AN ABSTRACT OF THE THESIS OF

Michelle L. Miller for the degree of Master of Science in Horticulture presented on November 1, 1996. Title: The Effect of Four Composts on the Establishment of Vesicular-Arbuscular Mycorrhizae in Soilless Media.

Abstract approved:

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The use of composts in the horticulture industry continues to grow nationwide as more regionally produced composts enter the market. Additionally, nursery growers are increasingly interested in the use of mycorrhizae to 1) enhance nutrient uptake which may reduce contamination of runoff water and 2) enhance the marketability of their crops. The possibility of utilizing mycorrhizae for resistance to soil-borne pathogens is also of interest at a time when pesticides are under stricter control.

The beneficial use of vesicular-arbuscular mycorrhizae (VAM) in mineral soils is well documented, but much less is known about the effect of soilless mixes on mycorrhizal colonization of roots. Previous research indicates that mycorrhizal colonization is affected by pH, soluble salts, phosphorus (P) levels, cation exchange capacity, percent organic matter, and some peats. No other research has been published, to our knowledge, on the role of commonly used horticultural composts and mycorrhizal establishment.

This study examined four different composts for their effect on VAM establishment using onion roots as an indicator. The composts used in this study were vermicompost, spent mushroom compost, yard waste compost and processed manure fiber

composted with wood wastes. A peat moss and perlite blend was the base medium and each compost was added at 35% of the total volume. Plant growth parameters, P levels and rate of desorption, and microbial populations were monitored and analyzed in relation to the percent of VAM colonization of the roots. Colonization increased the plant base caliper and increased plant height in mixes with low nutritional levels. Root/shoot ratios did not differ significantly except for the noninoculated control which was two and a half times greater than the other treatments. Increased rates of P released into the soil solution in a desorption study were negatively correlated with colonization. P levels in tissue at the end of the experiment were positively correlated with P levels in the compost blends but not with percent of VAM colonization. There was no significant correlation between number of microbial colonies antagonistic to *Thielaviopsis basicola* and percent of VAM colonization. Significant differences were found in percent VAM colonization between media types: control medium - 72.5%; mushroom compost medium -15.7%, the yard waste medium 53.1%; vermicompost medium 2.3%; and processed fiber medium - 8.8%.

This study also examined the effects of soluble salts on VAM colonization of onion roots. Mushroom compost was blended with peat moss and perlite at 10%, 20%, 30%, and 40%, with the 10% compost mix being the control. Electrical conductivity in the four blends ranged from 2.6 to 6.2 dS cm^{-1}. Soluble salt levels had no effect on either VAM colonization, shoot weight, shoot P levels, or root/shoot ratios. No significant correlation was found between soluble salt levels and percent VAM colonization.

In conclusion, the primary factors influencing VAM colonization of onions in compost blends were the initial levels of P in the blends and the rate and amount of P released, especially when VAM fungal spores may have been germinating. The experiment raised questions about the balance between mineralized P and organic P in composts and their effect on VAM fungal spore germination and subsequent colonization.

This should be further explored in other research as the ratio may explain the differences between composts and their ability to support mycorrhizae establishment.

The Effect of Four Composts on the Establishment of Vesicular-Arbuscular Mycorrhizae in Soilless Media

by

Michelle L. Miller

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented November 1, 1996
Commencement June 1997

Master of Science thesis of Michelle L. Miller presented on November 1, 1996.

APPROVED:

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I understand that my thesis will become part of the permanent collecton of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Michelle L. Miller, Author

ACKNOWLEDGMENT

I would like to gratefully acknowledge my professors, Leslie Fuchigami and Robert Linderman for their support and guidance during my work at Oregon State University. They encouraged and supported me, believed in me and structured a program to fit my needs and interests. Thanks also go to Dr. Sanliang Gu, Dr. Joseph Marlow, Anne Davis and Nola Mosier who graciously and patiently instructed me in the techniques of scientific research as well as the fundamental lab and greenhouse techniques needed for this project. To my lab partners, Brent Black, Sung-lee Guak, Lailiang Cheng, a special thanks for your humor and encouragement. To Steven Castagnoli and Abbas Shirazi, thank you for the extra help you both provided.

A very special thanks to my business partners, Nancy Cunningham and Steve Doolin, who believed that this was a worthwhile experience for both me and our company. They supported me financially, emotionally, and physically for the last three years and have given me a precious gift of knowledge and education. To my husband, John, thank you for believing in me and encouraging me every step of the way. To my children, Camille, Zachary and Benjamin, who have been willing to let Mom study and work even when they wanted her for other things, my deepest thanks.

I would like to acknowledge and express my appreciation to the USDA, Nursery Crops Research Center for funding and laboratory space during this study. And to all of my customers and business colleagues in the nursery industry, thank you for understanding when I could not always be there to take care of the business.

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DEDICATION

This thesis is dedicated to my brother, Lieutenant Samuel Walter Hanna, IV, USN, whose untimely death forced me to look at new horizons and changed my life forever.

The Effect of Four Composts on the Establishment of Vesicular-Arbuscular Mycorrhizae in Soilless Media

Chapter One

Introduction

The nursery industry in Oregon continues to grow steadily and is the leading agricultural industry in the state with 1995 sales of $419 million. Out of this total, $148 million came from containerized nursery crops and $83.5 million from greenhouse crops. Container nurseries occupied 4,400 acres and greenhouses occupied 600 acres in the state (Rowley et al., 1996). These two segments of the industry grow plants in some type of container system and have the potential to benefit from this research. Container mixes usually contain 75 to 80% organic materials and the potential to use composts in these mixes is large (Bettineski, 1996a).

Considerable pressure has been applied to the ornamental horticulture industry to improve the efficiencies in resource utilization. In 1991, the Oregon Association of Nurserymen adopted a nutrient and water runoff plan for all containerized nursery operations. The implemented plan on water runoff and groundwater contamination with nursery fertilizers has propelled the industry to examine different cropping systems. The possible use of mycorrhizae to increase nutrient use efficiency and the corresponding decrease in applied fertilizers is one way to achieve the goal of minimizing water and nutrient runoff. Additionally, the market demand for plants which better survive transplanting has led to nurseries growing more mycorrhizal plants and commanding more market share.

Composts in horticulture

Composting has been occurring naturally in the environment since the first plants grew in earth's soil. As vegetation decays, bacteria and fungi break down organic matter into humus. Composting is the ultimate method of recycling plant nutrients in nature. Organic matter returned to the soil repeats the cycle of decay and renewal. In order to reduce the tonnage of waste entering the nation's landfills, many communities have adopted active compost diversion programs. In 1993 and 1994, the percent of households recycling their yard trimmings for compost was 22% and 24%, respectively, nationwide. (EPA, 1995). In addition to yard trimmings, a varied group of waste products is being composted nationwide. According to the Dr. Charles Cannon of The Composting Council, a nationwide composting association in Alexandria, Virginia, (personal communication) these include fish wastes from canneries and processers, brewery waste from distilleries, post consumer food waste from restaurants and grocery stores, animal wastes and bodies from feedlots, and water hyacinths from the waterways of the South. As with any product, the market for consuming the product must exist before it is economically feasible to produce on a large scale.

The application of organic matter onto soils increases water holding capacity, aeration, cation exchange capacity, tilth and nutritional status, and decreases bulk density and crusting (Azevedo and Stout, 1974; Epstein. et al., 1976; Johnston, 1975; Levanon and Danai, 1995, Lund and Doss, 1980; Meek et al., 1982). Additionally, composts often can provide all of the essential trace elements for plant production (Bettineski, 1996a.).

Composted manures have historically been the main source of compost for sustainable agriculture and organic farming. They have not found ready usage in the horticulture industry due to problems with odor, moisture content, availability and ease of handling. Manures are used primarily to provide plant nutrients, especially nitrogen, and the timing and rate of application to soils is based on the release of nitrogen to the plant. Therefore, the rate of N mineralization in manures must be known to optimize its use by the crop and avoid nitrate runoff into the groundwater (Levanon and Danai, 1995). Evaluating composts is therefore based on the degree of decompostion (termed maturity) as measured by the carbon:nitrogen ratio, soluble constituents, humic content, microbial respiration rates and growth tests.

Phosphorus found in composts can be in organic or mineral forms. As compost stabilizes and matures, the organic P decreases and the precipitated mineral fraction increases (Fine et al., 1989; Banin et al., 1990).

A plant grown in artificial growth media in a container has different needs from a plant grown in mineral soil. The limited volume of the container puts restrictions on root growth and increases the demand for water, air and nutrients. Therefore, the growth medium has different requirements for physical and chemical properties (Handreck and Black, 1994). The artificial growth medium used in horticulture is traditionally based on peat moss, bark and wood wastes and various aggregates including vermiculite, perlite, pumice, styrofoam; and sand. Sphagnum peat moss is the most commonly used organic component in artificial media. Its physical and chemical characteristics are well documented but are often very different depending on the source. While peat moss is still

readily available in North America, it is at a high cost to many growers. In the early 1980's, composted barks began to be widely used in horticultural mixes to replace or supplement peat moss.. Environmental restrictions on timber harvesting have reduced the supply of bark and increased the price. The availability of organic composts coupled with increased recycling has expanded the interest in horticultural mixes which incorporate those composts which are regionally available. However the quality of the compost must be compared to peat moss and composted bark in physical and chemical characteristics. The final test is the growth of the plant (Inbar et al., 1993).

Composts are known for their potential capacity to suppress plant diseases (Hoitink and Grebus, 1994). Hoitink and Grebus stated that organic matter affects both the inoculum potential of plant pathogens and their biocontrol agents by providing a) better aeration of the soil or medium; b) a food base for antagonistic microflora; and c) secretion of fungistatic materials which may suppress pathogenic fungi. Many nursery growers are currently using composts to gain these benefits. The increasing restrictions on registered pesticides makes compost all the more attractive.

Vesicular-arbuscular mycorrhizae in horticulture

Vesicular-arbuscular mycorrhizae (VAM) are naturally occurring soil fungi which form symbiotic relationships with roots of host plants. The majority of plants are mycorrhizal with the exception of Cruciferae, Chenopodiaceae, Caryophyllaceae and Proteaceae families. VAM are characterized by the formation of branched haustorial structures, termed arbuscules, within the cortical cells and by mycelia that extend into the

surrounding soil. The arbuscules are the main sites of solute exchange. Additionally, many form vesicles as lipid-rich storage organs. The VAM fungi belong mainly to four genera: *Acaulospora, Gigaspora, Glomus,* and *Sclerocystis* (Marschner, 1995). Mycorrhizal associations can be mutualistic, neutral or parasitic, but generally, mutualism is the predominant response. VAM fungi are characterized by their need to be grown in association with a host plant in contrast to the ectomycorrhizal fungi which can be grown in culture with no host.

Woody ornamentals and greenhouse crops are propagated asexually and grown in soilless media. With high fertility levels and the absence of inoculum, these plants have little chance to have VAM. Marschner (1994) states that horticultural plants are a commercially feasible crop to inoculate with mycorrhizal fungi prior to outplanting due to the reduction of transplant shock and the increased survival and growth in the field. Research has demonstrated that nursery stock growth increases by VAM fungal inoculation (Hoepfner et al., 1983; Morrison et al., 1993; Nelson, 1987; Yeager et al., 1990). Morrison et al. (1993) found that growth responses to VAM fungal inoculation could be observed under nursery fertility levels in containers and upon transplant into a field site.

Soil factors which influence the establishment of VAM are pH, P levels, organic matter, cation exchange capacity, some types of peat moss, and liquid sludge (Menge et al., 1982; Biermann and Linderman, 1983). Continued interest in the use of VAM in commercial nurseries and greenhouses requires that growth media is conducive to VAM establishment in the host plant, yet questions remain about soilless media and its ability to

support VAM colonization in root systems. Research has been conducted on peat based mixes as well as bark mixes which yielded conflicting results (Biermann and Linderman, 1983; Menge et al., 1982; Nemec, 1992). Increasing use of composts as replacements or supplements to peat and bark mixes introduces unknown factors for successful VAM colonization. With the complexity and diversity of available composts, we cannot necessarily predict whether the common factors such as pH, P, and fertility will be the predominant indicators of a conducive growth medium to a VAM inoculated plant.

Studies on VAM establishment in soilless media have shown mixed results. Biermann and Linderman (1983) noted some inhibitory effects from sphagnum peat moss. Gaunt (1978) noted some inhibition when using 50% vermiculite on onions. Studies on composts and VAM have been limited. In 1982, Guttay published results from the development of ectomycorrhizae, endomycorrhizae and ericoid mycorrhizae in composts derived from corn, sugar maple and hemlock. He concluded that differences in compost effects on the development of mycorrhizae were due to the type and quantity of mycorrhizal propagules present in the composts. However, he did not inoculate the composts with a specific mycorrhizal fungus, but instead allowed any propagules that might be present to colonize the roots. A follow-up study by Guttay (1983) used the same composts with and without complete fertilizer additions. A similar pattern was followed as before, with no mycorrhizal fungal inoculum added to the composts but allowing native propagules to colonize the corn roots. Other studies (Maronek et al., 1980, Datnoff et al., 1991) have used composted bark as part of the growing substrate but have not looked at the compost as a separate factor in the establishment of mycorrhizae in the root system.

Datnoff et al. (1991) compared thirteen commercial mixes and their effect on mycorrhizal formation in tomatoes. Two of the mixes, Pro-Grow PX-2 and Ball Growing Mix #2, contained peanut hull compost and composted bark, respectively. Both mixes had VAM colonization below 30%. Both had similar levels of acid extractable P (27 and 29 ppm), but the peanut hull compost had lower (287 ppm) salt levels than the composted bark mix (1473 ppm). Work by Sweatt and Davies (1984) with geraniums on mycorrhizae and water relations utilized a growth medium of sand:sandy loam and composted cow manure. Their work was designed to reflect commercial growing conditions in respect to the P levels commonly found in growth media. They do not report the percent of VAM colonization in their published results but do note the increased drought tolerance and increased P levels in the plants. Similarly, Menge et al. (1982) looked at twenty six soils and mixes commonly used for citrus production in southern California. One of those mixes was a nursery blend used for containerized stock which consisted of top soil, rice hulls, mushroom compost and sand. Again, the percent of colonization is not given in the published results, but increased growth due to mycorrhizal dependency was reported and increased levels of P in the shoots were shown.

The objective of this research was to determine how composts would affect VAM establishment in a growth medium. We also hoped to see if the influencing factors would be similar to those already reported for other soilless mixes.

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CHAPTER TWO

Literature Review

Organic materials in soilless mixes

The majority of nursery container mixes use 75% to 80% organic materials including peat moss, bark, compost, and sawdust. Due to the lack of consistent and available mineral soils, few U.S. growers incorporate mineral soils in their growing mixes. Growth medium has the same functions as field soil, that is 1) it must be a reservoir for plant nutrients; 2) it needs to hold sufficient available water; 3) it must allow for the exchange of gases in the root zone; and 4) it should provide sufficient physical support for the plant (Nelson, 1991). Advantages of organic material in a horticultural growth medium include its readily availability, low bulk density, excellent water retention properties and high cation exchange capacity (Nelson, 1991; Handreck and Black, 1994). However, researchers have found that anions, especially PO_4, leach rapidly from soilless mixes regardless of the type of P used (Havis and Baker, 1985a, 1985b; Marconi and Nelson, 1982; Yeager and Barrett, 1984). The nutrient solubility increases in media with a pH below 6.0 (Peterson, 1982). Most soilless mixes have a pH between 5.5 and 6.0. Nursery growers, especially greenhouse growers, have reported problems with P retention over a wide variety of crops. While the standard practice in the soilless mix business is to add superphosphate at the time of blending, it is leached out before the plants can benefit from its presence according to Yeager and Barrett (1982).

Vesicular-arbuscular mycorrhizae

General characteristics of mycorrhizal plants

VAM symbiosis has been found to a) increase nutrient uptake, b) increase resistance to soil pathogens, c) increase transplant survival, d) increase drought tolerance, e) decrease toxicity of soil pollutants, and f) induce systemic resistance (Marschner, 1995, Pfleger and Linderman, 1994, Linderman, 1992; Fitter, 1991; Sweatt and Davies, 1984.). Additionally, VAM plants experience changes in auxins, cytokinins and gibberellins; photosynthetic rates increase; and the partitioning of photosynthates changes. Root cell membranes change as do the quality and quantity of root exudates. (Linderman, 1992; Marschner, 1995)

In general, VAM have been found to enhance uptake of nutrients of low mobility in the soil solution such as P, Zn and Cu. The external hyphae bridge the area of nutrient depletion around the root zone (Marschner, 1995). Additionally, VAM accumulate polyphosphates in their vacuoles which have been suggested as a vehicle to transport inorganic phosphate across the plasma membrane of the host root cell (Gianninazzi-Pearson, 1988, Ratnayake et al. 1978). The effectiveness of VAM to transport phosphorus to the host plant depends on the VAM fungal species (Marschner, 1995). Researchers have found that the tissue levels of P, Zn, and Cu are typically higher in mycorrhizal plants compared to non-mycorrhizal plants (Azaizeh et al., 1995; Furlan and Bernier-Cardou, 1989; Krishna and Bagyaraj, 1984; Marschner and Dell, 1994).

Researchers have found VAM plants are often more resistant to several soil-borne pathogens including *Phytophthora cinnamomi, Rhizoctonia solani, Fusarium oxysporum and Pythium spp.*(Fitter and Garbaye, 1994; Linderman, 1994). The mechanisms of the protective effect of VAM fungi include mechanical protection due to morphological changes in the root tissues, antibiotic production, phytoalexins, rhizosphere modification

especially in the microbial populations, and host mineral nutrition (Fitter and Garbaye, 1994; Linderman, 1994).

Research has also shown that VAM plants can survive on less water which has important implications for nursery growers needing to reduce water runoff. Sweatt and Davies (1984) found that root systems of mycorrhizal geraniums were more efficient at depleting soil water and that VAM plants acclimated more efficiently to water stress.

Plant growth responses

In general, most growth enhancement effects of VAM root infection are caused by increases in P absorption, according to Marschner and Dell (1994). Researchers have found that mycorrhizae facilitate P absorption especially in growth media with low P availability (Gerdemann, 1964; Menge et al., 1978; Menge et al, 1980; Mosse, 1973; Mosse et al., 1976). Numerous studies have examined the host plant response to mycorrhizal colonization of roots. Nemec (1992) found that *Glomus intraradix* caused plant growth increases of two to three-fold in his study of citrus rootstocks in various potting media. Additionally, he found up to fourfold root mass increases in sand-perlite mixes that had been inoculated. Other media increased root growth mass by 1 to 2.5-fold. Johnson et al. (1984) found similar responses in chrysanthemum when grown in inoculated soils.

Maronek et al. (1980) also noted increased growth in southern magnolia and Bar Harbor juniper when grown in composted hardwood bark - shale mix inoculated with *Glomus fasciculatus* (Thaxter) Gerdemann and Trappe. Crews et al. (1978) found that three woody ornamentals inoculated with either *Glomus fasciculatus* or *G. mossae* (Nicholson and Gerdemann) Gerdemann and Trappe showed improved growth in plant height, stem caliper, shoot and root fresh weight.

Biermann and Linderman (1983) found host growth response and more crop uniformity in geraniums inoculated with *Glomus fasciculatum* grown in soilless media or soil. When the plants grown in soilless media were transplanted into soil, host plant growth response was dramatic even if no responses was noted prior to transplanting. This may have important implications for the horticulture industry which may not notice significant differences in inoculated plants while in containers, but transplanting survival may be greater even for herbaceous species such as geraniums.

Problems with VAM in soilless media

According to Marschner (1995), limitations to inoculating with VAM include: lack of pathogen-free inoculum in sufficient quantities; poor knowledge of host plant/VAM fungal species interactions; competition with indigenous VAM fungal species; and the need to introduce VAM fungi at high densities to offset competition.

Additionally, it has been suggested that nutrients added to potting media may reduce the inoculum potential (Chambers et al., 1980; , Johnson et al., 1980, Johnson et al., 1984; Nemec, 1992). However, Morrison and Nicholl (1993) found that *Glomus intraradices* Schenck and Smith could colonize a variety of nursery stock even under normal nursery fertilization practices. Similarly, while Johnson (1980) found reduced infection rates under a high N fertilizer regime, the woody ornamentals still exhibited enhanced growth with mycorrhizal inoculation. He concluded that container production of woody ornamentals would lend itself to inoculation under a wide range of nursery fertilizer practices. Different plants grown under the same conditions can respond differently to colonization. Maronek et al. (1980) found differences in southern magnolia and Bar Harbor juniper, when inoculated with the same fungus and grown in the same growth medium, to be in marked contrast to each other. He suggested that the differences were due to the nature of the root system, the release of the fertilizer, or the type of

growth conditions used for the experiment. No eveidence of inhibition was found by using slow release fertilizers and Maronek et al. (1980) felt that slow release fertilizers might enhance colonization.

Other researchers have suggested that the pH of the substrate may be outside the limits tolerated by the fungus (Nemec, 1992; Menge et al., 1982, Hepper and Warner, 1983). Additionally, media components may be fungitoxic (Nemec, 1992).

Hepper and Warner found that organic matter played an important role in the growth of VAM fungal hyphae in soil. Their studies indicated that VAM fungal hyphae could grow saprophytically in soil if organic matter was present and that the infective material could survive up to 50 days prior to the presence of a host plant. However, Menge et al. (1982) found that mycorrhizal dependency of citrus was inversely correlated with the percent of organic matter in the soil. They pointed out that in the soils surveyed organic matter was closely correlated with extractable soil P and therefore, did not use organic matter in their predictive equation.

Nemec (1992) found that fungus development in the roots decreased as the proportion of sphagnum peat was increased in the mix. Likewise, infection levels of roots grown in the reed sedge peat/perlite experiment decreased with increasing levels of peat. The highest levels of infection were found in the roots of plants grown in sand-perlite blends. Biermann and Linderman (1983) found that all four sphagnum peats used in their study reduced the amount of colonization in geranium roots compared to mixes containing soils. They concluded that peats had an inhibitory effect on colonization in geraniums, and in two of the peats tested, mycorrhizal growth enhancement was not noted in any combination of soil and peat.

Types of compost

Many types of compost are available on a regional basis throughout the United States. As the landfill sites have been depleted, attention has focused on composting any organic matter previously disposed of in the waste stream. In this study, four regionally available composts were examined.

Vermicompost

Vermicompost is a mixture of worm castings and unused bedding produced by worms (usually *Eisenia foetida*) feeding on organic matter. The worms may be fed any or all of the following feedstocks - animal manures, post consumer vegetable waste, restaurant food waste, grass straw, paper, coffee grounds, farm vegetable or fruit wastes. The compost is generally of a granular nature and easy to blend into a soilless mix. Handreck and Black (1994) examined the chemical characteristics of vermicomposts derived from various feedstocks and found that pH levels ranged from 5.8 to 7.8; N ranged from 1.1 to 2.7% of dry matter; P ranged from 0.4 to 3.2 % of dry matter and K ranged from 0.1 to 1.2% of dry matter. Similarly, the levels of minor nutrients covered a broad range with some at toxic levels for plant growth.

Worm castings have been shown to have a beneficial effect on root growth and plant development (Grappelli et al., 1985; Edwards, 1980) Growth regulators, specifically gibberellins, cytokinins and auxins have been found in worm castings along with microbial populations that could possibly be antagonistic to pathogenic fungi (Grappelli et al., 1985; Brown, 1995). Additionally, worms have been found to transport VAM fungal propagules through their digestive tract (Harinikumar et al., 1991) Under the proper conditions, VAM fungal spores can germinate and grow in worm castings, infecting host plants (Ponge, 1991; McIlveen and Cole, 1976; Reddell and Spain, 1991).

Yard Waste Compost

The most widely used compost is derived from yard wastes: grass clippings, leaves, discarded plants and their soil, flowers, weeds, prunings and bark. Yard waste can vary and usually does depending on the time of year, resulting in a lack of consistency in both physical and chemical properties. In Oregon, it is estimated that 40 percent of the refuse brought to landfills is yard debris, and communities throughout the state are taking measures to keep this organic material out of crowded landfills. Consequently, the availability of composted yard waste is growing rapidly and with the larger amounts, some improvement in consistency is to be expected. Research has shown that woody ornamentals can be successfully grown in up to 80% yard waste compost (Rayner and Arnold, 1993).

Mushroom Compost

Mushroom compost is primarily derived from straw-bedded horse manure amended with gypsum, poultry litter, peat moss, straw, cottonseed or soya bean meal, and urea or ammonium nitrate. After composting, the composts are inoculated with the spawn of *Agaricus bisporus* which is grown for approximately 8 to 10 weeks. Following cropping, the spent substrate is usually pasteurized and then moved outside to be leached and weathered for up to six months. Spent mushroom compost has traditionally been discarded as waste, but in recent years agriculture, horticulture, soil reclamation projects and purification of contaminated soil or water projects have found a use for this product. As mushroom growers have been facing increasing environmental pressure over their discarding of the spent substrate, the demand for organic matter is increasing and mushroom compost is beginning to have many uses. Since much of the mushroom production is in urban areas, the social and environmental pressure to dispose of the substrate is high.

The chemical, physical and microbiological properties needed by each of the potential end users of the spent compost may vary. Potential difficulties with mushroom compost include high soluble salt levels, especially Na and K, and high pH levels (above 7.0). Chemical properties fall within the following ranges according to Szmidt and Chong (1995): pH - 8.2 to 7.3; electrical conductivity- 2.38 to 1.41 dS/cm; C:N ratio - 15:1 to 9:1; total N 9 - 4 g/kg; P 11.2 - 2.9 g/kg; K 18.2 to 3.3 g/kg. In a 1991 study, Chong et al.(1991) found that five woody ornamentals responded positively to the presence of mushroom compost in a container mix, while three others had a negative response. Only one species, privet, showed phytotoxic symptoms to the presence of mushroom compost. Previous recommendations for nursery crops included up to 15% of composts in the growing medium (Greenhouse Manager, 1985); 33% decomposed compost for greenhouse crops (Rathier, 1982); 30%-50% compost for vegetable crops (Wang et al. 1984); and 15-20% compost for landscape crops (Smith, 1982). Further studies by Chong et al in 1994, indicated that 25% mushroom compost would be a satisfactory level for containerized nursery crop production.

Processed Manure Fiber

Processed manure fiber is a relatively new product for the Northwest and still under development. It has been successfully produced in Hawaii and the Netherlands for several years. The fiber is a byproduct of dairy wastes anaerobically digested in a closed vessel system. After digestion, the processed fiber is composted with wood waste at a 1.5 fiber:1 wood ratio by volume. Following composting, it is screened through a 1/2" screen. It is characterized by having relatively high pH and high soluble salts levels. The primary salts come from the elevated levels of potassium, sodium, sulfate, and phosphate. The general recommendation for incorporation of manure fiber into growth medium is 10-15% by volume (Fonteno, 1996).

Factors influencing VAM establishment

Phosphorus levels and mycorrhizae

Increasing levels of P in the root zone have an inhibitory effect on mycorrhizal colonization. Schubert and Hayman (1986) showed that mycorrhizal colonization usually decreased with increasing amounts of P, but the rate of root colonization and the colonization plateau varied with different VAM fungal species. Menge et al. (1978) found that high P fertilization reduced colonization and hyphal production as well. Amijee et al (1989) discussed the negative correlation of soil P on mycorrhizal colonization which is in agreement with other researchers (Fay et al., 1995).

With increasing soil P levels, the growth enhancement effect of VAM decreases and may lead to reduced growth in the host plant. A shift in root/shoot ratios is often observed in favor of the shoot (Marschner and Dell, 1994). Biermann and Linderman (1983) determined that the extractable P concentration in peat was not related to mycorrhizal growth enhancement, but that the equilibrium solution P levels of peat were negatively correlated with host growth enhancement. Datnoff et al. (1991) looked at P levels in thirteen commercial mixes used in plug production of tomatoes. In determining the P levels in their mixes, they carried out both an acid extraction and a water extraction of P. Levels of P determined by acid extraction ranged from 4 to 154 lb/A and water extraction ranged from 2 to 63 lb/A. They observed a negative correlation between percent VAM fungal colonization of the roots and P ($r=-0.58$, $P=0.05$).

Soluble salts

Soluble salts are usually the various ions that are in soluble forms in substrates or soil, i.e. Na^+, K^+, Ca^{+2}, NH_4^+, NO_3^-, Cl^-, SO_4^{-2}, HPO_4^{-2}. The concentration of soluble salts in mixes is determined by measuring the electrical conductivity of the mix. Soluble salts

can be reported as ppm or dS/cm (dS/cm x 640 =ppm). High soluble salts are injurious to plants directly or indirectly by preventing uptake of water due to the lowered osmotic potential (Handreck and Black, 1994). Ideal conductivity of media for greenhouse plants range from 0.75 to 3.50 dS/cm according to Handreck and Black(1994).

Composts typically have a high initial soluble salts level between 3.5 to 15.0 dS/cm (Handreck and Black, 1994) In research by Datnoff et al. (1991) soluble salts ranged from 97 to 1511 ppm (0.14 to 2.13 dS/cm). They found a negative correlation ($r = -0.58$, $p = 0.05$) between soluble salts and colonization. However, work done on woody ornamentals by Verkade and Hamilton (1985) showed 30-50% colonization under high fertility levels (soluble salts-164 mg/l). Similarly, research by Johnson et al. (1980) showed that colonization was acceptable even under high levels of N, K, and Mg fertilization. The differing reports on mycorrhizal colonization and the soluble salt levels found in many composts made this area a logical focus of this research.

Microbial activity

The presence of bacteria have been reported to enhance germination of VAM fungal propagules and hyphal growth (Carpenter-Boggs et al., 1995, Mayo and Davis, 1985, Paulitz and Linderman, 1989). Mosse (1959) found that stimulation of VAM fungal spore germination in a soil extract required actively-growing microorganisms. Azcon-Aguilar and Barea (1985) showed that spores of *Glomus mosseae* contaminated with bacteria formed more mycorrhizae on roots grown in sterile soil than did spores that had been surface sterilized. Tylka et al. (1991) found that three *Streptomyces spp.* had different effects on the spore germination of VAM fungi. Stimulation of VAM fungi by microorganisms has been found to occur even though there was no direct contact between the two, suggesting the presence of volatile factors (Linderman, 1992). Mugnier and Mosse (1987) showed that the presence of *Streptomyces orientalis* on the bottom of an agar plate or in a divided petri dish was necessary to stimulate spore germination of *G.*

mosseae. Likewise, Carpenter-Boggs et al. (1995) found that volatiles from actinomycetes stimulated germination of *Gigaspora margarita* and suggested a mutually beneficial relationship between host plant, VAM and chitinaceous actinomycetes.

Compost from grass straw has actinomycetes in the 10^8 level (Horwath et al., 1995) as does mushroom compost (Miller et al., 1990) and worm castings (Grappelli et al., 1995). In spent mushroom compost, supplements added to the original compost may influence the microbial populations during cropping and composting. Microbial populations in various horticultural growing media have been shown to be sensitive to small quantities of supplements (Pattison et al.,1993).

It appears that many soil bacteria can affect VAM fungal spore germination. Additionally, some bacteria have been shown to have a beneficial effect on the VAM symbiosis, enhancing the growth response (Mosse, 1962; Meyer and Linderman, 1986; Ames, 1989). The term, mycorrhizal helper bacteria, has been coined for the bacteria suspected to stimulate mycorrhizal development (referred to as MHB). Much of the work in this field has been done with ectomycorrhizae rather than endomycorrhizae. The review by Garbaye (1994) details the research that has been carried out in this field. A few interesting notes include that the majority of MHB found to date are fluorescent pseudonomads and sporulating bacilli and they seem to be closely associated with mycorrhizal fungi under a broad range of conditions.

Inhibition of VAM fungi by soil microbes has also been suggested. Ross (1980) found that the addition of small amounts of non-sterile soil to sterilized soil inhibited *Glomus macrocarpum* compared to the sterilized soil. Wilson et al. (1988) found that spore germination of two VAM fungal species was inhibited in a non-sterile soil compared to sterilized soil and the addition of non-sterile soil to the sterilized soil produced inhibition as well.

As Linderman (1992) points out in his review of microorganisms and VAM fungi, there is still much to be learned about their interactions. The complexity of microbes in

the soil makes it difficult to draw conclusive statements about inhibition or enhancement and further research will likely address some of these areas.

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CHAPTER THREE

Establishment of VAM in onion roots and the relation to phosphorus levels and microbial activity in four composts

Abstract

Four composts - vermicompost, yard waste compost, mushroom compost and processed manure fiber- were blended with peat moss and perlite at a volume of 35% peat/35% compost/30% perlite. Half of the treatments were inoculated with *Glomus intraradices* prior to planting .

The mycorrhizal plants usually had greater caliper than the nonmycorrhizal plants. Height was greatest in the mycorrhizal plants in the control medium. Root/shoot ratios were greatest in the nonmycorrhizal plants in the control medium, but there were no differences for the remaining treatments.

Colonization of roots was related to the initial levels of phosphorus (P) present in the media as well as the release of P into the soil solution. No significant correlation was found between microbial populations and percent VAM colonization, but all four compost amendments significantly increased microbial counts, including number of antagonists against the root pathogen, *Thielaviopsis basicola.*

Introduction

Vesicular-arbuscular mycorrhizae establish readily in mineral soils but establishment in soilless media (e.g. peat moss) has been difficult (Biermann and Linderman, 1983 a,b; Menge et al., 1982; Nemec, 1981). Biermann and Linderman (1983) had reduced colonization in four sphagnum peats, though not in a hypnum peat, and linked

the inhibitory nature of peat moss to the equilibrium solution P concentration of the fertilized peats. Nemec (1992) found that VAM fungus development in the root system was inversely correlated with the amount of sphagnum peat in the mix. Menge et al. (1982) found that the commercial greenhouse and nursery mixes used as part of their trials had low percentages of VAM colonization.

In more recent years, research has been focusing on the relationship between mycorrhizae and other microorganisms in the rhizosphere (Linderman, 1982, 1983; Marschner, 1995; Garbaye, 1992). Garbaye (1992) reviewed the relationship between mycorrhizae helper bacteria (MHB) and mycorrhizal fungal growth and concluded that current research supports the theory that mycorrhizal fungi grow better in the presence of MHB. Composts typically have high levels of bacteria, actinomycetes and fungi, and some could be expected to enchance VAM fungal growth.

Limited work on composts and mycorrhizae (Guttay, 1982, 1983) has not addressed the effect of composts on VAM establishment. Guttay (1982, 1983) used inoculation of seeds or plants with native mycorrhizae in plant-derived compost. Other researchers (Sweatt and Davies, 1984; Maronek et al., 1990; Datnoff et al., 1991) have used composted bark or manure as a component in their plant mixes when examining VAM establishment, but have not addressed the question of compost effect. Nursery growers, in the Northwest, have asked what the use of compost will do to the mycorrhizal inoculum which is being added to their mixes. Therefore, our primary objective in this study was to see how composts would affect the establishment of VAM fungi, using onion roots as our bioassay. We were also interested in knowing if the addition of compost would improve the percentage of roots colonized compared to a peat moss/perlite growth medium. In this experiment, we hypothesized that the higher levels of P present in the compost blends would inhibit the formation of mycorrhizae. Additionally, we hypothesized that media which readily released P into the soil solution would inhibit mycorrhizae formation. Composts vary in the amount of P in an organic form compared

to a mineralized form depending on the maturity and stability of the compost. It is unknown how these proportions influence VAM fungal spore germination and establishment. Our final objectives were to look at the populations of antagonistic bacterial colonies and percent of mycorrhizal colonization to determine if the presence of these bacteria would correlate with improved mycorrhizal colonization and determine the antagonistic potential of the compost-amended media.

Materials and methods

Media blends and experimental design

Five growth media blends were prepared in the following combinations: a) control medium consisting of 70% Canadian sphagnum peat moss (Lakeland Peat, Edmonton, Alberta) with 30% horticultural grade perlite (Supreme Perlite, Portland, Oregon), supplemented with 8 lb. of dolomite lime per cubic yard of media; b) 35% Canadian sphagnum peat moss, 30% horticultural grade perlite, and 35% yard debris compost - primarily composted grass clippings and leaves (Valley Disposal, Corvallis, Oregon); c) 35% Canadian sphagnum peat moss, 30% horticultural grade perlite, and 35% composted earthworm castings (Resource Conversion, Yelm, Washington); d) 35% Canadian sphagnum peat moss, 30% horticultural grade perlite, and 35% composted spent mushroom substrate (Pictsweet, Salem, Oregon); and e) 35% Canadian sphagnum peat moss, 30% horticultural perlite, and 35% processed manure fiber (MEAD project, Tillamook, Oregon). A wetting agent (Aquagro 2000L, Aquatrol Corp., New Jersey) was added to each media blend at the rate of 2 ounces per cubic yard of material. No lime was added to the compost blends as the pH of each was between 6.2 and 7.2.

Each medium was analyzed for pH, soluble salts, cation exchange capacity, N, P, K, Ca, Mg, Na, and S by the Oregon State University Department of Soil Science Soil and Plant Analysis Lab (Table 3.2). The pH was by a 1:2 soil - water ratio. Soluble salts was from a saturated paste extract with a Solu-Bridge (RD-26, Industrial Instruments, Cedar Grove, New Jersey). Total N was determined spectrophotometrically by an Alpkem rapid flow analyzer, RF-300, (Alpkem Corporation, Wilsonville, Oregon) after a Kjehdahl digestion. Phosphorus was determined colorimetrically after dilute acid-fluoride extraction using the Alpkem rapid flow analyzer, RF-300. Calcium, magnesium,

potassium and sodium were extracted with ammonium acetate and determined with atomic absorption (Perkin-Elmer spectrophotometer model 372). Sulfur was determined by ion chromatography. All methods are described in Methods of Soil Analysis, Oregon State University, (1989).

Forty Ray Leach Supercell-10 (Steuwe and Sons, Corvallis, Oregon) were filled with 150 mls of growth media, 20 amended with live VAM fungal inoculum and 20 with killed inoculum that had been autoclaved.

The experiment was arranged as a complete randomized block design. For the 20 inoculated supercells, a cotton plug was placed at the bottom of each tube, and then filled with 150 ml of medium and tapped once on the greenhouse bench to settle the mix. The VAM fungal inoculum of 10 ml of *Glomus intraradices* (Tree of Life Nursery, San Juan Capistrano, California) in a calcined clay carrier, was placed on top of the medium. Onion seeds (*Allium cepa* cv. Yellow Sweet Spanish Utah) obtained from Lake Valley Seed, Boulder, Colorado were planted two per cell and then topdressed with 5 ml of the control medium (see medium a above).

The other 20 supercells of each medium blend were filled as above but amended with killed inoculum prepared by autoclaving for one hour at 121 degrees C (15 lbs. PSI) to kill all mycorrhizal fungal propagules. After autoclaving, the clay was covered and allowed to cool for 24 hours. To prepare the microbial control solution, one liter of distilled water was added to one liter of calcined clay inoculum of *G. intraradices*. The mixture of water and clay was poured through a 38μm sieve and the resulting liquid was collected (hereafter referred to as microbial extract). The extract was filtered through a Whatman #1 filter. The extract contained the microorganisms associated with the mycorrhizal fungi and they were added to the root zone. Each cell received 10 ml of killed inoculum and 1 ml of the collected microbial extract. Each cell was planted with two seeds as described above.

Ten days after germination, all cells were thinned to one seedling per cell and topdressed with one tablespoon of white quartz grit to minimize splashing of inoculum and prevent the growth medium from washing away from the developing roots. Tubes were watered from the top during the course of the experiment with a wand and breaker to help minimize splashing.

Plants were grown in a greenhouse with temperature controls set at 16° C (night) and 20 to 22° C (days) from March 25, 1996 to June 4, 1996. Plants were hand watered every two days and fertilized weekly with 40 ml per cell of a modified Long Ashton nutrient solution, without phosphorus (Hewitt, 1966). Supplemental lighting with an intensity of 200 $\mu E/m^2$ was provided from 6 am to 10 p.m. daily with high-pressure sodium vapor lamps.

The experiment was a completely randomized design with 20 container replications per treatment. After 4 weeks of growth, plants were measured for total height and caliper of shoot. Caliper measurements were taken just above the base of the first leaf. Plants were harvested at 10 weeks and roots and tops separated. Subsamples of roots were collected for clearing and staining of mycorrhizae (Phillips and Hayman 1970). Root samples were assessed for colonization levels on the 100 grid line intersection count using the gridline intersect method detailed by Giovannetti and Mosse (1980) with an estimated standard error of $\pm$4%.

The remaining roots and all of the tops were dried at 65 ° C for 3 days. Dry weights were recorded for roots and tops. Root to shoot ratios were calculated from dry weights. Dried tops were ground and three subsamples from each treatment were chemically analyzed except for the non-inoculated peat/perlite control which only had enough tissue for one sample, and the inoculated peat/perlite which had only enough tissue for two samples due to the small size of the plants at harvest. Tissue analysis for P was carried out by an inductively coupled plasma spectrometer (Jarrell-Ash ICP 9000, Waltham, Massachusetts).

Antagonistic Microbial Populations

Total antagonistic microbial populations were determined for each medium as follows. Soil extract agar medium was prepared according to Wollum (1982). One Kg of the control blend of peat/perlite was mixed with one liter of distilled water. The suspension was separated into two 1000 ml flasks and autoclaved for one hour at 121 °C (15 lbs. PSI). After cooling, the mixture was decanted and filtered though a Whatman #1 filter. The extract was centrifuged for 5 minutes at 7000 rpm, decanted and brought back to one liter of solution with distilled water and stored at room temperature in covered flasks until needed. To prepare the soil extract agar, 100 ml of soil extract was combined with 900 ml distilled water, 15 g agar, 0.5 g K_2PO_4 and 1 g of glucose and pH was adjusted to 6.9 with 1N NaOH. The mixture was autoclaved for 30 min at 121 ° C (15 lbs. PSI), then put in a water bath to cool. When sufficiently cool, petri plates were hand poured and the agar was allowed to solidify. Plates were stored at room temperature in plastic storage bags until used.

Ten g of each plant growth medium blend were added to 100 ml of 0.1M KPO_4 buffer and placed on the shaker table for 25 min at 200 rpm. Ten fold dilutions were made to concentrations of 10^{-4}, 10^{-5} and 10^{-6}. Then 0.1 ml of solution per plate was spread, using a sterilized glass spreader. Each dilution had two replicates and plates were inverted and stored at room temperature for 24 h.

Analysis for antagonistic bacterial colonies was performed by the pathogen-overspray method (Hoefnagels and Linderman, unpublished data). The pathogen used was *Thielaviopsis basicola* Berk.. The spore suspension was prepared by adding 10 ml of sterile distilled water to a *T. basicola* culture and the conidia were removed with a sterile glass spreader. This conidial suspension was poured into a plastic tube connected to a Sigma compressed air sprayer. A plastic bag was taped open inside the laminar flow hood and each dilution plate from growth medium extracts was held inside the bag and sprayed

once with spores. Immediately after spraying, the covers were replaced and the plates were inverted and stored at room temperature for 48 h. Bacterial colonies showing clear zones where the *T. basicola* conidial germination and growth were inhibited were then counted (Linderman and Marlow, unpublished data; Linderman, 1993).

Nutrient retention/phosphorus desorption

A comparison of the nutrient retention properties of the growth media blends was performed using phosphorus desorption as the indicator. Phosphorus desorption values were calculated using a modified Fox and Kamprath (1970) procedure. Thirty-five ml of medium were placed in a 250 ml flask with either 50 ml of 0.01M $CaCl_2$, or 50 ml of 0.01M $CaCl_2$ containing 250 ppm P from $Ca(H_2PO_4)_2$. The flasks were shaken for 11 days at room temperature to equilibrate. After equilibration, 36 ml of solution was removed and retained from each flask and replaced with an equivalent amount of 0.01M $CaCl_2$. The mixtures were shaken for 2 to 4 h and then solution was extracted and retained again. The process was repeated three times. All retained solutions were sent to the Oregon State University Soil Analysis Lab for determination of P levels by colorimetry.

Statistical analysis

The experiment was repeated once; data were similar for the two but are presented for the second experiment only. Planned statistical comparisons (F tests) were made between mycorrhizal and nonmycorrhizal plant growth parameters, between root/shoot ratios, between media blends using percent root length with VAM colonization, between P levels in tissue samples and P levels in compost blends, between percent VAM colonization and P desorption rates, and between antagonistic microbial colonies to *T.*

basicola between blends and percent VAM colonization. All statistical analysis was done with Statgraphics 7.0 (Manugistics, Rockville, Maryland).

Results and discussion

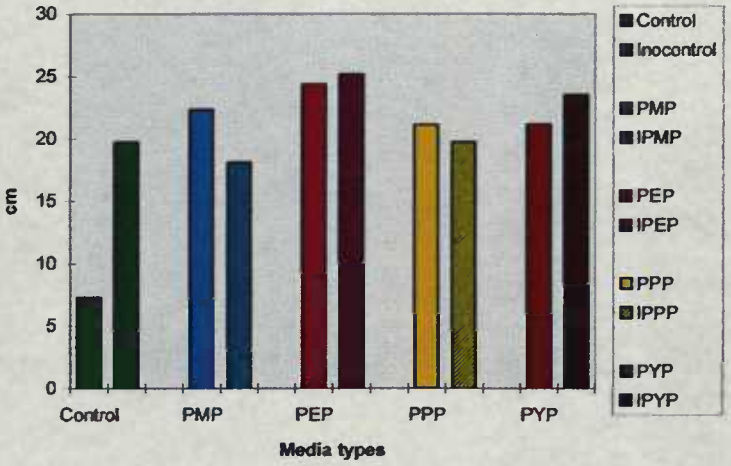
Plant growth parameters

There were significant differences (P=0.05) between the non-inoculated base medium and the inoculated base medium plants as well as the inoculated and noninoculated plants grown in the mushroom compost blend (Table A.1). The remaining plants showed few differences between the inoculated and noninoculated plants in each media type. Caliper measurements (Table A.2) showed significant differences (p=0.05) between inoculated and non-inoculated plants in every growth medium except the yard waste compost. Caliper was greater in inoculated plants than noninoculated seedlings.

Root and shoot measurements were taken at harvest on a dry weight basis then root/shoot ratios were analyzed. The only significant difference (P=0.01) in root/shoot ratios was the noninoculated control (Table A.3) compared to all the other treatments. The shoot dry weights were significantly different (P<.0001) between the inoculated and non-inoculated plants grown in the base medium. However, significant differences were not found between the inoculated and non-inoculated plants in the other growth media (Table A.4).

Mycorrhizal fungi are obligate biotrophs and depend on the organic carbon from the living plant roots. Their presence may change root exudation by the colonized plant (Azaizeh et al., 1995). Nevertheless, the beneficial effects of colonization may not be enough to overcome the loss of photosynthates to the fungi. Approximately 6-10% of the net fixed carbon is diverted to the roots and in some cases the carbon diverted to the fungi may decrease plant growth. In some cases, researchers (Wright et al., 1995; Fay et al., 1996) have found that the 'sink' demand of mycorrhizal metabolism may exceed the photosynthates produced.

Figure 3.1 Summary of height at 4 weeks after inoculation with *Glomus intraradices* in various potting mix blends.

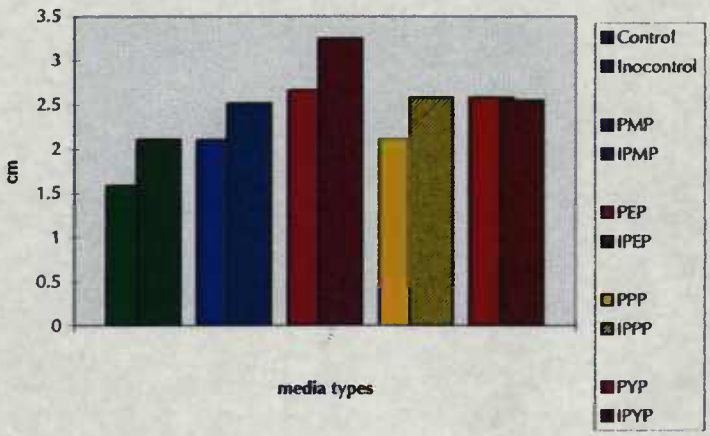


Marschner (1995) states that despite the benefits of enhanced nutrient uptake in mycorrhizal plants, the host root growth is less enhanced or even depressed compared with shoot growth and the root/shoot dry weight decreases accordingly. This corresponds to the results found here between the nonmycorrhizal and the mycorrhizal plants in the control medium of peat moss and perlite. He states that if nutrients are not limiting and the plants derive no other benefit from the presence of mycorrhiza, mycorrhization depresses root growth primarily by sink competition in that the mycorrhizal fungi draw photosynthates away from the roots. In this experiment, sufficient nutrients were provided through the growth medium in all the compost blends as well as the weekly

fertilization of the plants. However, in the control medium, the inherent nutritional content in the growth medium was lower than the other blends.

In this case, the plant may have benefited strongly from the mycorrhizal root system regardless of the sink demand. The mycorrhizal fungi remain a strong sink regardless of their contribution to host plant growth. Schubert and Hayman(1986) found a smaller root/shoot ratio in the mycorrhizal plants than in the controls with no added P. As P levels increased in the growth media, the root/shoot ratios were closer to the controls, though slightly lower. Their findings support the observations made here as well. Due to the sufficient levels of nutrients in the composted blends and the weekly fertilization, there may have been sufficient nutrients for both healthy plant growth and mycorrhizal roots.

Figure 3.2 Summary of caliper at 4 weeks after inoculation with *Glomus intraradices* in various potting mix blends.

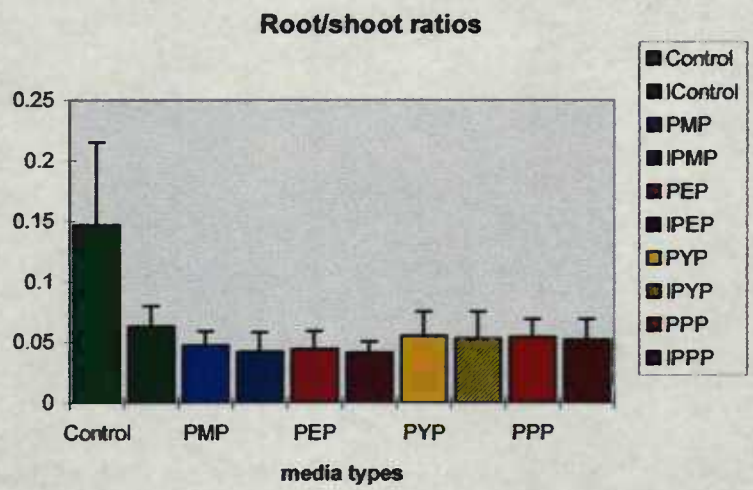


Berta et al. (1990) also found in *Allium porum* colonized with *Glomus mosseae*, that total root length decreased, but the number of lateral roots per unit root length increased as did branching. This was observed also in this study in all treatments except the vermicompost blend which had low colonization (see Table 3.1) Marschner (1995) reported that newly formed roots were shorter and the activity of their apical meristems declined rapidly, which explains the higher branching rate in mycorrhizal root systems.

He suggests that higher rates of phloem unloading of photosynthates and IAA at the sink sites of VAM colonization could explain this characteristic of mycorrhizal roots.

Biermann and Linderman (1983b) found that mycorrhizae increased shoot dry weight in soil-based mixes under low P fertilization. However, in soilless mixes, inoculation actually decreased the shoot dry weight even under low P fertilization. In our study, plants grown in the control medium of peat/perlite had a ten fold greater shoot dry weight when inoculated with mycorrhizal fungi (Figure 3.4). Interestingly, the yard waste compost blend caused a slight decrease in the mycorrhizal plant biomass while the mycorrhizal plants in other media had slight increases in biomass.

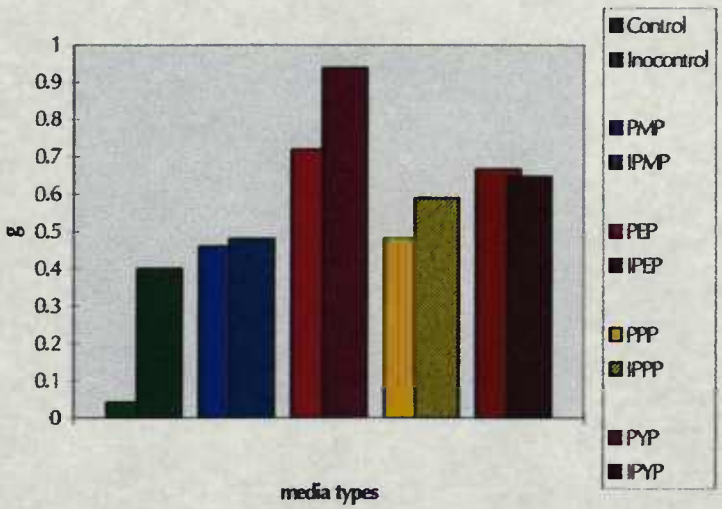
Figure 3.3 Root/shoot ratios at 10 weeks after inoculation with *Glomus intraradices* in various potting mix blends.



VAM colonization of roots and plant growth in relation to P levels in media

In this study, the highest levels of colonization were found in the base medium, followed by blends with yard waste compost, mushroom compost, processed manure fiber and finally vermicompost (Table 3.1). There was no colonization of noninoculated

Figure 3.4 Shoot dry weight of onion seedlings at 10 weeks.



plants. The vermicompost, which had previously been reported to be conducive to the germination and growth of VAM propagules, had the lowest root colonization rates. One possible reason for this was the presence of at least two other fungi found in association with the onion roots when stained and observed. Possibly these were direct competitors with the *Glomus intraradices* although they were not mycorrhizal fungi. It is well documented that earthworms feed primarily on fungal hyphae and pass fungal spores through their guts undigested (Brown, 1995; Dash et al., 1986; Edwards and Fletcher, 1988; Morgan, 1988) and this could have been the source of the competing fungi.

Table 3.1 Root colonization of onions in media blends inoculated with *Glomus intraradices.*

Media	P levels	% colonization	Standard error
Control	13	72.5a	8.5
PYP	77	53.1a	12.6
PMP	161	15.7b	5.3
PPP	644	8.9b	2.2
PEP	729	2.3b	1.2

Standard error n=7

Table 3.2 shows the nutrient analysis of the growth medium prior to planting. The variation in P levels was great, ranging from 729 ppm P for vermicompost to 13 ppm P for peat/perlite. A comparison of the P values with the percent VAM colonization seems to indicate that a direct correlation may exist (Table 3.1). A linear regression of colonization on P media levels provides a correlation coefficient of -0.69. The standard deviations in percent of roots colonized with VAM are not equal, so the use of a linear regression model is perhaps not appropriate for this data (Appendix Table A.5)

Biermann and Linderman (1983a) also found that increasing fertilizer P decreased the VAM colonization of geranium in soilless mixes. In this study, the two mixes with the highest initial levels of P (see Table 3.1) had the lowest colonization levels. However, they found in peat mixes that the colonization was sparse and limited to the outer cortical cells. In contrast, we found extensive colonization throughout the entire cortex on the plants grown in the peat/perlite blend.

Table 3.2 Nutrient analysis of unused growth media

Media	pH	Soluble Salts	CEC meq/100 g	total N %	P ppm	K ppm	Ca meq/100 g	Mg meq/100 g	Na meq/100 g	SO_4-S ppm
Control	7.0	0.5	97.1	0.79	13	113	47.0	43.0	0.75	144.0
PYP	5.7	1.1	61.9	0.89	77	2886	30.0	10.4	1.10	12.2
PEP	5.6	2.3	86.2	1.67	729	2730	39.0	18.6	3.74	199.0
PPP	6.9	17.0	86.9	1.74	644	17160	35.8	31.8	13.40	2377
PMP	6.1	7.5	63.4	1.24	161	5928	70.0	11.6	2.8	4170

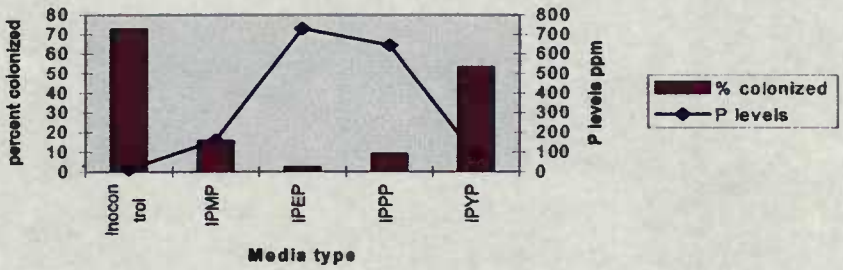
Control is peat/perlite blend, PYP is peat/yard waste compost/perlite; PEP is peat/vermicompost/perlite; PPP is peat/processed manure fiber/perlite; PMP is peat/mushroom compost/perlite.

Sanders (1975) demonstrated that mycorrhizal fungal growth in onion roots was affected by the internal root P level. Johnson et al. (1984) also indicated that increased P tissue levels were the predominant factor in reduction of root infection in

chrysanthemums. Menge et al. (1978) found that tissue levels of P were critical during the early stages of infection. Research by Graham et al. (1981) suggested that P inhibition of VAM colonization is associated with a membrane-mediated decrease in root exudation.

Datnoff et al. (1991) conducted a study to evaluate commercially available mixes and their influence on colonization of tomato seedlings by *Glomus intraradix*. They concluded that the infection and colonization rates were positively correlated with low P and low soluble salt concentrations in the mix. Plenchette et al. (1983) found a host growth response from inoculation with two different VAM fungal species at all levels of P applied to a calcined montmorillonite clay, though the colonization of the roots decreased with increasing levels of P. Interestingly, Plenchette et al. (1983) found that colonization was not always highest at the lowest levels of P.

Figure 3.5 Comparison between percent VAM colonization and media P levels.



P desorption/equilibrium rates

Biermann and Linderman (1983b) examined the equilibrium levels of P in soil solution for soil, bark , sand, peat, vermiculite, and perlite individually and in various combinations. Their technique was to add nutrient solution containing either 11 ppm P or

technique used in this study in that we looked at the desorption into soil solution with a calcium chloride extract (Figure 3.6). In their trials, the components which retained the least amount of P into solution were perlite, bark, vermiculite,and sphagnum peat. Soil, sand and hypnum peat released low amounts of P into solution and therefore had the highest retention. A peat/vermiculite blend had the same level of P in solution as did vermiculite alone, whereas release from a blend of soil/bark was relatively low.

Biermann and Linderman (1983b) found that VAM colonization, growth response of the host plant, and shoot P concentration were correlated negatively with the logarithm of equilibrium solution P levels. Significant growth enhancement by VAM did not occur in media with equilbrium levels above 4 ppm P. In our experiment with no added P to the solution, the blends with intial desorption levels of less than 11 ppm P were positively associated with VAM colonization. When 250 ppm P were added to the media and allowed to equilibrate, the same three blends had the lowest soil solutions of P upon extraction, indicating that the P was bound to the substrate. Organically bound P may be higher in a less mature compost than in a compost that has stabilized and matured (Fine et al., 1989; Banin et al., 1990). The amount of solution P may be a critical factor during the germination of the VAM fungal spores since many research reports have documented a reduced infection rate under high levels of solution P (Menge et al., 1978; Schubert and Hayman, 1986; Amijee et al., 1989; Fay et al., 1996). Since the majority of soilless mixes in nurseries and greenhouses use peat, bark, vermiculite, perlite, sand or compost, verification of this hypothesis is important to the use of mycorrhizae.

Table 3.3 which shows the nutrient analysis of the used growth media provides the levels of P at the time of harvest. No additional P had been added to the mixes during the experiment. The media blends with the highest colonization rates (e.g. control, PYP

Figure 3.6 Release of P from compost-amended potting mix into soil solution when extracted with 0.01M calcium chloride.

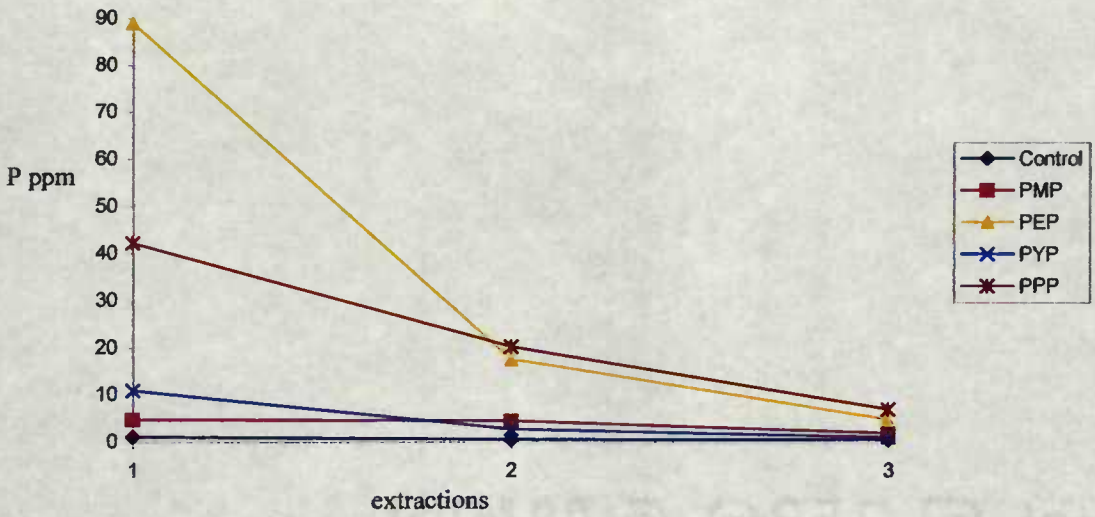


Table 3.3 Nutrient analysis of used growth media after harvest of onion seedlings

Media	pH	Soluble Salts	Total N %	P ppm	K ppm
Control	7.6	0.3	0.88	8	390
PYP	6.6	0.7	0.93	69	1092
PEP	6.4	1.0	1.40	132	421
PPP	7.0	1.8	1.37	439	803
PMP	7.1	1.2	1.24	160	530

Control is peat moss/perlite; PYP is peat moss/yard waste compost/perlite; PEP is peat moss/vermicompost/perlite PPP is peat moss/processed manure fiber/perlite; PMP is peat moss/mushroom compost/perlite

and PMP) still had close to initial levels of P at the end of the experiment. However, both PEP and PPP had released large amounts of phosphorus (597 ppm for PEP; 205 ppm for PPP) during the ten weeks. The differences between organic P and mineralized P may

have an effect on VAM colonization. As stated earlier, as a compost matures and stabilizes, more of the P is mineralized from the organic fraction. While we did not carry out a compost maturation study on this experiment, we observed that there was a wide range of stability and maturation in these four composts. We observed differences in temperature, texture and aroma between the composts which suggested the lack of full composting in some of the composts. Future studies should test these factors as well as the organic and mineral P present in the composts.

CEC/organic matter

Menge et al. (1982) noted an negative correlation between percent organic matter or cation exchange capacity and mycorrhizal dependence. Our study supports a negative correlation between CEC and mycorrhizal establishment and we concur with Menge et al. (1982) that the close correlation between CEC and P levels suggests that using CEC as a predictive value is unnecessary. Gaonker et al. (1993), using regionally available organic amendments, found that the principal effects of VAM on increased plant growth were related to the levels of N, P and C, as well as C:N ratios, in the organic amendments.

N and P in relation to VAM

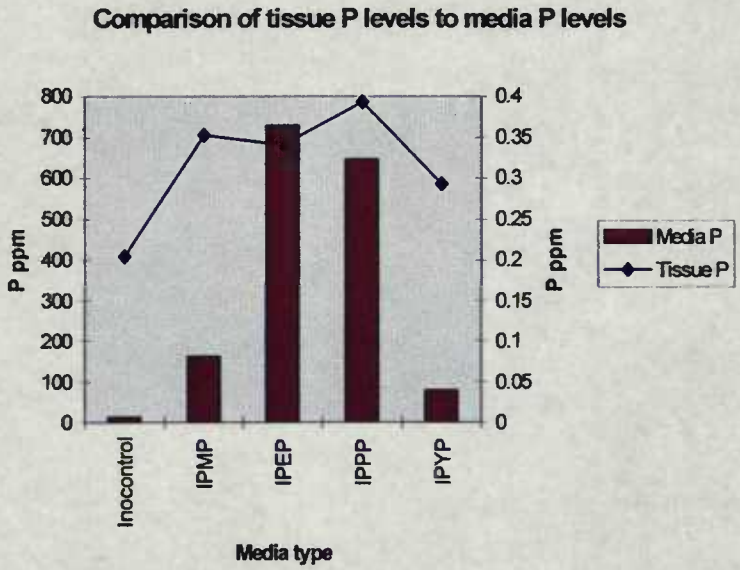
This research did not differentiate between NO_3^- and NH_4^+ ions in evaluating the total N available to the plant. However, there were differences in the total N available (see Table 3.2). Other researchers (Johnson et al., 1984; Chambers et al., 1980) have examined the effects of NO_3^- compared to NH_4^+ on VAM establishment and concluded that NH_4^+ has a negative effect on VAM colonization. High nitrogen supply reportedly depresses VAM colonization if combined with high phosphorus supply, especially if the N is present as ammonium.(Marschner 1995). In this experiment, the two composts with

high P levels also had high N levels. Previous tests (not shown) indicated that the total N levels in the processed manure compost were completely ammonium based. However, the N available in the worm casting compost was completely nitrate based.

P levels in tissue analysis

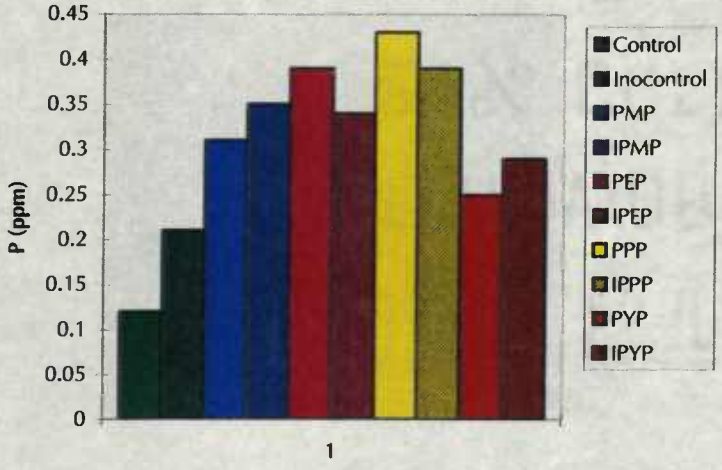
A significant difference (P=0.02) was found between media types when comparing media P levels and tissue P levels for the mycorrhizal plants (Table A.6). The levels of tissue P were lower for the mixes which had low inherent levels of P. However, no significant difference was found in tissue levels of P when comparing mycorrhizal and nonmycorrhizal plants, except for the control medium (Table A.7). Zajicek et al. (1987) found no significant differences in tissue P content between inoculated and noninoculated wildflower seedlings when grown in soil/peat/perlite or soil/sand media without additional P. In the soil/peat/perlite medium with 700 ppm P added, noninoculated seedlings had significantly higher tissue P content that inoculated seedlings, even though colonization was light. Our study found the opposite to be true. With low levels of P (e.g. 13 ppm in the base medium), there were significant differences in the tissue P content due to the

Figure 3.7 Comparison between inoculated media of tissue P levels with media P levels.



content due to the presence of VAM colonized roots. With VAM, the plants were able to compensate for the lower levels of P in the growth medium. But in the growth media with higher P, the mycorrhizal and nonmycorrhizal plants had similar levels of tissue P.

Figure 3.8 Comparison of tissue P levels in VAM and non-VAM plants.



Antagonistic microbial populations

Levels of bacterial colonies that were antagonistic to *Thielaviopsis basicola* in vitro were found to be not significant ($p=0.33$) in relation to percent of roots with VAM colonization (Appendix Table A.8). The control medium had no detectable antagonistic bacterial colonies although it had the highest levels of VAM colonization; the mushroom compost blend had 3.3×10^5; the yard waste blend had 3.5×10^5; the vermicompost blend had 1.7×10^5; and the processed manure blend had 4.0×10^4. Therefore, we concluded in this experiment that the percentage of VAM colonization was not correlated with populations of antagonistic bacteria.

All of the compost-amended blends showed increased antagonistic potential to *Thielaviopsis basicola* compared to the control medium. Since *T. basicola* is a prevalent disease in the ornamental nursery industry, the antagonistic potential of these mixes is promising. We did not test the antagonistic potential to other common nursery soilborne pathogens although that would be a worthy subject for future research.

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CHAPTER FOUR

Establishment of VAM in onion roots in relation to varying salt levels in mushroom compost

Abstract

Soluble salts and their relationship to colonization of onion roots by *Glomus intraradices* were examined in this experiment. Mushroom compost amounts from 10% to 40% (v:v) were mixed with peat and perlite for four different media blends. Twenty cells of each blend were inoculated with the VAM fungus, *Glomus intraradices,* and twenty cells were amended with autoclaved inoculum carrier and an extract of microorganisms from the inoculum. Onions were planted two seeds per cell and plants were grown for 10 weeks in a greenhouse.

Planned comparisons and regression analysis were made on percent VAM colonization, soluble salt levels, root/shoot ratios and levels of P in the shoots.

No significant difference was found between the four mixes on percent VAM colonization. Additionally, no significant correlation was found between soluble salts and percent of VAM colonization. No significant difference was found in root/shoot ratios between mycorrhizal and nonmycorrhizal plants.

Introduction

Onions are salt sensitive, showing a reduction in growth when soluble salt levels exceed 1.8 dS/cm in their growth medium (Handreck and Black, 1994). VA mycorrhizae have been shown to be sensitive to soluble salt levels depending on the species (Menge et al., 1982; Nemec, 1982). However, Verkade et al. (1985) found that *Liriodendron*

tulipifera inoculated with either *Glomus fasciculatum* or *G. mosseae* and grown under high fertility conditions had root colonization ranging from 30-60%. The seedlings were grown in a mix consisting of perlite, peat moss and loam soil. Likewise, Morrison and Nicholl (1993) found that inoculant mycorrhizal fungi were more tolerant of high soluble salts than indigenous mycorrhizal fungi and colonized woody ornamentals even at high fertility levels.

The composts used in the previous chapter had moderate to high levels of soluble salts. For the vermicompost, yard waste compost and mushroom compost, the primary source of salts came from the high levels of K rather than Na. The processed manure fiber had significant levels of both K and Na. Since most composts currently in use in the nursery industry come from yard waste or mushroom compost, we selected mushroom compost for studies on the effect of the soluble salts on VAM colonization. Our objective was to determine if higher soluble salts would be inversely correlated with VAM colonization. We did not exceed 40% compost by volume to avoid damage to the onions.

Materials and methods

Preparation of compost blends and experimental design.

Four media blends were prepared using composted mushroom substrate at volume levels of 10%, 20%, 30% and 40%. Perlite was held at 30% and peat moss was adjusted to bring the volume to 100% Blends were chemically analyzed by the Oregon State University Soil Science Analytical Lab (as detailed in Chapter 3) for pH, soluble salts, cation exchange capacity, N, P, K, Ca, Mg, Na, and S. (Table 4.1) Each media blend was either inoculated with *Glomus intraradices* or topdressed with autoclaved inoculum and microbial extract as detailed in Chapter 3. Ray Leach Supercell-10's were used with twenty cells per treatment. Two onion seeds were planted per cell and the cells were

completely randomized. Upon germination, plants were thinned to one seedling per cell and topdressed with one tablespoon of white quartz grit. Plants were hand watered every two days with wand and breaker and fertilized weekly with 40 ml of Long Ashton solution with no added phosphorus (Hewitt, 1966).

The onions were grown from April 19, 1996 to June 28, 1996 in a greenhouse with supplemental lighting as detailed in Chapter 3. At the end of 10 weeks, the plants were harvested and tops and roots separated. Subsamples of 8 to 10 roots were cleared and stained to determine VAM colonization (Phillips and Hayman 1970, Giovannetti and Mosse 1980). The remaining roots and the tops of the plants were dried in a forced air dryer for 3 days at 65 ° C, then weighed for biomass determination and root/shoot ratios. Tops were ground and combined to form two subsamples per treatment for P analysis, carried out by inductively coupled plasma spectrometer (Jarrell Ash, Waltham, Massachusetts).

Statistical analysis

Analysis of variance was carried out on the root/shoot ratios of mycorrhizal and nonmycorrhizal plants, on tissue P levels and on percent VAM colonization. Regression and correlation analysis were carried out to determine the relationship between percent VAM colonization and soluble salts. All statistical analysis was done using Statgraphics 7.0 (Manugistics, Rockville, Maryland).

Results and Discussion

Colonization of roots

No significant difference ($p = .73$) was found between the four media blends and the percent of roots colonized by VAM fungus (Table A.9). The percent of roots with VAM colonization ranged from 15.6 to 28.6 percent. Nearly all the roots had the same intensity of VAM colonization when observed under a dissecting scope, and the fungal structures were spread uniformly throughout the root system.

Tables 4.1 and 4.2 show the beginning and final levels of soluble salts, percent N, P, K, and pH in the blends. Initial soluble salt levels ranged from 2.6 to 6.2 dS/cm with the majority of the soluble salt levels coming from K^+. The salt levels of all four blends had fallen to acceptable levels for both plant and fungal growth by the end of the test period.

Table 4.1 Nutrient analysis of unused growth media

Media	pH	Soluble Salts dS/cm	CEC meq/100 g	total N %	P ppm	K ppm	Ca meq/100 g	Mg meq/100 g	Na meq/100 g	SO_4-S ppm
10MC	7.0	2.6	90.6	1.14	69	2184	54.0	26.0	1.34	1440
20MC	7.1	3.8	82.9	1.22	112	2808	61.0	25.4	1.21	2320
30MC	7.0	4.5	81.5	1.29	127	4056	67.0	24.0	1.84	3160
40MC	6.4	6.2	69.0	1.49	158	5226	76.0	18.4	2.22	3560

10MC is 10% mushroom compost/30% perlite/60% peat moss; 20MC is 20% mushroom compost/30% perlite/50% peat moss; 30MC is 30% mushroom compost/30% perlite/40% peat moss; 40MC is 40% mushroom compost/30% perlite/30% peat moss.

Table 4.2 Nutrient analysis of used growth media after harvest of onion seedlings

Media	pH	% colonization	Soluble salts dS/cm	Total N %	P ppm	K ppm
10MC	7.4	15.67	1.3	1.15	86	1123
20MC	7.2	21.22	1.9	1.24	183	889
30MC	7.1	28.56	2.2	1.31	169	1318
40MC	7.0	20.00	2.8	1.41	251	1256

10 MC is 10% mushroom compost/60% peat moss/30% perlite; 20 MC is 20% mushroom compost/50% peat moss/30% perlite; 30 MC is 30% mushroom compost/40% peat moss/30% perlite; 40 MC is 40% mushroom compost/30% peat moss/30% perlite

Smith and Bowen (1979) divided the development of mycorrhizas into two phases: a pre-infection phase in the soil and fungal growth phase within the root, and stated that effects of soluble salts in the root zone may affect either the pre-infection phase or the root growth phase. Chambers et al. (1980) found that presence of salts in the root zone inhibited the colonization by *Glomus mosseae* on *Trifolium subterraneum*. They found that both NH_4^+ and NO_3^- levels inhibited mycorrhizal fungal growth on the roots. They hypothesized that rhizosphere pH levels may have contributed to these results, but also found inhibition when using Na_2SO_4 and concluded that the soil phase of fungal establishment was inhibited by high soluble salts in the soil.

Wang et al. (1984) reported that yield of onions, a salt sensitive crop, decreased as the rate of mushroom compost increased due to the increased salt levels. Additionally they found increased uptake of K^+ and decreased Mg^{++} as the rate of mushroom compost increased. P levels were not affected by increasing amounts of mushroom compost in one year but did increase in proportion to the rate of compost in the next year. While Wang et al.(1984) did not look at VAM in their report, Furlan and Bernier-Cardou (1989) reported that increased K^+ uptake was beneficial to VAM fungal spore production. Little has been reported on the effect of K^+ on VAM colonization.

It is possible that the salts present in the mushroom compost in our study were readily leached with the first few waterings. Certainly the salts had dropped appreciably

by the end of the experiment (Table 4.2). This could explain the lack of difference in percent VAM colonization between the four compost blends. Yet all four inhibited VAM colonization comparably to results reported in Chapter 3 for the mushroom compost blend compared to the control. It is probable that even the 10% mushroom compost amendment with 2184 ppm K^+ was inhibitory to the fungus initially.

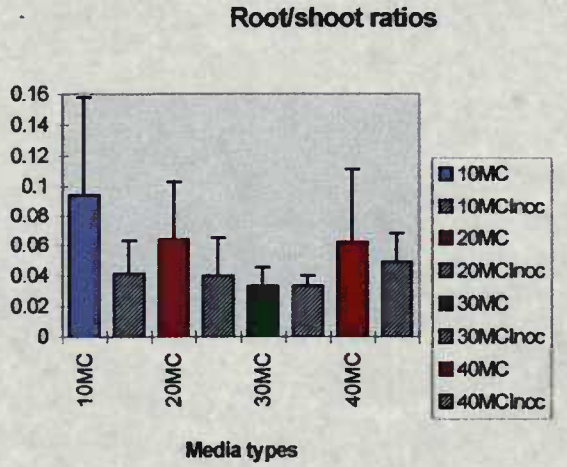
Correlation to soluble salt levels and tissue P levels

A simple linear regression model revealed no correlation (R^2=.36) between initial soluble salt levels and percent of VAM colonization of roots (Table A.9). Likewise, the analysis of variance for tissue P levels and percent colonization showed no significant difference (p=.55).

Root/shoot ratios

There were no significant differences (p=.35) in the root/shoot ratios (Table A.10; Figure 4.1). One possible explanation could be that the salts present were leached out before the plants could be damaged from exposure to the high levels. In the 40% mushroom compost blend, the inoculated plants had a lower root/shoot ratio (.049) than the non-

Figure 4.1 Root/shoot ratios between media



inoculated plants (.062). While this is not considered statistically significant, it may be biologically significant and follows a similar pattern to the root/shoot ratios reported in Chapter 3.

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CHAPTER FIVE

Summary and Conclusions

A significant difference between compost types as to their effects on VAM colonization of roots was evident from this study. Percent colonization was negatively correlated with P levels in the media blends. Additionally, a positive relationship was found between the amount of P bound on organic matter particles and the percent of roots with VAM colonization. Whether this is due to the ratio of organic P to mineral P is unclear. Marschner (1995) states that fungal hyphae take up both inorganic and organic forms of P and does not indicate whether VAM fungi have a preference.

Tissue levels of P in the mycorrhizal plants were closely correlated with media P levels in two of the five media (processed manure blend and worm casting blend). However, the remaining three mixes had higher levels of tissue P levels which did not correlate with the initial media P levels. No difference was observed in tissue P levels between VAM and non-VAM plants grown in the compost blends, all of which had sufficient P for plant growth. However, in the peat/perlite control medium which had only 13 ppm P, a difference in tissue level P was noted between the VAM and non-VAM plants. The tissue P in these VAM plants was close to that of plants grown in higher P media levels. Marschner (1995) discussed a link with nitrogen and VAM, but for this research, N levels were closely tied to P levels and could not be observed as a separate factor.

Plant growth parameters varied in their response to VAM colonization. The VAM plants had larger calipers at 4 weeks than the non-VAM plants, though height measurements were greater in the VAM plants only in the control medium. The root/shoot ratio was significantly less in the non-VAM plants compared to the VAM

plants grown in the control medium; no significant differences were found between VAM and non-VAM plants in the other growth media.

We did not observe any difference on VAM colonization due to soluble salt levels. We expect that the primary contributor, K^+, to the salt level was not fungitoxic at 2184 ppm or above and further research looking at soluble salt levels may find different results depending on the amount of Na^+ and K^+ present.

In conclusion, VAM fungi can establish in a compost blend but the colonization may not be as high as a peat based mix. However, amount of colonization needed to derive benefits for the host plant has not been established for nursery and greenhouse stock. It may be that a small percentage of root system with VAM colonization is sufficient for the plant to receive the benefits of the VAM symbiosis. This research suggests that composts will affect VAM establishment since all of the composts used reduced colonization. The use of compost as a medium component, when inoculation with VAM fungi is desirable, must primarily consider the levels of P and the amount bound to the organic matter. The question of organically bound versus mineralized P was not part of our hypothesis, yet its impact seems to be strong. Additionally, the hypothesis that antagonistic microbial populations would be positively correlated with percent VAM colonization was not supported here, but rather some interesting questions were raised about the type of bacterial colonies present in these mixes and the increase in antagonistic potential shown by all the compost blends.

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APPENDIX

Table A.1 Summary of height measurements at four weeks

	Mean	n=	SE pooled	95%	intervals	Groupings
Control	7.285	7	1.416	5.293	9.277	a
Cont Inoc	19.700	8	1.324	17.836	21.563	bc
PMP	22.300	10	1.184	20.633	23.966	cde
IPMP	18.090	10	1.184	16.423	19.756	b
PEP	24.309	11	1.129	22.719	25.898	de
IPEP	25.122	9	1.248	23.365	26.879	e
PPP	21.088	9	1.248	19.331	22.845	bcd
IPPP	19.713	8	1.324	17.849	21.575	bc
PYP	21.120	10	1.184	19.453	22.786	bcd
IPYP	23.500	9	1.248	21.743	25.256	de

Control mix is 70% peat moss/30% perlite; Cont Inoc is same amended with *G. intraradices.*
PMP is 35% mushroom compost/35% peat moss/30% perlite; IPMP is same amended with *G. intraradices*
PEP is 35% vermicompost/35% peat moss/30% perlite; IPEP is same amended with *G. intraradices.*
PPP is 35% processed manure fiber/35% peat moss/30% perlite; IPPP is same amended with *G. intraradices.*
PYP is 35% yard waste compost/35% peat moss/30% perlite; IPYP is same amended with *G. intraradices.*
Homogeneous groupings based on LSD (least significant difference).

Analysis of variance for height measurements

Dependent variable: height

Source	DF	Sum of squares	Mean square	F ratio	P value
Between groups	9	1761.4760	195.71956	13.952	.00001
Within groups	81	1136.2959	14.02834		
Corrected total	90	2897.7719			

Table A.2 Summary of caliper measurements

	Mean	n=	SE pooled	95%	intervals	Groupings
Control	1.584	17	.138	1.390	1.777	a
Cont Inoc	2.105	20	.128	1.926	2.284	b
PMP	2.097	20	.128	1.918	2.276	b
IPMP	2.504	19	.131	2.321	2.687	c
PEP	2.657	18	.134	2.469	2.846	c
IPEP	3.242	16	.143	3.042	3.442	d
PPP	2.113	18	.134	1.924	22.301	b
IPPP	2.578	16	.143	2.379	2.778	c
PYP	2.576	20	.128	2.398	2.754	c
IPYP	2.542	20	.128	2.362	2.720	c

Control mix is 70% peat moss/30% perlite; Cont Inoc is same amended with *G. intraradices.*
PMP is 35% mushroom compost/35% peat moss/30% perlite; IPMP is same amended with *G. intraradices*
PEP is 35% vermicompost/35% peat moss/30% perlite; IPEP is same amended with *G. intraradices.*
PPP is 35% processed manure fiber/35% peat moss/30% perlite; IPPP is same amended with *G. intraradices.*
PYP is 35% yard waste compost/35% peat moss/30% perlite; IPYP is same amended with *G. intraradices.*
Homogeneous groupings based on LSD (least significant difference).

Analysis of variance for caliper measurements

Dependent variable: caliper

Source	DF	Sum of squares	Mean square	F ratio	P value
Between groups	9	30.640430	3.4044923	10.401	.00001
Within groups	174	56.952347	.3273123		
Corrected total	183	87.592778			

Table A.3 Root/shoot ratios for four composts and control blends.

	Mean	n =	SE pooled	95%	intervals	Groupings
Control	.147	6	.010	.133	.161	a
Cont Inoc.	.063	8	.009	.051	.075	b
PMP	.047	8	.009	.035	.059	b
IPMP	.042	8	.009	.030	.055	b
PEP	.045	8	.009	.032	.057	b
IPEP	.041	9	.008	.028	.053	b
PPP	.055	8	.009	.043	.067	b
IPPP	.053	6	.010	.039	.067	b
PYP	.054	6	.010	.039	.068	b
IPYP	.052	7	.009	.038	.065	b

Control mix is 70% peat moss/30% perlite; Cont Inoc is same amended with *G. intraradices.*
PMP is 35% mushroom compost/35% peat moss/30% perlite; IPMP is same amended with *G. intraradices*
PEP is 35% vermicompost/35% peat moss/30% perlite; IPEP is same amended with *G. intraradices.*
PPP is 35% processed manure fiber/35% peat moss/30% perlite; IPPP is same amended with *G. intraradices.*
PYP is 35% yard waste compost/35% peat moss/30% perlite; IPYP is same amended with *G. intraradices.*
Homogeneous groupings based on LSD (least significant difference).

Analysis of variance for root/shoot ratios and treatments

Dependent variable: root/shoot ratio

Source	DF	Sum of squares	Mean square	F ratio	P value
Between groups	9	.0552980	.0061442	10.153	.00001
Within groups	64	.0387287	.0006051		
Corrected total	73	.0940267			

Table A.4 Summary of shoot dry weight statistics

Media	Mean	n=	SD	p-value*
Control	0.042	11	0.019	.00001
Control Inoculated	0.399	15	0.106	
PMP	0.459	16	0.204	0.76
IPMP	0.480	15	0.187	
PEP	0.717	13	0.408	0.07
IPEP	0.937	15	0.192	
PPP	0.482	15	0.250	0.79
IPPP	0.589	14	0.183	
PYP	0.665	15	0.198	0.20
IPYP	0.645	13	0.208	

*** p values** -indicate comparisons between inoculated and noninoculated plants in each pair.

Control mix is 70% peat moss/30% perlite; Cont Inoc is same amended with *G. intraradices.*
PMP is 35% mushroom compost/35% peat moss/30% perlite; IPMP is same amended with *G. intraradices*
PEP is 35% vermicompost/35% peat moss/30% perlite; IPEP is same amended with *G. intraradices.*
PPP is 35% processed manure fiber/35% peat moss/30% perlite; IPPP is same amended with *G. intraradices.*
PYP is 35% yard waste compost/35% peat moss/30% perlite; IPYP is same amended with *G. intraradices.*
Homogeneous groupings based on LSD (least significant difference).

Table A.5 Summary statistics of root colonization

Media	Mean	n =	SE pooled	95%	intervals	Groupings
Cont Inoc.	72.50	6	7.83	61.15	83.84	a
IPMP	15.71	7	7.25	5.21	26.21	b
IPEP	2.33	6	7.83	-9.01	13.68	b
IPPP	8.85	7	7.25	-1.64	19.36	b
IPYP	53.14	7	7.25	42.64	63.64	a

Cont Inoc. is 70% peat moss/30% perlite amended with 10 ml *G. intraradices.*
IPMP is 35% mushroom compost/35% peat moss/ 30% perlite amended with 10 ml *G. intraradices.*
IPEP is 35% vermicompost/35% peat moss/30% perlite amended with 10 ml *G. intraradices.*
IPPP is 35% processed manure fiber/35% peat moss/ 30% perlite amended with 10 ml *G. intraradices.*
IPYP is 35% yard waste compost/35% peat moss/30% perlite amended with 10 ml *G. intraradices.*
Homogeneous groupings based on LSD (least significant difference).

Analysis of variance for root colonization

Dependent variable: root colonization

Source	DF	Sum of squares	Mean square	F ratio	P value
Between groups	4	23736.751	5934.1878	16.126	.00001
Within groups	28	10303.976	367.9991		
Corrected total	32	34040.727			

Table A.6 Summary statistics for comparison of tissue P levels to media P levels

Media	media P level	Mean	n =	SE pooled	95%	intervals	Groupings
Cont Inoc.	13	.205	2	.034	.151	.259	a
IPMP	161	.353	3	.028	.309	.397	bc
IPEP	729	.340	3	.028	.296	.384	bc
IPPP	644	.393	3	.028	.349	.437	c
IPYP	77	.293	3	.028	.249	.337	ab

Cont Inoc. is 70% peat moss/30% perlite amended with 10 ml *G. intraradices.*
IPMP is 35% mushroom compost/35% peat moss/ 30% perlite amended with 10 ml *G. intraradices.*
IPEP is 35% vermicompost/35% peat moss/30% perlite amended with 10 ml *G. intraradices.*
IPPP is 35% processed manure fiber/35% peat moss/ 30% perlite amended with 10 ml *G. intraradices.*
IPYP is 35% yard waste compost/35% peat moss/30% perlite amended with 10 ml *G. intraradices.*
Homogeneous groupings based on LSD (least significant difference).

Analysis of variance for comparison of tissue P to media P

Dependent variable: tissue P levels

Source	DF	Sum of squares	Mean square	F ratio	P value
Between groups	4	.0489000	.0122250	5.328	.0176
Within groups	9	.0206500	.0022944		
Corrected total	13	.0695500			

Table A.7 Comparison of tissue P levels between mycorrhizal and non-mycorrhizal plants per media type

Media	n=	Mean	Std dev	p value
Control	1	0.12	n/a	
Inoculated control	2	0.21	0.02	.009
PMP	3	0.31	0.09	
Inoculated PMP	3	0.35	0.02	0.50
PEP	3	0.39	0.08	
Inoculated PEP	3	0.34	0.02	0.40
PPP	3	0.43	0.09	
Inoculated PPP	3	0.39	0.09	0.67
PYP	3	0.25	0.06	
Inoculated PYP	3	0.29	0.03	0.39

Control medium is 70% peat moss/30% perlite; PMP is 35% mushroom compost/35% peat moss/30% perlite; PEP is 35% vermicompost/35% peat moss/30% perlite; PPP is 35% processed manure fiber/35% peat moss/30% perlite; PYP is 35% yard waste compost/35% peat moss/30% perlite.
p-value is comparing pairwise means of same media.

Table A.8 Regression analysis for antagonistic microbial populations compared to percent VAM colonization of roots.

Media	n=	Mean	Std deviation
Control	4	0.00	n/a
PEP	4	1.7×10^5	8.8×10^4
PYP	4	3.5×10^5	4.5×10^5
PPP	2	4.0×10^4	n/a
PMP	5	3.3×10^5	3.8×10^5

Cont is 70% peat moss/30% perlite.
PMP is 35% mushroom compost/35% peat moss/ 30% perlite.
PEP is 35% vermicompost/35% peat moss/30% perlite.
PPP is 35% processed manure fiber/35% peat moss/ 30% perlite.
PYP is 35% yard waste compost/35% peat moss/30% perlite.

Analysis of variance

Dependent variable: percent root colonization
Independent variable: microbial population

Source	DF	Sum of squares	Mean square	F ratio	P value
Between groups	1	698.72862	698.72862	1.6456	0.32814
Within groups	2	424.60974	424.60974		
Corrected total	3	1547.9481			

Regression analysis - Linear model: Y = a+bx

Parameter	Estimate	Std. Error	T-value	P-value
Intercept	-3.24192	20.9546	-0.154712	0.89125
Slope	1.04964×10^{-4}	8.18239×10^{-5}	1.2828	0.32814

Correlation coefficient = 0.671856
Standard error of estimate = 20.061
R-squared = 45.14 percent

Table A.9 Summary of VAM root colonization and treatment.

Media	Soluble salts dS/cm	percent colonization	n=	Std. dev.
10% MC	2.6	15.67	9	17.72
20% MC	3.8	21.22	9	22.04
30% MC	4.5	28.56	9	27.26
40% MC	6.2	20.00	9	20.47

10% MC is 10% mushroom compost/60% peat moss/30% perlite.
20% MC is 20% mushroom compost/50% peat moss/30% perlite.
30% MC is 30% mushroom compost/40% peat moss/30% perlite.
40% MC is 40% mushroom compost/30% peat moss/30% perlite.

Analysis of variance of root colonization compared to soluble salts

Dependent variable: percent colonization

Source	Sum of Squares	DF	Mean square	F ratio	P value
Model	41.109750	1	41.109750	.11798	.73342
Residual	11499.062	33	348.456		
Lack of fit	89.283901	2	44.641950	.12129	.88619
Pure error	11409.778	31	368.057		
Corrected total	11540.171	34			

Regression analysis - Linear model Y=a+bx

Dependent variable: percent colonization
Independent variable: soluble salts

Parameter	Estimate	Std. Error	T-value	P-value
Intercept	16.1254	10.6761	1.51042	.14045
Slope	0.820694	2.38937	0.343477	.73342

Correlation coefficient = 0.0596851
Standard error of estimate = 18.667
R-squared = .36 percent

Table A.10. Root/shoot ratios for mushroom compost blends

	VAM	Mean	n=	SE(pooled)	95%	intervals	Homogeneous groups
10% MC	-	.094	9	.023	.062	.126	x
10% MC	+	.099	8	.024	.065	.133	x
20% MC	-	.064	10	.021	.034	.095	x
20% MC	+	.052	8	.024	.018	.086	x
30% MC	-	.035	9	.023	.003	.067	x
30% MC	+	.033	8	.024	-.0001	.067	x
40% MC	-	.062	8	.024	.028	.096	x
40% MC	+	.049	9	.023	.017	.081	x

10% MC is 10% mushroom compost/60% peat moss/30% perlite; 10% MC inoc is same amended with 10 ml *G. intraradices.*
20% MC is 20% mushroom compost/50% peat moss/30% perlite; 20% MC inoc is same amended with 10 ml *G. intraradices.*
30% MC is 30% mushroom compost/40% peat moss/30% perlite; 30% MC inoc is same amended with 10 ml *G. intraradices.*
40% MC is 40% mushroom compost/30% peat moss/30% perlite; 40% MC inoc is same amended with 10 ml *G. intraradices.*

Analysis of variance for root/shoot ratios and treatment.

Dependent variable: root/shoot ratio

Source	DF	Sum of squares	Mean square	F ratio	P value
Between groups	7	.0363731	.0051962	1.128	.3576
Within groups	61	.2809527	.0046058		
Corrected total	68	.3173258			

Table A.11 Summary of shoot weight and tissue P levels on plants colonized or not with *G. intraradices* after 10 weeks growth.

Media	VAM inoc.	Tissue P levels	Std deviation	Shoot weight	Std. deviation
10 MC	-	0.305	0.092	0.164	0.112
10 MC	+	0.295	0.007	0.206	0.089
20 MC	-	0.370	0.00	0.195	0.098
20 MC	+	0.320	0.014	0.208	0.100
30 MC	-	0.375	0.035	0.236	0.137
30 MC	+	0.315	0.007	0.324	0.109
40 MC	-	0.330	0.028	0.350	0.140
40 MC	+	0.330	0.028	0.340	0.034

10% MC is 10% mushroom compost/60% peat moss/30% perlite; 10% MC inoc is same amended with 10 ml *G. intraradices.*
20% MC is 20% mushroom compost/50% peat moss/30% perlite; 20% MC inoc is same amended with 10 ml *G. intraradices.*
30% MC is 30% mushroom compost/40% peat moss/30% perlite; 30% MC inoc is same amended with 10 ml *G. intraradices.*
40% MC is 40% mushroom compost/30% peat moss/30% perlite; 40% MC inoc is same amended with 10 ml *G. intraradices.*

Analysis of variance of tissue P levels and colonization

Dependent variable: tissue P levels

Source	DF	Sum of squares	Mean square	F ratio	P value
Between groups	22	.0070769	.00032167	.967	.5459
Within groups	12	.0039917	.00032639		
Corrected total	34	.0110686			

Analysis of variance of shoot dry weight on colonization

Dependent variable: shoot dry weight

Source	DF	Sum of squares	Mean square	F ratio	P value
Between groups	22	.4843873	.0220176	1.264	.3448
Within groups	12	.2089710	.0174142		
Corrected total	34	.6933583			