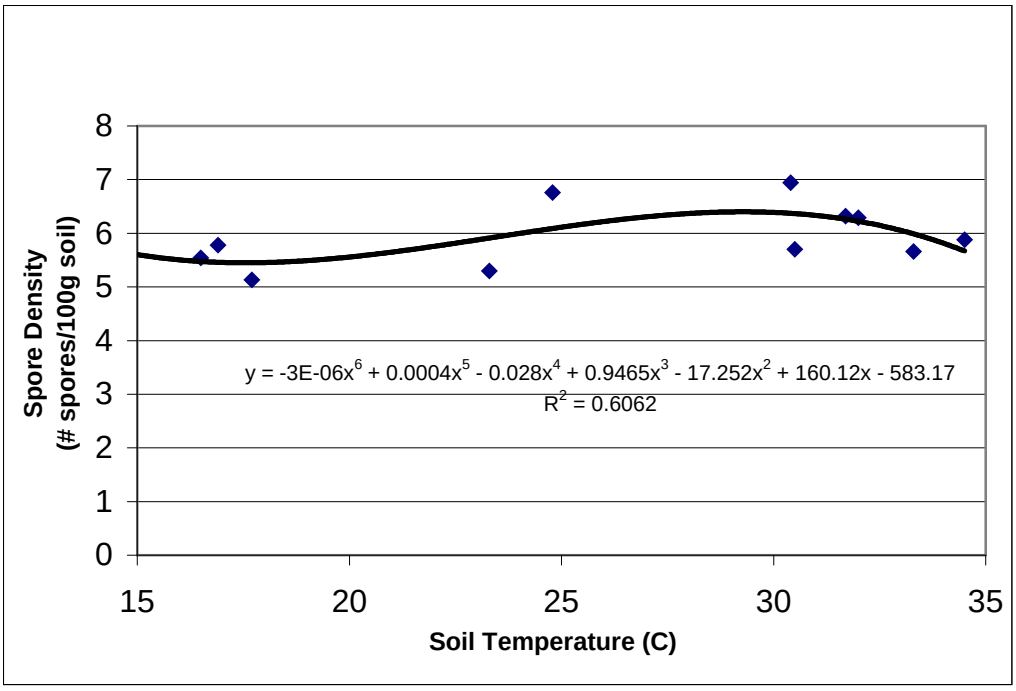


Fig. 2-32. Spore density with increasing soil temperatures over a 12-month period at the Starke sod farm location.

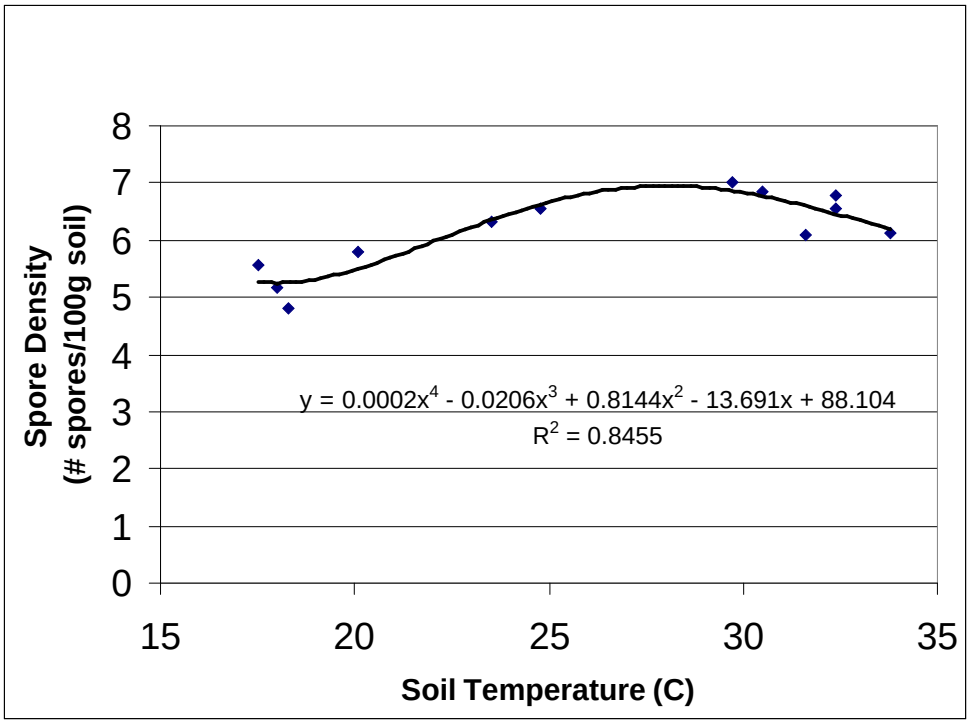


Fig. 2-33. Spore density with increasing soil temperatures over a 12-month period at the Fort McCoy sod farm location.

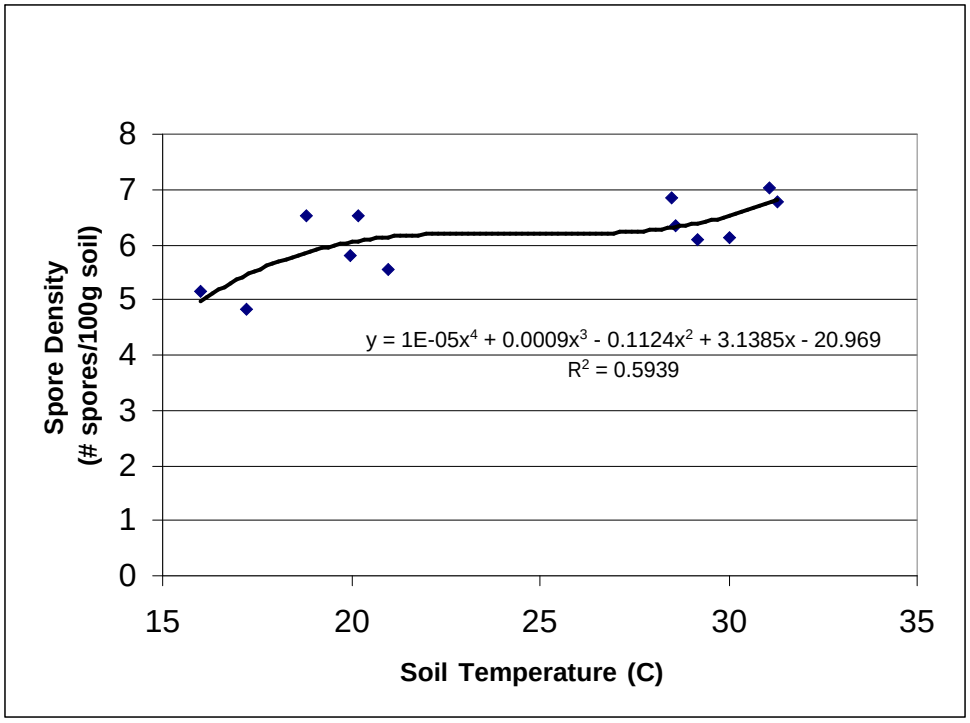


Fig. 2-34. Spore density with increasing soil temperatures over a 12-month period at the Starke sod farm location.

Table 2-4. Evaluation of analysis of variance data for percent root length colonized from each sod farm location.

Date	Location		%Colonization (GIM)
Dec. '04	Fort McCoy		
			27.226†
	Lake Butler		25.47
	Starke		25.69
		mean	26.13 ab‡
Jan '05	Fort McCoy		28.28
	Lake Butler		28.05
	Starke		30.69
		mean	29.01 a
Feb '05	Fort McCoy		24.86
	Lake Butler		29.91
	Starke		31.79
		mean	28.85 a
March '05	Fort McCoy		26.96
	Lake Butler		24.01
	Starke		23.58
		mean	24.84 abc
April '05	Fort McCoy		26.98
	Lake Butler		30.03
	Starke		28.74
		mean	28.58 a
May '05	Fort McCoy		24.62
	Lake Butler		28.35
	Starke		27.21
		mean	26.73 ab
June '05	Fort McCoy		25.25
	Lake Butler		29.54
	Starke		22.97
		mean	25.92 ab
Aug '05	Fort McCoy		23.00
	Lake Butler		22.76
	Starke		22.14
		mean	22.63 bcd
Sept '05	Fort McCoy		23.81
	Lake Butler		18.27
	Starke		22.96
		mean	21.68 bcd
Oct '05	Fort McCoy		21.13
	Lake Butler		18.87
	Starke		18.51
		mean	19.50 cd
Nov '05	Fort McCoy		18.08

	Lake Butler		20.94
	Starke		20.71
		mean	19.91 cd
Dec '05	Fort McCoy		18.87
	Lake Butler		19.90
	Starke		17.29
		mean	18.68 d

†Each value is the average of three sample plots/location (10 sub-samples/plot).
‡Means followed by the same letter are not significantly different according to Tukey's (HSD) Studentized Range Test (P = 0.0001).

Table 2-5. Chemical characteristics of soils sampled for AMF at three north central Florida sod farm locations during January, April, August, and November 2005.

		Soil Nutrient Levels					
Date	Location	P/g soil	Ca	K	Mg	pH†	OM‡
Jan '05	FM*	9	883	13	46	5.5	1.57
Jan '05	LB**	112	455	75	28	5.8	2.31
Jan '05	Starke	38	306	117	38	5.7	2.02
April '05	FM	12	830	16	47	7.0	1.70
April '05	LB	91	418	91	26	5.8	2.53
April '05	Starke	27	359	103	44	5.7	2.01
Aug '05	FM	35	260	37	30	5.9	1.34
Aug '05	LB	88	1065	97	91	6.3	2.97
Aug '05	Starke	56	903	87	87	6.2	2.08
Nov '05	FM	55	392	82	27	5.4	1.99
Nov '05	LB	47	414	91	37	5.7	2.07
Nov '05	Starke	45	370	83	30	5.4	1.93

†Soil pH, nutrient level, and organic matter content based on the mean of three composite samples/location.
‡OM = Organic matter content.
*FM = Fort McCoy location.
**LB = Lake Butler location.

CHAPTER 3
THE EFFECT OF ARBUSCULAR MYCORRHIZAL FUNGI ON GAEUMANNOMYCES GRAMINIS VAR. GRAMINIS AND RHIZOCTONIA SOLANI COLONIZATION OF ST. AUGUSTINEGRASS SOD IN NORTH CENTRAL FLORIDA SOILS

Take-all root rot and brown patch are two of the more common and devastating diseases of St. Augustinegrass sod throughout Florida. Take-all root rot, caused by *Gaeumannomyces graminis* (Sacc.) Arx & D. Olivier var. *graminis*, is a disease of both grasses and cereals (Nilsson, 1969; Huber and McCay-Buis, 1993). Take-all root rot was first described in Sweden in the early 1800's infecting grasses (Mathre, 1992). It is one of several *G. graminis* varieties which infect many important crops worldwide (Rovira and Whitehead, 1983). This particular variety of the fungus infects all cultivars of St. Augustinegrass (Elliott, 1995; Datnoff et al., 1997). In the late 1980's, large, chlorotic patches of St. Augustinegrass were observed on sod farms in South Florida and were confirmed as the first disease symptoms of *G. graminis* var. *graminis* infection observed in this species (Elliott, 1993). The disease was found in St. Augustinegrass throughout Alabama, Florida, and Texas (Fig. 3-1) and it is notably more severe in the summer and fall months, especially during periods of increased precipitation (Elliott, 1993). Early studies suggested that the fungus preferred alkaline or high pH soil, mild winters, thatch-accumulation and frequent light irrigation, however the conditions that predisposed the stand to disease or prompted disease escape are not known (Guyette, 1994).

Management recommendations included elimination of low areas where water accumulates, watering only when needed, and the use of pH decreasing

fertilizers in the fall, as well as thatch prevention and aeration (Guyette, 1994). Fungicides were recommended as preventative but not curative treatments, which limited management options to growers (Guyette, 1994). The effect of systemic fungicides on *G. graminis* var. *graminis* infection and colonization of turfgrasses was evaluated; but results indicated that preventative and/or curative rates of fungicides did not limit take-all root rot disease or increase turfgrass quality (Elliott, 1995). Biological controls were explored in an attempt to decrease take-all root rot in wheat and turfgrasses. The effects of bacterial isolates, actinomycetes, and fluorescent pseudomonads on the roots of wheat were evaluated as antagonists against *G. graminis* var. *tritici* (Sivasithamparam and Parker, 1978). These organisms make up a large portion of the microbial community of soils and researchers expected their production of antibiotics or toxic metabolites would inhibit take-all in wheat in suppressive soils. While combinations of these microorganisms reduced disease, none were successful alone (Sivasithamparam and Parker, 1978). To date, no effective curative or preventative controls for take-all root rot are recognized for use in St. Augustinegrass.

In order to determine the impact of arbuscular mycorrhizal fungi (AMF) on take-all root rot in St. Augustinegrass sod, it is necessary to accurately diagnose *G. graminis* var. *graminis* and determine its population within the field. The diagnosis of take-all root rot involves several characteristics and diagnostic tools for isolation and identification. The pathogen is somewhat elusive and may be easily confused with other fungi if the scientist is not familiar with the morphology of the fungus and patterns of infection. The ascomycete, *G. graminis* var. *graminis*, is classified in the order Diaporthales because it produces ascospores in black, flask-shaped, ostiolate perithecia, which are fully enclosed

and lined with hyaline periphyses (Landschoot, 1997; Walker, 1973). The perithecia are typically 200-400 µm x 150-300 µm in length, with the neck portion 100-400 µm in length and 70-100 µm wide (Landschoot, 1997). The asci, clavate in shape, are unitunicate, are formed in a hymenium, and range in length from 80-140 µm and 10-15 µm in width. The apex of the ascus, which has a refractive apical ring, is generally yellowish en masse. Each ascospore is typically 70-110 µm in length, 2-4 µm in width and they usually contain 3-8 septa, but there may be 11 or 12 septa produced. The anamorphic state, which is rarely observed, is a *Philaphora* species that produces conidia 5-14 µm in length x 2-4 µm in width. The use of conidia as taxonomic criterion is not recommended due to variation between isolates and their non-descript morphology. In culture, mycelia range from short to aerial, white to gray, green to brown, or black (Landschoot, 1997).

Dark runner hyphae are typically observed on and around the crown portion of the plant, with extension onto the stem and stolons. The roots usually have relatively fewer dark surface runner hyphae, compared to the foliar portion of the plant, which may remain green. Instead of dark runner hyphae, the roots are often covered with dark brown to black lesions and subsurface hyaline hyphae. The cortical browning of roots is thought to be a host defense mechanism, while the discoloration of shoots is a necrotic symptom of disease (Penrose, 1992). The name “take-all root rot,” implies that the roots are the first plant parts to be severely affected whether facilitated by feeding damage from nematodes or mole crickets, mechanical damage from sod production, cultural techniques, or through natural openings.

After the initial invasion, the seminal roots are colonized internally by more hyaline and infectious, secondary hyphae usually right behind the root tip (Henson et al., 1999; Gilligan, 1983), which is were AMF usually colonize root tissue. Pathogenic colonization causes an occlusion of vascular tissues resulting in the characteristic gradual decline in plant health and potential death. Dark runner hyphae may continue up the plant in search of more juvenile and susceptible tissue while producing deeply lobed and melanized hyphopodia.

The hyphopodia are considered by most as superficial hyphal structures (Henson et al., 1999) since they originate from the hyphae, however they behave much in the same way as appressoria, which develop from the germ tube of germinating fungi providing infection pressure and anchoring the fungus to plant tissue (Agrios, 2004). Hyphopodia cluster and develop into an infection cushion which provides the added structural stability while helping to maintain the turgor pressure required for colonization (Henson et al., 1999). The force of exertion of *G. graminis* var. *graminis* is associated with reduced cell wall permeability, turgor, and wall rigidity (Bastmeyer et al., 2002). The deeply lobed hyphopodia are unique to *G. graminis* var. *graminis* and may exist to allow the fungus to overcome plant resistance mechanisms. Plants of St. Augustinegrass may benefit from AMF colonization in the presence of *Gaeumannomyces graminis* var. *graminis*. But, it is possible for AMF to have a negative impact on plants in some situations, or they may even be neutral in nature (Johnson et al., 1997).

Brown patch or *Rhizoctonia* blight, caused by *Rhizoctonia solani* Kühn (Figs. 3-2, 3-3), is most active in St. Augustinegrass from November to May when temperatures average

25 C and below (Elliott and Simone, 2001). Brown patch is typically worse in periods of excessive rainfall or irrigation, or when grass leaves remain wet for more than 48 hours (Elliott and Simone, 2001). In the field, small chlorotic patches of sod gradually turn brown as infected leaf blades die, hence the name brown patch (Elliott and Simone, 2001). As patches expand, they may coalesce into large rings of yellow-brown sod with dark and wilted margins. It is not uncommon for sod to appear green and healthy in the center of the rings. Grass blades are killed near the crown due to restriction of water and nutrient transport, which creates a dark rot near the base of the blade. Infected blades can easily be pulled from the leaf sheath due to the soft rot (Elliott and Simone, 2001). Most usually the stolons and leaves are affected more than the roots themselves. A barrage of chemical controls, such as azoxystrobin, fluotanil, and mancozeb offer effective brown patch control when used as preventatives. Cultural controls include irrigating only when necessary between 2 and 8 AM and removal of mower clippings from the site. However, the use of quick release nitrogen during periods of *R. solani* activity seems most beneficial (Elliott and Simone, 2001). The use of chemicals in sod production has been controlled in recent years and these restrictions will continue according to state and federal regulations. Effective disease prevention strategies including the use of biological controls, such as AMF, are essential research objectives in an industry where quality is of utmost importance to buyers and growers.

Brown patch was first described in St. Augustinegrass in the 1980's (Hurd and Grisham, 1983; Martin and Lucas, 1984) as an aerial type of pathogen common to a variety of crops including corn, soybean, and rice (Sneh et al., 1991). Other pathogenic species of *Rhizoctonia* affecting St. Augustinegrass include *R. oryzae* Ryker & Gooch

and *R. zeae* Voorhees which cause a sheath rot or spot, but the two species are rare (Martin and Lucas, 1984; Haygood and Martin, 1990). The telomorph, *Thanatephorus cucumeris* Frank, is assigned to the Basidiomycota (Ainsworth et al., 1973). Mycelia of *R. solani* appear buff to dark brown in culture with irregularly shaped light to dark brown sclerotia (Sneh et al., 1991). *Rhizoctonia solani* is identified by its characteristic right angle (90^{o}) branching between the primary and secondary hypha (Duggar, 1915) with branches forming acute (45^{o}) angles to main hypha (Butler and Bracker, 1970). Identification is made easier by the presence of a septum at the branches near hyphal constrictions at the base of right angles (Duggar, 1915). Additionally, the older, main runner hypha of *R. solani* are more than 7 µm in diameter with more than two nuclei per cell (Sneh et al., 1991).

Arbuscular mycorrhizal fungi have been associated with increased nutrient and water acquisition in plants for many years. Mycorrhizal symbiosis often results in increased plant vigor and the use of AMF has been studied in many crops as potential antagonists to root pathogens (Schenck, 1987; Sylvia and Williams, 1992; Smith and Read, 1997; Yao et al., 2002). *Glomus etunicatum* Becker & Gerdemann and *G. intraradices* Schenck & Smith are two of the more common AMF species investigated as potential biological controls and chemical alternatives against *R. solani* in crops such as potato (Yao et al., 2002) and species of *Fusarium* in tomato crops and alfalfa (Caron et al., 1986; Hwang et al., 1992). In several cases, *G. intraradices* provided significant control of soilborne pathogens (Niemira et al., 1996; Khalil et al., 1994; Viyanak and Bagyaraj, 1990). Newsham et al., (1995) reported that mycorrhizal fungi are capable of protecting annual grasses from soilborne fungi. In other surveys, researchers found that

G. intraradices significantly reduced take-all root rot caused by *G. graminis* var. *graminis* in cool-season bentgrasses on greens with low soil P levels (Koske et al., 1995).

Reductions in take-all disease severity in mycorrhizal wheat may be due to increased P uptake, increased root cell wall lignification, pathogen exclusion, production of antagonistic compounds, or altered root exudates (Graham and Menge, 1992). However, baseline information concerning pathogen colonization and potential effects of AMF on disease in the field is necessary before experiments concerning mechanisms of resistance and inoculation can be undertaken.

The objective of this survey was to determine the extent of *R. solani* and *G. graminis* var. *graminis* colonization in production fields of 'Floratam' St. Augustinegrass sod in north central Florida and to determine whether populations of AMF are having any effect on disease incidence in the field. Many researchers may feel that the effects of AMF in turfgrass systems may be outweighed by the benefits of added nutrients, pesticides, and irrigation. However, in St. Augustinegrass sod systems where inputs are limited, AMF may serve a greater role in plant resistance to soilborne pathogens or soil suppressiveness.

Materials and Methods

Root Pathogen Sampling. – 'Floratam' St. Augustinegrass stolons and roots were collected on a bimonthly basis from the three north central Florida sod farms described in chapter 2 in January through December 2005. The roots and stolons were surveyed for take-all root rot and brown patch. From each of the three (3 m^2) plots described in chapter 2, ten subsamples of root and stolon tissue (1-5 cm above the crown) were randomly dissected from collected plants and cut into 100 pieces of tissue 2-5 cm in length, in order to quantify the extent of root rot disease and to isolate and identify the

causal organisms. The pieces were washed, surface-sterilized for 1 min in a 10% sodium hypochlorite and deionized water solution, rinsed twice for 1 min with sterile deionized water, and blotted dry.

Pathogen Identification. - Forty pieces of tissue from each of the 100 segments/plot were randomly selected for isolation of *G. graminis* var. *graminis* and forty for isolation of *R. solani* and aseptically plated into selective agar media (Appendix-A) in 15 x 100 mm Petri dishes. Selective media (Appendix A) were used to isolate the pathogens from tissue and to slow growth of other soilborne fungi not associated with diseased tissue. The Petri dishes were incubated at 24 C under a 12 h diurnal cycle. Fungal growth was monitored by light microscopy for 5-8 d or until opportunistic fungal growth required colony transfer to sterile media, in order to isolate the desired root pathogens. Samples of fungal colonies suspected of being *R. solani* or *G. graminis* var. *graminis* were mounted in water on glass slides and viewed with a Nikon Optiphot compound microscope to identify fungal structures microscopically. *Gaeumannomyces graminis* var. *graminis* colonies were readily identified in media by the presence of deeply-lobed hyphopodia (Figs. 3-4, 3-5) within melanized mycelium (Landschoot, 1997). *Rhizoctonia solani* colonies (Figs. 3-6, 3-7) were identified based on the auburn to light brown color and 90^{o} branching of the mycelium (Sneh et al., 1991).

Pathogen Quantification and Statistical Analysis. – The number of colonies of *G. graminis* var. *graminis* and *R. solani* observed emerging from root or stolon pieces were used to quantify the amount of infection of these root pathogens at each sod farm location (Figs. 3-5, 3-6). The mean colonization data were expressed as the percentage of sampled root or stolon pieces colonized by *G. graminis* var. *graminis* or *R. solani* on

selective agar media (Appendix A). The survey was performed using a random model in a randomized complete block design with multiple samplings at multiple locations. The percent colonization data were analysed using the Generalized Linear Model (SAS Institute, Version 9.0, 2004) (Appendix F-2; Appendix F-3). Arbuscular mycorrhizal fungi sampling data, as described in chapter 2, were used in this survey since root pathogen sampling occurred simultaneously in the same plot locations as the survey of AMF in the previous chapter. Significant interactions ($P < 0.05$) were separated using Tukey's Studentized Range Distribution test, and correlations between AMF percent colonization and spore density to percent colonization of each root pathogen were done in SAS using Pearson product-moment correlation coefficients.

Results and Discussion

No correlation between AMF spore density or percent colonization in relation to *R. solani* or *G. graminis* var. *graminis* colonization were found. Additionally, no location effects were detected in the analysis of variance among or within the sampling months ($P < 0.001$). However, pathogen colonization did vary significantly between sampling months ($P < 0.001$), which suggested a seasonal influence on pathogen activity in north central Florida soils at each sod farm location. Mean values of root colonization by *R. solani* were greatest in December 2004 at 24.40% and lowest in June 2005 at 10.71 percent (Fig. 3-8). The warmer months of June and August had the lowest *R. solani* colonization percentages but the values were not significantly different from values in March, January, or October. The cooler months of December and April had the highest percentages of *R. solani*, although the April mean was not significantly different ($P < 0.05$) from October, January, or March (Fig. 3-8). This finding is not surprising since *R. solani* has optimal growth below 26 C therefore it is typically more

active in cooler weather (Elliott and Simone, 2001). Interestingly, as noted in chapter 2, AMF spore density (Table 2-2) was generally lowest during the cooler months of December, January, and April and highest during warmer weather, with percent colonization highest during the cooler months when *R. solani* is most active in these soils (Table 2-4).

Mean values of root colonization by *G. graminis* var. *graminis* were highest in the warmer months of August 2005 at 20.01% and lowest in December 2004 at 5.35 percent (Fig. 3-9). The months of August, June, and October had the highest percentages of *G. graminis* var. *graminis* colonization, with the lowest mean values occurring in December, January, March, and April. However, there were no significant differences ($P < 0.05$) between mean values in June and October, or October, April, March, and January. Again, this finding is not surprising because *G. graminis* var. *graminis* is most active in warm, markedly wet conditions where there is excessive thatch accumulation (Elliott, 1993; Guyette, 1994). During the warm, humid days of summer, St. Augustinegrass sod is often heavily irrigated and mowed, which produces favorable growth conditions for *G. graminis* var. *graminis* because of surplus moisture and accumulating clippings which add to thatch layers. In this survey, the pathogen is most active during periods when AMF percent colonization is lowest suggesting a limited role for AMF in take-all root rot disease suppression in these soils. More controlled studies might shed light on potential AMF effects on soilborne pathogens which may be confounded during field evaluations due to rhizosphere variability and environmental effects. If these criteria can be evaluated under less variable conditions, beneficial AMF effects could be evaluated and

perhaps manipulated for optimal disease suppression and concurrent decreases in pesticide use.

Fig. 3-1. ‘Floratam’ St. Augustinegrass sod mat infected with *Gaeumannomyces graminis* var. *graminis*. Insert in bottom right-hand corner depicts underside of a mat with rotting roots.

Figs. 3-2 – 3-3. Comparison of healthy ‘Floratam’ St. Augustinegrass sod mat and sod affected by brown patch.

Fig. 3-2. Healthy ‘Floratam’ St. Augustinegrass sod mat.

Fig. 3-3. ‘Floratam’ St. Augustinegrass sod mat infected with *R. solani* causing brown patch.

Fig. 3-4. Deeply-lobed hyphopodia isolated from *Gaeumannomyces graminis* var. *graminis* in 'Floratam' St. Augustinegrass sod samples. Scale bar = 40 µm.

Fig. 3-5. Medium isolation plate depicting a *Gaeumannomyces graminis* var. *graminis* colony isolated from 'Floratam' St. Augustinegrass sod samples. Arrow points to colony.

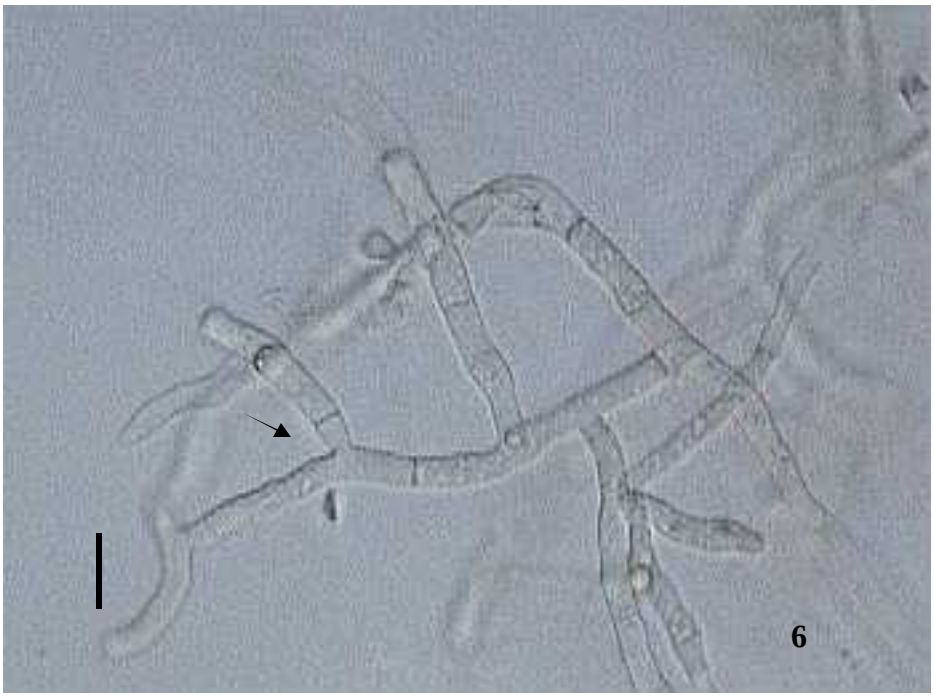


Fig. 3-6. *Rhizoctonia solani* hyphae isolated from 'Floratam' St. Augustinegrass sod exhibiting diagnostic 90^{o} branching at constriction points and characteristic septa. Scale bar = 40 µm. Arrow points to branching pattern.

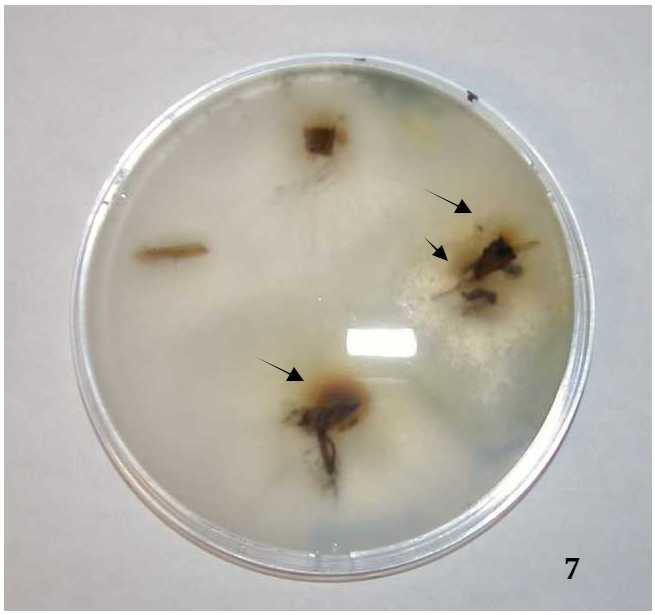


Fig. 3-7. Medium isolation plate depicting light brown *Rhizoctonia solani* colony isolated from 'Floratam' St. Augustinegrass sod samples. Arrows point to colonies.

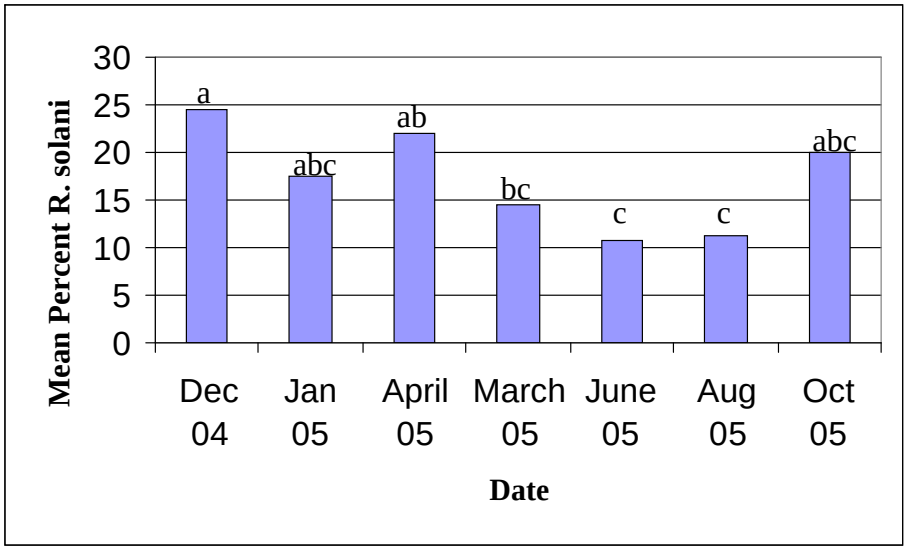


Fig. 3-8. Mean percent of *Rhizoctonia solani* colonization of 'Floratam' St. Augustinegrass in north central Florida. Means followed by the same number are not significantly different according to the Tukey's mean separation test ($P < 0.05$). The percent colonization is based on the mean number of colonies where *R. solani* was recovered.

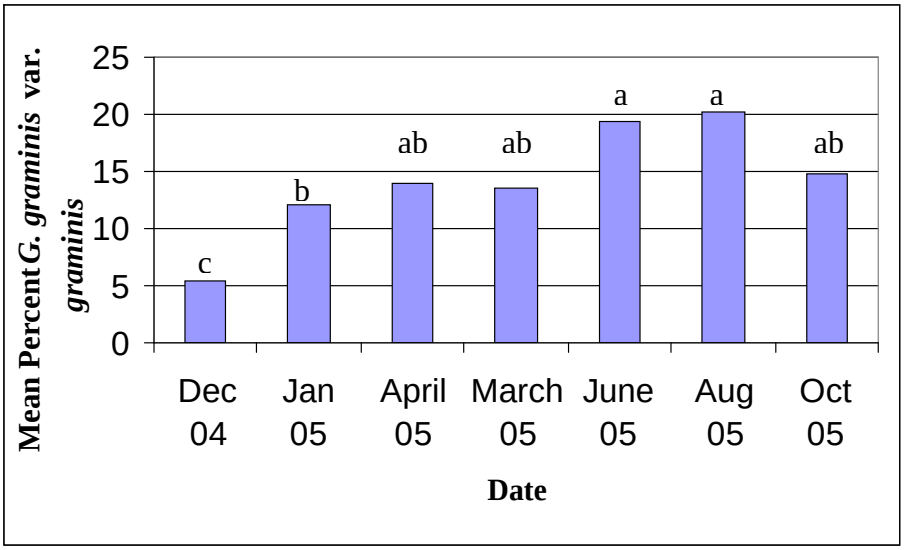


Fig. 3-9. Mean percent of *Gaeumannomyces graminis* var. *graminis* colonization of 'Floratam' St. Augustinegrass in north central Florida. Means followed by the same number are not significantly different according to the Tukey's mean separation test ($P < 0.05$). The percent colonization is based on the mean number of colonies where *G. graminis* var. *graminis* was recovered.

CHAPTER 4
EFFECT OF GLOMUS INTRARADICES ON THE EXTENT OF DISEASE CAUSED BY GAEUMANNOMYCES GRAMINIS VAR. GRAMINIS AND RHIZOCTONIA SOLANI IN ST. AUGUSTINEGRASS

Arbuscular mycorrhizal fungi (AMF) are widespread symbionts in the majority of plant species; and are associated with increased plant vigor via improved nutrient uptake, especially P, and increased water acquisition (Smith and Read, 1997). The beneficial effects of AMF on crop yield have been thoroughly documented (Harley and Smith, 1983). There is much debate on whether or not AMF alter plant resistance to pathogens by an indirect mechanism or simply interact directly with the pathogens themselves.

When AMF act as pathogen antagonists, there are likely one or more mechanisms of resistance. For example, AMF may be deterring pathogen infection by increasing plant vigor through improved nutrient acquisition, the AMF themselves may be producing anti-microbial metabolites, or the AMF may be stimulating the plant's own natural defense response to colonization by increasing phytoalexin production (Schenck, 1970). Previous studies have indicated that AMF symbiosis greatly improves plant resistance to abiotic pressures such as water stress (Sylvia and Williams, 1992) and transplant shock (Menge et al., 1978) in various crops. AMF have also been evaluated as biological controls against biotic stresses such as bacterial pathogens (Weaver and Wehunt, 1975), parasitic nematodes (Baltruschat et al., 1973; Schenck and Kellam, 1978), viral pathogens (Daft and Okusanya, 1973; Giannakis and Sanders, 1989), and soilborne fungal pathogens (Jeffries, 1987; Schenck, 1987; Hooker et al., 1994; Linderman, 1994; Azcón-Aquilar and Barea, 1996).

The vast majority of evaluations concerning the effects of AMF on disease severity involve fungal pathogens (Schenck and Kellam, 1978). The first report of an interaction between mycorrhizal fungi and fungal pathogens involved soybean (*Glycine max* L. Merr) and *Phytophthora* root rot, where the mycorrhizal plants actually had higher rates of disease versus the nonmycorrhizal plants (Ross, 1972). In other reports, AMF had no effect on disease at all (Ramirez, 1974; Sherinkina, 1975). Depending on the stage of host plant development, plant and mycorrhizal fungal species, and the complexities between biotic and abiotic rhizosphere factors, there is evidence that mycorrhizal interactions lie along a continuum ranging from mutualistic to parasitic, commensal, amensal, and potentially even neutral (Johnson et al., 1997). However, there are many reports of mycorrhizal colonization reducing disease severity in many plant systems such as pea, tomato, soybean, wheat, and peanut involving such fungal pathogens as *Fusarium solani* Mart. (Sacc.), *G. graminis* (Sacc.) Arx & Olivier var. *tritici* J. Walker, *Sclerotium rolfsii* (Sacc.), *Pythium* spp., *Phytophthora parasitica* Dastur, and *R. solani* Kühn (Graham and Menge, 1992; Dehne, 1982; Krishna and Bagyaraj, 1983; Zambolim and Schenck, 1983; Hedge and Rai, 1984; Vigo et al., 2000; Yao et al., 2002).

In fact, the effects of mycorrhizal colonization on disease severity is potentially so important that Newsham et al. (1995) suggested that the benefits of AMF to disease suppression may be as important as the nutritional benefits derived from the symbiosis in some instances. For example, in temperate grasslands, the effects of a direct AMF interaction with root pathogens reduced disease severity and increased plant vigor and fecundity greatly (Newsham et al., 1995). Soilborne pathogen suppression by AMF includes both physical and physiological mechanisms (Sharma et al., 1992). Physical

plant defense responses against pathogen penetration are: increased lignification (Dehne and Schoenbeck, 1978), greater mechanical strength and nutrient flow within vascular systems (Schoenbeck, 1979), and direct competition with the pathogen for cortical infection courts and resources (Graham, 2001). Becker (1976) observed that pathogen penetration of root cells was directly reduced by the presence of AMF and not indirectly by a systemic plant resistance based on thickening cell walls. In some cases the direct influence of AMF may be the only reason for observations of disease resistance. It is important to establish whether or not particular plant systems benefit, suffer, or remain unaltered by mycorrhizal colonization. If the relationship appears to be beneficial, Gerdemann (1975) remarked that the effect of mycorrhizal fungi on disease should be determined whether resistance is due to direct or indirect mechanisms.

The host-pathogen relationship can be greatly influenced by indirect or physiological effects of AMF through increased P nutrition, enhanced mycorrhizal root growth which aids in disease escape, or up-regulation of pathogenesis-related proteins (Gianinazzi-Pearson and Gianinazzi, 1989; Blee and Anderson, 2000; Graham, 2001). AMF may also be responsible for lowering disease severity in complex reactions involving host physiology such as the production of rhizosphere leachates from mycorrhizal plant roots. These leachates have been observed to substantially limit the production of zoospores and sporangia of *Phytophthora cinnamomi* Ronds in sweet corn and chrysanthemum (Meyer and Linderman, 1986).

There appears to be no information concerning the effects of AMF, if any, on disease severity in St. Augustinegrass. If there is a direct or indirect beneficial effect of AMF on disease severity of St. Augustinegrass in relation to brown patch or take-all root

rot, several questions will remain concerning the actual mechanism of observed resistance. However, without basic information and techniques to differentiate between direct and indirect effects and to determine what extent disease severity may or may not be lowered, further evaluations would not be warranted.

The economic importance of AMF in soils of north central Florida St. Augustinegrass sod fields may be considerable where diseases such as brown patch and take-all root rot reduce harvestable hectares. Arbuscular mycorrhizal fungi can stimulate plant vigor and possibly interact directly or indirectly with soilborne pathogens to limit disease. AMF have been observed colonizing St. Augustinegrass (see Chapter 2), and they might benefit sod production. The potential AMF benefits to sod growers include reduced loss of sod and revenue to soilborne pathogens, and lowered management costs through reduced fungicide use. The potential advantages of AMF inoculation or field manipulation with specialized techniques may also benefit the environment by decreasing soil and water pollution through reduced of fungicide use. For these reasons, it is prudent to evaluate the potential benefits of AMF to disease resistance whether by direct or indirect mechanisms in St. Augustinegrass sod. As part of ongoing research on the effect of AMF on disease severity in St. Augustinegrass, the objective of this study was to determine the effect of *G. intraradices,* on St. Augustinegrass in disease development by challenging it both directly and indirectly with *G. graminis* var. *graminis* or *R. solani*.

Material and Methods

Direct Experiments

St. Augustinegrass Sprig Propagation and Stock Plants.- ‘Floratam’ St. Augustinegrass sprigs having no apparent signs or symptoms of disease were obtained from Hendrick’s Turf Farm (Lake Butler, Florida). The sprigs were rooted in flat, plastic

nursery trays or 18 cm clay pots in a sterilized Arrodondo fine sand medium supplemented with a nutrient solution (Appendix B) every three weeks. The sprigs were grown and maintained in a growth chamber at 25-27 C under cool-white fluorescent bulbs with irradiance at 25 $\mu E/m^2/s$ and a 15 h photoperiod/day. Sprigs were watered every other day throughout the experimental period with water adjusted to pH 6.0-6.5. After approximately 6 weeks of propagation, selected sprigs, not in direct contact with soil, were excised from the edge of the flat trays and replanted as sterile stock plantlets. These sub-cultured plants were maintained as described above until additional sprigs, not touching the soil and hanging from the edge of the tray, were collected for experimentation.

R. solani Inoculum Production.- A virulent strain of *R. solani* (PDC 7884) (Fig. 4-1) isolated from diseased St. Augustinegrass submitted by a homeowner in Leon County, Florida was provided by the Plant Disease Clinic (Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida). The isolate was cultured at 4 C and stored on potato dextrose agar (Difco Laboratories, Inc., Detroit, Michigan) for approximately 2 weeks. An oat (*Avena sativa* L.) inoculum was prepared according to Sneh et al. (1991) and Gaskill (1968) with modifications (Appendix C) and inoculated with agar plugs from actively growing *R. solani* (PDC 7884) mycelium or with sterile agar plugs (control). The inoculum substrate was incubated at 21 C with a 12 h photoperiod for 4 weeks and shaken 2-3 times/week to prevent packing of the oat seeds. The inoculated seeds were then air-dried, sealed in plastic zip-lock bags, and stored at room temperature until use.

G. graminis var. graminis Inoculum Production.- A virulent strain of *G. graminis* var. *graminis* (JK2) was collected and identified from diseased St. Augustinegrass (Fig. 4-2) from the lawn of Dr. James Kimbrough (Gainesville, Florida) and isolated on selective media amended with antibiotics (Appendix A). Actively growing *G. graminis* var. *graminis* mycelium from a single Petri dish was chopped and combined with sterilized ryegrass seed as described by Datnoff and Elliott (1997) with modification (Appendix C). The inoculated flasks of sterile ryegrass seed substrate and uninoculated control flasks were incubated in total darkness at 21 C for 4 weeks prior to use. The flasks were shaken 2-3 times/week to prevent packing of the inoculated ryegrass seed.

Mycorrhization of 'Floratam' St. Augustinegrass Sprigs.- Sprigs of 'Floratam' St. Augustinegrass were selected from the edge of sterile stock plants in flat trays, as previously described. Sprigs were inspected visually for any signs or symptoms of potential pathogens or diseases, and if healthy, were selected for experimental use. The sprigs were then planted into 6.8 cm wide by 18 cm deep conetainers (Steuwe and Sons, Inc., Corvallis, Oregon) filled with a sterilized low P soil, as mentioned in Chapter 2 (Fig. 4-3). The sprigs were then placed in a controlled growth room with a 15 h photoperiod/d at 21-25 C, watered daily with pH adjusted 6.0-6.5 deionized water, and maintained for approximately 3 weeks to allow root development to occur and transplant shock to subside. After the 3 week growth period, the sprigs, with approximately 8 cm of root length, were inoculated with approximately 20 spores of *G. intraradices* (FL 208A) (Fig. 4-4) obtained from the INVAM Culture Collection (Morgantown, West Virginia) or noninoculated water controls. The FL 208A isolate was selected because it was first isolated in a citrus grove in central Florida, near Orlando, in 1978 in 7.0-7.5 pH soil,

which is similar to that of the sod fields in north central Florida. The sprigs were then acclimatized for approximately 4 weeks in the growth room to allow the AMF time to colonize the sprig roots, which was determined at 2 and 4 weeks in extra experimental units.

Pathogen Inoculation.- The AMF colonized sprigs were inoculated with either *R. solani* (PDC 7884) or the *G. graminis* var. *graminis* (JK2) isolate or uninoculated as controls by gently pushing the soil aside to expose a portion of the roots near the crown of the sprig. Approximately 3-5 infected seeds of either the *R. solani* inoculated oat substrate or *G. graminis* var. *graminis* inoculated ryegrass seed substrate were placed equidistant from the crown in each conetainer at a 1-2 cm distance from the plant. The soil was carefully replaced following inoculation. Inoculated sprigs were maintained in the growth room for approximately 4 weeks with a 15 h photoperiod/d at 21-25 C. Each cone was supplied with a nutrient solution devoid of P on two occasions at 50 ml/conetainer (Appendix B). Plants were watered daily with 50 ml water/conetainer adjusted to 6.0-6.5 pH.

Mycorrhizal Evaluation.- Roots from the sprigs were rinsed in tap water and separated with a scalpel from the plant crown. Selected roots were cut into 1-2 cm long segments, put into porous nylon sleeves, inserted in small, plastic clips (Fig. 4-5), and the cell and wall components cleared in 10% KOH (w/v) under pressure in an autoclave for approximately 20 min at 121 C psi (Brundrett et al., 1996). The root segments were cooled, then rinsed in tap water, and placed into 0.05% trypan blue in 25% glycerol overnight to stain mycorrhizal structures (Bevenge, 1968; Phillips and Hayman, 1970; Kormanik and McGraw, 1982). Excess stain was rinsed from the root segments with tap

water and then the roots were mounted in water on glass slides to view vesicles, intraradical hyphae, and arbuscules (Fig. 4-6).

Root segments from each replicate were pooled from each treatment, and evaluated for intensity of colonization. Mycorrhizal structures on glass slides were viewed with a Nikon Optiphot compound microscope at 200, 400, and 1000x magnifications, and photographs were taken with a Nikon CoolPix 990 digital camera. In order to judge the amount of mycorrhizal root colonization, the grid line intersect method was used to approximate the total root length colonized by AMF (Newman, 1966; Tennant, 1975; Giovannetti and Mosse, 1980).

Direct Experiment Disease Assessment.- Disease severity (root and shoot rot) was rated at the conclusion of a 3 week growth period on both the AMF inoculated, pathogen inoculated, and control sprigs. Disease severity was assessed using an arbitrary disease scale from 1 to 6 with 1 = no symptoms of disease; 2 = 1-25% disease; 3 = 26-50% disease; 4 = 51-75% disease; 5 = 76-100% disease; and 6 = plant death (Figs. 4-7; 4-8). The presence of either the *R. solani* or *G. graminis* var. *graminis* pathogens on each infected sprig was confirmed by re-isolation of each pathogen (Figs. 4-9; 4-10) on selective media (Appendix A). For each sprig, the percent colonization of the pathogen and/or AMF was recorded as described in Chapters 2 and 3.

Direct Experiment Design and Statistical Analysis.- The experiment was performed using a factorial arrangement (1 cultivar of St. Augustinegrass) x (1 AMF + uninfected pathogen control) x (1 *R. solani*-infected + 1 AMF) x (1 *R. solani*- infected – AMF) and (*1 G. graminis* var. *graminis*- infected + 1 AMF) x (1 *G. graminis* var. *graminis* – AMF) and (uninfected pathogen control + uninoculated AMF control) in a randomized complete

block design with four replicates/treatment (Fig. 4-11). Regression analyses were performed with the regression procedure in SAS (SAS Institute, 2004) (Appendix F-4). All data presented are the means of four replicates. As there were no differences between trials based on the ANOVA, all data presented were combined for the purpose of presenting the results and discussion more easily.

Indirect Experiments

St. Augustinegrass sprigs were produced and maintained in the same manner as described above in the Direct Experiment section as were mycorrhization and pathogen inoculum production, inoculation, and quantification. However, in this experiment, the potential effects of indirect AMF interactions with soilborne pathogens were evaluated instead of the potential direct impacts of mycorrhization. Instead of a direct challenge between AMF and pathogen in one container, indirect effects were investigated using a split-root assay.

Indirect AMF Challenge Split-Root Assay.- Sterile, 4 week old 'Floratam' St. Augustinegrass sprigs with approximately 8 cm of healthy root tissue were placed into two adjacent conetainers with one rooted end of the sprig in one conetainer and the other rooted end in another conetainer (Fig. 4-12). Holes (1 cm in diameter) were drilled 2.5 cm from the top of each 6.5 cm wide by 18 cm deep conetainer (Steuwe and Sons, Inc., Corvallis, Oregon) prior to planting, on one side of the conetainer (Appendix E-1). A cut was made from the top of the drilled hole to the top of each conetainer to allow the sprig to be inserted into the hole without tissue damage. Sprigs were planted into conetainers filled with sterile low P soil as previously described and maintained in the growth room for 3 weeks to limit transplant shock and acclimatize the sprigs. Sprigs were then inoculated with the *G. intraradices* isolate (FL 208A) as described in the direct

experiment above, or a control substrate in one conetainer, with either the *G. graminis* var. *graminis* isolate (JK2) or *R. solani* isolate (PDC 7884) inoculated or an uninoculated control substrate in the adjacent conetainer occupied by the other rooted end of that same sprig (Fig. 4-13). The conetainers were watered daily with 50 ml water/conetainer adjusted to pH 6.0-6.5 and supplied with a nutrient solution on two occasions (Appendix B). The sprigs were maintained for 3 weeks in the growth chamber at 21-25 C with a 15 h photoperiod. The sprigs were visually inspected every 2-3 d for the presence of invading pathogenic mycelia along the stolon portion of the sprig to prevent cross contaminiation. The presence of the pathogen used to inoculate one conetainer was not observed in any of the adjacent experimental units (conetainers) based on the lack of recovery of the pathogen from adjacent conetainers by selective media isolation (Appendix A). The stolon portion spanning the distance between the two adjacent conetainers was approximately 5 cm in length. Percent *G. intraradices* colonization was measured using the gridline intersect method described in the previous section.

Indirect Experiment Design and Statistical Analysis.- The experiment was performed using a factorial arrangement (1 cultivar of St. Augustinegrass) x (1 AMF + uninfected pathogen control) x (1 *R. solani*-infected + 1 AMF) x (1 *R. solani*- infected – AMF) and (*1 G. graminis* var. *graminis*- infected + 1 AMF) x (1 *G. graminis* var. *graminis* – AMF) and (uninfected pathogen control + uninoculated AMF control) split-root assay in a randomized complete block design with four replicates. The entire experiment was setup three times from January – May 2006. Regression analyses were performed with the regression procedure in SAS (SAS Institute, 2004) (Appendix F-5). All data presented

are the means of four replicates/treatment. No differences were found between trials based on the ANOVA, therefore, data were pooled for analysis.

Results

Direct Experiments

Mycorrhizal Colonization.- In the direct experiment, mean values of root colonization by the AMF, *Glomus intraradices*, were 10% for the *R. solani*-infect + AMF treatment, 11.3% for the AMF inoculated control treatment (no pathogen), and 11.7% for the *G. graminis* var. *graminis*-infected + AMF treatment, respectively, after mycorrhizal inoculation. Root colonization of AMF was not significantly affected by the direct presence of either pathogen nor did the AMF control treatment (no pathogen) have any direct effect, either positive or negative, on disease severity itself (Appendix D-1). In this study, the colonization of plants by AMF, *G. intraradices*, apparently had a neutral effect on the St. Augustinegrass plants without the direct presence of either pathogen nor did the AMF affect plant growth.

Disease Development.- The direct effect of *G. intraradices* on brown patch (caused by *R. solani*) disease severity was evaluated by first investigating the relationship of the *R. solani*-infected control (no AMF) treatment (Appendix D-2) to disease severity. The mean percent colonization of the *R. solani*-infected control treatment was 60%, but the disease severity (mean = 3.8 on a scale of 1 to 6) was not significantly correlated with the mean colonization percentage of *R. solani* using the regression procedure in SAS (SAS Institute, 2004). Since there was no definitive relationship between plant disease severity and the percentage of *R. solani* colonization with this treatment, there was no need to assume that *G. intraradices* in the *R.solani*-infected + AMF treatment would have a beneficial effect on disease severity. This was supported by the regression analysis

comparing the relationship of disease severity to percent *R. solani* colonization (mean colonization = 57%) in the *R. solani*-infected + AMF treatment (Appendix D-3) where disease severity (3.3 on a scale of 1 to 6) was not correlated to the mean percentage of AMF colonization (mean colonization = 18%). In this study, the AMF treatments had no effect on disease severity in the direct presence of *R. solani* regardless of the mean colonization of the pathogen or AMF.

The direct effect of *G. intraradices* on disease severity was also evaluated in this study for take-all root rot caused by *G. graminis* var. *graminis*. Based on regression, the relationship between disease severity and the *G. graminis* var. *graminis*-infected control (no AMF), it appears that the pathogen (mean colonization = 42.8%) had a significant relationship ($r^2 = 0.65$) with disease severity (2.4 on a scale of 1 to 6). This model shows that as disease severity increases so does *G. graminis* var. *graminis* percent colonization in a direct pathogenicity challenge (Fig. 4-14). This finding suggests that the AMF could potentially have a direct effect on disease severity and that the relationship could be evaluated since the percent colonization of *G. graminis* var. *graminis* had a measurable effect on disease severity. The regression analysis of disease severity (mean = 3.3 on a scale of 1 to 6) to the *G. graminis* var. *graminis*-infected + AMF inoculated treatment revealed a highly correlated relationship between the treatment and disease severity ($r^2 = 0.81$). As disease severity increased according to this treatment, so did the percent colonization of *G. graminis* var. *graminis* even in the direct presence of AMF (mean = 8.6%) (Fig. 4-15). There was no apparent reduction or increase in disease severity. Therefore, the AMF have no direct beneficial effect on take-all root rot disease severity. Additionally, the AMF treatment alone could not be correlated to a reduction in percent

G. graminis var. *graminis* colonization (data not shown) nor did the treatment have a direct effect on lowering take-all root rot disease severity since the disease severity trend did not differ from that of the *G. graminis* var. *graminis*-infected – AMF treatment.

Since disease severity was not affected by *G. intraradices* in the *G. graminis* var. *graminis*-infected + AMF treatment or correlated to the percent of *G. graminis* var. *graminis* colonization in the control uninoculated with AMF, it appears that the AMF colonization had no direct negative or positive impact on the pathogen or disease severity. In this study, the interaction between AMF and the plant in the direct presence of the pathogens, *G. graminis* var. *graminis* and *R. solani* would thus be considered neutral in nature.

Discussion

More importantly, this study demonstrates that mycorrhization with the AMF, *G. intraradices*, did not reduce development of *R. solani* or *G. graminis* var. *graminis* in direct contact nor did the AMF treatment reduce or increase disease severity of brown patch or take-all root rot in 'Floratam' St. Augustinegrass, as has been observed in other mycorrhizal studies (Ross, 1972; St. Arnaud et al., 1994; Mark and Cassells, 1996). Arbuscular mycorrhizal fungi have been associated with increased disease severity in some instances with *R. solani*, so analysis based on this assumption was as necessary as assuming the AMF treatment would lower disease severity (Ramirez, 1974; Sherinkina, 1975; Johnson et al., 1997; Yao et al., 2002). No beneficial effects of AMF inoculation on take-all root rot or brown patch disease severity in St. Augustinegrass were observed. This is perhaps due to the relatively low levels of mycorrhizal root colonization. Possibly AMF inoculation would be more beneficial to plants with a higher level of mycorrhizal colonization.

In summary, the results show that the purported beneficial effects of direct AMF interactions with plant roots such as increased cell wall lignification or the production of antagonistic mycorrhizal root exudates did not play a role in this study (Becker, 1976; Dehne and Schoenbeck, 1978; Graham, 2001). Thus, inoculation with *G. intraradices* will not improve disease severity or reduce disease development. The effects of such an interaction within field trials could potentially yield contradictory results, and the microbial and environmental variability within the rhizosphere would make such experiments difficult at best.

Results

Indirect Experiment

In order to thoroughly evaluate the potential effects of AMF on disease severity and/or soilborne pathogen development, another series of studies involving a more indirect method was performed simultaneously with the direct experiment described above. This assay was designed to isolate potential systemic resistance responses from mycorrhization which have been documented (Gianinazzi-Pearson and Gianinazzi, 1989; Blee and Anderson, 2000; Graham, 2001).

In this assay, the *R. solani* control (no AMF) treatment revealed a significant correlation between pathogen colonization and disease severity (Fig. 4-16). In this instance, as percent colonization of *R. solani* (mean = 54.9%) increased so did disease severity (mean = 3.5 on a scale of 1 to 6; $r^2 = 0.75$). Since there was a significant relationship between the pathogen and disease severity, the regression procedure in SAS was also used to analyze the indirect effects of the *R. solani* + *G. intraradices* treatment on disease severity. The combination of this pathogen and AMF in an indirect assay, where one conetainer was inoculated with *R. solani* and the other conetainer containing

the other end of that same sprig was inoculated with *G. intraradices* showed no correlation (r^2 = 0.33) between AMF (mean colonization = 10.2%) and the pathogen (mean colonization = 35.4%) on disease severity (mean = 2.4 on a scale of 1 to 6) (Appendix E-4).

As in the direct experiment, described above, the percent of *G. intraradices* (mean colonization = 6.75%) did not have an impact on disease severity of take-all root rot (mean = 1.33 on a scale of 1 to 6) or brown patch as a treatment alone (r^2 = 0.34) (Appendix E-3). In this assay, the indirect effect of the AMF, *G. intraradices* (mean colonization = 9.7%), had no significant effect on take-all root rot disease severity (mean = 2.33 on a scale of 1 to 6) nor did the *G. graminis* var. *graminis*-infected + AMF treatment (r^2 = 0.33) have an impact on pathogen colonization (mean colonization = 22.9%) (Appendix E-5). In the *G. graminis* var. *graminis*-infected control treatment (no AMF), there was no significant correlation (r^2 = 0.32) based on the regression analysis between the percent of *G. graminis* var. *graminis* colonization (mean = 59.6%) and take-all root rot disease severity (mean = 3.0 on a scale of 1 to 6) (Appendix E-2).

Discussion

Based on this indirect assay and on the direct challenge between the AMF, *G. intraradices* and *R. solani* or *G. graminis* var. *graminis*, there is no correlation between AMF colonization and disease severity. Disease severity does not increase or decrease, which is important considering that mycorrhizal benefits lie along a continuum ranging from mutualistic to parasitic (Johnson et al., 1997). If there is an interaction between this AMF and either of these two pathogens in St. Augustinegrass sod field soils, it is most likely neutral in nature. Based on these results, AMF colonization of St. Augustinegrass

in north central Florida soils are neither harmful nor beneficial to the plants when infected with these pathogens.

In summary, these results provide a foundation for future field trials in relation to direct and indirect impacts of AMF in St. Augustinegrass sod. This is the first study that attempts to correlate take-all root rot or brown patch disease severity to potential direct or indirect AMF effects. Since no influences were observed in either experiment, the proposed mechanisms of direct resistance or indirect systemic resistance were not examined.

Fig. 4-1. *Rhizoctonia solani* isolate (PDC 7884) colony used to prepare inoculum in direct and indirect experiments.

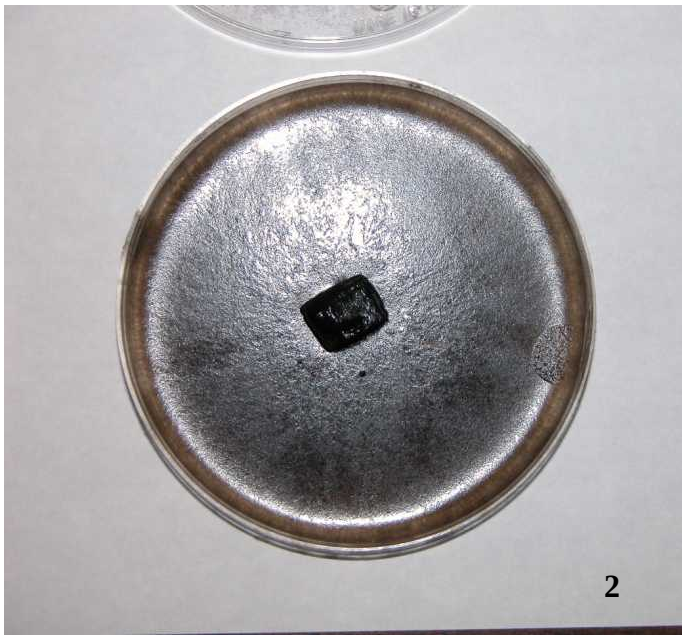


Fig. 4-2. *Gaeumannomyces graminis* var. *graminis* isolate (JK2) used to prepare inoculum in direct and indirect experiments.

Fig. 4-3. Conetainers filled with low P soil and 'Floratam' St. Augustinegrass sprigs inoculated in trial 1 of the direct experiment.

Fig. 4-4. *Glomus intraradices* isolate (FL 208 A) used in direct and indirect assays to inoculate 'Floratam' St. Augustinegrass sprigs. Bar scale = 40 µm.

Fig. 4-5. Photo showing nylon sleeves and plastic clips used in direct and indirect experiments to clear and stain root segments from treatment replicates.

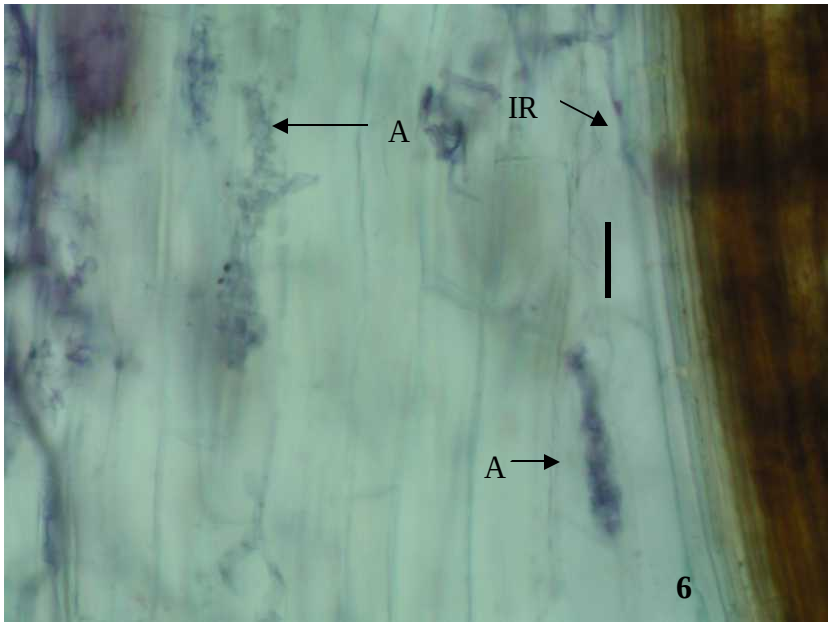


Fig. 4-6. Photo of mycorrhizal St. Augustinegrass root with arbuscules and intraradical hypha of *Glomus intraradices* stained with 0.05% trypan blue from the direct experiment *G. intraradices* inoculated control sprigs. Arrows pointing to A = arbuscule; Arrow pointing to IR = intraradical hypha. Bar scale = 40 µm.

Fig. 4-7. ‘Floratam’ St. Augustinegrass sprigs after inoculation with *Rhizoctonia solani* depicting disease severity rating scale (1-6). Respective numbers below each sprig signify the disease severity rating of that sprig.

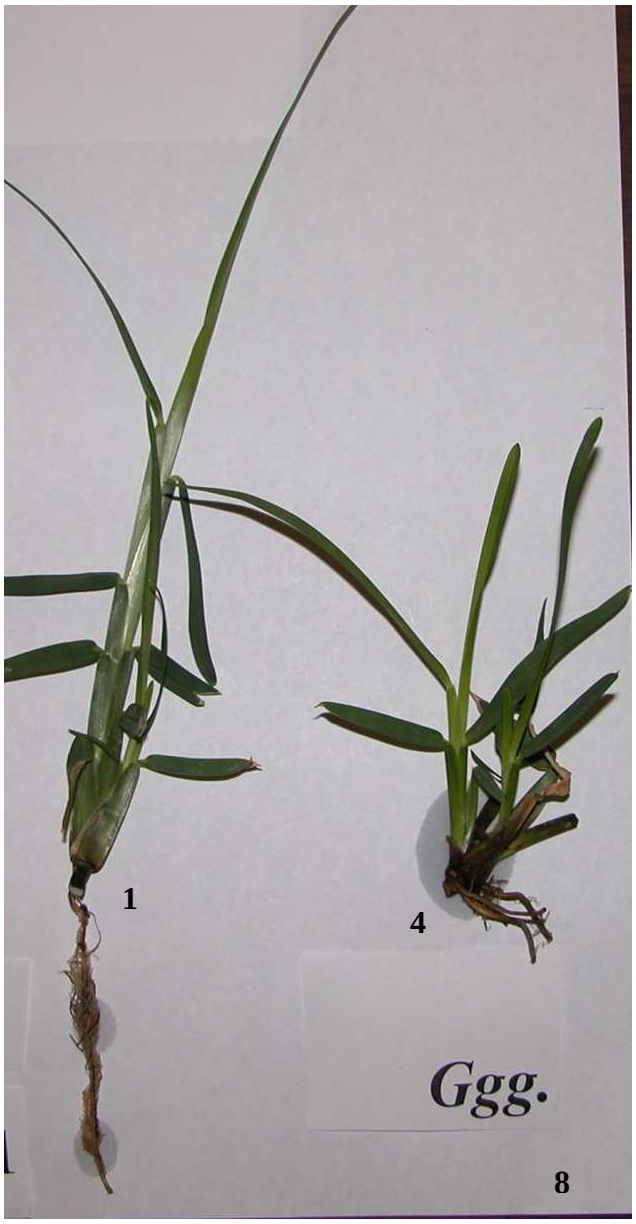
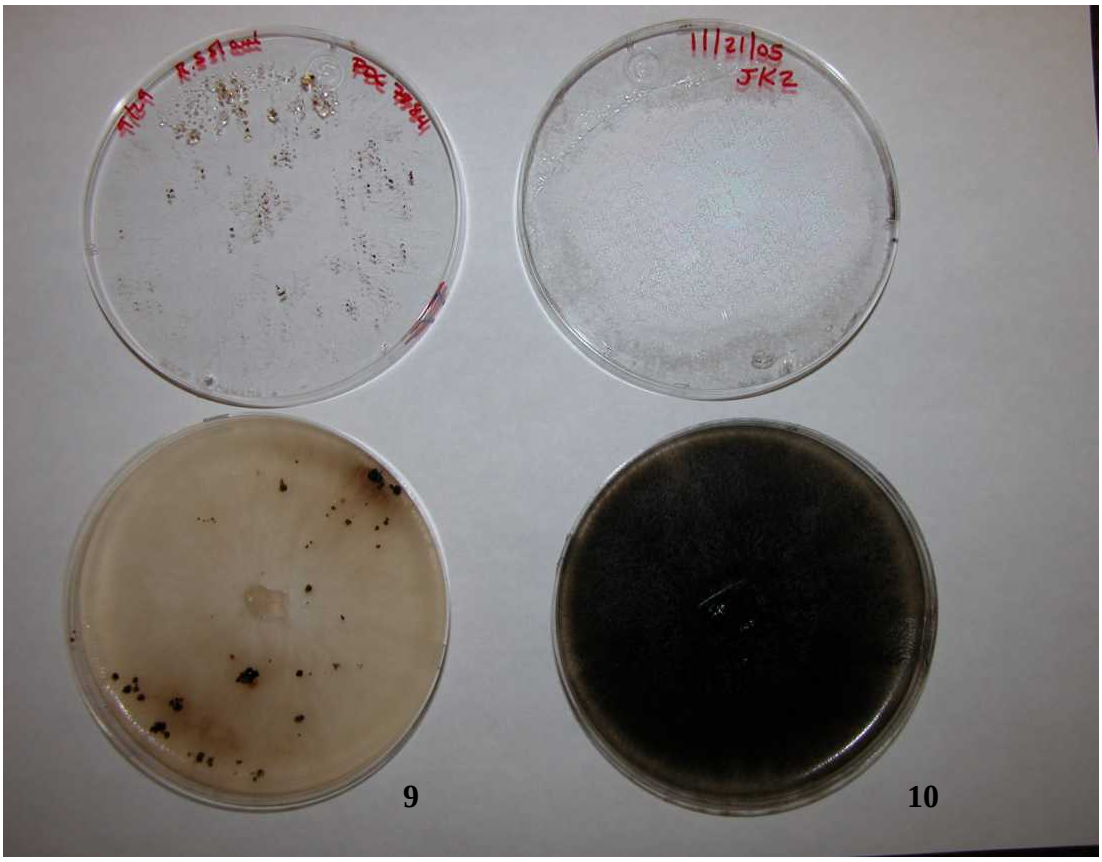


Fig. 4-8. ‘Floratam’ St. Augustinegrass sprigs after inoculation with *Gaeumannomyces graminis* var. *graminis* depicting disease severity rating scale (1-6). Respective numbers below each sprig signify the disease severity rating of that sprig.



Figs. 4-9 – 4-10. Photo depicting re-isolation plates of the two pathogenic isolates used to challenge *Glomus intraradices* in both the direct and indirect experimental trials.

Fig. 4-9. *Rhizoctonia solani* (PDC 7884) re-isolation plate with selective medium from the *R. solani*-infected without *Glomus intraradices* treatment in the indirect experimental trial 2.

Fig. 4-10. *Gaeumannomyces graminis* var. *graminis* (JK2) re-isolation plate with selective medium from the *G. graminis* var. *graminis*-infected with *Glomus intraradices* treatment in the direct experimental trial 2.

Fig. 4-11. Photo of the indirect experimental trial 3 conetainers arranged in a randomized complete block design with four replicates per treatment.

Fig. 4-12. Photo showing a close-up view of the experimental units of the indirect experimental trial 1 depicting the split-root assay.

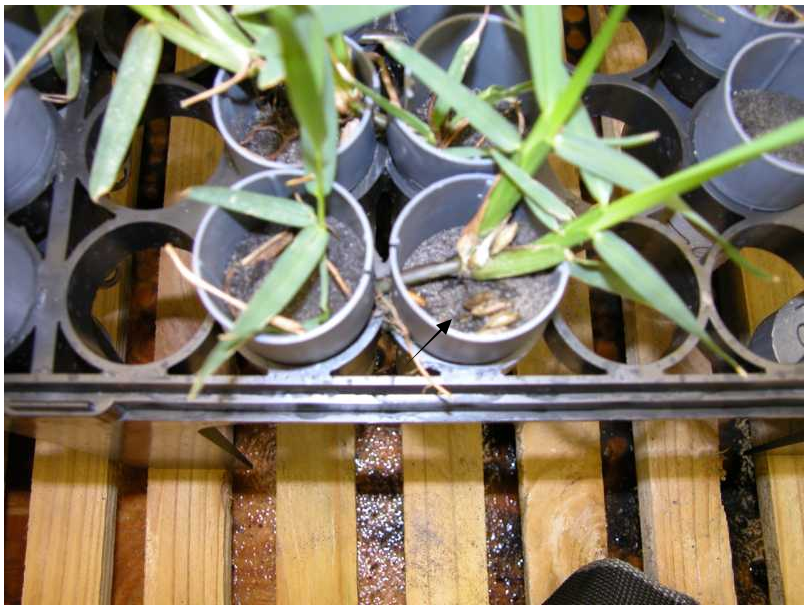


Fig. 4-13. Photo showing the split-root assay of the indirect experimental trial 2 after inoculation with ryegrass seeds inoculated with *Gaeumannomyces graminis* var. *graminis* (JK2). Arrow points to the inoculum.

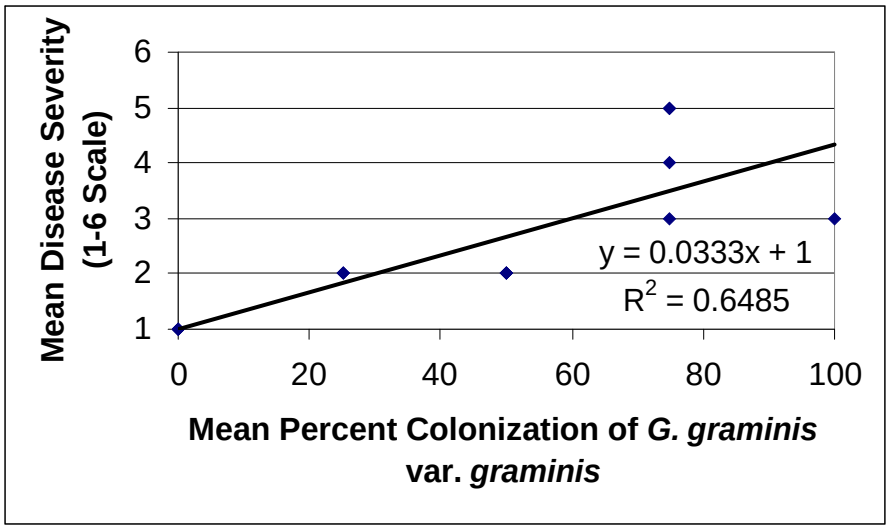


Fig. 4-14. The direct effect of *G. graminis* var. *graminis* on St. Augustinegrass take-all root rot disease severity without *G. intraradices*.

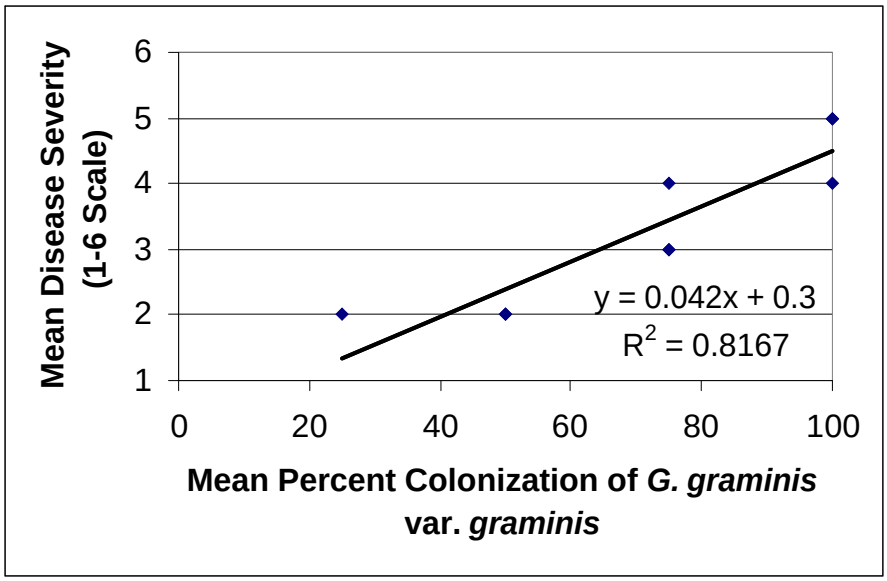


Fig. 4-15. The direct effect of *G. graminis* var. *graminis* on St. Augustinegrass take-all root rot disease severity with *G. intraradices*.

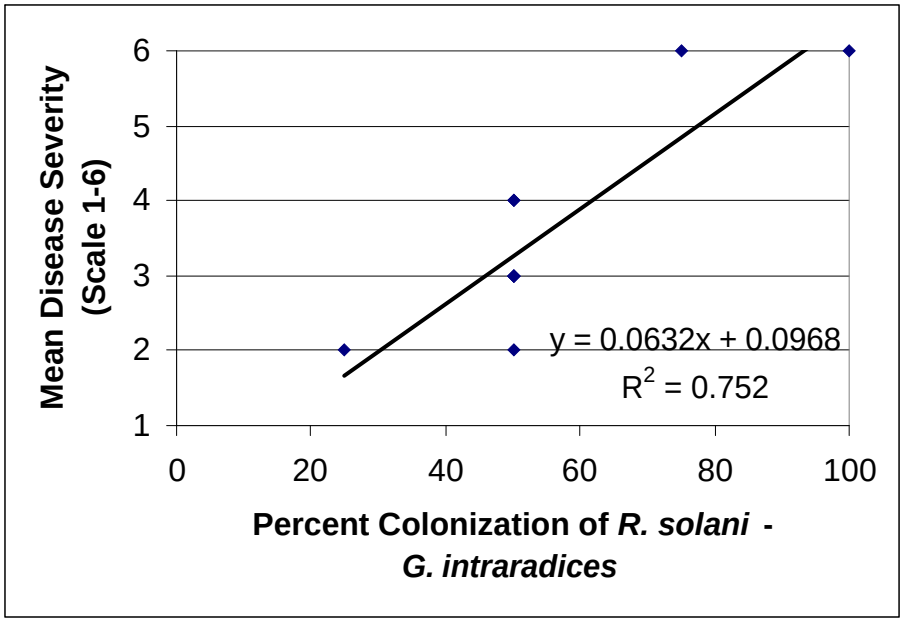


Fig. 4-16. The indirect effect of *R. solani* without *G. intraradices* on St. Augustinegrass brown patch disease severity in an adjacent split sprig system.

CHAPTER 5
SUMMARY AND CONCLUSIONS

From Frank's (1885) initial report of "fungus-roots" in the forests of Germany there has been great interest in the potential benefits of arbuscular mycorrhizal fungi colonization in a vast array of crops. Many documented evaluations suggest a positive role for AMF in the reduction of disease severity and increased uptake of limited nutrients and water which all contribute to improved vigor and fecundity (Newsham et al., 1995). However, there are also a number of reports suggesting a parasitic role for AMF in plant disease (Ross, 1972; Graham and Menge, 1992; Dehne, 1982; Krishna and Bagyaraj, 1983; Zambolim and Schenck, 1983; Hedge and Rai, 1984; Vigo et al., 2000; Yao et al., 2002). In fact, there appears to be a range of mycorrhizal effects from positive or negative to neutral, commensal, or amensal (Johnson et al., 1997). The impact of AMF must be evaluated, whether the result is positive or negative, within each plant system so that further research can be undertaken to determine the best strategies for maximizing their benefits or for minimizing their damage in the ecology of the cropping system (Gerdemann, 1975).

Prior to these studies, there was no information concerning St. Augustinegrass and the role of AMF in sod production, or even if there was a mycorrhizal association between the two types of organisms. In Chapter 1, an overview of past and present research objectives concerning AMF and their role in various hosts was highlighted for the purpose of detailing their potential effects and to report on the vast amount of information from previous research studies. In Chapter 2, a survey of three St.

Augustinegrass sod farms in north central Florida revealed a moderate level of AMF colonization as well as a diverse population of arbuscular mycorrhizal fungi. There was no correlation between AMF spore density and percent colonization of the St. Augustinegrass plants, or to soil P levels, as previously documented in other crops (Hayman and Stovold, 1979; Giovannetti and Nicolson, 1983; Medina-Gonzalez et al., 1998).

In these soils, there was a correlation to soil moisture and temperature. Spore density and percent colonization fluctuated in relation to soil moisture with spore density tending to decrease at temperature above 28 C and soil moisture levels above 7 cm. The overall trend of percent AMF colonization was to decrease during warmer months increase in cooler weather; however, there was no highly correlated response to soil moisture levels or temperature observed. Based on this survey, AMF prefer warmer months for spore production and cooler months for colonization in these soils, perhaps due to physiological effects of seasonal change on the host plant that leave the plant more susceptible to colonization during less than optimal growing conditions. These results suggest a potentially harmful role for AMF in St. Augustinegrass based on the continuum of mycorrhizal symbiosis proposed by Johnson et al. (1997) and the fact that AMF colonization is highest when St. Augustinegrass is least active increasing carbon depletion in the relationship.

In Chapter 3, a survey of the amount of *Rhizoctonia solani* and *Gaeumannomyces graminis* var. *graminis* colonization in St. Augustinegrass was documented in an effort to highlight the importance of evaluating the potential benefits of AMF on disease severity in this plant system. In the field, no correlation was observed between AMF spore

density or percent colonization of the plants in relation to either of the pathogens. However, disease severity did vary greatly for each pathogen based on seasonal variations. Results suggest *R. solani* colonizes St. Augustinegrass at higher rates in cooler weather, as do the arbuscular mycorrhizal fungi in the field soils surveyed in Chapter 2. This observation suggests a greater potential role for AMF in lowering brown patch disease severity since both the beneficial fungi and the pathogen are active during the same seasons, which happens to be the time when St. Augustinegrass is under the most seasonal stress. Conversely, the rate of *G. graminis* var. *graminis* colonization was highest in the survey during warmer months. During this time period AMF spore density was highest, but percent colonization of the plant was lowest. This finding suggests less of a potentially beneficial role for AMF in lowering take-all root rot disease severity since the AMF and pathogen are not most active in the same season.

Based on the findings presented in Chapters 2 and 3, it was pertinent to evaluate the potential effects of AMF on brown patch and take-all root rot disease severity in a more controlled environment in order to evaluate the interaction more thoroughly. Any role that AMF might have in soilborne pathogen disease severity whether positive or negative is important to document since the mycorrhizal interaction might be manipulated in a field situation to lower disease severity and possibly fungicide use and cost. In Chapter 4, both direct and unique indirect assays were designed to investigate the role of AMF in controlled growth room experiments where *R. solani* and *G. graminis* var. *graminis* were challenged by the common AMF, *Glomus intraradices*. In the direct experiments, no correlation between both pathogens and *G. intraradices* were observed, which suggests limited impact of AMF in a direct interaction. Apparently, AMF were

not producing antimicrobial metabolites, occupying infection courts, or improving plant health enough to reduce brown patch or take-all root rot disease severity in a highly controlled environment as suggested in previous studies (Becker, 1976; Dehne and Schoenbeck, 1978; Schoenbeck, 1979). Furthermore, the indirect assay using a split-rooted sprig system where *G. intraradices* was used to challenge *R. solani* and *G. graminis* var. *graminis* in separate conetainers revealed no correlation between AMF colonization and disease severity in the case of either pathogen. Based on the results of this experiment, there is no systemic defense response afforded to the St. Augustinegrass plant by AMF colonization.

While neither the direct nor indirect experiments revealed a positive role for AMF in St. Augustinegrass root disease severity, the evaluations did provide valuable information about AMF that was previously unknown. Arbuscular mycorrhizal fungi do colonize St. Augustinegrass with a diversity of species, but the relationship appears to be neutral role in this species. Based on this information, the focus of future research on AMF in St. Augustinegrass sod should involve a thorough evaluation of AMF species and their individual effects on the host. Additionally, field trials designed to evaluate various sod management strategies and their effects on AMF for the purpose of manipulating the symbiosis into a mutually beneficial relationship would be worthwhile. While no positive effects of AMF on disease severity were observed in these studies, the potential for reduced pesticide use and cost with the use of mycorrhizae justifies further evaluations.

APPENDIX A
SELECTIVE MEDIA RECIPES FOR ISOLATION OF *G. GRAMINIS* VAR. *GRAMINIS* AND *R. SOLANI* FROM PLANT TISSUE

A. Semi-selective media recipe for isolation of *G. graminis* var. *graminis* from plant tissue (Gooch, 2002).

    a. 500 ml deionized water in 1000 ml Erlenmeyer flask
    b. 4.8 g PDA (potato dextrose agar) – (Difco Laboratories, Inc., Detroit, Michigan)
    c. 2 g solidifying agar – Difco Laboratories, Inc.
    d. Autoclave for 20 min at 121 C and 15 psi
    e. Amend with:
        a. 0.01 g rifampicin
        b. 0.01 g streptomycin sulfate

B. Semi-selective media recipe for isolation of *R. solani* from plant tissue (Adapted from Adams and Butler, 1983).

    a. 500 ml deionized water in 1000 ml Erlenmeyer flask
    b. 3.8 g granulated agar – Difco Laboratories, Inc.
    c. 0.5 g KH_2PO_4
    d. 1 ml $MgSO_4.7H_2O$
    e. Autoclave for 20 min at 121 C and 15 psi
    f. Amend with:
        a. 0.35 g neomycin sulfate
        b. 0.1 g casein hydrolsyate
        c. 1 ml Benlate
        d. 1 ml tannic acid
        e. 2 drops Ridomil (metalayxyl)

APPENDIX B

NUTRIENT SOLUTION (20-0-20) USED IN DIRECT AND INDIRECT TRIALS DESCRIBED IN CHAPTER IV

Total N = 20 %

1.97 % Nitrate N

18.03 % Urea N

Soluble Potash (K_2O) = 20 %

1. $MgSO_4 \cdot 7H_2O$ (0.34 mg)
2. $CuSO_4 \cdot 5H_2O$ (0.1 mg)
3. Fe-EDTA (150 mg)
4. $MnSO_4 \cdot H_2O$ (0.05 mg)
5. $(NH_4)_4\ Mo_7O_{24} \cdot 4H_2O$ (400 mg)
6. $ZnSO_4 \cdot 7H_2O$ (0.6 mg)
7. KNO_3 (190 mg)
8. $Ca\ (NO_3)_2 \cdot 4H_2O$ (50 mg)
9. NaCl (1.0 mg)

*All contained in 1 litre of water

APPENDIX C
RHIZOCTONIA SOLANI AND *G. GRAMINIS* VAR. *GRAMINIS* INOCULUM PRODUCTION PROTOCOLS

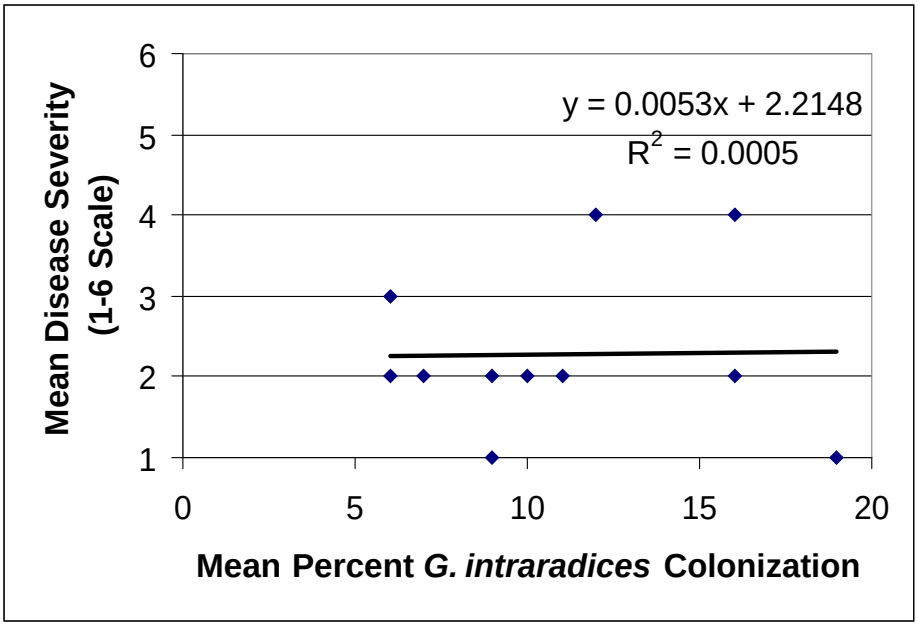
A. Sterile substrate inoculation with JK2 (*G. graminis* var. *graminis*) isolate (Adapted from Datnoff and Elliott, 1997).

a. 250 ml perennial ryegrass (BrightStar II) seed/500 ml wide-mouth Erlenmeyer flask
b. 125 ml deionized water/flask
c. Autoclave substrate two consecutive days for 90 min/d at 121 C and 15 psi
d. Aseptically chop 7 d old Petri dish of JK1 *G. graminis* var. *graminis* isolate and mix into sterile substrate in flask with 30 ml sterile deionized water
e. Incubate substrate at 25 C for four weeks with 24 h darkness
f. Shake flasks twice weekly to prevent substrate packing

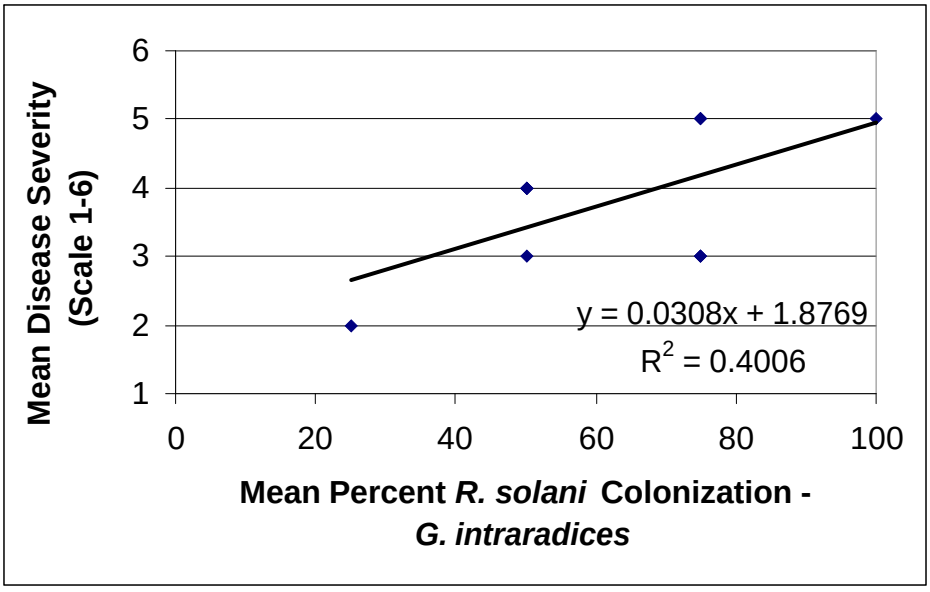
B. Sterile substrate inoculation with PDC 7884 (*R. solani)* isolate (Adapted from Sneh et al., 1991 and Gaskill, 1968).

a. 25 g oat seed/250 ml Erlenmeyer flask
b. 25-30 ml deionized water; soak overnight
c. Autoclave substrate three consecutive days for 90 min/day at 121 C and 15 psi
d. Once cooled, inoculate flask with three to four 7 mm plugs of actively growing *R. solani* mycelium
e. Incubate substrate at 25-30 C for two to three weeks
f. Shake flasks to loosen seeds and prevent packing
g. After three weeks incubation, pour seed into sterile Petri dishes; allow to air dry uncovered for two weeks
h. Store in sterile vials at 4 C until inoculation

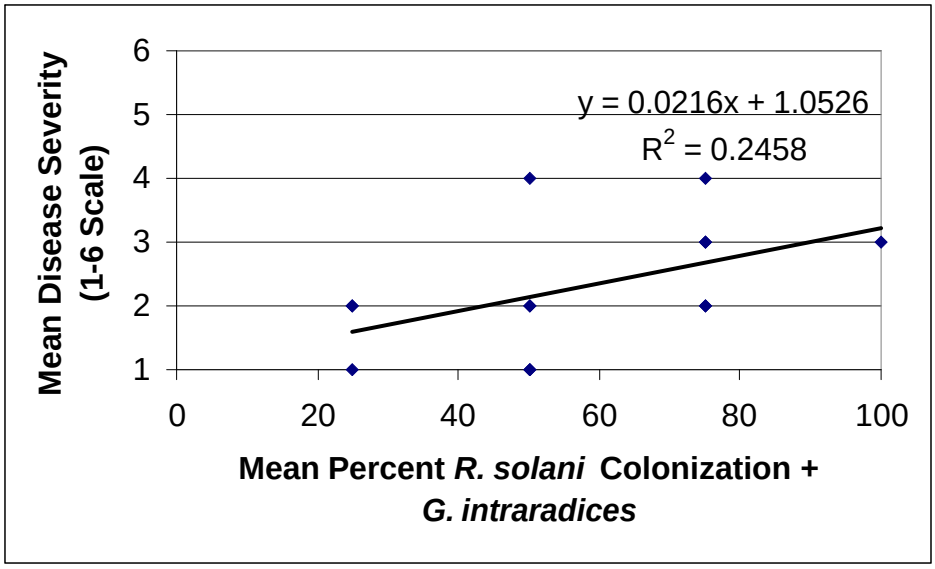
APPENDIX D
ADDITIONAL DATA ANALYSIS RESULTS REFERENCED IN CHAPTER IV
DIRECT EXPERIMENTS



Appendix D-1. The direct effect of *G. intraradices* colonization on take-all root rot disease severity in 'Floratam' St. Augustinegrass. Values represent the mean of three trials with four replicates/trial.

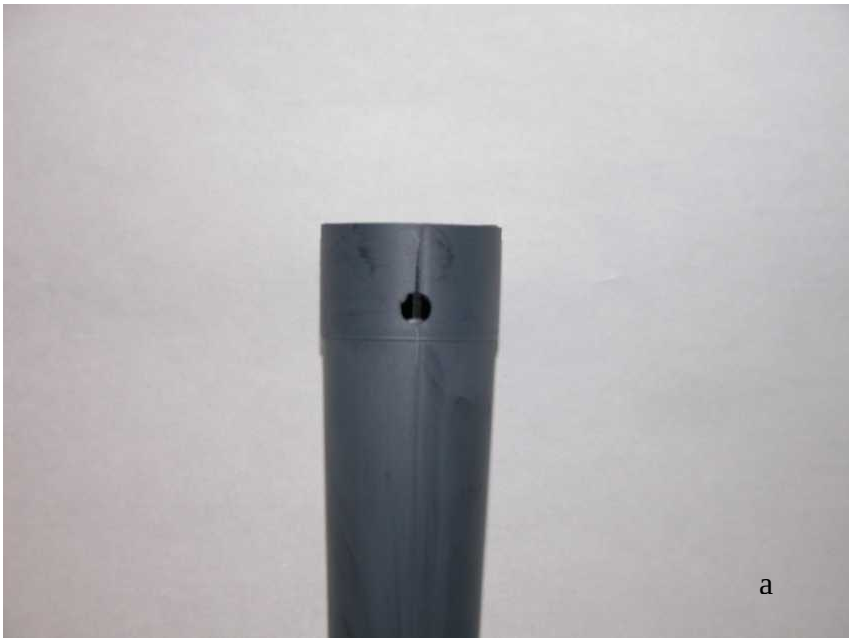


Appendix D-2. The relationship between *R. solani* colonization and brown patch disease severity in 'Floratam' St. Augustinegrass. Values represent the means of three trials with four replicates/trial.

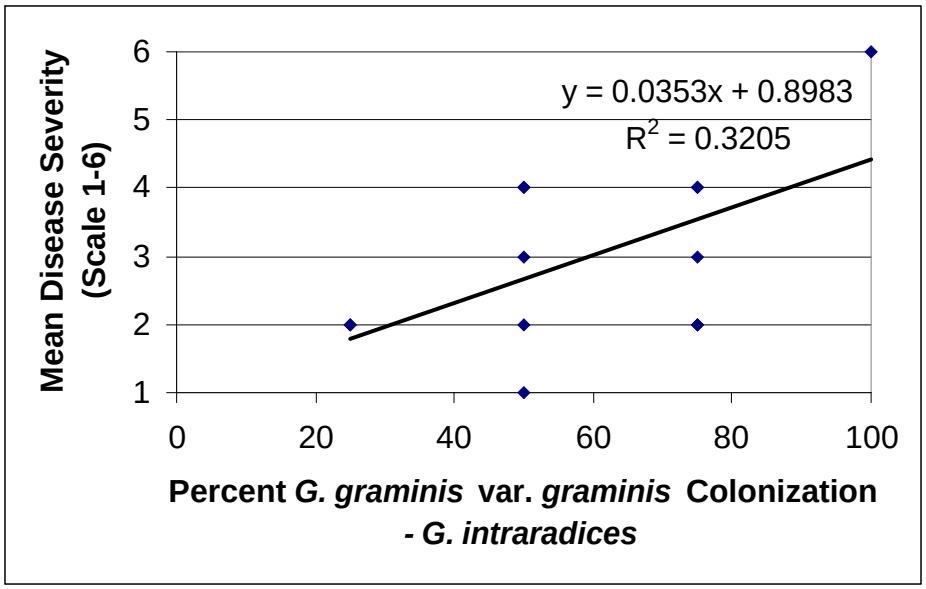


Appendix D-3. The relationship between *R. solani* colonization and *G. intraradices* on brown patch disease severity in 'Floratam' St. Augustinegrass. Values represent the means of three trials with four replicates/trial.

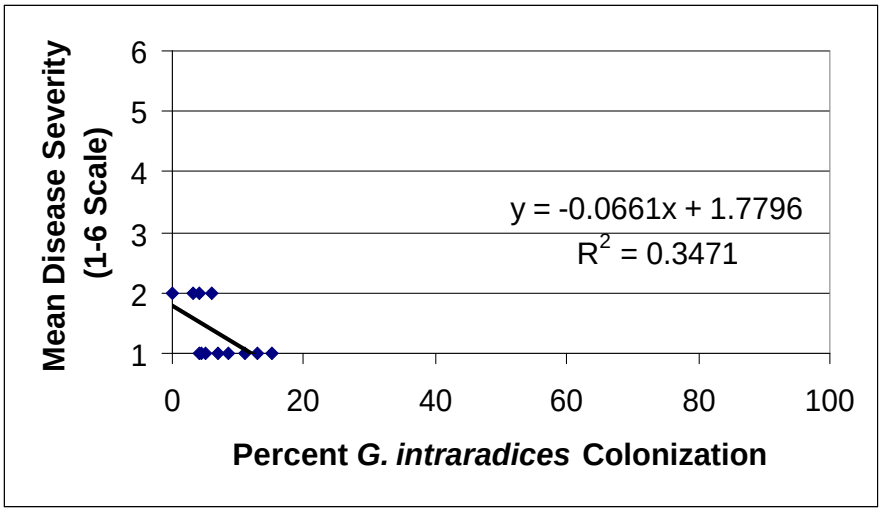
APPENDIX E
ADDITIONAL DATA ANALYSIS RESULTS REFERENCED IN CHAPTER IV
INDIRECT EXPERIMENTS



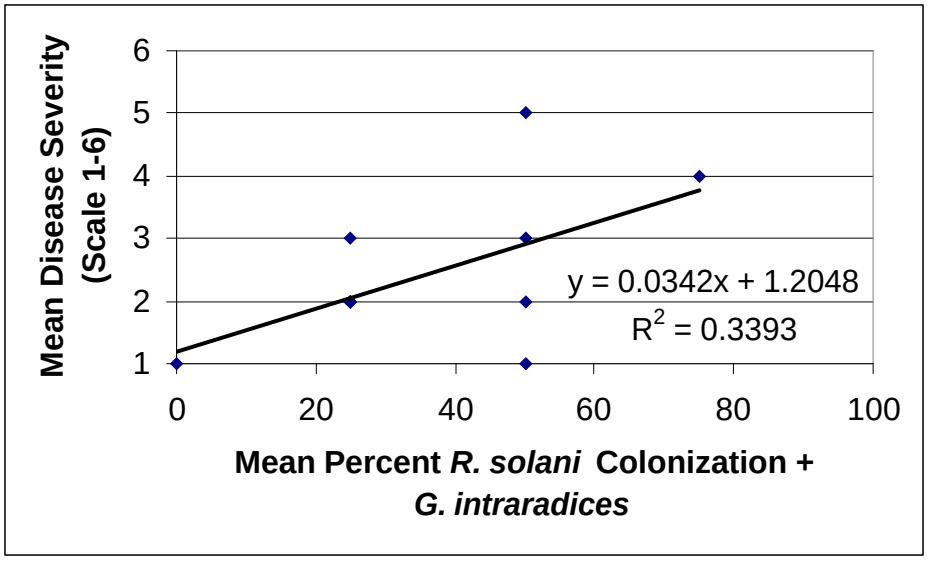
Appendix E-1. Photograph depicting a conetainer used in the indirect experiment with drilled hole and cut to allow for sprig to be inserted without tissue damage.



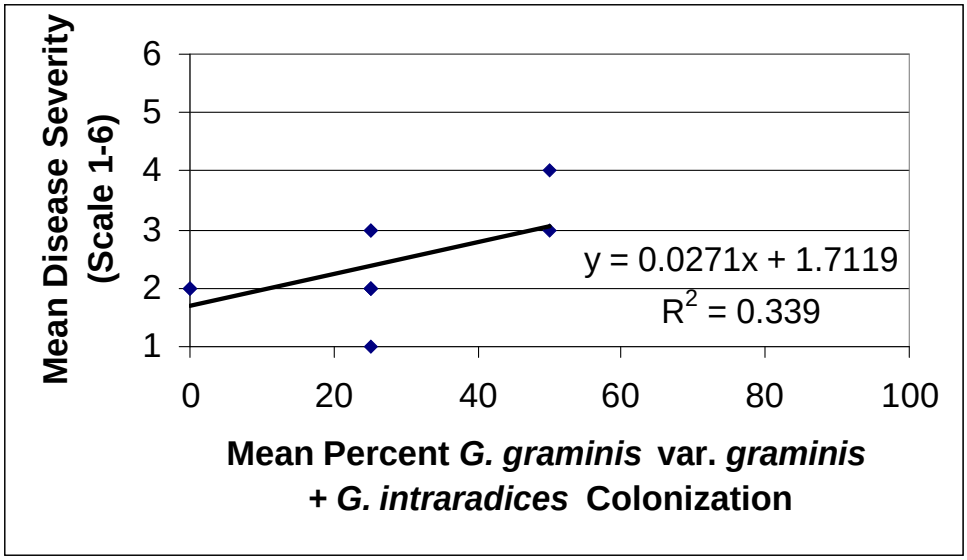
Appendix E-2. The indirect effect of *G. graminis* var. *graminis* on take-all root rot diease severity in 'Floratam' St. Augustinegrass without *G. intraradices*. Values represent the means of three trials with four replicates/trial on an adjacent split sprig system.



Appendix E-3. The effect of *Glomus intraradices* colonization on brown patch and take-all root rot disease severity in 'Floratam' St.Augustinegrass on plants in the split sprig assay. Values represent the means of three trials with four replicates/trial.



Appendix E-4. The indirect effect of *R. solani* on disease severity in 'Floratam' St. Augustinegrass with *G. intraradices* on an adjacent split sprig system. Values represent the means of three trials with four replicates/trial.



Appendix E-5. The indirect effect of *G. graminis var. graminis* on disease severity in 'Floratam' St. Augustinegrass with *G. intraradices*. Values represent the means of three trials with four replicates/trial.

APPENDIX F
ANALYSIS OF VARIANCE TABLES FOR CHAPTERS 2, 3, AND 4

The SAS System 22:22 Wednesday, July 19, 2006 2

The GLM Procedure

Dependent Variable: sporeden

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	41	38.55619444	0.94039499	3.94	<.0001
Error	66	15.73407222	0.23839503		
Corrected Total	107	54.29026667			

R-Square	Coeff Var	Root MSE	sporeden Mean
0.710186	8.054095	0.488257	6.062222

Source	DF	Type I SS	Mean Square	F Value	Pr > F
date	11	28.08166667	2.55287879	10.71	<.0001
location	2	0.49388889	0.24694444	1.04	0.3606
rep(location)	6	1.21259444	0.20209907	0.85	0.5379
rainfall	1	0.02743228	0.02743228	0.12	0.7355
soiltemp	1	0.00744901	0.00744901	0.03	0.8602
date*location	20	8.73316316	0.43665816	1.83	0.0349

Source	DF	Type III SS	Mean Square	F Value	Pr > F
date	11	12.88105106	1.17100464	4.91	<.0001
location	2	0.52131593	0.26065796	1.09	0.3411
rep(location)	6	1.21259444	0.20209907	0.85	0.5379
rainfall	0	0.00000000	.	.	.
soiltemp	0	0.00000000	.	.	.
date*location	20	8.73316316	0.43665816	1.83	0.0349

Tests of Hypotheses Using the Type III MS for date*location as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
date	11	12.88105106	1.17100464	2.68	0.0267

The GLM Procedure

Tukey's Studentized Range (HSD) Test for sporeden

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	66
Error Mean Square	0.238395
Critical Value of Studentized Range	4.79129
Minimum Significant Difference	0.7798

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	date
A	6.7344	9	May
A	6.6622	9	June
A	6.5511	9	April
A	6.5100	9	Nov
B A	6.2689	9	August
B A	6.1467	9	Oct
B A C	6.0522	9	Sept
B A C	5.9922	9	5-Dec
B C	5.7244	9	March
B D C	5.7189	9	Feb
D C	5.3800	9	Jan
D	5.0056	9	4-Dec

The SAS System 22:22 Wednesday, July 19, 2006 5

The GLM Procedure

Dependent Variable: percolon

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	41	1744.970096	42.560246	3.71	<.0001
Error	66	757.324989	11.474621		
Corrected Total	107	2502.295085			

R-Square	Coeff Var	Root MSE	percolon Mean
0.697348	13.89690	3.387421	24.37537

Source	DF	Type I SS	Mean Square	F Value	Pr > F
date	11	1410.787241	128.253386	11.18	<.0001
location	2	4.613424	2.306712	0.20	0.8184
rep(location)	6	23.386811	3.897802	0.34	0.9134
rainfall	1	11.152745	11.152745	0.97	0.3278
soiltemp	1	7.560355	7.560355	0.66	0.4199
date*location	20	287.469520	14.373476	1.25	0.2430

Source	DF	Type III SS	Mean Square	F Value	Pr > F
date	11	1315.392404	119.581128	10.42	<.0001
location	2	5.718640	2.859320	0.25	0.7802
rep(location)	6	23.386811	3.897802	0.34	0.9134
rainfall	0	0.000000	.	.	.
soiltemp	0	0.000000	.	.	.
date*location	20	287.469520	14.373476	1.25	0.2430

Tests of Hypotheses Using the Type III MS for date*location as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
date	11	1315.392404	119.581128	8.32	<.0001

The SAS System 22:22 Wednesday, July 19, 2006 6

The GLM Procedure

Tukey's Studentized Range (HSD) Test for percolon

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	66
Error Mean Square	11.47462
Critical Value of Studentized Range	4.79129
Minimum Significant Difference	5.41

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	date
A	29.007	9	Jan
A	28.854	9	Feb
A	28.584	9	April
B A	26.731	9	May
B A	26.133	9	4-Dec
B A	25.923	9	June
B A C	24.849	9	March
B C	22.636	9	August
B D C	21.680	9	Sept
D C	19.914	9	Nov
D C	19.504	9	Oct
D	18.688	9	5-Dec

The SAS System 22:22 Wednesday, July 19, 2006 7

The CORR Procedure

2 Variables: percolon sporeden

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum
percolon	108	24.37537	4.83590	2633	12.71000	38.32000
sporeden	108	6.06222	0.71231	654.72000	4.36000	7.66000

Pearson Correlation Coefficients, N = 108
Prob > |r| under H0: Rho=0

	percolon	sporeden
percolon	1.00000	-0.00758 0.9380
sporeden	-0.00758 0.9380	1.00000

The SAS System 22:22 Wednesday, July 19, 2006 8

The CORR Procedure

8 Variables: percolon soiltemp percolon rainfall sporeden soiltemp sporeden rainfall

Simple Statistics

Variable	N	Mean	Std Dev	Sum	Minimum	Maximum
percolon	108	24.37537	4.83590	2633	12.71000	38.32000
soiltemp	108	3.74222	2.35241	404.16000	0	8.69000
percolon	108	24.37537	4.83590	2633	12.71000	38.32000
rainfall	108	25.23417	6.55045	2725	11.77000	34.54000
sporeden	108	6.06222	0.71231	654.72000	4.36000	7.66000
soiltemp	108	3.74222	2.35241	404.16000	0	8.69000
sporeden	108	6.06222	0.71231	654.72000	4.36000	7.66000
rainfall	108	25.23417	6.55045	2725	11.77000	34.54000

Pearson Correlation Coefficients, N = 108
Prob > |r| under H0: Rho=0

	percolon	soiltemp	percolon	rainfall	sporeden	soiltemp	sporeden	rainfall
percolon	1.00000	0.02488	1.00000	-0.14948	-0.00758	0.02488	-0.00758	-0.14948
		0.7983		0.1226	0.9380	0.7983	0.9380	0.1226
soiltemp	0.02488	1.00000	0.02488	0.61090	0.48819	1.00000	0.48819	0.61090
	0.7983		0.7983	<.0001	<.0001		<.0001	<.0001
percolon	1.00000	0.02488	1.00000	-0.14948	-0.00758	0.02488	-0.00758	-0.14948

		0.7983		0.1226	0.9380	0.7983	0.9380
0.1226							
rainfall	-0.14948	0.61090	-0.14948	1.00000	0.45921	0.61090	0.45921
1.00000							
	0.1226	<.0001	0.1226		<.0001	<.0001	<.0001
sporeden	-0.00758	0.48819	-0.00758	0.45921	1.00000	0.48819	1.00000
0.45921							
	0.9380	<.0001	0.9380	<.0001		<.0001	
<.0001							
soiltemp	0.02488	1.00000	0.02488	0.61090	0.48819	1.00000	0.48819
0.61090							
	0.7983		0.7983	<.0001	<.0001		<.0001
<.0001							
sporeden	-0.00758	0.48819	-0.00758	0.45921	1.00000	0.48819	1.00000
0.45921							
	0.9380	<.0001	0.9380	<.0001		<.0001	
<.0001							
rainfall	-0.14948	0.61090	-0.14948	1.00000	0.45921	0.61090	0.45921
1.00000							
	0.1226	<.0001	0.1226		<.0001	<.0001	<.0001

Appendix F-1. Analysis of variance tables for spore density and percent colonization data in Chapter 2, and Pearson's product moment correlation coefficients for attempted correlations between variables and soil chemical characteristics and soil moisture and soil temperature.

The SAS System 22:13 Wednesday, July 19, 2006 2

The GLM Procedure

Dependent Variable: rsolani

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	28	23746.3116	848.0826	1.98	0.0023
Error	559	240032.3874	429.3960		
Corrected Total	587	263778.6990			

R-Square	Coeff Var	Root MSE	rsolani Mean
0.090024	120.3404	20.72187	17.21939

Source	DF	Type I SS	Mean Square	F Value	Pr > F
date	6	13979.59184	2329.93197	5.43	<.0001
location	2	1001.27551	500.63776	1.17	0.3124
rep(location)	6	644.48696	107.41449	0.25	0.9592
amfcolon	1	818.37429	818.37429	1.91	0.1680
amfsd	1	132.41846	132.41846	0.31	0.5789
date*location	12	7170.16457	597.51371	1.39	0.1653

Source	DF	Type III SS	Mean Square	F Value	Pr > F
date	6	13183.23554	2197.20592	5.12	<.0001
location	2	634.32230	317.16115	0.74	0.4782
rep(location)	6	564.06333	94.01055	0.22	0.9707
amfcolon	1	39.58906	39.58906	0.09	0.7615
amfsd	1	3.47586	3.47586	0.01	0.9283
date*location	12	7170.16457	597.51371	1.39	0.1653

Tests of Hypotheses Using the Type III MS for date*location as an Error Term

Source	DF	Type III SS	Mean Square	F Value	Pr > F
date	6	13183.23554	2197.20592	3.68	0.0262

The SAS System 22:13 Wednesday, July 19, 2006 3

The GLM Procedure

Tukey's Studentized Range (HSD) Test for rsolani

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	559
Error Mean Square	429.396
Critical Value of Studentized Range	4.18483
Minimum Significant Difference	9.4616

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	date
A	24.405	84	29-Dec
A			
B A	22.024	84	29-Apr
B A			
B A C	19.940	84	19-Oct
B A C			
B A C	17.560	84	5-Jan
B C			
B C	14.583	84	31-Mar
C			
C	11.310	84	31-Aug
C			
C	10.714	84	21-Jun

Appendix F-2. Analysis of variance tables for *Rhizoctonia solani* percent colonization data in Chapter 3.

The SAS System 22:07 Wednesday, July 19, 2006 1

The REG Procedure
Model: MODEL1
Dependent Variable: dissev

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	14.08211	14.08211	27.28	0.0005
Error	9	4.64516	0.51613		
Corrected Total	10	18.72727			

Root MSE	0.71842	R-Square	0.7520
Dependent Mean	3.54545	Adj R-Sq	0.7244
Coeff Var	20.26316		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.09677	0.69486	0.14	0.8923
rsolcolon	1	0.06323	0.01210	5.22	0.0005

The SAS System 22:07 Wednesday, July 19, 2006 3

The REG Procedure
Model: MODEL1
Dependent Variable: dissev

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	7.05085	7.05085	5.19	0.0437
Error	11	14.94915	1.35901		
Corrected Total	12	22.00000			

Root MSE	1.16577	R-Square	0.3205
Dependent Mean	3.00000	Adj R-Sq	0.2587
Coeff Var	38.85892		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.89831	0.97771	0.92	0.3779
gggcolon	1	0.03525	0.01548	2.28	0.0437

The SAS System 22:07 Wednesday, July 19, 2006 5

The REG Procedure
Model: MODEL1
Dependent Variable: dissev

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.92562	0.92562	5.32	0.0438
Error	10	1.74105	0.17410		
Corrected Total	11	2.66667			

Root MSE	0.41726	R-Square	0.3471
Dependent Mean	1.33333	Adj R-Sq	0.2818
Coeff Var	31.29439		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.77961	0.22797	7.81	<.0001
amfcolon	1	-0.06612	0.02867	-2.31	0.0438

The SAS System 22:07 Wednesday, July 19, 2006 7

The REG Procedure
Model: MODEL1
Dependent Variable: dissev

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	5.06124	5.06124	5.14	0.0469
Error	10	9.85542	0.98554		
Corrected Total	11	14.91667			

Root MSE	0.99274	R-Square	0.3393
Dependent Mean	2.41667	Adj R-Sq	0.2732
Coeff Var	41.07909		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.20482	0.60671	1.99	0.0751
rsolcolon	1	0.03422	0.01510	2.27	0.0469

The SAS System 22:07 Wednesday, July 19, 2006 9

The REG Procedure
Model: MODEL1
Dependent Variable: dissev

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2.25989	2.25989	5.13	0.0470
Error	10	4.40678	0.44068		
Corrected Total	11	6.66667			

Root MSE	0.66384	R-Square	0.3390
Dependent Mean	2.33333	Adj R-Sq	0.2729
Coeff Var	28.45011		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.71186	0.33472	5.11	0.0005
gggcolon	1	0.02712	0.01198	2.26	0.0470

The SAS System 22:07 Wednesday, July 19, 2006 12

The GLM Procedure

Dependent Variable: ggg

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	24	16766.7893	698.6162	3.32	<.0001
Error	563	118364.1273	210.2382		
Corrected Total	587	135130.9167			

R-Square	Coeff Var	Root MSE	ggg Mean
0.124078	102.9557	14.49959	14.08333

Source	DF	Type I SS	Mean Square	F Value	Pr > F
date	6	12105.21429	2017.53571	9.60	<.0001
location	2	501.73810	250.86905	1.19	0.3040
rep	2	81.93466	40.96733	0.19	0.8230
amfcolon	1	57.95802	57.95802	0.28	0.5998
amfsd	1	1.82548	1.82548	0.01	0.9258
date*location	12	4018.11879	334.84323	1.59	0.0895

Source	DF	Type III SS	Mean Square	F Value	Pr > F
date	6	9889.269923	1648.211654	7.84	<.0001
location	2	570.223651	285.111825	1.36	0.2585
rep	2	73.695910	36.847955	0.18	0.8393
amfcolon	1	98.114370	98.114370	0.47	0.4948
amfsd	1	174.670045	174.670045	0.83	0.3624
date*location	12	4018.118793	334.843233	1.59	0.0895

The SAS System 22:07 Wednesday, July 19, 2006 13

The GLM Procedure

Tukey's Studentized Range (HSD) Test for ggg

NOTE: This test controls the Type I experimentwise error rate, but it generally has a higher Type II error rate than REGWQ.

Alpha	0.05
Error Degrees of Freedom	563
Error Mean Square	210.2382
Critical Value of Studentized Range	4.18472
Minimum Significant Difference	6.6204

Means with the same letter are not significantly different.

Tukey Grouping	Mean	N	date
A	20.012	84	31-Aug
A			
A	19.345	84	21-Jun
A			
B A	14.881	84	19-Oct
B A			
B A	13.393	84	31-Mar
B A			
B A	13.393	84	29-Apr
B			
B	12.202	84	5-Jan
C	5.357	84	29-Dec

Appendix F-2. Analysis of variance tables for *Gaeumannomyces graminis* var. *graminis* percent colonization data in Chapter 3.

The SAS System 22:16 Wednesday, July 19, 2006 1

The REG Procedure
Model: MODEL1
Dependent Variable: dissev

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	3.57143	3.57143	3.13	0.1075
Error	10	11.42857	1.14286		
Corrected Total	11	15.00000			

Root MSE	1.06904	R-Square	0.2381
Dependent Mean	3.50000	Adj R-Sq	0.1619
Coeff Var	30.54414		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.71429	1.05624	1.62	0.1357
rsolcolon	1	0.02857	0.01616	1.77	0.1075

The REG Procedure
Model: MODEL1
Dependent Variable: dissev

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	7.07065	7.07065	4.39	0.0600
Error	11	17.69858	1.60896		
Corrected Total	12	24.76923			

Root MSE	1.26845	R-Square	0.2855
Dependent Mean	2.69231	Adj R-Sq	0.2205
Coeff Var	47.11381		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.63830	0.61365	2.67	0.0218
gggcolon	1	0.02284	0.01089	2.10	0.0600

The SAS System 22:16 Wednesday, July 19, 2006 5

The REG Procedure
Model: MODEL1
Dependent Variable: dissev

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00526	0.00526	0.00	0.9471
Error	9	10.17656	1.13073		
Corrected Total	10	10.18182			

Root MSE	1.06336	R-Square	0.0005
Dependent Mean	2.27273	Adj R-Sq	-0.1105
Coeff Var	46.78771		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	2.21483	0.90713	2.44	0.0373
amfcolon	1	0.00526	0.07714	0.07	0.9471

The SAS System 22:16 Wednesday, July 19, 2006 8

The REG Procedure
Model: MODEL1
Dependent Variable: wgtai

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.50760	0.50760	3.02	0.1130
Error	10	1.68209	0.16821		
Corrected Total	11	2.18969			

Root MSE	0.41013	R-Square	0.2318
Dependent Mean	0.85583	Adj R-Sq	0.1550
Coeff Var	47.92203		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.52905	0.40522	3.77	0.0036
rsolcolon	1	-0.01077	0.00620	-1.74	0.1130

The SAS System 22:16 Wednesday, July 19, 2006 10

The REG Procedure
Model: MODEL1
Dependent Variable: wgtai

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00729	0.00729	0.03	0.8644
Error	11	2.62282	0.23844		
Corrected Total	12	2.63011			

Root MSE	0.48830	R-Square	0.0028
Dependent Mean	1.00615	Adj R-Sq	-0.0879
Coeff Var	48.53145		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.04000	0.23623	4.40	0.0011
gggcolon	1	-0.00073333	0.00419	-0.17	0.8644

The SAS System 22:16 Wednesday, July 19, 2006 12

The REG Procedure
Model: MODEL1
Dependent Variable: wgtai

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00796	0.00796	0.03	0.8576
Error	9	2.10153	0.23350		
Corrected Total	10	2.10949			

Root MSE	0.48322	R-Square	0.0038
Dependent Mean	0.95091	Adj R-Sq	-0.1069
Coeff Var	50.81681		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.02212	0.41223	2.48	0.0350
amfcolon	1	-0.00647	0.03506	-0.18	0.8576

Appendix F-4. Analysis of variance tables for the direct assay in the split-sprig challenge including *Gaeumannomyces graminis* var. *graminis* and *Rhizoctonia solani* data in Chapter 4.

The SAS System 22:14 Wednesday, July 19, 2006 1

The REG Procedure
Model: MODEL1
Dependent Variable: dissev

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	14.08211	14.08211	27.28	0.0005
Error	9	4.64516	0.51613		
Corrected Total	10	18.72727			

Root MSE	0.71842	R-Square	0.7520
Dependent Mean	3.54545	Adj R-Sq	0.7244
Coeff Var	20.26316		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.09677	0.69486	0.14	0.8923
rsolcolon	1	0.06323	0.01210	5.22	0.0005

The SAS System 22:14 Wednesday, July 19, 2006 3

The REG Procedure
Model: MODEL1
Dependent Variable: dissev

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	7.05085	7.05085	5.19	0.0437
Error	11	14.94915	1.35901		
Corrected Total	12	22.00000			

Root MSE	1.16577	R-Square	0.3205
Dependent Mean	3.00000	Adj R-Sq	0.2587
Coeff Var	38.85892		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	0.89831	0.97771	0.92	0.3779
gggcolon	1	0.03525	0.01548	2.28	0.0437

The SAS System 22:14 Wednesday, July 19, 2006 5

The REG Procedure
Model: MODEL1
Dependent Variable: dissev

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.92562	0.92562	5.32	0.0438
Error	10	1.74105	0.17410		
Corrected Total	11	2.66667			

Root MSE	0.41726	R-Square	0.3471
Dependent Mean	1.33333	Adj R-Sq	0.2818
Coeff Var	31.29439		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.77961	0.22797	7.81	<.0001
amfcolon	1	-0.06612	0.02867	-2.31	0.0438

The SAS System 22:14 Wednesday, July 19, 2006 7

The REG Procedure
Model: MODEL1
Dependent Variable: dissev

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	5.06124	5.06124	5.14	0.0469
Error	10	9.85542	0.98554		
Corrected Total	11	14.91667			

Root MSE	0.99274	R-Square	0.3393
Dependent Mean	2.41667	Adj R-Sq	0.2732
Coeff Var	41.07909		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.20482	0.60671	1.99	0.0751
rsolcolon	1	0.03422	0.01510	2.27	0.0469

The SAS System 22:14 Wednesday, July 19, 2006 9

The REG Procedure
Model: MODEL1
Dependent Variable: dissev

Analysis of Variance

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2.25989	2.25989	5.13	0.0470
Error	10	4.40678	0.44068		
Corrected Total	11	6.66667			

Root MSE	0.66384	R-Square	0.3390
Dependent Mean	2.33333	Adj R-Sq	0.2729
Coeff Var	28.45011		

Parameter Estimates

Variable	DF	Parameter Estimate	Standard Error	t Value	Pr > |t|
Intercept	1	1.71186	0.33472	5.11	0.0005
gggcolon	1	0.02712	0.01198	2.26	0.0470

Appendix F-4. Analysis of variance tables for the indirect assay in the split-sprig challenge including *Gaeumannomyces graminis* var. *graminis* and *Rhizoctonia solani* data in Chapter 4.

LIST OF REFERENCES

Abbott, L.K. and A.D. Robson. 1977. The distribution and abundance of vesicular-arbuscular endophytes in some Western Australian soils. Aust. J. Bot. 25:515-522.

Adams, G.C. and E.E. Butler. 1983. Influence of nutrition on the formation of basidia and basidiospores in *Thanatephorus cucumeris*. Phytopathol. 73:147-151.

Agrios, G.N. Plant Pathology 5th Edition. Academic Press. San Diego, CA. 2004.

Ainsworth, G.C., Sparrow, F.K., and A.S. Sussman. 1973. The fungi, an advanced treatise. Vol. IV B. Academic Press, New York and London. p 504.

Angle, J.S. and R.J. Heckman. 1986. Effect of soil pH and sewage sludge on VA mycorrhizal infection of soybeans. Plant and Soil. 93:437-441.

Auge, R.M., Schekel, K.A., and B.L. Wample. 1986. Greater leaf conductance of well-watered VA mycorrhizal rose plants is not related to P nutrition. New Phytol. 103:107-116.

Azcón-Aguilar, C. and J.M. Barea. 1996. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens – an overview of the mechanisms involved. Mycorrhiza. 6:457-464.

Bagyaraj, D.J. 1991. Ecology of vesicular-arbuscular mycorrhizae. Handbook of Applied Mycology. *In*: Arora, D.K., Rai, B., Mukerji, K.G. and G.R. Knudsen (eds.) Vol. I Soil and Plants. Marcel Dekker, Inc., New York. p 3-34.

Bagyaraj, D.J., Manjunath, A., and R. Mohan, 1980. Root length in relation to endomycorrhizal infection in grasses. Mysore J. Agric. Sci. 14: 318-320.

Baltruschat, H., Sikora, R.A., and F. Schoenbeck. 1973. Effect of VA mycorrhizae (*Endogone mosseae*) on the establishment of *Thielaviopsis basicola* and *Meloidogyne incognita* in tobacco. 2nd Inter. Congr. Plant Path. Abstr. No. 0661.

Barrett, J.T. 1958. Synthesis of mycorrhiza with pure cultures of *Rhizophagus*. Phytopathol. 48:391.

Bastmeyer, M., Deising, H.B., and C. Bechinger. 2002. Force exertion in fungal infection. Ann. Rev. Biophys. Biomol. Struct. 31:321-341.

Baylis, G.T.S. 1959. The effect of vesicular-arbuscular mycorrhizas on growth of *Griselinia littoralis* (Cornaceae). New Phytol. 58:274-280.

Baylis, G.T.S. 1967. Experiments on the ecological significance of phycomycetous mycorrhizas. New Phytol. 66:231-243.

Baylis, G.T.S. 1975. The magnolioid mycorrhizal and mycotrophy in root systems derived from it. *In*: Sander, F.E., Mosse, B., and B.B. Tinker (eds.) Endomycorrhizas. Academic Press, London. p 373-389.

Becker, W.N. 1976. Quantification of onion vesicular-arbuscular mycorrhizae and their resistance to *Pyrenochaeta terrestris*. Ph.D. Diss. Univ. Illinois, Urbana. 72 p.

Beever, R.E. and D.J.W. Burns. 1980. Phosphorus uptake, storage and utilization by fungi. Adv. Bot. Res. 8:128-219.

Bethlenfalvay, G.F., Dakessian, S., and R.S. Pacovsky. 1984. Mycorrhizae in a southern California desert: Ecological implications. Can. J. Bot. 62:519-524.

Bevege, D.I. 1968. A rapid technique for clearing tannins and staining intact roots for detection of mycorrhizas caused by *Endogone* spp. and some records of infection in Australasian plants. Trans. Brit. Mycol. Soc. 51:808-810.

Bird, G.W., Rich, J.R. and S.U. Glover. 1974. Increased endo-mycorrhizae of cotton roots in soil treated with nematicides. Phytopathol. 64:48-57.

Blee, K.A. and A.J. Anderson. 2000. Defense responses in plants to arbuscular mycorrhizal fungi. *In*: Podila, G.K. and D.D. Douds (eds.) Current advances in mycorrhizae research. APS Press. St. Paul, Minnesota. p 27-44.

Bonfante-Fasolo, P. Vian, B. and F. Faccio. 1990. A texture of host cell walls in mycorrhizal leeks. Agric. Ecosys. Environ. 29:51.

Bowen, G.D. 1980. Mycorrhizal roles in tropical plants and ecosystems. *In*: Mikola. E.T. (ed.) Tropical mycorrhizae research. Clarendon Press, Oxford. p 165-190.

Bracker, C.E. and L.J. Littlefield. 1973. Structural concepts of host pathogen interfaces. *In*: Byrde, R.J.W. and C.V. Cutting (eds.) Fungal pathogenicity and plants response. Academic Press, London, New York. p 159-317.

Brejda, J.J., Yocum, D.H., Moser, L.E., and S.S. Waller. 1993. Dependence of 3 Nebraska sandhills warm-season grasses on vesicular-arbuscular mycorrhizae. J. Range Manage. 46:14-20.

Brundrett, M.C., Piche, Y. and R.L. Peterson. 1984. A developmental study of the early stage in vesicular-arbuscular mycorrhizae formation. Can. J. Bot. 63:184.

Brundrett, M.C. and W.B. Kendrick. 1988. The mycorrhizal status, root anatomy, and phenology of plants in a sugar maple forest. Can. J. Bot. 66**:**1153-1173.

Butler, E.E. and C.E. Bracker. 1970. Morphology and cytology of *Rhizoctonia solani*. *In*: Parmeter, J.R. (ed.) Biology and pathology of *Rhizoctonia solani*. Univ. of California Press. Berkeley, CA. p 32-51.

Cade-Menun, B.J., Berch, S.M. and A.A. Bomke. 1991. Seasonal colonization of winter wheat in south coastal British Columbia by vesicular-arbuscular mycorrhizal fungi. Can. J. Bot. 69:78-86.

Caron, M., Fortin, J.A., and C. Richard. 1986. Effects of *Glomus intraradices* on infection by *Fusarium oxysporum* f. sp. *radicis-lycopersici* in tomatoes over a 12-week period. Can. J. Bot. 64:552-556.

Cavagnaro, T.R., Gao, L-L., Smith, F.A., and S.E. Smith. 2001. Morphology of arbuscule mycorrhizas is influenced by fungal identification. New Phytol. 151:469-475.

Charest, C., Clark, G., and Y. Dalpe. 1997. The impact of arbuscular mycorrhizae on phosphorus status on growth of two turfgrass species. J. Turfgrass Manage. 2:1-14.

Cox, G. and F.E. Sanders. 1974. Ultrastructure of the host-fungus interface in a vesicular-arbuscular mycorrhizae. New Phytol. 73:901-912.

Cronquist, A. 1981. An integrated system of classification of flowering plants. Columbia University Press, New York, USA. p 1262.

Crush, J.R. 1974. Plant growth responses to vesicular-arbuscular mycorrhizae. VII. Growth and nodulations of some herbage legumes. New Phytol. 73:743-749.

Daft, M.J. and B.O. Okusanya. 1973. Effect of *Endogone* mycorrhiza on plant growth. Influence of infection on the multiplication of viruses in tomato, petunia, and strawberry. New Phytol. 72:975-983.

Daft, M.J., Hacskaylo, E. and T.H. Nicolson. 1975. Arbuscular mycorrhizas in plant colonizing coal spoils in Scotland and Pennsylvania. *In*: Sanders, F.E., Mosse and P.B. Tinker (eds.) *Endomycorrhizas*. Academic Press, London, UK. p 561-80.

Daft, M.J. and T.H. Nicolson. 1966. Effect of *Endogone* mycorrhizae on plant growth. I. New Phytol. 65:343-350.

Daniels, B.A. and H.D. Skipper. 1982. Methods for the recovery and quantitative estimation of propagules from soil. In: Schenck, N.C. (ed.) Methods and principles of mycorrhizal research. APS Press. St. Paul, MN. p 29-35.

Daniels, B.A. and J.M. Trappe. 1980. Factors affecting spore germination of the vesicular-arbuscular mycorrhizal fungus, *Glomus epigaeus*. Mycologia. 72:457-471.

Datnoff, L.E., Elliott, M.L., and J.P. Krausz. 1997. Cross pathogenicity of *Gaeumannomyces graminis* var. *graminis* from bermudagrass, St. Augustinegrass, and rice in Florida and Texas. Plant Dis. 81:1127-1131.

De Bary, A. 1879. In: Trubner, K.J. (ed.) Vortrag auf der Versammlung der Naturforscher und Artze zu Cassel. Strassberg, Germany. P 1-30.

Dehne, H.W. and F. Schoenbeck. 1978. Investigation on the influence of endotrophic mycorrhiza on plant diseases. 3. Chitinase activity and ornithine cycle. Zeitschrift für Pflanzenkrankheiten und Pflanzenschuty. 85:666-678.

Dehne, H.W. 1982. Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. Phytopathol. 72:1114-1119.

Dickson, S., Schweiger, P.F., Smith, F.A., Söderström, B., and S. Smith. 2003. Paired arbuscules in the *Arum*-type arbuscular mycorrhizal fungus Glomus *intraradices*. Mol. Plant-Microbe Inter. 11:489-497.

Dickson, S. 2004. The *Arum-Paris* continuum of mycorrhizal symbioses. New Phytol. 163:187-200.

Duggar, B.M. 1915. *Rhizoctonia crocorum* (Pers.) D.C. and *R. solani* Kühn (*Corticium vagum* B. & C.), with notes on other species. Ann. Missouri Bot. Garden. 2:403-458.

Elliott, M.L. 1993. Association of *Gaeumannomyces graminis* var. *graminis* with a St. Augustinegrass root rot disease. Plant Dis. 77:206-209.

Elliott, M.L. 1995. Disease response of bermudagrass to *Gaeumannomyces graminis* var. *graminis*. Plant Dis. 79:699-702.

Elliott, M.L. and G.W. Simone. 2001. Brown Patch. Florida Lawn Handbook, SP-45.

El-Giahmi, A.A., Nicolson, J.H. and M.J. Daft. 1976. Effects of fungal toxicants on mycorrhizal maize. Trans. Brit. Mycol. Soc. 67:172-173.

El-Kherbawy, M., Angle, J.S., Heggo, A. and R.L. Chaney. 1989. Soil pH, rhizobia, and vesicular-arbuscular mycorrhizae inoculation effects on growth and heavy metal uptake of alfalfa (*Medicago sativa* L.). Biol. Fert. Soils. 8:61-65.

Faber, B.A., Zaroski, R.J., Munns, D.A. and K. Shackel. 1991. A method of measuring hyphal nutrient and water uptake in mycorrhizal plants. Can. J. Bot. 69:87-94.

Fitter, A.H. 1991. Costs and benefits of mycorrhizae; Implications for functioning under natural conditions. Expermentia. 47:350-355.

Frank, A.B. 1885. Uber die auf Wurzelsymbiose berudende Ernahrung gewisser Baiimedurch Unteridsdischr Pilze. Berichte Des Deutscher Botanischem Gersellschaft. 3:128-145.

Friese, C.F. and Koske, R.E. 1991. The spatial dispersion of spores of vesicular-arbuscular mycorrhizal fungi in a sand dune: microscale patterns associated with the architecture of American beachgrass. Mycol. Res. 95:952-957.

Furlan, V. and J.A. Fortin. 1973. Formation of vesicular-arbuscular endomycorrhizas by *Endogone calospora* on *Allium cepa* under three temperature regimes. Naturaliste Canadian. 100:467-477.

Gallaud, I. 1904. Études sur les mycorrhizes Endotrophs. Lille, France: le Bigot Frères.

Gallaud, I. 1905. Études les mycorrhizes endotrophs. *Revue Generale de Botanique*. p 17, 5-48, 66-83, and 123-135.

Gandolfi, A., Sanders, I.R., Rossi, V., and P. Menozzi. 2003. Evidence of recombination in putative ancient asexuals. Mol. Biol. and Evol. 20:754-761.

Garriock, M.L., Peterson, R.L. and C.A. Ackerley. 1989. Early stages in colonization of *Allium porruno* (Peck) roots by the VAM fungus, *Glomus versiforme*. New Phytol. 112:85.

Gaskill, J.O. 1968. Breeding for *Rhizoctonia* resistance in sugar beet. J. Amer. Soc. Sugar Beet Technol. 15:107-119.

Gerdemann, J.W. 1955. Relation of a large soil-borne spore to phycomycetous mycorrhizal infection. Mycologia. 47:619-632.

Gerdemann, J.W. 1964. The effect of mycorrhizas on the growth of maize. Mycologia. 56:342-349.

Gerdemann, J.W. 1965. Vesicular-arbuscular mycorrhiza formed on maize and tuliptree by *Endogone fasciculata*. Mycologia. 57:562-575.

Gerdemann, J.W. 1968. Vesicular-arbuscular mycorrhizae and plant growth. Ann. Rev. Phytopathol. 6:397-418.

Gerdemann, J.W. 1975. Vesicular-arbuscular mycorrhizae. *In*: Torrey, J.G. and D.J. Clarkson (eds.) The development and function of roots. Academic Press. New York, USA. p 575-591.

Gerdemann, J.W. and T.H. Nicolson. 1963. Spores of mycorrhizal Endogone species extracted from soil by wet sieving and decanting. Trans. Brit. Mycol. Soc. 46:235-244.

Gerdemann, J.W. and J.M. Trappe. 1974. The Endogonaceae in the Pacific Northwest. Mycologia Memoir. 5:1-76.

Gianinazzi-Pearson, V. and S. Gianinazzi. 1989. Cellular and genetical aspects of interactions between hosts and fungal symbionts in mycorrhizae. Genome. 31:336-341.

Giannakis, N. and F.E. Sanders. 1989. Interactions between mycophagous nematodes, mycorrhizal and other soil fungi. Agric. Ecosystems and Environ. 29:163-167.

Gildon, A. and P.B. Tinker. 1983. Interactions of vesicular arbuscular mycorrhizal infections and heavy metals in plants. II. The effects of infection on uptake of copper. New Phytol. 95:263-268.

Gilligan, C.A. 1983. Dynamics of root colonization by the take-all fungus, *Gaeumannomyces graminis*. Soil Biol. Biochem. 12:507-512.

Giovannetti, M. 1985. Seasonal variations of vesicular-arbuscular mycorrhizas and endogonaceous spores in a maritime sand dune. Trans. Brit. Mycol. Soc. 84:679-684.

Giovannetti, M. and B. Mosse. 1980. An evaluation of technique for measuring vesicular-arbuscular mycorrhizal infection in roots. New Phytol. 84:489-500.

Giovannetti, M. and T.H. Nicolson. 1983. Vesicular-arbuscular mycorrhizas in Italian sand dunes. Trans. Brit. Mycol. Soc. 80:552-557.

Gogala, N. 1991. Regulation of mycorrhizal infection by hormonal factors produced by hosts and fungi. Experimentia. 47:331-340.

Gooch, M.A. 2002. Personal Communication.

Goto, B.T. and L.C. Maia. Glomerospores: a new denomination for the spores of Glomeromycota, a group molecularly distinct from the Zygomycota. Mycotaxon (in publication). 1-3.

Graham, J.H. 2001. What do root pathogens see in mycorrhizas? New Phytol. 149:357-359.

Graham, J.H. and J.A. Menge. 1992. Influence of vesicular-arbuscular mycorrhizae and soil phosphorous on take-all disease of wheat. Phytopathol. 72:95-98.

Green, N.E., Graham, S.O. and N.C. Schenck. 1976. The influences of pH on the germination of vesicular-arbuscular mycorrhizal spores. Mycologia. 68:929-933.

Griffiths, R.P. and Caldwell, B.A. 1992. Mycorrhizal mat communities in forest soils. *In*: Read, D.J., Lewis, D.H., Fitter, A.H. and I.J Alexander (eds.) *Mycorrhizas in Ecosystems* Alexander CAB International, Wallingford, UK. p 98-105.

Guyette, J.E. 1994. Take-all patch springs up on Southern golf courses. Land. Manage. 33:34.

Hardie, K. and L. Leyton. 1981. The influence of vesicular-arbuscular mycorrhiza on growth and water relations of red clover. I. In phosphate deficient soil. New Phytol. 89:677-684.

Harley, J.L. 1989. The significance of mycorrhizae. Mycol. Res. 92:129-139.

Harley, J.L. and S.E. Smith. 1983. Mycorrhizal Symbiosis. Academic Press. London.

Haydu, J.J., Satterthwaite, L.N. and J.L. Cisar. 2002. An agronomic and economic profile of Florida's sod industry in 2000. Economic information report.

Haygood, R.A. and S.B. Martin. 1990. Characterization and pathogenicity of species of *Rhizoctonia* associated with centipedegrass and St. Augustinegrass in South Carolina. Plant Dis. 74:510-514.

Hayman, D.S. 1974. Plant growth responses to vesicular-arbuscular mycorrhizae. VI. p 1-25. Effect of light and temperature. New Phytol. 73:71-80.

Hayman, D.S. 1975. The occurrence of mycorrhizae in crops as affected by soil fertility. *In*: Sanders, F.E., Mosse, B. and P.B. Tinker (eds.) Endomycorrhizas. Academic Press, London. p 495-509.

Hayman, D.S. 1978. Mycorrhizal populations of sown pastures and native vegetation in Otago, New Zealand. New Zealand J. Agr. Res. 21:271-275.

Hayman, D.S., Barea, J.M. and R. Azcon. 1976. Vesicular arbuscular mycorrhizae in southern Spain; its distribution in crops growing in soil of different fertility. Phytopathol. Mediterranea. 151 p.

Hayman, D.S. and B. Mosse. 1971. Plant growth responses to vesicular arbuscular mycorrhizae. I. Growth of *Endogone*-inoculated plants in phosphate deficient soils. New Phytol. 70:19-22.

Hayman, D.S. and G.E. Stovold. 1979. Spore populations and infectivity of vesicular-arbuscular mycorrhizal fungi in New South Wales. Aust. J. Bot. 27:227-233.

Hedge, S.V. and P.V. Rai. 1984. Influence of *Glomus fasciculatum* on damping-off of tomato. Curr. Sci. 53:588-589.

Helgason, T. and A. Fitter. 2005. The ecology and evolution of the arbuscular mycorrhizal fungi. Mycologist. 19:96-101.

Henson, J.M., Butler, M.J. and A.W. Day. 1999. The dark side of the mycelium: Melanins of Phytopathologenic fungi. Ann. Rev. Phytopathol. 37:447-471.

Hetrick, B.A.D. 1984. Ecology of VA mycorrhizal fungi. *In*: Powell, C.L.I. and D.J. Bagyaraj (eds.) VA mycorrhizal. CRC Press, Boca Raton, Florida. p 351-355.

Hetrick, B.A.D., Hartnett, D.C., Wilson, G.W.T., and D.J. Gibson. 1994. Effects of mycorrhizae, phosphorus availability, and plant density on yield relationships among competing tallgrass prairie grasses. Can. J. Bot. 72:168-176.

Hetrick, B.A.D., Kitt, D.G., and G.T. Wilson. 1988. Mycorrhizal dependence and growth habit of warm-season and cool-season tallgrass prairie plants. Can. J. Bot. 66:1376-1380.

Hetrick, B.A.D., Wilson, G.W.T., and J.F. Leslie. 1991. Root architecture of warm and cool-season grasses: relationship to mycorrhizal dependence. Can. J. Bot. 69 112-118.

Hetrick, B.A.D., Wilson, G.W.T., and C.E. Owensby. 1990a. Mycorrhizal influences on big bluestem rhizome regrowth and clipping tolerance. J. Range Manage. 43: 286-290.

Hetrick, B.A.D., Wilson, G.W.T., and T.C. Todd. 1990b. Differential responses of C_3 and C_4 grasses to mycorrhizal symbiosis, phosphorus fertilization, and soil microorganisms. Can. J. Bot. 68:461-467.

Holevas, C.D. 1966. The effect of vesicular-arbuscular mycorrhizae on the uptake of soil phosphorus by strawberry (*Fragaria* sp. var. Cambridge Favourite). J. Hort. Sci. 41:57-64.

Hooker, J.E., Jaizme-Vega, M., and D. Atkinson. 1994. Biocontrol of plant pathogens using arbuscular mycorrhizal fungi. *In*: Gianinazzi, S. and H. Schüepp (eds.) Impact of arbuscular mycorrhizas on sustainable agriculture and natural ecosystems. Birkhäuser, Basel. p 191-200.

Huber, D.M. and T.S. McCay-Buis. 1993. A multiple component analysis of the take-all disease of cereals. Plant Dis. 77:437-447.

Hurd, B. and M.P. Grisham. 1983. *Rhizoctonia* spp. associated with brown patch of St. Augustinegrass. Phytopathol. 73:1661-1665.

Hwang, S.F., Chang, K.F., and P. Chakravarty. 1992. Effects of vesicular-arbuscular mycorrhizal fungi on the development of *Verticillium* and *Fusarium* wilts of alfalfa. Plant Dis. 76:239-243.

Jarvis, M.C., Forsyth, W. and H.J. Duncan. 1988. A survey of the pectic contents of nonlignified monocot cell walls. Plant Physiol. 88:309.

Jeffries, P. 1987. Use of mycorrhizae in agriculture. Crit. Rev. Biotech. 5:319-357.

Jenkins, W.R. 1964. A rapid centrifugal-flotation technique for separating nematodes from soil. Plant Dis. Reptr. 48:692.

Johnson, N.C., Graham, J.H., and F.A. Smith. 1997. Functioning of mycorrhizal associations along the mutualistic-parasitic continuum. New Phytol. 135:575-585.

Johnston, A. 1949. Vesicular-arbuscular mycorrhizae in Sea Island Cotton and other tropical plants. Trop. Agr. Trinidad. 26:118-121.

Khalil, S. Loynachan, T.E., and H.S. McNabb, Jr. 1992. Colonization of soybean by mycorrhizal fungi and spore populations in Iowa soils. Agron. J. 84:832-836.

Khalil, S., Loynachan, T.E., and M.A. Tabatabai. 1994. Mycorrhizal dependency and nutrient uptake by improved and unimproved corn and soybean cultivars. Agron. J. 86:949-958.

Khan, A.G. 1974. The occurrence of mycorrhizas in halophytes, soils. J. Gen. Microbiol. 81:7-14.

Koide, R.T. 1985. The nature of growth depressions in Sunflower caused by vesicular-arbuscular mycorrhizal infection. New Phytol. 99:449-462.

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

Kormanik, P.P. and A.C. McGraw. 1982. Quantification of vesicular-arbuscular mycorrhizae in plant roots. *In*: Schenck, N.C. (ed.) Methods and principles of mycorrhizal research. APS Press. St. Paul, MN. p 37-45.

Koske, R., Gemma, J.N., and N. Jackson. 1995. Mycorrhizal fungi benefit putting greens. USGA Green Section Record. 33(6):12-14.

Krishna, K.R. and D.J. Bagyaraj. 1983. Interaction between *Glomus fasciculatum* and *Sclerotium rolfsii* in peanut. Can. J. Bot. 41:2349-2351.

Landschoot, P.J. 1997. Turfgrass Patch Diseases Caused by Ectotrophic Root-Infecting Fungi. APS Press. St. Paul, Minnesota. 72-84.

Lawley, R.A., Newman, E.I., and R. Campbell. 1982. Abundance of endomycorrhizas and root-surface microorganisms on three grasses grown separately and in mixtures. Soil Biol. Biochem. 14:237-240.

Linderman, R.G. 1994. Role of VAM fungi in biocontrol. *In*: Pfleger, F.L. and R.G. Linderman (eds.) Mycorrhizae and plant health. APS Press. St. Paul, Minnesota. p 1-25.

Mark, G.L. and A.C. Cassells. 1996. Genotype-dependence in the interaction between *Glomus fistulosum*, *Phytophthora fragariae* and the wild strawberry (*Fragaria vesca*). Plant Soil. 185:233-239.

Marks, G.C. and R.C. Foster. 1973. Structure, morphogenesis, and ultrastructure of ectomycorrhizae [Mycorrhizae]. *In*: G.C. Marks (ed.) Ectomycorrhizae: Their ecology and physiology. p 1-41.

Marschner, H. and B. Dell. 1994. Nutrients uptake in mycorrhizal symbiosis. Plant and Soil. 159:89-102.

Martin, S.B. and L.T. Lucas. 1984. Characterization and pathogenicity of *Rhizoctonia* spp. and binucleate *Rhizoctonia*-like fungi from turfgrasses in North Carolina. Phytopathol. 74:170-175.

Mathre, D.E. 1992. *Gaeumannomcyes*. *In*: Singleton, L.L, Mihail, J.D., and C.M. Rush (eds.) Methods for research on soilborne phytopathologenic fungi. APS Press. St. Paul, Minnesota. p 200.

Medina-Gonzalez, O.A., Sylvia, D.M., and A.E. Kretscher, Jr. 1988. Growth response of tropical forage legumes to inoculation with *Glomus intraradices*. Trop. Grasslands. 21:24-27.

Menge, J.A., Johnson, E.L.V., and R.G. Platt. 1978. Mycorrhizal dependency of several citrus cultivars under three nutrient regimes. New Phytol. 81:553-559.

Meyer, J.R. and R.G. Linderman. 1986. Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomycetes by mycorrhizas formed by *Glomus fasciculatum*. Soil Bio. & Biochem. 18:191-196.

Miller, R.H., Cardoso, E.J.B.N., and C.O.N. Cardoso. 1979. Some observations on mycorrhizal infection of tropical forage legumes and grasses in Brazil. Summa Phytopathol. 5:168-172.

Morton, J.B. n.d. INVAM website. http://invam.caf.wvu.edu/methods/spores/enumeration.htm. Accessed: August 2005.

Morton, J.B. and G.L. Benny. 1990. Revised classification of arbuscular mycorrhizal fungi (Zygomycetes): a new order, Glomales, two new suborders, Glomineae and Gigasporneae and two new families, Acaulosporaceae and Gigasporaceae with an emendation of Glomaceae. Mycotaxon. 37:471-491.

Morton, J.B., Frank, M. and G. Cloud. 1992. The nature of fungal species in Glomales (Zygomycetes). *In*: Read, D.J., Lewis, D.H., Filler, A.H. and I.J. Alexander (eds.) Mycorrhizae in ecosystems. CAB International, Oxon, UK. p 65-73.

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. 159:47-60.

Mosse, B. 1957. Growth and chemical composition of mycorrhizal and non-mycorrhizal apples. Nature. 179:922-924.

Mosse, B. 1973. Advances in the study of vesicular-arbuscular mycorrhizas. Ann. Rev. Phytopathol. 11:170-196.

Munns, D.N. and B. Mosse. 1980. Mineral nutrition of legume crops. *In*: Summer-field, R.J. and A.H. Bunting (eds.) Advances in legume science University of Reading Press, Reading. p 115-125.

Murdoch, C.L., Jackobs, J.A. and J.W. Gerdemann. 1967. Utilization of phosphorus sources of different availability by mycorrhizal and non-mycorrhizal maize. Plant and Soil. 27:329-334.

Neimira, B.A., Hammerschmidt, R., and G.R. Safir. 1996. Postharvest suppression of potato dry rot (*Fusarium sambucinum*) in prenuclear minitubers by arbuscular mycorrhizal fungal inoculum. Am. Potato J. 73:509-515.

Nelsen, C.E. 1987. The water relations of vesicular-arbuscular mycorrhizal systems. *In*: G.K. Safir (ed.) Ecophysiology of VA mycorrhizal plants. CRC Press,

Boca Raton, Florida, USA. P 71-92.

Nemec, S. and J.H. O'Bannon. 1979. Responses of *Citrus aurantium* to *Glomus etunicatus* and *Glomus mosseae* after soil treatment with selected fungicides. Plant and Soil. 53:351-359.

Newman, E.I. 1966. A method of estimating the total length of root in a sample. J. App. Ecol. 3:139-145.

Newsham, K.K., Fitter, A.H., and A.R. Watkinson. 1995. Arbuscular mycorrhizae protect an annual grass from root pathogenic fungi in the field. J. Ecology. 83:991-1000.

Newsham, K.K., Fitter, A.H., and A.R. Watkinson. 1995. Multi-functionality and biodiversity in arbuscular mycorrhizas. Trends Ecol. Evol. 10:407-411.

Nicolson, T.H. 1955. The mycotrophic habit in grasses. Ph.D. Thesis. University of Nottingham. London.

Nicolson, T.H. 1956. Mycorrhizae in grasses and cereals. University of Nottingham. School of Agriculture. Report of the School of Agriculture. p 33-36.

Nicolson, T.H. 1967. Vesicular-arbuscular mycorrhizae – a universal plant symbiosis. Sci. Prog. Oxford. 55:561-581.

Nicolson, T.H. 1975. Evolution of vesicular-arbuscular mycorrhizae. *In*: Sander, F.E., Mosse, E. and P.B. Tinker (eds.) Endomycorrhizas. Academic Press, New York. p 25-34.

Nilsson, H.E. 1969. Studies of root and foot rot diseases of cereals and grasses. 1. On resistance to *Ophiobolus graminis* Sacc. Lantb – Hogsk. Ann. Rev. 35:275-807.

Nye, P. and P.B. Tinker. 1977. Solute movement in the Soil-Root System. Oxford, Blackwell Scientific. p 342.

Penrose, L.D.J. 1992. Interpretive value of symptoms of infection by *Gaeummanomyces graminis* in wheat seedlings grown in sand culture. Ann. Appl. Biol. 121:545-557.

Phillips, J.M. and D.S. Hayman. 1970. Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. Trans. Brit. Mycolo. Soc. 55:158-160.

Pirozynski, K.A. and Y. Dalpe. 1989. Geological history of the Glomales 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.

Rabatin, S.C. 1979. Seasonal and edaphic variation in vesicular-arbuscular mycorrhizal infection of grasses by *Glomus tenuis*. New Phytol. 83:95-102.

Ramirez, B.N. 1974. Influence of endomycorrhizae on the relationship of inoculum density of *Phytolphthora palmivora* in soil to infection of papaya roots. M.S. Thesis. Univ. Florida. Gainesville, Florida. 45 p.

Rani, S.S., Kunwar, I.K., Prasad, G.S., and C. Manoharachary. 2004. *Glomus hyderabadensis*, a new species: its taxonomy and phylogenetic comparison with related species. Mycotaxon. 89:245-253.

Raymer, M.C. 1927. Mycorrhizae. New Phytol. Reprint 15. p 246.

Read, D.J., Koucheki, H.K. and J. Hodgson. 1976. Vesicular-arbuscular mycorrhizae in natural vegetation systems. I. The occurrence of infection. New Phytol. 77: 641-653.

Remy, W., Taylor, T.N., Hass, N. and H. Kerp. Dec. 1994. Four hundred-million-year-old vesicular arbuscular mycorrhizae. Proc. Natl. Acad. 91:11841-11843.

Rhodes, L.H. 1981. Effects of fungicides on mycorrhizal development of creeping bentgrass. Plant Dis. 65:145-147.

Rhodes, L.H. and P.O. Larsen. 1979. Effects of fungicides on mycorrhizal development of creeping bentgrass. Plant Dis. 65:145-147.

Rilling, M.C., Ramsey, P.W., Morris, S., and E.A. Paul. 2003. Glomalin, an arbuscular-mycorrhizal fungal soil protein, responds to land-use change. Plant and Soil. 245:293-299.

Rives, C.S., Baswa, M.I. and A.E. Liberta. 1990. Effects of topsoil storage during surface mining on the viability of VA mycorrhizae. Soil Science. 129:253-257.

Ross, J.P. 1972. Influence of *Endogone* mycorrhiza on *Phytophthora* rot of soybean. Phytopathol. 62:896-897.

Rovira, A.D. and D.G. Whitehead. 1983. Activity of fungicides in soil against infection of wheat roots by *Gaeumannomyces graminis* var. *tritici*. Proceedings of Section 5 of the Fourth International Congress of Plant Pathology. APS Press. St. Paul, Minnesota.

Rusell, E.W. 1973. Soil conditions and Plant Growth, Tenth Edition. Longman, London and New York. p 64-67.

Ryan, M.H. and J.H. Graham. 2002. Is there a role for arbuscular mycorrhizal fungi in production agriculture? Plant and Soil. 244:263-271.

Saif, S.R. and A.G. Khan. 1975. The influence of season and stage of development of plant on *Endogone* mycorrhiza of field-grown wheat. Can. J. Microbiol. 21:1021-1024.

Sanders, F.E. and P.B. Tinker. 1973. Phosphate flow into mycorrhizal roots. Pest. Sci. 4:383-395.

SAS Institute Inc. 2004. The SAS system for Windows. Version 9.0. SAS Institute. Cary, North Carolina.

Schenck, N.C. 1970. Mycorrhizal Fungi. Sunshine State Agr. p 12-14.

Schenck, N.C. 1987. Vesicular-arbuscular mycorrhizal fungi and the control of fungal root diseases. *In*: Chet, I. (ed.) Innovative approaches to plant disease control. Wiley, New York. p 179-191.

Schenck, N.C. and M.K. Kellam. 1978. The influence of vesicular arbuscular mycorrhizae on disease development. Bulletin 798. Institute of Food and Agricultural Sciences. Univ. Florida. Gainesville, Florida. 16 p.

Schenck, N.C. and R.A. Kinloch. 1980. Incidence of mycorrhizal fungi on six field crops in monoculture on a newly cleared woodland site. Mycologia. 72:445-456.

Schenck, N.C. and Y. Pérez. 1988. Manual for the identification of VA mycorrhizal fungi. 2nd Edition. Synergistic Publications. Gainesville, Florida. 250 p.

Schenck, N.C. and V.N. Schroder. 1974. Temperature response of Endogone mycorrhizae on soybean roots. Mycologia. 66:600-605.

Schenck, N.C. and G.S. Smith. 1981. Distribution and occurrence of vesicular-arbuscular mycorrhizal fungi on Florida agricultural crops. Soil and Crop Sci. Soc. of Florida, Proceedings. 40:171-175.

Schoenbeck, F. 1979. Endomycorrhiza in relation to plant diseases. *In*: Schippers, B. and W. Gams (eds.) Soil borne plant pathogens. Academic Press. New York, USA. p 271-280.

Schussler, A., Schwarzott, D. and C. Walker. 2001. A new fungal phylum, the Glomeromycota: phylogeny and evolution. Mycol. Res. 105(12):1413-1421.

Sharma, A.K., Johri, B.N., and S. Gianinazzi. 1992. Vesicular-arbuscular mycorrhizae in relation to plant disease. World J. Microbio. Biotech. 8:559-563.

Sheikh, N.A., Saif, S.E. and A.G. Khan. 1975. Ecology of *Endogone* II. Relationship of *Endogone* spore population with chemical soil factors. Islamabad J. Sci. 2:6-9.

Shirinkina, L.G. 1975. Intensity of mycorrhizal infection in healthy and loose smut infected wheat plants. Ann. Rev. Appl. Plant Path. 56:142.

Sivasithamparam, K. and C.A. Parker. 1978. Effects of certain isolates of bacteria and actinomycetes on *Gaeumannomyces graminis* var. *tritici* and take-all of wheat. 26:773-782.

Smith, S.E. and G.D. Be. 1979. Soil temperature, mycorrhizal infection, and nodulation of *Medicago truncate* and *Trifolium subteranneum*. Soil Biol. Biochem. 11:469-473.

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

Smith, S.E. and D.J. Read. 1997. Mycorrhizal symbiosis, 2nd Ed. Academic Press, San Diego, London. p 13, 127, 147, 151-153, and 159.

Smith, F.A. and S.E. Smith. 1997. Structural diversity in (vesicular)-arbuscular mycorrhizal symbiosis. New Phytol. 137:373-388.

Sneh, B., Burpee, L, and A. Ogoshi. 1991. Identification of *Rhizoctonia* species. APS Press. St. Paul, Minnesota. 584 p.

Sondergaard, M. and S. Laegaard. 1977. Vesicular-arbuscular mycorrhizae in some aquatic vascular plants. Nature. London. 168:232-233.

Sreenivasa, L.N. and D.J. Bagyaraj. 1988. *Chloris gayana* (Rhodes grass), a better host for the mass production of *Glomus fasciculatum* inoculum. Plant and Soil. 106: 289-290.

Srivastava, D., Kapoor, R., Srivastava, S.K. and K.G. Mukerji. 1996. Vesicular arbuscular-mycorrhizae- an overview. *In*: K.G. Mukerji (ed.) Concepts in mycorrhizal research. Kluwer Academic Publishers, The Netherlands. p 2-24.

St. Arnaud, M., Hamel, C., Caron, M., and J.A. Fortin. 1994. Inhibition of *Pythium ultimum in* roots and growth substrate of mycorrhizal *Tagetes patula* colonized with *Glomus intraradices*. Can. J. Plant Pathol. 16:187-194.

St. John, T.V. 1980. Root size, root hairs, and mycorrhizal infection: a re-examination of Baylis's hypothesis with tropical trees. New Phytol. 84:483-487.

St. John, T.V. and H.W. Hunt. 1983. Statistical treatment of VAM infection data. Plant and Soil. 73:307-313.

Stuessy, T.F. 1992. The systematics of arbuscular mycorrhizal fungi in relation to current approaches to biological classification. Mycorrhizae. 1:667-677.

Sylvia, D.M. 1986. Spatial and temporal distribution of vesicular-arbuscular mycorrhizal fungi associated with *Uniola paniculata* in Florida foredunes. Mycologia. 78:728-734.

Sylvia, D.M. and J.N. Burks. 1988. Selection of a vesicular-arbuscular mycorrhizal fungus for practical inoculation of *Uniola paniculata*. Mycologia. 80:565-568.

Sylvia, D.M. and S.E. Williams. 1992. Vesicular-arbuscular mycorrhizae and environment stress. *In*: Bethlenfalvay, G.J. and R.G. Linderman (eds.) Mycorrhizae in sustainable agriculture. Am. Soc. Agr. Madison, Wis. p 101-124.

Sylvia, D.M., Wilson, D.O., Graham, J.H., Maddox, J.J., Millner, P., Morton, J.B., Skipper, H.D., Wright, S.F., and A.G. Jarstfer. 1993. Evaluation of vesicular-arbuscular mycorrhizal fungi in diverse plants and soils. Soil Biol. and Biochem. 25:705-713.

Taylor, T.N., Remy, W., Hass, H., and H. Kerp. 1995. Fossil arbuscular mycorrhizae from the early Devonian. Mycologia. 87:560-573.

Tennant, D. 1975. A test of a modified line intersection method of measuring root length. J. Ecol. 63: 995-1001.

Tinker, P.B. 1978. Effect of vesicular-arbuscular mycorrhizae on plant nutrition and plant growth. Physiol. Veg. 16:743-775.

Trappe, J.M. 1981. Mycorrhizae and productivity of arid and semiarid rangelands. *In*: Manassah, J.T. and E.J. Briskey (eds.) Advances in food producing systems for arid and semiarid lands Academic Press, New York. p 581-599.

Trappe, J.M. 1987. Phylogenic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint. *In*: G.R. Safir (ed.) Ecophysiology of VA mycorrhizal plants CRC Press, Boca Raton, Florida, USA. p 5-25.

Trenholm, L.E. 2004. Turfgrass Cultivars for Home Lawn Use: Less Work and Better Looking. Florida Turf Digest. 21:4, 6.

Vandenkoornhuyse, P., Ridgway, K.P., Watson, I.J., Fitter, A.H., and J.P.W. Young. 2003. Co-existing grass species have distinctive arbuscular mycorrhizal communities. Mol. Ecol. 12:3085-3095.

Vigo, C., Norman, J.R., and J.E. Hooker. 2000. Biocontrol of the pathogen *Phytolphthora parasitica* by arbuscular mycorrhizal fungi is a consequence of effects on location loci. Plant Pathol. 49:509-515.

Viyanak, K. and D.J. Bagyaraj. 1990. Selection of efficient VA mycorrhizal fungi for trifoliate orange. Biol. Agric. Hortic. 6:305-311.

Walker, J. 1973. *Gaeumannomyces graminis* var. *graminis*. Commonwealth Mycological Institute. No. 381. The Eastern Press Ltd., London and Reading. 607 p.

Walker, C., Blaszkowski, J., Schwarzott, and A. Schussler. 2004. *Gerdemannia* gen. nov., a genus separated from *Glomus*, and *Gerdemanniaceae* fam. nov., a new family in the *Glomeromycota*. Mycological Res. 108:707-718.

Warner, A. and B. Mosse. 1980. Independent spread of vesicular-arbuscular mycorrhizal fungi in soil. Trans. Brit. Mycol. Soc. 74:407-410.

Weaver, D.J. and E.J. Wehunt. 1975. Effect of soil pH on susceptibility of peach to *Pseudomononas syringae*. Phytopathol. 65:984-989.

Wu, Chi-Guang and Lin, Suh-Jen. 1997. Glomales of Taiwan: VII. *Jimtrappea* and *J. Macrospora*, new taxa of Acaulosporaceae (Glomaceae). Mycotaxon. 53:283-294.

Yao, M.K., Tweddell, R.J., and H. Désilets. 2002. Effect of two vesicular-arbuscular mycorrhizal fungi on the growth of micropropagated potato plantlets and on the extent of disease caused by *Rhizoctonia solani*. Mycorrhiza. 12:235-242.

Yocums, D.H., Larsen, H.J., and M.G. Boosatis. 1985. The effects of tillage treatments and a fallow season on VA mycorrhizae of winter wheat. *In*: R. Molina (ed.) Proc. of 6th North American Conference on Mycorrhizae Bend, Oregon, June 25-29, 1984. Forest Res. Lab. Corvallis, Oregon, USA. p 297.

Young, C.C., Juang, T.C., and H.Y. Guo. 1986. The effect of inoculation with vesicular-arbuscular mycorrhizal fungi on Soybean yield and mineral phosphate utilization in subtropical-tropical soil. Plant and Soil. 95:245-253.

Zambolim, L., and N.C. Schenck. 1983. Reduction of the effects of pathogenic root rot infecting fungi on soybean by the mycorrhizal fungus *Glomus mosseae*. Phytopathol. 73:1402-1405.

BIOGRAPHICAL SKETCH

Whitney Colleen Elmore, youngest daughter of Malcolm and Donna Elmore of Lucas, Kentucky, attended Barren County High School in Glasgow, Kentucky, and graduated in 1994. Whitney grew up with a sister, Emilee, and later two nephews, Ryan and Dustin Mosier. As an active member of FFA, Whitney served as vice president of her chapter, lettered in varsity golf and track and field, and participated in the BETA Club as well as many other clubs and activities. In 1994, she began her collegiate career at Western Kentucky University in Bowling Green, Kentucky where she received an Associate of Science degree in turf grass management in 1997 and Bachelor of Science degree in agriculture in 1998.

Finishing her undergraduate degree, she pursued her Master of Science Degree in turfgrass science/agriculture working on hydrophobic soils with Dr. Haibo Liu. Before becoming a recipient of the Sigma Xi Award for the Outstanding Graduate Research Paper in 2001, she became a member of the Golden Key National Honor Society. Upon the completion of her master's program in 2001, Whitney began her Ph.D. in plant pathology working on diseases of turfgrasses, at the University of Florida under the guidance of Dr. James Kimbrough. While pursuing her doctorate, she became a member of Gamma Sigma Delta, the Honor Society of Agriculture, in 2003 and received scholarships from the Florida Turfgrass Association and the Florida Nursery Growers Association. Whitney is currently teaching classes at Santa Fe Community College in

Gainesville, Florida, and has accepted a faculty position at Macon State College in Macon, Georgia.