MECHANISMS OF ENHANCED PHOSPHORUS UPTAKE BY VA MYCORRHIZAL SWEET POTATO

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

MECHANISMS OF ENHANCED PHOSPHORUS UPTAKE BY VA MYCORRHIZAL SWEET POTATO

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Vesicular-arbuscular mycorrhizal (VAM) fungi and root hairs directly affect phosphorus (P) uptake by roots. Experiments were conducted to determine (i) the interaction among sweet potato (*Ipomea batatas* (L.) Lam. cv White Star) mycorrhizae (*Glomus etunicatum* Becker and Gerdemann, and *Acaulospora rugosa* Morton), root hairs, and soil P; (ii) the chronology and mechanisms of the VAM plant-growth response; and (iii) the seasonal variation in sweet potato growth, nutrient content, root hairs, and colonization by VAM fungi. Colonization was inversely related to soil-P level. The degree of suppression varied with VAM isolate. Increasing the P level increased the root length with root

hairs and root hair length. Colonization by VAM fungi suppressed root hair formation in one experiment. A proposed chronology of the VAM plant-growth response is (i) colonization by the fungi, (ii) proliferation of external hyphae, (iii) an increase in P inflow, and (iv) a growth increase. Sweet potato did not follow this chronology. There was an increase in colonization by VAM fungi and P inflow before there was a substantial increase in external hyphae. Hyphae of VAM fungi were of a diameter that would allow them to penetrate pores that hold water at water contents less than field capacity while root and root hairs would be excluded from these pores. There was no evidence that sweet potato roots, regardless of colonization by VAM fungi, could alter the organic acid composition of the rhizosphere. Roots colonized by VAM fungi maintained a higher proportion of metabolically active soil hyphae than noncolonized roots. Hyphal distribution around roots colonized by VAM fungi was not consistently different from hyphal distribution around noncolonized roots. Nonetheless, in some instances, hyphal densities were higher than the control at distances beyond calculated P-depletion zones. In a field study, tissue-P concentration, root density, percent colonization by VAM fungi, and yield reached their

highest levels at different periods in the growing season. Amounts of roots, root hairs, and mycorrhizal colonization changed with depth and distance from the plant. Seasonal changes were most pronounced proximal to the main stem.

CHAPTER ONE
INTRODUCTION

My overall objective was to study how vesicular-arbuscular mycorrhizal (VAM) fungi increase phosphorus uptake by sweet potato (*Ipomea batatas* (L.) Lam. cv White Star). My approach was to (i) define the soil conditions where colonization by VAM fungi enhance P uptake; (ii) identify the chronology of events involved in the process of P-uptake enhancement by VAM fungi in order to evaluate several hypotheses to explain the VAM plant-growth response; and (iii) evaluate the VAM plant-growth response in terms of the seasonal dynamics of sweet potato growth, root characteristics, and root distribution in the field.

The process of P uptake by plants is affected by root hairs as well as VAM fungi. In terms of P uptake, root hairs and hyphae of VAM fungi may be thought of as functionally analogous. Furthermore, both root hairs and colonization by VAM fungi may be affected by plant interactions with P-availability. The role of VAM fungi and roots hairs in P uptake by plants is reviewed in Chapter Two of this dissertation.

Characterization of the soil-P levels where sweet potato responds to inoculation with VAM fungi and the interactions among soil-P level, root hairs, and VAM fungi are described in Chapter Three. Root colonization by VAM fungi is often suppressed by elevated levels of soil P (Sanders and Tinker, 1973; Abbott and Robson, 1979; Same et al., 1983; Schubert and Hayman, 1985). Evidence concerning the effect of soil P on root hairs is less conclusive and there are no data on the affect of VAM fungi on root hair development.

Sweet potato is dependent on VAM fungi for adequate P uptake (Hung et al., submitted) under P-limiting conditions, and possesses abundant root hairs. It was my purpose to determine if there is a functional interaction between colonization by VAM fungi, P fertilization, and root hair development. My specific objectives in Chapter Three were to (i) establish P-response curves for sweet potato in a high-P-fixing soil to ensure that the study was conducted within biologically relevant P conditions and (ii) investigate the relationships among colonization by VAM fungi, root hair development, soil-P level, and plant P uptake.

The chronology of events involved in the process of enhanced P uptake of mycorrhizal plants as well as hypotheses to explain the VAM plant-growth response are addressed in Chapter Four. To understand the mechanism of the VAM plant-growth response, the timing of the response must be determined (Abbott and Robson, 1984). A VAM plant-growth response occurs when P inflow is greater for a mycorrhizal plant than for a nonmycorrhizal plant, assuming that the mycorrhizal status is the only difference between the plants, or when P inflow exceeds the diffusion limits to the root alone (Sanders and Tinker, 1973). Growth increases in young plants may persist throughout their lives, therefore, multiple harvests (Sanders et al., 1977; Abbott and Robson, 1984) or repeated, nondestructive measurements are necessary to identify the onset of the VAM plant-growth response. In the short-term, such as growth chamber and glasshouse studies, the response may be the result of either a transitory event or a continuous phenomenon. In long-term situations, as in natural plant communities, the VAM effect may be transitory and vary with drought stress (Fitter, 1986) or season (Douds and Chaney, 1986). The focus of this research was the short-term response. A proposed chronology of events leading to the VAM plant-growth response is (i)

infection of the root, (ii) followed by proliferation of external hyphae, (iii) leading to increased P inflow and elevation of tissue-P levels, and (iv) resulting in a plant-growth increase. Once the chronology of the VAM plant-growth response is determined, the mechanisms of this response can be most efficiently studied. A reasonable, and generally accepted theory, is that the external hyphae of VAM roots are distributed in such a way as to improve the P-uptake characteristics of the root (Sanders and Tinker, 1971; Mosse, 1973). If mycorrhizae are to be effective in increasing P uptake, their external hyphae must be distributed beyond the P-depletion zones around roots and root hairs. Consequently, P-depletion zones are a reasonable spatial reference point for studying the distribution of hyphae around mycorrhizal roots. Phosphorus depletion around roots is radial, so one purpose of this research was to evaluate the radial distribution of hyphae around the root relative to P-depletion zones.

Soil pores vary in size and consequently, access to nutrients is partially dependent on the radii of the nutrient-absorbing structure. The small diameter of hyphae allows them access to smaller pore spaces than roots and root hairs. The mycorrhizal root system should therefore be

able to explore a greater volume of soil. Furthermore, the physical environment of the smaller pore spaces may be different from the larger pores, providing more favorable P-supply characteristics. For instance, volumetric water content will be higher in small pores than in large pores, resulting in greater P-diffusion coefficients. Estimates of the radii of roots, root hairs, and hyphae of VAM fungi, relative to pore size distribution of the soil, are needed to evaluate the relative effects of roots, root hairs, and hyphae of VAM fungi in exploration of the soil for P.

Plants can increase P solubility and uptake by chemically modifying the rhizosphere (Jungk, 1987; Marschner et al., 1987; Nye and Kirk, 1987). One such modification is the exudation of organic acids. The extent to which VAM and non-VAM roots differ with regard to organic acid production is not known. However, as a group, fungi possess the capacity to alter P availability in the soil by the same mechanisms as higher plants (Beever and Burns, 1980). However, there is no evidence on quantitative or qualitative changes in exudation of organic acids by roots following colonization by VAM fungi.

My specific objectives in Chapter Four were to (i) establish the chronology of the VAM plant-growth response

with sweet potato, (ii) determine the distribution of external hyphae of VAM fungi in relation to the root, root hairs, and P-depletion zones at the time when the VAM plant-growth response first becomes apparent, (iii) evaluate the pore size distribution of the soil relative to the radii of roots, root hairs, and hyphae of VAM fungi in terms of the significance of the role of hyphae of VAM fungi in exploration of larger volumes of soil and access to smaller pore spaces, and (iv) determine if sweet potato mycorrhizae alter the organic acid composition of the rhizosphere.

The VAM plant-growth response in terms of the seasonal dynamics of sweet potato growth, root hairs, and root distribution in the field is evaluated in Chapter Five. To understand the function of VAM fungi in plant-growth responses, it is necessary to document the symbiosis over the life of the plant. Plant nutrient and water demand, allocation of carbohydrates and mineral nutrients, and benefits of colonization by VAM fungi can vary considerably through the growing season (Douds and Chaney, 1985; Fitter, 1986; Dunne and Fitter, 1989). It is generally believed that yield increases attributable to inoculation with VAM fungi at planting are due to colonization by the introduced fungus followed by increased uptake of P or other diffusion-

limited nutrients such as Zn (Abbott and Robson, 1984). However, this relationship may be obscured in field experiments. There may be little difference in root colonization among plants inoculated with VAM fungi and noninoculated controls due to the presence of indigenous VAM fungi (Mosse, 1976; Medina et al., 1988). This would be true particularly in nonfumigated soils, late in the growing season, and far away from the point of inoculum placement. Furthermore, the P concentrations of plants among various inoculation treatments may be different only during a portion of the growing season (Fitter, 1986; Dunne and Fitter, 1988). As plant-growth rate increases dilution effects may obscure treatment differences in initial P concentrations while differences in total-P content may be carry-over effects of early growth. Plant partitioning of P may also change with plant-growth stage (Dunne and Fitter, 1989). Furthermore, there may be interactions of mycorrhizae with soil horizon, soil water content, and proximity to the point of inocula placement. If the full extent of the root system, along with root hairs and mycorrhizae, can be determined, root sampling approaches can be made with more confidence that the sampling is spatially and temporally representative.

My specific objectives in Chapter Five were to (i) determine the changes in distribution of roots, root hairs, and mycorrhizae of sweet potato over a growing season and (ii) evaluate the relationships among seasonal variation in root length, colonization by VAM fungi, P and Zn uptake, vine growth, and yield.

CHAPTER TWO
LITERATURE REVIEW

Introduction

Plant responses to infection by VAM fungi have received several explanations; nonetheless, improved P uptake has consistently proved to be the primary factor providing a benefit. The mechanism of P uptake is, therefore, the focus of this review. The principles examined may also be applied to other poorly-mobile elements in the soil, such as Cu and Zn. The relationships among infection by VAM fungi, micronutrients, and P uptake are discussed below. Nonnutritional plant responses to infection by VAM fungi, such as disease resistance, improved water relations, salinity tolerance, and hormone production, have been proposed; however, these will not be discussed in this review, except in the sense that most of these responses are secondary effects of improved plant nutrition (Graham, 1987).

Harley (1969) listed the following hypotheses to account for plant growth responses to infection by VAM fungi: (i) assimilation of gaseous nitrogen; (ii) conduction of material from the soil to the host; (iii) secretion of substances into the soil which affect the availability of nutrients; (iv) changes in the nature or area of the absorbing surface; and (v) production of vitamins or growth stimulators by the fungus within plant tissues.

By 1973 the search for the primary mechanism of the mycorrhizal growth response had been narrowed to improved P nutrition. Sanders and Tinker (1973) listed the following hypotheses to account for the underlying mechanism of plant growth promotion: (i) morphological changes in the plant root system; (ii) the ability of infected roots to absorb P more rapidly than noninfected roots; (iii) the ability of infected roots to absorb P from sources not available to noninfected roots; and (iv) uptake of P by the external mycelium from labile or other sources, and transport of P to the root by the fungus.

Sanders and Tinker's (1971, 1973) work supported primarily the fourth hypothesis. In addition, these authors noted the importance of the distribution of external hyphae beyond the P-depletion zones of roots and root hairs for

enhancement of P uptake by VAM-infected roots. They went on to explain that acceptance of the fourth hypothesis was contingent on the demonstration of uptake of P and its translocation to the host by VAM hyphae. The capacity of VAM hyphae to transport P to the plant was demonstrated by Hattingh, Gray, and Gerdemann (1973) and by Rhodes and Gerdemann (1975). Sanders and Tinker (1973) also stated that VAM hyphae must have P fluxes high enough to account for any P increase in the mycorrhizal versus nonmycorrhizal plant. Based on calculated P-inflow rates, the number of hyphal entry points to the root, and the average diameters of these entry points, Sanders and Tinker (1973) calculated P flux in the hyphae at the entry points to be 3.8×10^{-8} mol cm^{-2} s^{-1}. In a subsequent experiment, Pearson and Tinker (1975) measured maximum P fluxes of 10^{-9} mol cm^{-1} s^{-1} in external hyphae. Tinker (1975a) concluded that the difference between these values was reasonable, based on the difference in experimental conditions and distance from the root where the flux was estimated.

The above-mentioned work supports the fundamental theory for the mycorrhizal plant growth response, as paraphrased from Sanders and Tinker (1971) and Mosse (1973), that external hyphae form a better-distributed surface for

absorbing P from the soil solution than do roots alone. The ability of these fungi to increase the solubility of P in the rhizosphere, as suggested by Harley's third hypothesis, is still plausible. Sanders and Tinker's second hypothesis, that the uptake kinetics of the mycorrhizal root system are different from those of the root alone, also deserves further consideration.

Silberbush and Barber's (1983) work, using a mathematical model to investigate P-uptake processes, demonstrates the importance of distribution of the absorbing surface for P uptake. However, additional parameters were identified in their model, such as the growth (extension) rate and radius of the absorbing organ. The role of radii was implicit in Sanders and Tinker's (1971,1973) and Tinker's (1975a,1975b) papers. Silberbush and Barber (1983), however, more fully detailed this relationship.

While the fundamental hypothesis that the VAM-infected root system is a "better distributed surface for absorbing P from the soil solution" is undoubtedly correct, Harley's fourth hypothesis (1969), stating that "a change in the nature or area of the absorbing surface occurs," is more flexible. The latter hypothesis does not exclude the possibilities of (i) rhizosphere/hyphasphere alterations,

(ii) differences in extension rates between roots and hyphae, and (iii) extended longevity of the P-absorbing-capacity of mycorrhizal roots relative to nonmycorrhizal roots. Additionally, it would be beneficial to expand on the concept of a better-distributed hyphal network since little is known about the distribution of external hyphae.

Mechanisms that have been contemplated for improved P uptake by VAM-infected roots since Tinker's (1975a,1975b) reviews include (i) better distribution of the absorbing network, (ii) more favorable geometry (smaller radii) of hyphae relative to roots, (iii) greater surface area, (iv) different uptake kinetics, (v) faster extension rate, (vi) chemical alteration of rhizosphere/hyphasphere, (vii) increased functional longevity, (viii) mineralization of organic P, (ix) exploration of smaller pore spaces, (x) greater carbon-use efficiency of VAM hyphae vs. root hairs, (xi) and differences in rhizosphere populations. This review will summarize current knowledge relevant to these hypotheses, and will also attempt to identify pertinent areas for future research.

Temporal Considerations

In order to understand the mechanism of the VAM plant-growth response, the timing of the response must be determined (Abbott and Robson, 1984). Growth increases in young plants may persist throughout their lives; therefore, multiple harvests (Abbott and Robson, 1984, Sanders et al., 1977) or repeated, nondestructive measurements are necessary to identify the onset of the VAM plant-growth response.

To emphasize the importance of timing, the VAM plant-growth response can be considered in two time frames. The simplest case would be a short-term, glasshouse or growth-chamber study. The other extreme would be the long-term response in natural environments. The underlying mechanism may or may not be the same for long and short-term responses. In the short-term, the VAM response may be the result of either a transitory event or a continuous phenomenon. In the case of natural environments, there is evidence that the VAM effect is transitory and varies with the degree of drought stress (Fitter, 1986) or with the season (Douds and Chaney, 1986).

A chronology of events leading to the VAM plant-growth response, adapted from Gueye et al. (1987) and Snellgrove et

al. (1982), might be as follows: (i) infection and proliferation of external hyphae; (ii) elevation of tissue-P levels; and (iii) growth increase. While this sequence of events is simplistic and perhaps obvious, it points to the need to take measurements prior to the time the growth response becomes apparent in the shoot.

The response of the cowpea to infection by VAM fungi appears to follow this proposed chronology (Figure 2-1). The proliferation of external hyphae at 20 to 25 d after planting is in agreement with observations by Owusu-Bennoah and Wild (1979), who observed that changes in P-depletion zones between mycorrhizal and nonmycorrhizal onion roots occurred within 21 d. Sanders et al. (1977) reported that P inflow was higher in some VAM-inoculated treatments than for noninoculated controls after 17 d, followed by shoot dry mass differences between 30 and 40 d. Interestingly, the authors noted that increased P inflow occurred while colonization intensity and external hyphae proliferation were relatively low. This suggests that even small increases in external hyphae can increase P-inflow. In their experiments, as well as in the experiments of Sanders and Tinker (1973), P inflow into mycorrhizal roots exceeded that into nonmycorrhizal roots throughout the experiment,

suggesting that, at least in this case, the mycorrhizal response may be a continuous phenomenon. The mycorrhizal response occurs when P inflow is greater for the mycorrhizal plant than for the nonmycorrhizal plant, and also when P inflow exceeds the diffusion limits to the root alone (1973).

Mechanistic Hypotheses for Improved P-Uptake

Comparative Geometries of Roots and Hyphae

Nye (1973) theorized that, when a nutrient is deficient in the soil solution, the critical root parameter for its uptake is surface area and not volume. Factors that increase the surface area of the root, such as root hairs and mycorrhizal hyphae, will be advantageous to the absorption of nutrients present in low concentration in the soil solution. This relationship is demonstrated in the model of Silberbush and Barber (1983) which predicts nutrient uptake based on nutrient supply characteristics of soil and several root parameters (Table 2-1). A sensitivity analysis of their model demonstrated that root growth rate (K) and root radius (r_o) are the two most important root parameters for P uptake (Figure 2-2). Phosphorus uptake

increases with r_o because surface area increases with r_o. However, increasing surface area by increasing root radii is not an efficient strategy for uptake of nutrients having low diffusion coefficients because the steepness of the diffusion gradient is inversely related to the radius of the absorbing unit, and consequently the soil solution should be less depleted at the surface of a VAM hyphae compared to the surface of a root. These authors calculated the P-gradients around mycorrhizal hyphae, root hairs, grass, and corn roots (Figure 2-3), concluding that the relatively high concentration of P at the hypha surface was due to the effect of the radial geometry on nutrient flux to the root. As Barber noted (1984), an absorbing structure of larger radius will have greater uptake per unit length than an absorbing structure of smaller radius, but the narrower absorbing unit will be more efficient on a surface area basis. Mycorrhizal hyphae should, therefore, be less P-stressed than roots under identical soil conditions. For this reason the relative uptake kinetics of the absorbing surfaces may be of only secondary importance to nutrient uptake. The reader should refer also to our subsequent section on uptake kinetics.

The smaller diameter of hyphae should allow them to explore smaller pore spaces than roots and root hairs. This affords the mycorrhizal root system two advantages over the nonmycorrhizal root system. First, the mycorrhizal root system can explore a greater volume of soil. Second, the physical environment of the smaller pore spaces may be different from the larger pores. It is reasonable to speculate that P-supply characteristics are more favorable in the small pore spaces accessible to mycorrhizal hyphae than in the larger pore spaces accessible to roots and root hairs. For instance, it is likely that volumetric water content will be higher in small pores than in large pores. Higher volumetric water contents will lead to increased P-diffusion coefficients. The advantage of a long, thin absorptive system is diminished considerably if soil volume is held constant, because depletion zones then will begin to overlap (Silberbush and Barber, 1983, Baldwin et al., 1972). Therefore, the mycorrhizal effect should decrease with pot size. For example, in a glasshouse experiment we found that mycorrhizal dependency (dry mass mycorrhizal plant/dry mass nonmycorrhizal plant) was reduced from 8.13 to 1.80 and from 5.67 to 1.72 using *Glomus etunicatum* and *Aculospora rugosa*, respectively, when pot size was reduced from 11,350 cm^3 to

574 cm^3 (O'Keefe and Sylvia, unpublished results). Similarly, in an experiment using field beans, Kucey and Janzen (1987) concluded that P concentration and dry matter production due to infection by VAM fungi decreased with pot size. That restricted root growth will diminish the mycorrhizal growth response should be kept in mind when interpreting data from pot studies.

Due to the advantages in P uptake inherent in narrower absorbing structures, the possibility that VAM strains that have narrow hyphae may be more effective in promoting P uptake than strains with wide hyphae is intriguing, but has yet to be demonstrated. In fact, Lopez-Aguillon and Mosse (1987) reported that *Glomus tenue*, with a hyphal diameter of < 2 μm, was less effective in promoting growth of clover and sorghum than *G. fasciculatum* and *Gigaspora margarita* which had hyphal diameters of 2 to 5 and 5 to 10 μm, respectively.

Surface Area

Hyphae clearly increase the surface area of a VAM-infected root system. The reported increase in surface area due to VAM hyphae, assuming average diameters of 8 μm and 250 μm for hyphae and roots, respectively, ranges from >

1,800% to < 1% (Table 2-2). The proportion of hyphae that were metabolically active in these studies ranges from 2 to 96%. Metabolically active hyphae may be a more relevant measure than total hyphae for understanding P uptake, since P uptake by fungi is believed to be an active process (Beever and Burns, 1980). The wide range in the proportion of active external hyphae suggests that the proportion of VAM external hyphae involved in P uptake can vary considerably. This should be considered when using values for total hyphal length in nutrient-uptake models. The P-inflow values reported by Sanders and Tinker (1973) allow comparison of the relative increase in surface area due to VAM hyphae to the relative increase in P-inflow; a mean increase of 477% in P inflow occurred with a mean increase in surface area of only 3%. The large increase in P inflow due to a small increase in surface area indicates that there is more to the mycorrhizal growth response than a simple increase in absorptive surface area.

The high surface to volume ratio of VAM hyphae compared to roots should make mycorrhizal uptake a more carbon-efficient mechanism for P uptake. Assuming the same radii for hyphae and root used above (8.0 μm and 250.0 μm for the hyphae and root, respectively), the surface to

volume ratios are 25:100 for hypha and 8:1000 for roots. While the carbon cost of symbiosis may be high (Pang and Paul, 1982), allocation of carbon on a surface-area basis should be lower for VAM fungus hyphae than for roots.

The portion of the metabolically active hyphae that absorb P is not known. It is possible that only the growing hyphal tips actively take up P. If this is the case, an area for future research would be the role of hyphal branching in determining the effectivity of VAM isolates.

Distribution of VAM hyphae

If mycorrhizae are to be effective in increasing P uptake, their external hyphae must be distributed beyond the P-depletion zone around roots and root hairs. Interestingly, a model for nutrient uptake by root hairs, developed by Itoh and Barber (1983), demonstrates that root hair length has a large effect on P-uptake. If VAM hyphae can be considered as extended root hairs, it follows from their model that VAM hyphae will increase P uptake considerably. Within the root-hair cylinder, i.e., the zone around the root occupied by root hairs, P is depleted uniformly and root hairs compete for P (Bhat and Nye, 1973). Within the root-hair cylinder, it is not clear whether the

hyphae of VAM fungi would add to P uptake (see section on uptake kinetics and C_{min} values). Using the quantitative autoradiographic technique of Bhat and Nye (1973), Owusu-Bennoah and Wild (1979) found that VAM hyphae increased the measurable P-depletion zone around the roots of onion from 0.1 to 0.2 cm. The authors point out that the method used could not detect differences in P concentration of less than 10 to 20%, and consequently the P-depletion zone may have extended further than 0.2 cm from the root. If a hyphal system is very diffuse, it is probable that depletion gradients around roots due to hyphae will not be detected with current methods. The authors also commented on the possible role of soil physical properties in affecting the extent of hyphal spread from the root. For example, increased bulk density could restrict the spread of external hyphae.

In artificial systems, P uptake by VAM hyphae at distances much greater than 0.2 cm have been demonstrated (Hattingh, et al., 1973, Rhodes and Gerdemann, 1975, Alexander, et al., 1984). Furthermore, Warner and Mosse (1983) found that VAM hyphae could extend up to 2.0 cm from an infected root system and thereby spread to another host plant.

The above discussion assumes that the soil is homogeneous and that hyphae will grow out from the root in a uniform cylinder. However, St. John and coworkers (1983) found that VAM hyphae proliferated at nutrient-rich sites and, in effect, provided the root system with an efficient strategy for exploiting soil-heterogeneity. Their experiment examined heterogeneity of organic matter distribution; however, P distribution in soils is also heterogeneous and hyphae may proliferate at sites of higher P concentration. In a discussion of mycorrhizal response to rock phosphate application, Tinker (1975) proposed that VAM hyphae proliferated at sites of low-solubility sources of P such as rock phosphate grains.

Uptake Kinetics

The central question concerning P-uptake kinetics of mycorrhizal roots compared to nonmycorrhizal roots is, do mycorrhizal roots have more absorbing surface area, higher P uptake per unit surface area, or a combination of the two? If V_{max} (the maximum uptake rate) is larger for mycorrhizal roots than for nonmycorrhizal roots, then one may conclude that the mycorrhizal roots have more absorbing sites. If, on the other hand, K_m (the phosphate ion concentration at

which the uptake rate is half the maximum rate) for mycorrhizal roots is lower than for nonmycorrhizal roots, then one may conclude that mycorrhizal roots have greater uptake per unit surface area. Kinetics data from two research reports are presented in Table 1-3. Cress et al. (1979) reported that mycorrhizal roots of tomato had similar V_{max} values, but lower K_m values than nonmycorrhizal roots. They concluded that the mycorrhizal effect was due, at least in part, to a greater affinity of sites for P-uptake in mycorrhizal roots, and that the increased P-absorbing surface due to external hyphae was not a major contributing factor to P uptake. Working with soybeans, Karunaratne et al. (1986) came to the opposite conclusion. These authors suggested that the discrepancy between results was due to the fact that Cress et al. (1979) used plants which were physiologically impaired due to age (124 d) and to severe P starvation. Karunaratne et al. (1986) observed that P efflux at low-P solution concentrations was lower for mycorrhizal than for nonmycorrhizal roots. This suggests that mycorrhizal roots can remove P from soil solutions having lower P concentrations than can nonmycorrhizal roots. In effect, a mycorrhizal root would have a lower C_{min} value (the solution concentration of an ion at which efflux equals

influx) in Silberbush and Barber's (1983) model. The C_{min} may be important for understanding uptake kinetics and mycorrhizal response. A lower C_{min} value for mycorrhizal roots, as compared to nonmycorrhizal roots, may explain the finding of Owusu-Bennoah and Wild (1979) that P concentrations in depletion zones close to mycorrhizal roots were lower than for nonmycorrhizal roots. Mycorrhizal roots also had wider depletion zones than did nonmycorrhizal roots. If C_{min} values are identical for mycorrhizal and nonmycorrhizal roots, then the zones of depletion in Owusu-Bennoah and Wild's (1979) study which were attributed to uptake by the root alone (i.e. a 0.1 cm cylinder surrounding the root) should have had equal concentrations of P in the mycorrhizal and nonmycorrhizal treatments, but they did not.

Hyphal Growth Rates

As mentioned in a previous section, nutrient uptake will increase with an increase in the extension rate of the absorbing unit (Silberbush and Barber, 1983). With rapid growth through the soil, there will be less time available for formation of depletion zones around the root apex. The faster the absorbing unit elongates, the longer a portion of the absorbing surface will be in undepleted or less severely

depleted soil. While there are no reported measurements of actual extension rates for the hyphae of VAM fungi in soil, I suggest from the data presented in Table 2-2 that the rate of hyphal extension ranges from 59,000 to 5 times the rate of root growth. Excluding the exceptionally high value reported by Sylvia (1986), the average hyphal extension rate would be 823 times the extension rate of roots. This substantial increase in the extension rate of the absorbing unit should have a significant effect on P uptake. The importance of VAM hyphae extension rates needs further investigation.

Chemical Alteration of the Rhizosphere/Hyphasphere

It is generally accepted that plants can increase P uptake by chemically modifying the rhizosphere (Marschner et al., 1987, Jungk, 1987, Nye and Kirk, 1987). Basic mechanisms by which this occurs include (i) anion exchange of organic acids for adsorbed phosphate anions; (ii) formation of Fe- and Al-organic acid complexes (chelation); and (iii) pH alteration through excretion of H^+ and HCO_3^-. The extent to which mycorrhizal and nonmycorrhizal roots differ with regard to these mechanisms is not known. However, as a general group, fungi possess the capacity to

alter P availability in the soil by the same mechanisms outlined above for higher plants (Beever and Burns, 1980).

There is extensive evidence that mycorrhizal and nonmycorrhizal plants draw from the same pools of soil P, such as organic vs inorganic P (Sanders and Tinker, 1973, Mosse, 1973, Hayman and Mosse, 1972, Owusu-Bennoah and Wild, 1980, Powell, 1975). This suggests that mycorrhizal and nonmycorrhizal root systems do not differ with regard to the mechanisms of solubilization discussed above. However, these scientists all compared L values between inoculated and noninoculated plants to arrive at their conclusions. An L value is a measurement of isotopic dilution or specific activity of ^{32}P in a plant grown on ^{32}P-amended soil (Larsen, 1967). If L values are similar between mycorrhizal and nonmycorrhizal plants, then the plants are thought to be drawing from the same pool of soil P. Bolan et al. (1984) argued that forms of P that may differ in terms of availability to mycorrhizal and nonmycorrhizal plants may still be equally labeled by applied ^{32}P. The authors demonstrated that, of five mechanisms for the increased uptake of P by mycorrhizal plants, only solubilization of organic P would result in different specific activities between mycorrhizal and nonmycorrhizal plants (Figure 1-4).

Therefore, the conclusion that mycorrhizal and nonmycorrhizal root systems are drawing from the same pool of P may not be valid (Bolan et al., 1987).

Tinker (1975a) provided a thorough discussion of the various hypotheses used to explain the differences between mycorrhizal and nonmycorrhizal plants in terms of uptake from insoluble P sources. One hypothesis was the chemical alteration of the rhizosphere by mycorrhizae; however, this ability has yet to be demonstrated. Exudation of organic acids, presence of specific chelators, and pH changes attributed to infection by VAM fungi each need to be demonstrated in order to change current thinking on the role of mycorrhizae in enhanced P-uptake through chemical alteration of the rhizosphere.

The role of mineralization of organic P by the phosphatases of mycorrhizae is uncertain. Mycorrhizal fungi do produce phosphatases, and there have been repeated, measurable differences between mycorrhizal and nonmycorrhizal root systems of the same plant species. MacDonald and Lewis (1978), using a cytochemical method, demonstrated that VAM have acid phosphatases both inside and outside the roots. Azcon et al. (1982) found that infection by VAM fungi reduced root-surface-phosphatase activity of

lavender compared to nonmycorrhizal plants, but that infection by VAM fungi had no effect on wheat phosphatase activity. Gianinazzi-Pearson and Gianinazzi (1986) found that VAM had no effect on root-surface acid-phosphatases of clover. Dodd et al. (1987) reported that acid-phosphatase activity of wheat and onion varied with VAM species, whereas phosphatase activity increased over the control for two fungi (*Glomus geosporum* and *G. mosseae*) and was not different from the control for a third fungus (*G. monosporum*). The authors concluded that increased phosphatase activity in the rhizosphere of colonized plants was not due to excretion by the fungal hyphae, but rather to increased root-surface phosphatase activity and subsequent enhanced diffusion away from the root. The degree to which these phosphatases may be important in increasing P-uptake has not been assessed quantitatively; nonetheless, it appears that infection by VAM fungi has little effect on the uptake of organic P.

Increased Longevity of the Absorbing Unit

Bowen (1973, 1967) discussed the possibility that ectomycorrhizae increase the time during which a section of root functions in ion uptake. He explained that, as the

radius affected by P depletion increases with time, the volume of soil supplying P would increase, allowing for continued P uptake. The root must retain its capacity to take up nutrients over an extended period in order to take advantage of this expanding zone of P supply. Presumably, the presence of hyphae promotes extended uptake capacity. It is reasonable to assume that VAM fungi may also increase the longevity of P-uptake capacity of the root, though there is as yet no direct experimental evidence for this. Extended uptake capacity would become important especially as plants reach maturity and as root extension slows.

Role of VAM-Altered Rhizosphere Populations

Mycorrhizal infection has been shown to affect rhizosphere populations (Ames et al., 1984, Meyer and Linderman, 1986a). Furthermore, rhizosphere organisms have been shown to affect plant growth through hormone production and changes in nutrient availability (Bowen and Rovira, 1976, Rovira, 1965, Tinker, 1984). It is uncertain, though, if VAM-mediated changes in rhizosphere populations have a significant effect on P uptake by plants.

There are two basic mechanisms by which infection by VAM fungi may affect nutrient uptake through effects on

rhizosphere populations. Firstly, hormone production by rhizosphere bacteria can alter root morphology (Tien et al., 1979). However, there is no evidence to suggest that VAM increase the total number or proportion of hormone-producing bacteria in the rhizosphere. Secondly, it is well established that rhizosphere bacteria can solubilize soil P. A possible synergistic effect on P-uptake among mycorrhizal fungi and P-solubilizing bacteria has been demonstrated (Azcon et al., 1976; Raj, et al., 1981) but, in another study, synergism was not reported (Azcon-Aguilar, et al., 1986). However, research to date has not demonstrated that VAM alter populations or activities of P-solubilizing bacteria. Meyer and Linderman (1986b) found that a plant-growth promoting bacterium (*Pseudomonas putida*) proliferated equally in rhizoplanes of both mycorrhizal and nonmycorrhizal clover.

The increase in nutrient-absorbing surface area (Table 2-2) due to the hyphae of VAM fungi should increase potential surfaces for bacterial colonization, thus supporting a larger rhizosphere population on a per-plant basis. It is possible that a larger rhizosphere population would increase the total amount of organic acids exuded from mycorrhizae and associated microorganisms into the soil.

The degree to which this influences the VAM plant response would be difficult to quantify. On the other hand, Rozycki and Strzelczyk (1986), working with ectomycorrhizae, found that *Streptomyces spp.* extracted from the mycorrhizosphere of pine actually produced less organic acid than did populations isolated from the rhizosphere or bulk soil.

Other Poorly-Mobile Elements

Several micronutrients have been implicated in the VAM plant response. Like P, these micronutrients have low diffusion coefficients and low soil-solution levels (Table 2-3), so uptake of these ions could be diffusion-limited and infection by VAM fungi should aid in their uptake. Tinker and Gildon (1983) reviewed the literature on this subject and concluded that there was clear evidence that VAM take up Zn (Cooper and Tinker, 1978) and Cu (Timmer and Leyden, 1980). Munjunath and Habte (1988) demonstrated the role of VAM in increasing the concentration of Cu and Zn in *Leucaena leucocephala*. Pacovsky (1986) found that VAM increased Cu and Zn concentrations in soybeans, but actually decreased Mn and Fe concentrations relative to P-fertilized, nonmycorrhizal plants. Kucey and Janzen (1987) measured P,

Zn, Cu, Fe, and Mn uptake by wheat and field beans. The infection by VAM fungi increased uptake of P, Zn, Cu, and Fe by field beans and of P and Zn by wheat. Raju et al. (1987) reported increased total uptake of P, Mn, Fe, Cu, and Zn by sorghum; however, they did not report tissue concentrations of the micronutrients. Therefore, the increase in these nutrients may have been a secondary effect of increased P-uptake. A recalculation of Raju's data (1987), on a concentration basis, indicates that Cu and Zn concentrations in shoots were increased by infection by VAM fungi, while Mn and Fe concentrations were decreased.

It is likely that the mechanisms responsible for increased P uptake also apply to uptake of other diffusion-limited micronutrients. Using ^{65}Zn, Swaminathan and Verma (1979) found that mycorrhizal and nonmycorrhizal roots utilized the same pool of soil Zn (labile, soil-solution Zn), as is the case with P. In addition, Lambert et al. (1979) demonstrated that, in cases where high soil P decreased mycorrhizal development, Cu and Zn concentrations in corn and soybean were reduced correspondingly. Singh et al. (1986) demonstrated a similar effect with Zn deficiency for wheat grown in the field. In their study, plants from high-P plots contained high concentrations of P but a

significant decrease in Zn concentration compared to non-P-fertilized plants. Roots from the high-P plots had reduced VAM colonization compared to the low-P plots. In addition, Zn concentration in the shoot was correlated positively (r=0.84 to 0.85) with VAM colonization.

Increased uptake of Fe due to VAM, on the other hand, may be due in part to the production of specific Fe chelators (siderophores). Cress and coworkers (1986) found siderophore activity associated with infection by VAM fungi of four species of fungi but no activity in nonmycorrhizal roots of the host plant, *Hilaria jamesii*.

Conclusion

Since Tinker's reviews (1975a, 1975b) a better understanding of the effects of infection by VAM fungi on the uptake kinetics of plant nutrients has been achieved. However, additional study, especially on relative C_{min} values between VAM-infected and noninfected roots is required. The question of how the hyphae of VAM fungi are distributed around roots is still unclear, but new methods for studying external hyphae should improve our understanding (see Sylvia, In Press). Data are needed on external hyphae;

their distribution, lengths, growth rates, viability, radii, and the physiology of P uptake. Mycorrhizal research will be more meaningful if considered in light of the P chemistry of soil. To this end, diffusion coefficients and soil solution levels of relevant nutrients should be reported, along with references to the methods employed. In addition to putting VAM research data in a proper physical and chemical perspective, it is also necessary to understand the temporal framework of the VAM effect (Abbott and Robson, 1984).

At present, work concerning VAM-mediated alteration of rhizosphere chemistry and microbial populations is inconclusive. This research should benefit considerably from more quantitative considerations of hyphal length, absorbing surface area, and chemical changes. Attempts should be made to incorporate these values into current nutrient-uptake models. Evaluation of rhizosphere changes with respect to nutrient stresses is required to evaluate the relevance of data concerning organic acid production, phophatase activity, and qualitative and quantitative shifts in microbial populations caused by infection by VAM fungi.

A relatively unexplored area is the mechanism of VAM function in field situations where soil properties are

heterogeneous. While this will undoubtedly prove to be difficult, it may suggest new mechanistic hypotheses for the VAM growth response, such as decreased P-availiblilty due to water stress, salinity, or aluminum toxicity. Sufficient groundwork has been laid in artificial systems to begin applying mechanistic concepts to the understanding of VAM effects in the field.

Table 2-1. Plant and soil parameters for simulation of P uptake; their definition and initial values for Williams soybeans growing on Raub silt loam. (Used with permission from Silberbush and Barber (1983).

Symbol	Parameter	Initial value
D_e	Effective diffusion coeficient	2.3×10^{-9} cm^2 s^{-1}
b	Buffer Power	163
C_{li}	Initial conc. in soil soln.	0.0136 mol cm^{-3}
v_o	Water influx to root	5.0×10^{-7} cm^{-3} cm^{-2} s^{-1}
r_o	Root radius	0.015 cm
r_l	Half distance between roots	0.2 cm
I_{max}	Maximal influx rate	6.43×10^{-7} mol cm^{-2} s^{-1}
C_{min}	Minimal conc. where I_n=0	0.2×10^{-4} mol cm^{-3}
K_m	Conc.-Cmin when I_n=1/2 Imax	5.45×10^{-3} mol cm^{-3}
L_o	Initial root length	250 cm
k	Root growth rate	0.03 cm s^{-1}

I_n=net influx at r_o

Table 2-2. Increase in nutrient-absorbing surface area due to the hyphae of VAM fungi, assuming radii of hyphae and roots to be 8 µm and 250 µm, respectively.

Rhizoplane Hyphae (cm/cm IRL*)	Soil Hyphae (cm/cm RL*)	(%) Active Hyphae	(%) Surface Area Increase 96% Hyphae Active	2% Hyphae Active	Reference
	592.0		1,818.6	37.9	(Sylvia, 1986)
32.0		96-2	98.3	2.1	(Sylvia, 1988)
6.0			18.4	0.4	(Diem et al., 1986)
	30.0		92.2	1.9	(Abbott and Robson, 1985)
	9.0		27.7	0.6	(Abbott et al., 1984)
	1.3		4.0	0.1	(Tisdall and Oades. 1979)
	0.8		2.5	0.1	(Sanders and Tinker, 1973)
		96-15			(Schubert et al., 1987)
	0.05		0.15	0.003	(Gueye et al., 1987)

*Infected root length

*Root length

Table 2-3. Phosphorus-uptake kinetics for mycorrhizal and nonmycorrhizal soybean and tomato plants.

	Soybean[a]		Tomato[b]	
	Km	V_{max}	K_m	V_{max}
	(μM)	(nmol sec^{-1} m^{-2})	(μM)	(μM P g^{-1} fresh weight x h)
Mycorrhizal	20.0	58.0	1.6	0.10
Nonmycorrhizal	3.5	19.0	3.9	0.10
Difference(%)	-83.0	-67.0	+144.0	0.00

[a]Source: Karunaratne et al., 1986; Solution P range was 79-30 μM KH_2PO_4.

[b]Source: Cress et al., 1979; Solution P range was 79-20 μM KH_2PO_4.

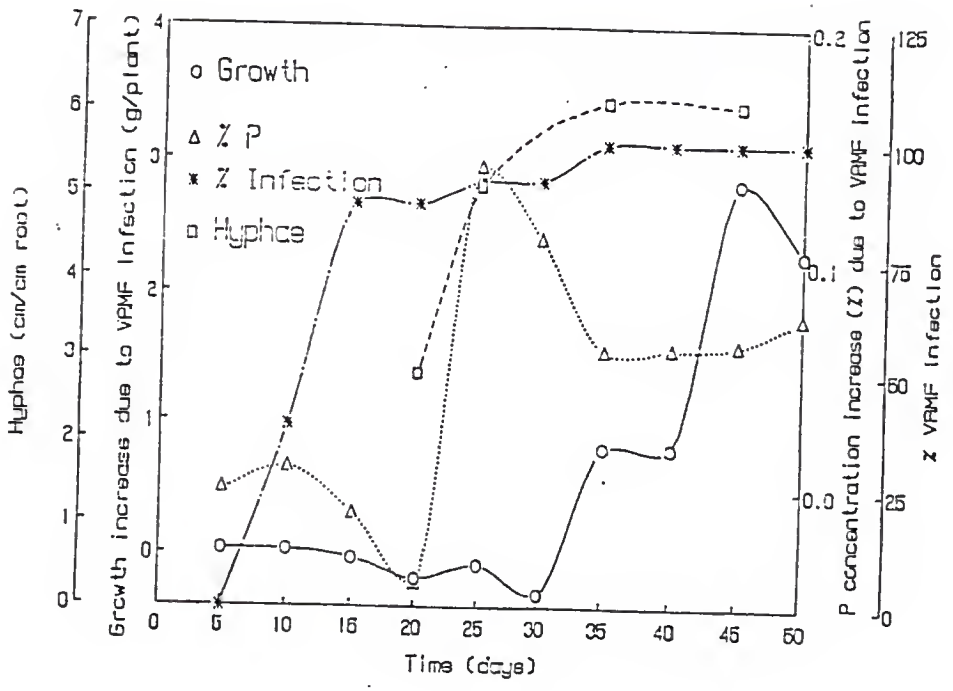
Table 2-4. Diffusion coefficients for P and selected micronutrients.

Ion	Medium[a]	Diffusion Coefficient ($cm^2\ s^{-1}$)	Reference
$H_2PO_4^-$	Asv	10^{-8}-10^{-11}	(Barber, 1984)
Zn^{2+}	Asv	10^{-7}-10^{-11}	
Mn^{2+}	Asv	5.0×10^{-7}-1×10^{-9}	
Fe	Asv	3.0×10^{-10}	
Fe^{2+}, Cu^{2+}			(Wilkinson, 1972)
Zn^{2+}, Mn^{2+}	M	1.0×10^{-6}	
Fe^{3+}	M	2.5×10^{-7}	
Zn^{2+}, Cu^{2+}	W	7.2×10^{-6}	(Parsons, 1959)
H_2PO_4	W	8.9×10^{-6}	(Drew and Nye, 1970)

[a]Asv = Average soil value; M = Montmorillonite; W = Water.

Figure 2-1. Chronology of the mycorrhizal response in cowpea. Data from Gueye et al. (1987).

Figure 2-2. Results of a sensitivity analysis for P uptake showing the relative effect of changing each model parameter separately, from 0.5 to 2.0x the initial value, while leaving all other parameters at the initial levels shown in Table 2-1. Used with permission from Silberbush and Barber, (1983).


o Growth
Δ % P
* % Infection
□ Hyphae
Hyphae (cm/cm root)
Growth increase due to VAMF Infection (g/plant)
P concentration increase (%) due to VAMF Infection
% VAMF Infection
Time (days)

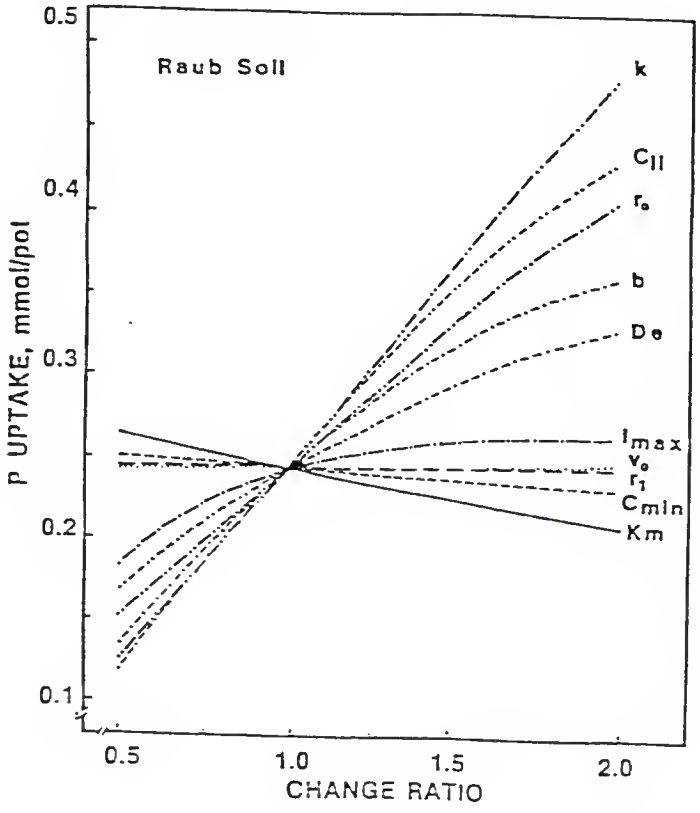
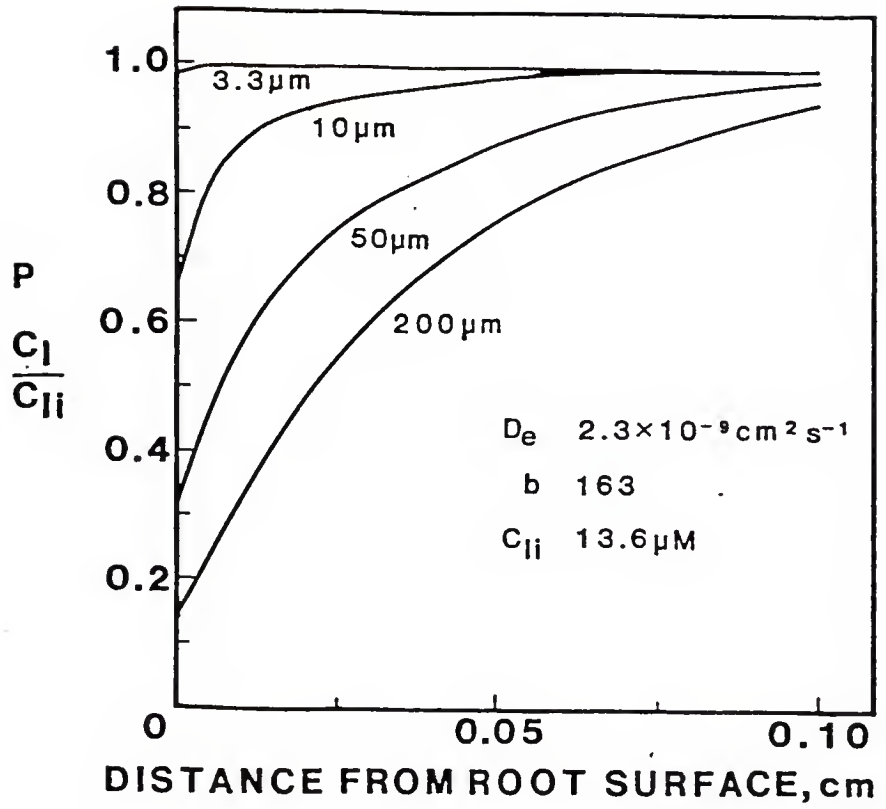
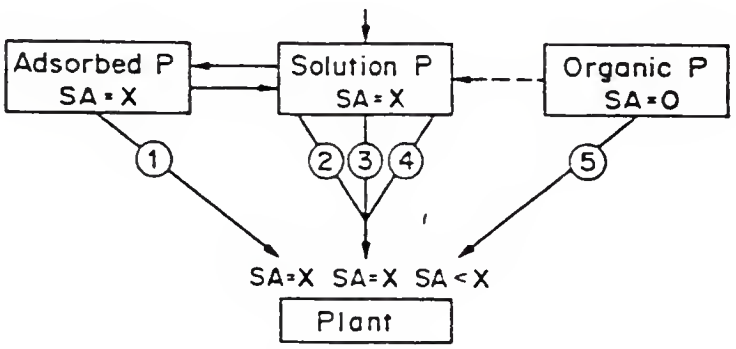

Raub Soil
k
C_{II}
r_o
b
De
I_{max}
v_o
r_1
C_{min}
Km
P UPTAKE, mmol/pot
CHANGE RATIO

Figure 2-3. The effect of root radius on depletion of the relative P concentration in the soil next to the root surface after 10 d of uptake. Soil and plant parameters, other than root radius, were held constant. See Table 2-1 for definitions. Used with permission from Silberbush and Barber, (1983).

Figure 2-4. Mechanisms for the increased uptake of P by mycorrhizal plants and their effects on specific activity (SA) of P in the plants. Used with permission from Bolan et al. (1984).





① Chemical modification
② Physical exploration
③ Lowering threshold
④ Higher affinity
⑤ Solubilization

CHAPTER 3
FUNCTIONAL INTERACTION OF SWEET POTATO MYCORHIZAE, ROOT HAIRS, AND SOIL PHOSPHORUS

Introduction

The role of VAM fungi and roots hairs in P uptake by plants is well documented (see Chapter Two). Baylis (1972) proposed that plants with numerous, long root hairs are less dependent on VAM fungi for P uptake than are plants with few, short root hairs. This relationship has been confirmed (St. John, 1980; Chilvers and Daft, 1981). Root colonization by VAM fungi is often suppressed by elevated levels of soil P (Sanders and Tinker, 1973; Same et al., 1983; Schubert and Hayman, 1986). Evidence concerning the effect of soil P on root hairs is less conclusive. Mackay and Barber (1984) found that growth of corn (*Zea maize*) root hairs decreased as soil P increased; the proportion of root length with root hairs, the length of root hairs, and the density of root hairs were all affected. The differences between their high-P and low-P treatments was most pronounced at low soil moisture where P diffusion would be most limited. On the other hand, Anderson et al. (1987)

found no relationship between P fertilization and density or length of corn root hairs in a field study.

In terms of P uptake, root hairs and hyphae of VAM fungi may be thought of as functionally analogous. Furthermore, both root hairs and colonization by VAM fungi may be affected by plant interactions with P-availability. Sweet potato has been shown to be dependent on colonization by VAM fungi for adequate P uptake under P-limiting conditions (Hung et al., submitted), and possesses abundant root hairs. It was my purpose to determine if there is a functional interaction between colonization by VAM fungi, soil-P levels, and root hair development. My specific objectives were to (i) establish P-response curves for sweet potato in a high-P-fixing soil to ensure that the study was conducted within biologically relevant P conditions and (ii) investigate the relationship between colonization by VAM fungi, root hair development, soil-P level and plant P uptake.

Materials and Methods

Experiment One

A three by four factorial experiment was done using a randomized complete block design with three replications per treatment. The factors and levels were: three fungal treatments (a noninoculated control; *Glomus etunicatum* Becker and Gerdemann, INVAM LETC216; and *Acaulospora rugosa* Morton, INVAM 981) and four P levels (0.01, 0.20, 0.30 and 1.0 μg P mL^{-1}). Soil-solution-equilibrium P levels were determined from P-adsorption curves after the method of Fox and Kamprath (1970). Phosphorus adsorption curves were determined by shaking 50 g of soil in 100 mL of 0.01 M $CaCl_2$ solutions containing varying concentrations of $Ca(H_2PO_4)_2$ for 6 d at 30°C. The soil was from the Bt horizon of an Orangeburg series (Typic Paleudult) and had the following characteristics after pasteurization: organic carbon, 0.04%; sand, 70%; clay, 17%; pH in H_2O, 5.3; and Mehlich I-extractable P, 3.2 $\mu g^{-1} g^{-1}$. From P-adsorption curves I determined the soil had a high P-fixing capacity (784 μg P mL^{-1}) and a low native soil-solution-equilibrium P level (0.005 μg P mL^{-1}). The soil was screened through a 2.0-mm sieve and pasteurized at 75°C for 4 h to eliminate indigenous populations of VAM fungi.

The VAM fungus inocula consisted of soil, roots, and spores from pot cultures maintained on *Desmodium etunicatum* D.C. growing in Arrendondo loamy sand (loamy, siliceious, hyperthermic, Grossarenic Paleudult). Cultures were harvested after three months and were stored at 4°C. Inoculum potential was determined prior to the experiment by the most probable number (MPN) method (Daniels and Skipper, 1982). Sweet potato was used as the host and dilutions of 10^{-1} to 10^{-4} were done using the Orangeburg soil as the diluent. Results of the MPN determination were used to adjust the inocula to give 3.8 propagules per pot. An additional MPN was performed on the adjusted inoculum at the beginning of the experiment. The second MPN assay gave inoculum potentials of 31 and 680 propagules per pot for *G. etunicatum* and *A. rugosa*, respectively. To inoculate plants a 2-cm-diam. by 4-cm-deep core was removed from the center of each pot (Deepot inserts 656 cm^3 capacity, J. M. McConkey & Co, Inc, Sumner, WA) and a 5-g pad of inoculum was placed in the hole and covered with the soil from the original core. A slurry, consisting of 20 g of pot culture material from both isolates, suspended in 2-L tapwater was passed through a 1.0-mm-mesh sieve and then five times through a 0.22-µm-mesh sieve. This slurry was applied, at a rate of

10 mL per pot, to all pots in an attempt to standardize bacterial populations.

Phosphorus levels were adjusted with $Ca(H_2PO_4)_2H_2O$ at rates of 8.0, 46.25, 54.38, and 83.33 mg P kg^{-1} soil to give 6 d soil-solution-equilibrium P levels of 0.01, 0.20, 0.30, and 1.00 µg P mL^{-1}, respectively. Nitrogen, as NH_2NO_3, and potassium, as KCl, were added at an initial rate of 30.0 mg N kg^{-1} and 49.8 mg K kg^{-1}. Additional fertilizer was added every two weeks at a rate of 7.5 mg N kg^{-1} and 12.5 mg K kg^{-1} plus 0.01 mL Hoagland's micronutrients (Hoagland and Arnon, 1950) per pot.

Stem cuttings of sweet potato were placed in the soil, over the inoculum in the case of the inoculated treatments, and were grown in a nonshaded glasshouse with average mean maximum and minimum temperatures of 31 and 22°C, respectively, and mean maximum photosynthetically active radiation (PAR) of 1,784 µE m^2 s^{-1}.

Plants were harvested after 9 wk. Shoots were dried at 70°C, ground in a Wiley mill to pass through a 2.0-mm screen, and a 0.2-g subsample was ashed at 500°C for 2 h. Samples were digested in 10 mL of 1.0 N HCl on a warm hotplate, evaporated to dryness and then resuspended in 5 mL concentrated HCl and digested a second time. The resulting

digest was dried and then resuspended in 20 mL of 0.1 N HCL. The final solution was analyzed colometrically for P (Murphy and Riley, 1962).

Total root fresh mass was determined and a 0.5-g subsample was analyzed for root hairs and colonization by VAM fungi using the gridline-intersect method (Giovanetti and Mosse, 1980). Total root length, colonized root length, percent of the root colonized by VAM fungi, length of the root with root hairs, and percent of the root length with root hairs were determined.

Data were subjected to analysis of variance using the General Linear Models Procedure (SAS, 1985) and vine mass data that had a significant phosphorus response were fitted to the Von Bertalanffy function (Draper and Smith, 1981). Total colonized root length (log), length of the root with root hairs (log), percent of the root length with root hairs (arc sine), and percent of the root colonized by VAM fungi (arc sine) were transformed according to the guidelines of Gomez and Gomez (1976) before analysis.

Experiment Two

The experiment was a three (VAM fungus inoculation) by five (P level) factorial with a randomized complete block

design and nine replications per treatment. The inoculation treatments were as described in the first experiment. Inoculum levels, as determined by the MPN method, were 5.6 and 2.8 propagules per pot for *G. etunicatum* and *A. rugosa*, respectively. Six-day soil-solution-equilibrium P levels were 0.005, 0.1, 0.2, 0.5 and 1.0 $\mu g\ mL^{-1}$, which corresponded to P additions of 0.0, 37.50, 46.25, 68.00, and 83.33 mg P kg^{-1}, respectively. Experimental procedures and conditions were as described in the first experiment except that larger pots were used (16-cm diam.). Each pot was filled with 3.5 kg of soil. Average maximum and minimum temperatures were 29 and 22°C, respectively, and the average daily maximum PAR was 1,679 $\mu E\ m^{-2}\ s^{-1}$.

Root measurements were made as in the first experiment, and several additional parameters were measured also. Average root hair length was determined by removing randomly five, 1-cm-long root segments containing root hairs from each plant, measuring the length of ten root hairs per segment using a microscope (300 X) and an eyepiece micrometer, and then averaging the 50 values for each plant. Average root hair density was determined by counting the number of root hairs in five randomly selected fields of view at 600 X (area of 1 field of view was 0.0158 mm^2) from

each of the five segments used for the root hair length measurements. Values were averaged for each plant. Total number of entry points of VAM fungi, and entry point densities, were determined on five randomly selected, colonized, 1-cm-long root segments per plant. This value was multiplied by two to account for entry points on the underside of the root and averaged for each plant.

Vine and root dry masses were measured and root and vine tissues analyzed for P concentration. Phosphorus-inflow rates were calculated from plant-P data and root length measurements (Sanders et al., 1977).

Data were subjected to analysis of variance using the General Linear Models Procedure (SAS, 1985). Data having a significant P or P by inoculation effect were subjected to regression analyses, except for root and vine dry mass data which were fit to the Von Bertalanffy function. Significant fungal effects were further analyzed by single-degree-of-freedom contrast procedures that compared the effect of each VAM fungus to the control. Average root hair length (log), average root hair density (log), total number of entry points of VAM fungi (log), and entry point densities (log) were transformed according to the guidelines of Gomez and Gomez (1976).

Results

Growth Response

Inoculation with VAM fungi and P addition significantly affected vine and root dry mass (Table 3-1). Vines had a typical VAM plant-growth response in both experiments; inoculation with VAM fungi improved growth, relative to the control, more at low-P levels than at high-P levels (Figure 3-1a and Appendix D). Root dry mass had a similar response with *A. rugosa*, but the other two treatments did not reach a level asymtote (Figure 3-1b). Growth responses were similar in both experiments so curves are presented only from the second experiment.

P Uptake

In the first experiment, there were no differences in vine-P concentrations due to P level or inoculation with VAM fungi (Table 3-1). In the second experiment, P concentration and total P in roots and vines had highly significant P-level X fungus effects, except for the interaction of total vine P which was significant at $P \leq 0.05$. Only in the second experiment did inoculated plants have greater vine-P content than the controls at all soil-P

levels (Figure 3-2a). This relationship was similar for total root P (Figure 3-2b) except at soil-P levels of 0.5 and 1.0 μg P mL^{-1} where the controls had higher root P than plants inoculated with *A. rugosa*. Vine-P concentration had either no relationship or a weak positive relationship ($P \leq 0.01$ and $R^2 \leq 0.20$) to soil-P levels, with the exception of plants inoculated with *A. rugosa* which had a weak negative relationship ($R^2 = 0.33$). There was a significant interaction between root-P concentration and VAM fungus inoculation (Table 3-1 and Figure 3-3).

Phosphorus inflow increased linearly ($P \leq 0.0001$, $R^2 = 0.39$) with soil-P level in control plants while P inflow did not change with increased P level for plants colonized with VAM fungi. Both treatments inoculated with VAM fungi had higher ($P \leq 0.05$) P inflow across all P levels than the control. Mean P inflow rates were 11.71, 15.15, and 19.75 X 10^{-4} μM cm^{-1} wk^{-1} for control, *A. rugosa*, and *G. etunicatum* treatments, respectively.

Colonization

All controls remained noncolonized and were excluded from the analysis of VAM colonization. In the first experiment, the interaction of fungus and P level affected total, but not percent colonized root length (Table 3-1). In the second experiment the effect of P level on percent colonized root length, total number, and density of VAM fungus entry points was analyzed by fungus treatment because there were significant fungus by P-level interactions for these variables. Phosphorus level had no effect on total root length by colonized by *G. etunicatum* (transformed mean = 8.5 cm, S.E.M. = 0.21) while plants colonized by *A. rugosa* (transformed mean = 7.0 cm, S.E.M. = 0.34) had a significant ($P \leq 0.01$), but weak ($R^2 = 0.20$) inverse relationship to P level. Percent colonization had an inverse relationship to P level with both VAM fungus treatments (Figure 3-4). The inverse relationship between percent colonization and P level was stronger for *A. rugosa* than *G. etunicatum,* judging from the significance level ($P = 0.0001$ vs 0.05) and R^2 values (0.51 vs 0.13) for *A. rugosa* and *G. etunicatum,* respectively. Phosphorus level had no effect on either total number or density of entry points of *G. etunicatum* (mean transformed total number of entry points = 10.24,

S.E.M.= 0.86, mean transformed entry point density = 2.26 no. cm^{-2}, S.E.M. = 0.28), while both these parameters were reduced with *A. rugosa* (mean transformed total number of entry points = 7.79, S.E.M. = 1.1, mean transformed entry point density = 1.92 cm^{-2}, S.E.M. = 0.33) (Figure 3-5).

Root Hairs

In the first experiment, the only root hair parameter to show any affect was total root length with root hairs which increased with P level (Table 3-1). In the second experiment, total length of roots with root hairs and percent of the root with root hairs were affected by fungus and P level with no interaction. Control plants had a greater ($P \leq 0.05$) proportion of their roots with root hairs (49%) than plants inoculated with *A. rugosa* (34%) or *G. etunicatum* (43%) across all P levels. The proportion of the root with root hairs responded cubically with P level ($P = 0.0001$, $R^2 = 0.38$) (Figure 3-6). Root hair length had a significant ($P \leq .01$), but weak ($R^2 = 0.15$) positive relationship to P level. Root hair density was not affected by P level or inoculation.

Discussion

Growth Response

The typical response of a mycorrhiza-dependent plant to P fertilization includes a significant inoculation by P fertilization interaction with a VAM-induced growth response only at the low-P levels (Abbott and Robson, 1984). In my first experiment, there was no inoculation by P level interaction. I attributed this to the preponderance of low-P treatments relative to high-P treatments which may have obscured the interaction. In the second experiment, an additional high-P treatment (0.5 μg P mL^{-1}), and additional replications were added which improved the significance level for the interaction term. Curves of root dry mass did not reach an asymptote within the P range used in this experiment. Nonetheless, the pattern of VAM-induced growth enhancement at the low-P levels is reflected in the root data as well.

P Uptake

The discrepancy between the vine-P concentrations in the two experiments may be due to differences in treatment replications or pot size. The larger pots in the second

experiment produced larger plants than in the first experiment which may have accentuated differences between the VAM fungus treatments. In the second experiment VAM fungus inoculation affected the P status of vines and roots. An anomaly in the data was the greater root dry mass and P content in the controls compared with the roots of plants inoculated with VAM fungi at the highest P level. A possible explanation for this is that colonization by VAM fungi altered the carbohydrate allocation between root and shoot (Hung et al., submitted; Miller et al., 1987) so that at P levels where neither control or colonized plants were P-stressed, control plants had larger root systems than colonized plants.

Plants inoculated with VAM fungi had greater P-inflow rates than the controls. In addition, P level had no effect on P-inflow rates of plants inoculated with VAM fungi while P-inflow rates increased with P level in control plants. This supports the concept than VAM fungi improve P uptake by maintaining adequate P inflow under soil conditions which limit P supply to roots (Sanders and Tinker, 1971). Colonization by VAM fungi allowed the plants to overcome limitations in P supply to the roots over most of the P-response curve. In a parallel study (Hung et al.,

submitted), sweet potato responded to inoculation with VAM fungi in a low P-fixing soil at low P-fertilization rates. Phosphorus-inflow rates were ten- and 100-fold lower in the first and second experiments, respectively, than values reported for onion by Sanders and Tinker (1973). This may have been due to the more fibrous nature of the sweet potato root system, lower P-availibility or degree of external hyphae in the experiments with sweet potato.

Colonization of sweet potato by *G. etunicatum* was less sensitive to P inhibition than colonization by *A. rugosa* in the second experiment. This was supported by the finding that plants inoculated with *A. rugosa* were the only inoculated treatments to have a significant decrease in total colonized root length. This is generally a better indicator of P inhibition of VAM colonization than percent colonization because it reduces the confounding effect of P-induced increases in total root length. The difference between the two fungi in sensitivity to P inhibition was also apparent in the entry point data.

In the first experiment, there was no affect of fungi or P level on percent of root length with root hairs. The total length of roots with root hairs was increased by P fertilization; however, this was likely a secondary effect

of a P-induced increase in total root length, and not a direct effect on root hair proliferation. Based on the possibility that other root hair parameters may be more sensitive to VAM and P effects, and that more replications may be needed due to the high variability in root hair distribution (cv > 30%) found in the first experiment, in the second experiment the number of replications was increased and additional root hair parameters were measured. In both experiments, total length of roots with root hairs increased with treatments that increased total root length (i.e., P fertilization and VAM fungus inoculation) and consequently the effects may be secondary. Phosphorus fertilization increased the percent of the root length with root hairs but not root hair density. This is in contrast to Mackay and Barber's findings that density of root hairs decreased with P level (1984), but in agreement with Anderson et al. (1987) who found no relationship between soil-P availability and root hair density. Root hair length also increased with P level which is the opposite of Mackay and Barber's results (1984). This discrepancy may be due to differences between plant species or the soil-P ranges used in the experiments as the effect of P on root hair length varies with the range of P considered.

If one accepts the idea that VAM hyphae and root hairs are functionally analogous, the question arises as to why soil-solution-equilibrium P level can have the opposite effect on VAM fungi and root hairs. The mechanisms of plant control of root hairs and VAM fungi may not related. In addition, considering root hairs and VAM fungi as functionally analogous in terms of P-uptake does not preclude additional roles that may not be shared between the two structures. The suppression of root hairs by VAM formation seen in the second experiment is a logical extension of the hypothesis put forth by Baylis (1972); however, a direct cause and effect relationship has yet to be established. Allen (1982) found that mycorrhizal *Bouteloua gracilis* had fewer and shorter root hairs than nonmycorrhizal plants. Additional work is needed to determine how this relationship changes with soil characteristics, host, and VAM fungus isolate.

Table 3-1. Summary of *P* values from analysis of variance of two experiments with sweet potato.

Effect	Vine Dry Mass	Root Dry Mass	Vine P Conc.	Root P Conc.	Total Vine P	Total Root P	Root Length With Root Hairs	%Root Length With Root Hairs	P Inflow	Colonized Root Length	% Colonized Root Length	Root Hair Density	Root Hair Length	Entry Point Density	Total Entry Points
								Experiment One							
F	0.0120	ND	0.5880	ND	ND	ND	0.5075	0.6869	ND	0.1768	0.4545	ND	ND	ND	ND
P	0.0001	ND	0.2300	ND	ND	ND	0.0001	0.4811	ND	0.0960	0.6697	ND	ND	ND	ND
F*P	0.5292	ND	0.1030	ND	ND	ND	0.2889	0.6254	ND	0.0946	0.3546	ND	ND	ND	ND
CV(%)	24		50				4	38		30	76				
								Experiment Two							
F	0.0001	0.0091	0.0001	0.0001	0.0001	0.0001	0.0401	0.0001	0.0001	0.0001	0.0001	0.5673	0.5191	0.3598	0.0406
P	0.0001	0.0001	0.0141	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0630	0.0001	0.2290	0.0852	0.0221	0.0520
F*P	0.1181	0.0016	0.0001	0.0004	0.0486	0.0035	0.5053	0.3944	0.0476	0.0777	0.1143	0.6047	0.2570	0.0666	0.0486
CV(%)	29	36	21	17	35	37	8	39	42	24	67	7	4	47	36

'ND-not determined.

Figure 3-1. Phosphorus response curves for sweet potato vine (a) and root (b) dry mass from Experiment Two. Symbols represent the means of nine replicates. Data were fit to the Von Bertalanffy function. Equations and R^2 values for the curves are:
Vines;
Control, $Y = 5.5 * (1 - 0.77 * \exp(-1.84 * X))$, $R^2 = 0.46$;
G. etunicatum, $Y = 5.5 * (1 - .97 * \exp(-107.89 * X))$, $R^2 = 0.58$;
A. rugosa, $Y = 5.25 * (1 - 0.76 * \exp(-14.21 * X))$, $R^2 = 0.49$;
Roots;
Control, $Y = 22.38 * (1 - 0.94 * \exp(-0.55 * X))$, $R^2 = 0.72$;
G. etunicatum, $Y = 24.27 * (1 - 0.83 * \exp(-0.3 * X))$, $R^2 = 0.47$;
A. rugosa, $Y = 8.64 * (1 - 0.75 * \exp(-8.64 * X))$, $R^2 = 0.57$.

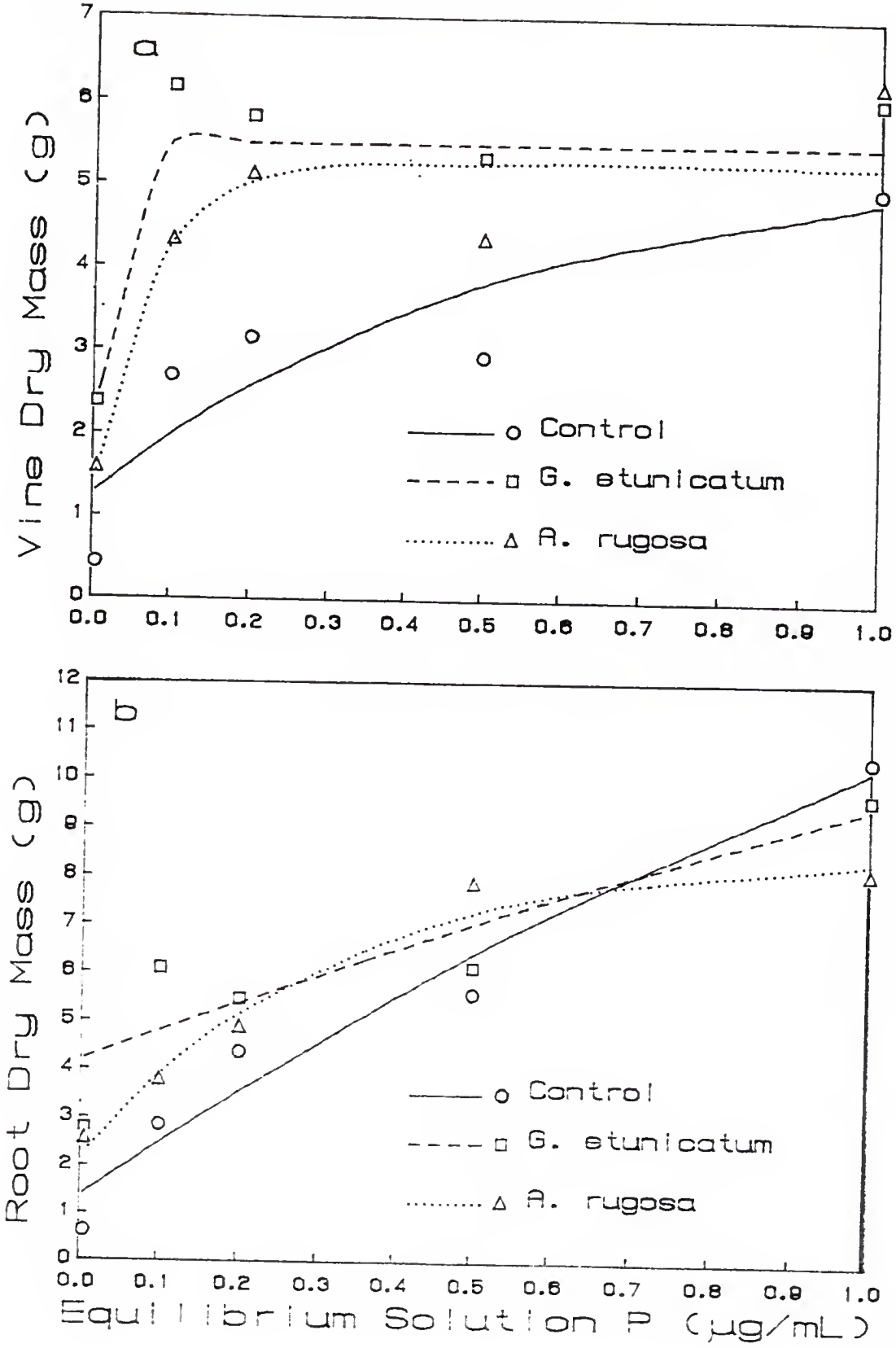
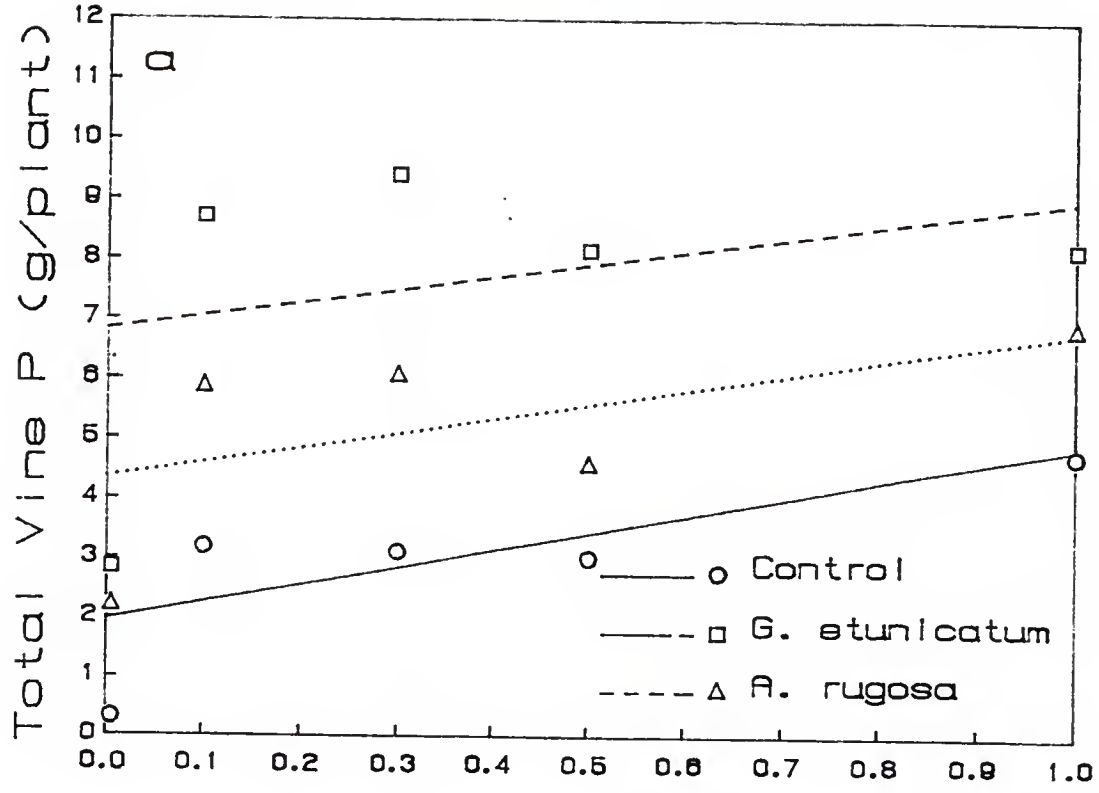

a
Vine Dry Mass (g)
Control
G. etunicatum
A. rugosa
b
Root Dry Mass (g)
Control
G. etunicatum
A. rugosa
Equilibrium Solution P (µg/mL)

Figure 3-2. Total vine (a) and total root (b) phosphorus content of sweet potato grown in a glasshouse for 9 wk from Experiment Two. Symbols represent means of nine replicates. Equations and R^2 values for the lines are:
Vine phosphorus;
Control, $Y = (2.97 * X) + 1.89$, $R^2 = 0.25$;
G. etunicatum, $Y = (2.22 * X) + 6.77$, $R^2 = 0.08$;
A. rugosa, $Y = (2.5 * X) + 4.30$, $R^2 = 0.16$;
Root phosphorus;
Control, $Y = (7.71 * X) + .868$, $R^2 = 0.75$;
G. etunicatum, $Y = (6.41 * X) + 4.25$, $R^2 = 0.42$;
A. rugosa, $Y = (4.63 * X) + 2.99$, $R^2 = 0.51$.


a
Total Vine P (g/plant)
Control
G. etunicatum
A. rugosa

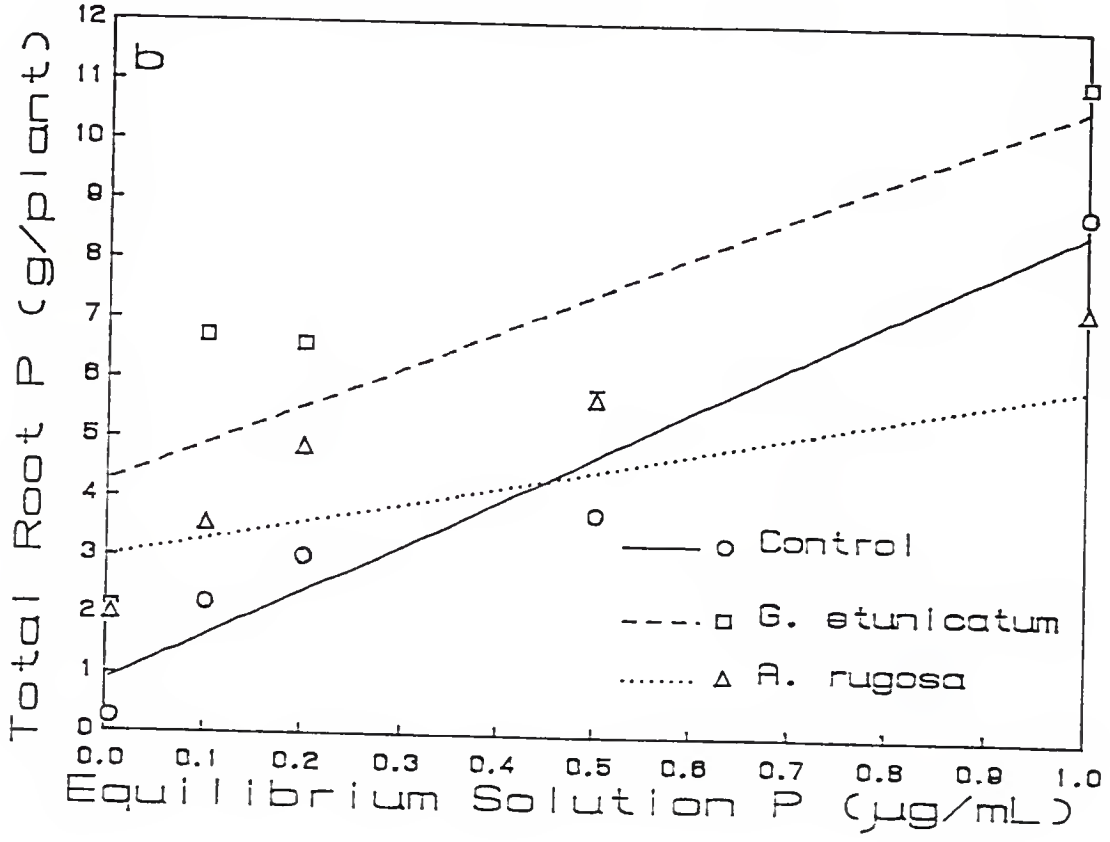
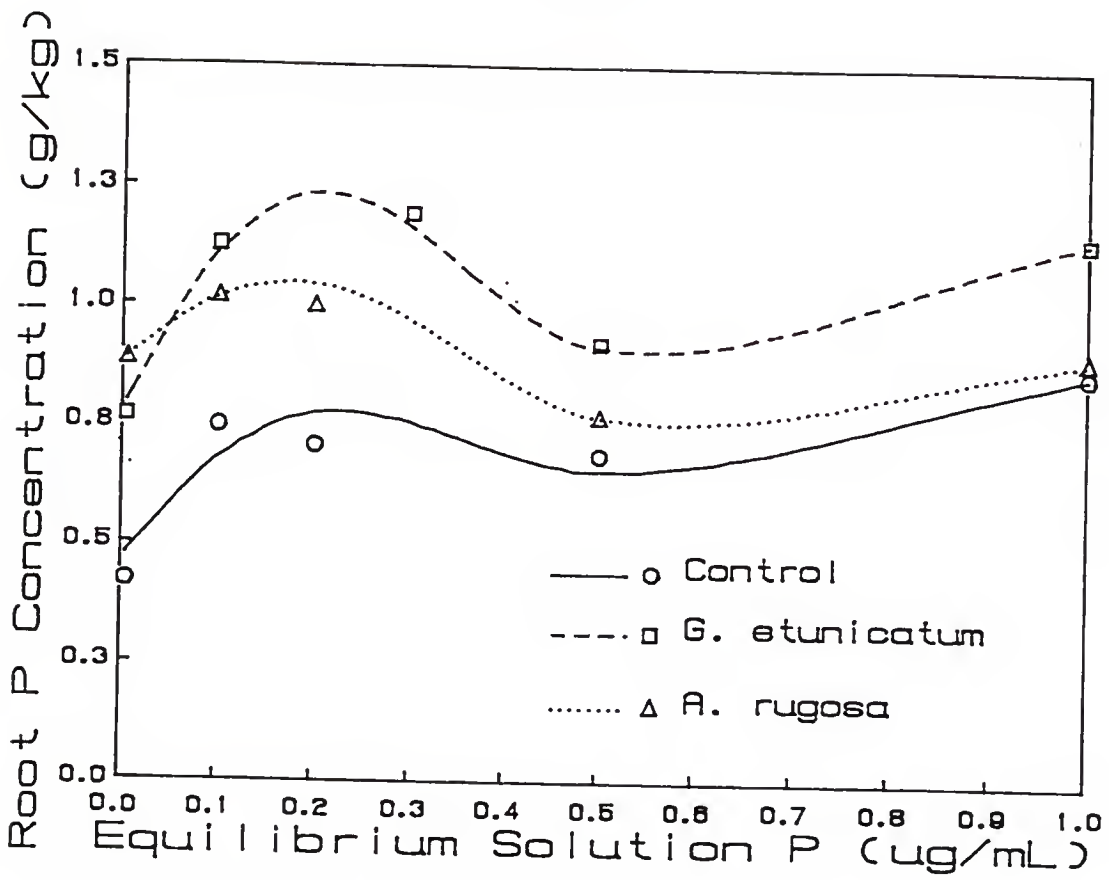

b
Total Root P (g/plant)
Control
G. etunicatum
A. rugosa
Equilibrium Solution P (µg/mL)

Figure 3-3. Root phosphorus concentration of sweet potato grown in a glasshouse for 9 wk from Experiment Two. Symbols represent means of nine replicates. Equations and R^2 values for the lines are:
Control, $Y = (2.83 * X) + (-7.36 * X^2) + (4.93 * X^3) + .462$, $R^2 = 0.49$;
G. etunicatum, $Y = (4.54 * X) + (-12.89 * X^2) + (8.72 * X^3) + .774$, $R^2 = 0.55$;
A. rugosa, $Y = (2.01 * X) + (-6.95 * X^2) + (4.95 * X^3) + .879$, $R^2 = 0.24$.

Figure 3-4. Arc sine transformed percent root length of sweet potato grown in a glasshouse for 9 wk colonized by VAM fungi from Experiment Two. Symbols represent means of nine replicates. Equations and R^2 values for the lines are:
G. etunicatum, $Y = \{ (-.15 * X) + .33 \} * 100$, $R^2 = 0.10$;
A. rugosa, $Y = \{ (-.82 * X) + (.62 * X^2) + .26 \} * 100$, $R^2 = 0.51$.


Root P Concentration (g/kg)
1.5
1.3
1.0
0.8
0.5
0.3
0.0
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
Equilibrium Solution P (ug/mL)
o Control
G. etunicatum
A. rugosa

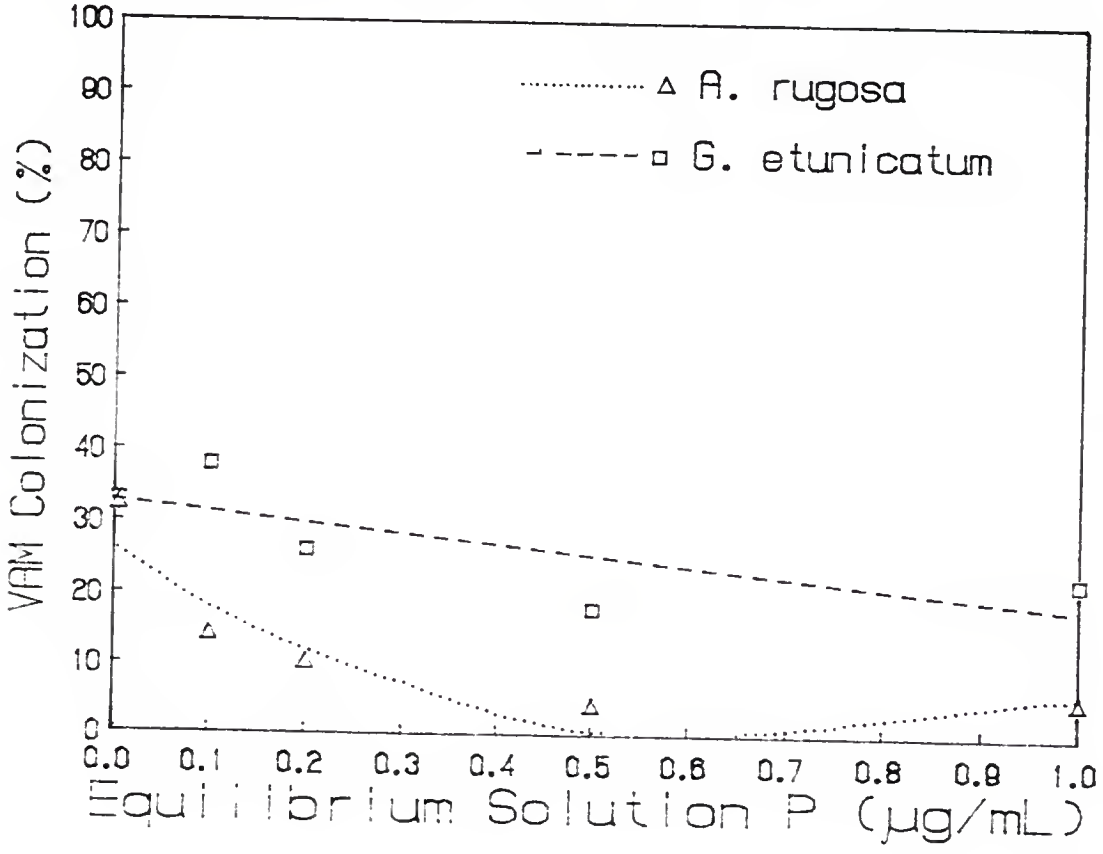
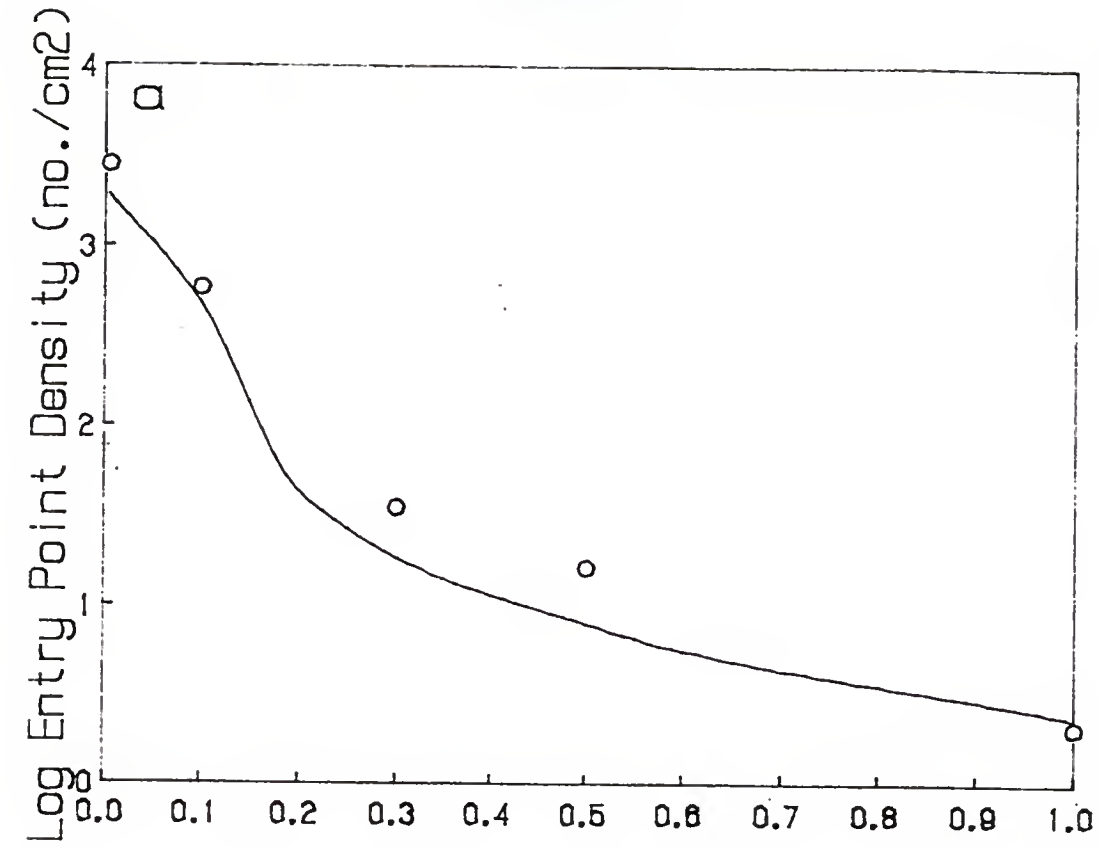

VAM Colonization (%)
100
90
80
70
60
50
40
30
20
10
0
0.0 0.1 0.2 0.3 0.4 0.5 0.6 0.7 0.8 0.9 1.0
Equilibrium Solution P (µg/mL)
A. rugosa
G. etunicatum

Figure 3-5. Log transformed density (a) and total number (b) of VAM fungi entry points in plants inoculated with *A. rugosa*. Equation and R^2 value for the lines are:
entry point density; $Y = (-6.73 * X) + (3.8 * X^2) + 3.31$, $R^2 = 0.69$;
total entry points per plant; $Y = (-12.33 * X) + (3.16 * X^2) + 11.27$, $R^2 = 0.38$.


a
Log Entry Point Density (no./cm2)
0
1
2
3
4
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0

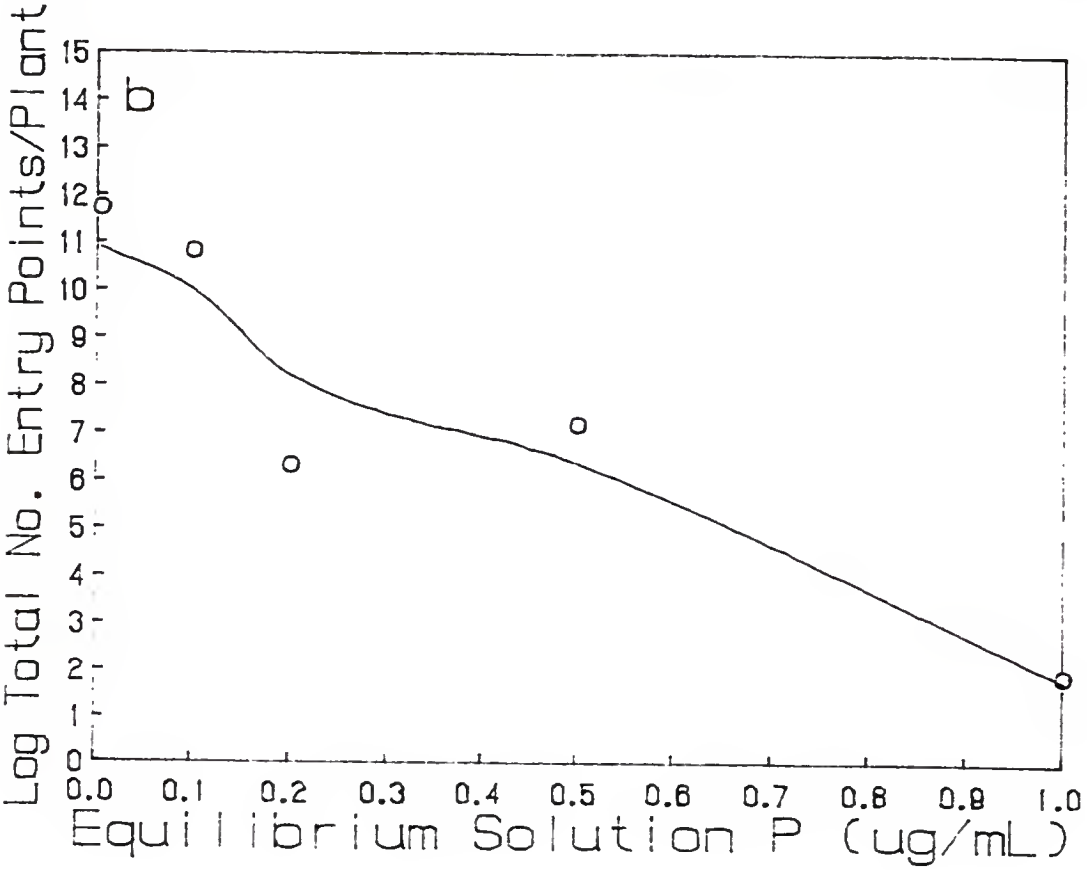
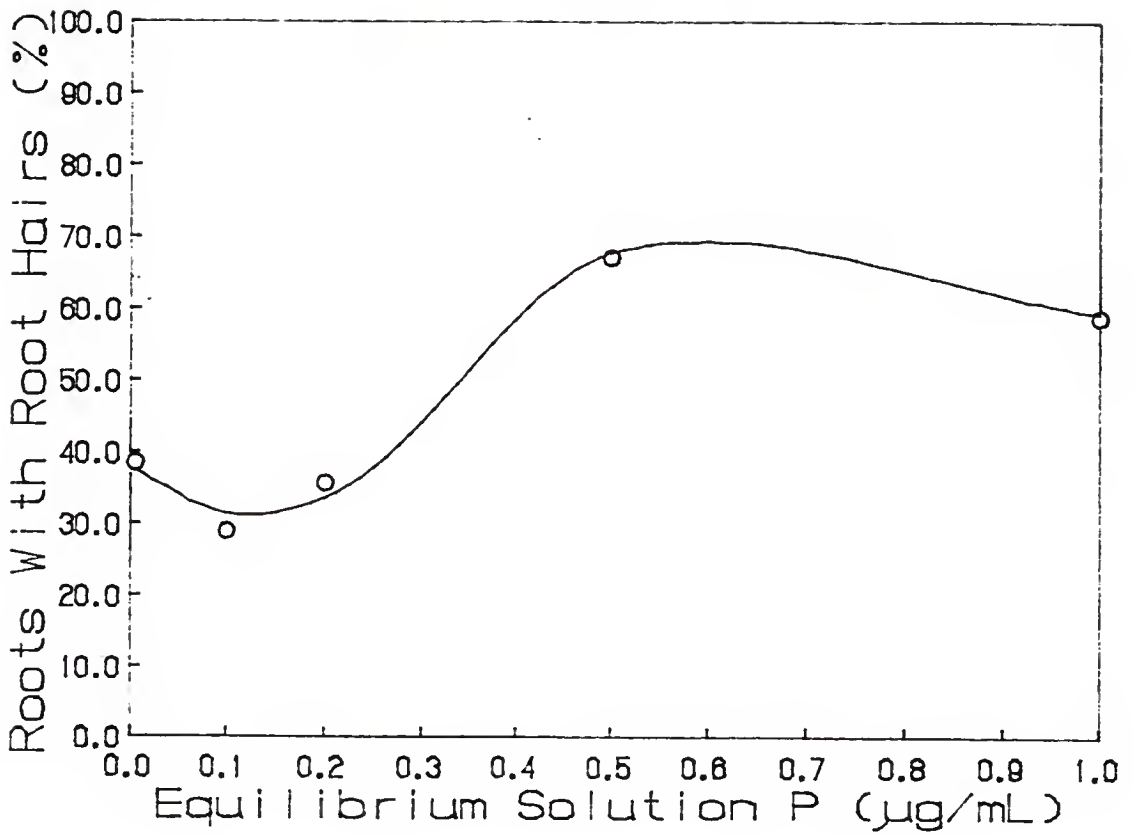

b
Log Total No. Entry Points/Plant
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Equilibrium Solution P (ug/mL)

Figure 3-6. Arc sine transformed percent of sweet potato root length with root hairs. Equation and R^2 value for the line is:
$Y = \{ (-1.20 * X) + (5.76 * X^2) + (-4.35 * X^3) + 0.38\} * 100$, $R^2 = 0.38$.


Roots With Root Hairs (%)
100.0
90.0
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Equilibrium Solution P (µg/mL)

CHAPTER FOUR
THE CHRONOLOGY AND MECHANISMS OF THE VESICULAR-ARBUSCULAR MYCORRHIZAL PLANT-GROWTH RESPONSE FOR SWEET POTATO

Introduction

To understand the mechanism of the VAM plant-growth response, the timing of the response should be determined (Abbott and Robson, 1984). Growth increases in young plants may persist throughout their lives, therefore, multiple harvests (Sanders et al., 1977; Abbott and Robson, 1984) or repeated, nondestructive measurements are necessary to identify the onset of the VAM plant-growth response. A VAM plant-growth response occurs when P inflow is greater for a mycorrhizal plant than for a nonmycorrhizal plant, assuming the mycorrhizal status is the only difference between the plants, or when P inflow exceeds the diffusion limits to the root alone (Sanders and Tinker, 1973). In the short-term, such as growth chamber and glasshouse studies,the response may be the result of either a transitory event or a continuous phenomenon. In long-term situations, as in

natural plant communities, the VAM effect may be transitory and vary with drought stress (Fitter, 1986) or season (Douds and Chaney, 1986). The focus of this research was the short-term response.

A proposed chronology of events leading to the VAM plant-growth response is: (i) infection of the root; (ii) followed by proliferation of external hyphae; (iii) leading to an increased P inflow and elevation of tissue-P levels; and (iv) resulting in a plant growth increase. Gueye et al. (1987) studied the response of cowpea to colonization by a VAM fungus and reported data that fit the above described chronology. Their finding, that proliferation of external hyphae occurred 20 to 25 d after planting is in agreement with observations of Owusu-Bennoah and Wild (1979) who reported that differences in P-depletion zones between mycorrhizal and nonmycorrhizal onion roots occurred within 21 d. Sanders et al. (1977) reported that P inflow was higher in some plants inoculated with VAM fungi compared to noncolonized controls after 17 d, while dry mass differences became apparent 30 to 40 d after planting. In that same experiment, and a similar experiment with onions (Sanders and Tinker, 1973), P inflow into mycorrhizal roots exceeded that into nonmycorrhizal roots throughout the experiment,

suggesting that in these short-term experiments, the mycorrhizal response was a continuous phenomenon.

Once the chronology of the VAM plant-growth response is determined, the mechanisms of this response can be most efficiently studied. Proposed mechanisms for the VAM plant-growth response have been reviewed (see Chapter Two). A reasonable and generally accepted theory is that the hyphae of VAM roots are distributed in such a way as to improve the P-uptake characteristics of the root (Sanders and Tinker, 1971; Mosse, 1973). If mycorrhizae are to be effective in increasing P uptake, their external hyphae must be distributed beyond the P-depletion zones around roots and root hairs. Consequently, P-depletion zones are a reasonable spatial reference point for studying the distribution of hyphae around mycorrhizal roots. Using the quantitative autoradiographic technique of Bhat and Nye (1973), Owusu-Bennoah and Wild (1979) found that hyphae of VAM fungi increased the measurable P-depletion zone around the roots of onion from 0.1 to 0.2 cm. In artificial systems, P uptake by hyphae of mycorrhizal fungi at distances much greater than 0.2 cm have been demonstrated (Hattingh et al., 1973; Rhodes and Gerdemann, 1975; Alexander et al., 1984). Since P depletion around roots is

radial, one purpose of my research was to evaluate the radial distribution of hyphae around the root relative to P-depletion zones.

Soil pore sizes vary, and as a result, access to nutrients is partially dependent on the radii of the nutrient absorbing structure. Estimates of the radii of roots, root hairs, hyphae of VAM fungi, and pores of the soil are needed to evaluate the relative effects of root hairs and hyphae of VAM fungi in exploration of the soil for P. The small diameter of hyphae should allow them access to smaller pore spaces than roots and root hairs and thereby to explore a greater volume of soil. Furthermore, the physical environment of the smaller pore spaces may be different from the larger pores, providing more favorable P-supply characteristics. For instance, at a given bulk-soil moisture content, volumetric water content will be higher in small pores than in large pores, resulting in greater P-diffusion coefficients.

Plants can increase P solubility and uptake by chemically modifying the rhizosphere (Jungk, 1987; Marschner et al., 1987; Nye and Kirk, 1987). One such modification is the exudation of organic acids. The extent to which VAM and non-VAM roots differ with regard to organic acid production

is not known. However, as a group, fungi possess the capacity to alter P availability in the soil by the same mechanisms as higher plants (Beever and Burns, 1980). Nonetheless, there is no evidence on quantitative or qualitative changes in exudation of organic acids from plant roots due to VAM fungal infections.

The objectives of this research were to (i) establish the chronology of the VAM plant-growth response with sweet potato, (ii) determine the distribution of external hyphae of VAM fungi in relation to the root, root hairs, and P-depletion zones at the time when the VAM plant-growth response first becomes apparent, (iii) evaluate the pore size distribution of the soil relative to the radii of roots, root hairs, and hyphae of VAM fungi in terms of the significance of the role of hyphae of VAM fungi in exploration of larger volumes of soil and access to smaller pore spaces, and (iv) determine if sweet potato mycorrhizae alter the organic acid composition of the rhizosphere.

Materials and Methods

Experiment One

This experiment was designed to establish the chronology of the VAM plant-growth response in sweet potato. A three (VAM treatment) by four (harvest date) factorial experiment was established using a completely randomized design with five replications per treatment.

The Orangeburg soil (described in Chapter Three) was fertilized with 37 mg P kg^{-1} soil to give a 6 d soil-solution-equilibrium P concentration (Fox and Kamprath, 1970) of 0.10 µg P mL^{-1}. This P concentration was conducive to a VAM plant-growth response (Chapter Three). Pots (16 cm diam.) were filled with 3.5 kg of pasteurized soil. Twenty mL of a nutrient solution containing 12.87 g of NH_2NO_3, 14.27 g of KCL, and 1 mL Hoagland's (Hoagland and Arnon, 1950) micronutrients per L was added initially to each pot. Every two weeks thereafter 10 mL of the nutrient solution was added to each pot. Plants were grown in a glasshouse with average a maximum PAR over the 8-wk growing period of 1,746 µE m^2 s^{-1} and average maximum and minimum temperatures of 32 and 24°C, respectively. Pots were watered to maintain approximate field capacity.

Inoculation treatments were as described in Chapter Three. Maintenance of cultures of *G. etunicantum* and *A. rugosa* was also described in Chapter Three.

Plants were harvested at 2, 4, 6, and 8 wk after planting and the following plant parameters were measured or calculated: vine and root dry mass; phosphorus concentration in vines, roots, and the first fully expanded leaf and petiole; root length; length of root with root hairs; percent of the root with root hairs; root hair length, diameter, and density; and P-inflow rates. Phosphorus concentration of vine cuttings was analyzed at the beginning of the experiment to obtain initial-P concentrations for P-inflow calculations. Root and root hair diameter were determined by taking the mean of ten measurements collected at random on each of five 1-cm-long segments per plant of roots with root hairs. Methods for estimating the other root parameters were described in Chapter Three. Vine lengths were measured twice weekly as a means of nondestructively monitoring the growth of plants.

At each harvest, the following fungal parameters were also measured: total and percent length of root colonized by VAM fungi; diameter of hyphae of VAM hyphae; and density and total number of entry points of VAM-fungal hyphae. Methods

for estimation of the other parameters are described in Chapter Three. Hyphal diameter was measured using an eyepiece micrometer at 600x. Five, 1-cm-long root segments colonized by VAM fungi were examined per plant and five measurements per segment were made. Values were averaged for each plant. Two plants from each treatment inoculated with VAM fungi at the first harvest, and one plant inoculated with *A. rugosa* at the second harvest were not colonized. These plants were eliminated from the experiment.

At each harvest, soil was analyzed for total and active soil hyphae (Sylvia, 1988). Soil hyphae measurements were divided into two samples, cores A and B. Core A was taken directly over the stem and inoculum. Core B was sampled by pooling three cores collected 5 cm from the central core. Cores were taken to the bottom of the pot using a sharp, 20-mm diam. stainless steel corer. Each sample was suspended in 5 L of tap water, blended for 15 sec, allowed to settle for 5 min, and then a 25-mL subsample was removed and placed on filter paper. Hyphae were then stained and quantified according to the procedure of Sylvia (1988). Coarse, brown, septate hyphae were not included in hyphal counts.

An additional study was done to test the efficiency and variability of the soil hyphae extraction technique. My specific objective was to determine the degree to which blending and settling times of the blended soil suspension affected the length of hyphae measured. Orangeburg soil was placed in 7- by 12-cm plastic trays to a depth of 3 cm, amended with 500 mL of a 1.0 g L^{-1} malt-extract solution, and incubated uncovered at 21°C, for 4 wk to allow saprophytic fungal populations to increase. After incucubation, 25-g soil samples were processed as above except that the blending time (5, 15, and 60 s) and settling time (0, 0.5, 3, 5, and 10 min) were varied. There were five replications per treatment combination.

Soil-solution P was determined every 2 wk by solution lysimeters (internal diam. of 22 mm) made from polyvinyl-chloride tubing cut to 6-cm lengths and closed at the bottom with scintered glass discs. The top end was closed with a rubber stopper fitted with a glass tube, vacuum hose, and clamp. Lysimeters were placed in two pots from each inoculation treatment and the solution was removed by a hand-held vacuum pump. The extracted solution was removed from the lysimeter with a pipette and kept at 3°C with 3 drops of toluene to inhibit microbial growth until the

completion of the experiment when the solutions were analyzed for P concentration (Murphy and Riley, 1962).

Experiment Two

This experiment was designed to determine the distribution of hyphae around sweet potato roots and to quantify rhizosphere organic acids at the time when the VAM plant-growth response was first detected. The experiment was a three (VAM treatment) by two (harvest date) factorial. The experimental protocol was as described in the first experiment except that P was applied 6 wk before planting. Harvests were made at 2 and 4 wk. Mean maximum and minimum temperatures were 29 and 23°C with a mean maximum natural PAR of 1,162 $\mu E\ m^2\ s^{-1}$ supplemented with metal halide lamps supplying 670 $\mu E\ m^2\ s^{-1}$ for 14 hr d^{-1}. Vine dry masses, vine-P concentrations, and P concentration of the first fully expanded leaf and petiole were determined at both harvests. Root systems were recovered completely, except for portions contained in soil cores (coring procedure described below), and analyzed for VAM-fungal colonization. Multiple, 0.5 g samples were taken (up to four) from each root system and analyzed for total root length, root length and percent colonization by VAM fungi, and percent of the root with root

hairs. Because the entire root system was used in this way, P-inflow values were based on vine P only. Soil-solution-P concentrations were analyzed at 0, 2, and 4 wk.

At the final harvest, four cores were removed from each pot in the same pattern as in the first experiment. Cores were collected using 22-mm diam. by 18-cm long, beveled PVC tubes. Cores were impregnated with a 50 g L^{-1} gelatin solution that contained 25 mL L^{-1} of a trypan blue stain {(0.5 g trypan blue L^{-1}), H_2O (500 mL L^{-1}), lactic acid (170 mL L^{-1}) and glycerin (330 mL L^{-1})}. Cores were packed at both ends with polyester fiber, rubber stoppers with glass tubes were placed in each end of the core, and the cores were placed upright in an incubator at 34°C to keep gelatin from solidifying. The gelatin solution was placed above the incubator on a hot plate and allowed to run down through a tube connected to the bottom of the core. The solution flowed upwards through the core until the color of the solution coming out through the top of the core was the same as the solution going in. The cores were then clamped at the bottom, removed from the incubator, and placed at 4°C to solidify. Impregnated soil was pushed out of the PVC tubes in 5-mm increments using a tight-fitting wooden dowel and sectioned with a razor blade. Sections were inspected with

a dissecting microscope and those with roots visible on the surface were examined at 60 X using a compound scope. Hyphae adjacent to roots were quantified using an eyepiece whipple disc by the gridline-intersect method (Newman, 1966). The grid was divided into five, 0.22-mm-wide zones. The root was aligned with the edge of the whipple disk and hyphae were quantified in each zone (Figure 4-1). Values from the three outer cores were averaged for each zone to give one value per zone per plant. Values from the outer cores were analyzed separately from the center core.

Bulk soil (soil not adhering to roots) and rhizosphere soil were extracted at 4 wk by shaking soil in plastic bottles containing 100 mL of deionized water at room temperature for 2 h. Rhizosphere soil was collected by dipping roots with attached soil into the deionized water. Extracts were then filtered and frozen (-20°C) until they were analyzed. After extraction soil was dried at 70°C for 24 h and weighed to adjust values to a dry mass basis. Extracts were thawed and analyzed for organic acids using high-pressure liquid chromatography (HPLC) (Lee and Lord, 1986). The HPLC system consisted of a Gilson single piston high pressure pump and pressure module (Gilson Medical Electronics, Inc., Middleton, Wisconsin), a Rheodyne model

7125 injection valve fitted with a 20 µL injection loop (Rheodyne, Inc., Cotati, California), a Hamilton PRP-X300 150 x 4.1 mm organic acid column (Hamilton Co., Reno, Nevada), a Gilson Holochrom variable wavelength uv detector and a Gilson computerized integrator. Organic acids were separated at ambient temperature with H_2SO_4 as the eluent at a flow rate of 2 mL min^{-1} and detected at 210 nm. Organic acids were quantified by comparing peak areas with an external standard curve.

Soil pore-size distribution was determined using water release curves that relate volumetric water content at varying pressure heads to soil pore size (Swanson and Peterson, 1941). Bulk densities were determined by the method of Blake and Harge (1986). Soil water content was determined gravimetrically throughout the experiment, averaged and adjusted to volumetric water content (ϕ was used to calculate the soil P-diffusion coefficient (D_e) ($D_e = D_w \phi f b^{-1}$) where D_w is the P-diffusion coefficient in water (Schenk and Barber, 1979). Buffer power (b) was calculated from P adsorption curves (Barber, 1984). The tortuosity factor (f) was taken from Warncke and Barber (1972) as 0.15. From these values the width of the depletion zone at 7 d around the root and root hair cylinder was calculated from

$\sqrt{2}$Det (Baldwin et al., 1972) and used as a reference point for measuring hyphal distribution.

Data Analysis

Data were subject to analysis of variance using the General Linear Models Procedure (SAS, 1985). Data having a significant harvest or harvest x inoculation effect were subjected to regression analysis. Significant inoculation effects were analyzed by single-degree-of-freedom contrast procedures that compared the effect of inoculation with each VAM fungus to the control. Root length (log), length of root with root hairs (log), percent of the root with root hairs (square root), root hair length (log), root hair density (log), total length of root colonized by VAM fungi (log), and percent of the root colonized by VAM fungi (square root) were transformed according to the guidelines outlined in Gomez and Gomez (1976). Means for the length of active and total soil hyphae, and hyphal density values were positivley correlated with the variance and were therefore power transformed according to the methods of Glenn et al. (1987). Exponents used in the transformations were 0.45, 0.30, and 0.10 for hyphal density, total hyphal length, and active hyphal length, respectively.

Results

Soil Solution P Levels

Mean soil-solution-P levels in the first experiment were initially 0.05 μg mL^{-1}, dropped to 0.02 μg mL^{-1} at the 2-wk harvest, and then stabilized at 0.01 μg mL^{-1}. Soil-solution-P levels in the second experiment were stable throughout the experiment at 0.003 μg mL^{-1}. The lower value in the second experiment was probably due to the 6-wk equilibration period. It is important to note that the soil-solution-P level from the 6-d adsorption curve (0.10 μg mL^{-1}) is considerably higher than soil-solution-P values obtained from soil-solution samples taken after the experiment began, indicating that equilibrium-solution-P level is a misnomer.

Experiment One: Plant and VAM Fungus Reponses

Vine and root dry mass increased linearly with harvest (vine dry mass, $P = 0.0001$, $R^2 = 0.71$; root dry mass, $P = 0.0001$, $R^2 = 0.69$). Inoculation had no affect on root dry mass. Combined analysis indicated fungus inoculation effects (Table 4-1), but contrast procedures revealed no differences between *G. etunicatum* (mean = 4.2 g, *P* for

contrast = 0.1017) and *A. rugosa* (mean = 3.4 g, P for contrast = 0.1477) when compared to the control (mean = 3.4 g). There was no significant interaction between fungus inoculation and harvest for vine and root dry mass. Differences in vine length between plants inoculated with *A. rugosa* and the controls first appeared between 3 and 4 wk, while differences between plants inoculated with *G. etunicatum* and the controls first appeared between 4 and 5 wk (Figure 4-2). There was a significant interaction between harvest and inoculation for total root and vine P. Total root (Figure 4-3a) and vine P (Figure 4-3b) increased with harvest. Vine-P concentration increased in plants inoculated with *G. etunicatum*, but decreased in plants inoculated with *A. rugosa* and controls (Figure 4-3c). Phosphorus concentration in the first fully expanded leaf, on the other hand, decreased with harvest for all VAM treatments (Figure 4-3d). There was no significant interaction between VAM treatment and harvest for the concentration of phosphorus in the root; however, both fungi had higher concentrations ($P \leq 0.01$) than the control with means of 684, 852, and 736 μg P g^{-1} for the control, *A. rugosa*, and *G. etunicatum*-inoculated plants, respectively. For root length, there was a significant interaction between

For root length, there was a significant interaction between inoculation and harvest (Figure 4-3e).

Phosphorus-inflow increased until 6 wk for the two treatments inoculated with VAM fungi, while P inflow decreased until 6 wk in the control treatment (Figure 4-3f).

Root hair density and length were not affected by fungus or harvest (Table 4-1). Percent of the root length with root hairs decreased linearly with time ($P = 0.0001$, $R^2 = 0.42$), but was not affected by inoculation with VAM fungi. Colonized root length (total and percent) increased with time and there was no difference between the two VAM fungi. Roots were colonized at the first harvest (4%) and colonization increased linearly ($P = 0.0001$ $R^2 = 0.54$) to a maximum of 18%. There were no significant ($P \leq 0.05$) treatment effects on either the density or total number of entry points of VAM fungi, except for a linear increase ($P = 0.0155$, $R^2 = 0.17$) in the total number of entry points with harvest.

Experiment One: Soil Hyphae

In Core A, length of active hyphae increased linearly with harvest (Figure 4-4a), but was not affected by inoculation with VAM fungi (Table 4-2). Total length of

hyphae in Core A was not affected by inoculation with VAM fungi or harvest. The only significant difference in soil hyphae in Core A between the treatments inoculated with VAM fungi and the controls was the percentage of active hyphae; *G. etunicatum* maintained higher percent active hyphae than the control with means of 24, 73, and 52% for the control, *A. rugosa*, and *G. etunicatum* inoculated treatments, respectively (*P* values for the single degree of freedom contrasts comparing the inoculated treatments to the control were 0.0060 for *G. etunicatum* and 0.1142 for *A. rugosa*). Percent active hyphae increased linearly with harvest in both Core A and B (Figure 4-4b), but the rate of increase was greater in Core B. Settling time affected the amount of hyphae measured using this technique (Table 4-3). With a settling time of five min, 56% of the maximum amount of soil hyphae recoverable using this technique (i.e. after three min) was obtained. Unfortunately, with shorter settling times, suspended clay that collected on the filter interfered with observation of the vital stain. The amount of hyphae lost in the first three min of settling was not determined.

Experiment One: Chronology

Phosphorus inflow was the first parameter to show an increase between 2 and 4 wk (Figure 4-5). After 4 to 6 wk, colonization by VAM fungi and vine dry masses began to increase sharply. Active hyphae did not begin to increase sharply until after 6 wk.

Experiment Two: Plant and VAM Fungus Reponses

Phosphorus-inflow, total vine P, and vine-P concentration were greater for the VAM-fungus inoculated treatments than controls at 4 wk, but not at 2 wk (Table 4-4). Differences between plants inoculated with VAM fungi and controls for other plant parameters were not apparent by 4 wk. Colonization by *G. etunicatum* was greater than *A. rugosa* at the 4-wk harvest while at the 2-wk harvest there was no difference between the two fungi.

Experiment Two: Hyphal Distribution

The spatial relationships among sampled zones, root diameter, root hair length, and the calculated 1-wk P-depletion zones are illustrated in Figure 4-1. There was a slight, linear decline in hyphal length ($P = 0.0151$ $R^2 = 0.05$) away from the root in Core B. Within each zone the

only differences in hyphal density were the outermost zone in zone in core A and in the middle zone in core B (Table 4-5). Hyphae were observed primarily traversing soil pores as hyphae directly on soil-particle surfaces were difficult to see.

Experiment Two: Organic Acids

Oxalate was the only organic acid found in quantifiable amounts in the soil extracts. There was no difference between rhizosphere and nonrhizosphere soil, nor was there any difference between plants inoculated with VAM fungi and noninoculated plants. The mean concentration of oxalate in the soil was 20 $\mu g\ g^{-1}$.

Experiment Two: Relationship of Pore Size Distribution, Root, Hypha and Root Hair Radii

The pore size distribution in the soil in relation to the average radii of hyphae, root hairs, and roots is shown in Figure 4-6. The average volumetric water content during the second experiment was $0.18 \pm 0.09\ cm^3\ cm^{-3}$. By the relationship between volumetric water content and pore size outlined by Swanson and Peterson (1941), pores greater than 20 µm radii were, on the average, drained.

Discussion

Conclusions drawn from the measurment of soil hyphae using existing techniques must be considered tentative due to the high variability associated with such measurements and the inability to distinguish between hyphae of VAM fungi and the hyphae of other soil fungi. Nonetheless, the chronology of events observed in these experiments appeared to differ from the proposed chronology presented in the introduction in that P inflow increased rapidly before colonization by VAM fungi and the percent active hyphae began to increase sharply. Both inoculated treatments followed the same basic chronology. The mycorrhizal response was continuous, not transitory, for the duration of this experiment. The evidence for this is that, once it first became apparent, the P inflow to roots colonized with VAM fungi was greater than noninoculated controls throughout the experiment.

The active hyphae that were measured here were not necessarily hyphae of VAM fungi and there were no differences between the inoculated and control treatments in terms of total amounts of active hyphae. The increase in P inflow due to inoculation with VAM fungi appeared to occur

before there was appreciable proliferation of external hyphae or near-maximal levels of internal colonization. There is the possibility, however, that the hyphae of VAM fungi represented only a fraction of the total hyphae present and therefore the response of VAM hyphae could not be discerned. Nevertheless, Sylvia (1988) observed that abundant proliferation of active external hyphae associated with the roots of *Uniola paniculata* followed increased root colonization by several weeks. Sanders et al. (1977) also observed that P inflow increased while intensity of colonization by VAM fungi and proliferation of external hyphae were relatively low. This phenomenon may be due in part to a qualitative difference in the nature of contact between the soil solution and the root system due to the presence of hyphae of VAM fungi. Hyphae are in closer contact with the surface of soil particles, and therefore the soil solution, than root hairs or roots. Hyphae can explore smaller pore spaces than roots or root hairs. In addition, while measuring hyphal density in gel-impregnated soil cores, I observed that fungal hyphae follow the contour of, and are in direct contact with, the soil particles. This is also seen in micrographs published by Foster et al. (1983). While past researchers (Hattingh et

al., 1973; Rhodes and Gerdemann, 1975; Alexander et al., 1984) worked on the scale of centimeters when addressing the question of how far the hyphae of VAM fungi extend from the root, P-depletion zones around roots occur on the scale of micrometers to millimeters. Calculated P-depletion zones around roots in this study were 468 µm while Owusu-Bennoah and Wild measured P-depletion zones of 1 to 2 mm (1979). This is why I measured hyphal distributions on a scale < 1.0 cm. Nonetheless, only in roots from Core B, which should have a higher proportion of younger roots, was there a detectable hyphal-density gradient away from the root. If this is the case it will be difficult, and perhaps useless, to attempt to determine hyphal distribution around single roots as hyphal gradients around individual roots will overlap, obscuring any discernable distribution patterns. There were also some increases in hyphal densities outside the calculated P-depletion zone around roots inoculated with a VAM fungus compared to noninoculated roots. Thus, it appears that VAM hyphae are distributed beyond P-depletion zones around roots. The presence of external hyphae of VAM fungi should allow VAM colonized roots access to more mobile pools of P than noncolonized roots. Pores with radii greater than 15 µm will generally be drained at field

capacity (Marshall, 1979). In the soil used in these experiments, roots would be excluded from all undrained pores at field capacity while, both root hairs and hyphae of VAM fungi, would have direct access to undrained pores when the soil water content was below field capacity. Within these undrained pores, P availability should be higher than in drained pores due to the positive linear relationship between volumetric water content and soil-P diffusion which follows from the equation given by Schenk and Barber (1979). By this mechanism root hairs and hyphae of VAM fungi afford the plant access to a more mobile pool of P. This alone does not explain why P inflow is higher in roots with root hairs and VAM fungi than in roots with root hairs alone. Hyphae were approximately one-third the diameter of roots hairs in this study, but the magnitude of increased P inflow (> 10 fold) cannot be explained solely by the small difference in accessibility to undrained pores between root hairs and hyphae. More likely, the difference lies in a combination of: (i) the radial extension of hyphae of VAM fungi; (ii) the nature of the contact between hyphae and the soil surface; and (iii) access of the hyphae of VAM fungi to smaller pore spaces than roots and root hairs.

Table 4-1. Experiment One *P* values from ANOVA of plant parameters for sweet potatoes grown in a glasshouse for 8 wk.

Effects	P Inflow	Root Length	%Root Length With Root Hairs	Root Hair Density	Root Hair Length	Vine Dry Mass	Root Dry Mass	Vine P Conc.	Total Vine P	Root P Conc.	Total Root P	First Leaf P
Fungus	0.0001	0.0108	0.2869	0.4654	0.8262	0.0137	0.2977	0.0151	0.0003	0.0043	0.0001	0.0018
Harvest	0.0002	0.0001	0.0001	0.5049	0.3462	0.0001	0.0001	0.1854	0.0001	0.0156	0.0001	0.0001
Fungus*Harvest	0.0001	0.0556	0.4438	0.3775	0.8196	0.2330	0.7579	0.0001	0.0002	0.1504	0.0115	0.1022
CV(%)	45	3	24	11	8	28	34	22	41	20	29	29

Table 4-2. Experiment One *P* values from ANOVA of VAM fungus parameters for sweet potatoes grown in a glasshouse for 8 wk.

Effects	Colonized Root Length	%Colonized Root Length	Entry Point Density	Total Entry Points	Hyphal Length Core A	Hyphal Length Core B	Active Hyphal Length Core A	Active Hyphal Length Core B	%Active Hyphal Length Core A	%Active Hyphal Length Core B
Fungus	0.4864	0.6594	0.8144	0.7157	0.6029	0.6914	0.2712	0.8634	0.8011	0.9128
Harvest	0.0001	0.0446	0.3105	0.0877	0.2346	0.1069	0.0604	0.5171	0.0065	0.0125
Fungus*Harvest	0.5284	0.8860	0.8976	0.8993	0.6025	0.4504	0.8907	0.7908	0.6201	0.9999
CV(%)	17	52	48	190	55	90	88	136	69	76

Table 4-3. Means and ANOVA summary of a test of the efficiency of the soil-hyphae-extraction technique.

I. Means summary

Settling Time (min)	Hyphal Density (mm g^{-1})	Transformed Hyphal Density (mm g^{-1})$^{.43}$	Standard Error	Coefficient Of Variation
0.0	1.61	0.27	0.92	88.78
0.5	1.74	0.16	1.18	40.35
3.0	2.45	0.25	1.29	57.36
5.0	0.92	0.21	0.72	87.43
10.0	0.66	0.23	0.39	178.03

II. Anova summary

Source of variation	*P* Value
Blending Time (BT)	.7262
Settling Time (ST)	.0640*
BT*ST	.3872
CV(%)	76

*Regression equation for settling time effect:
$Y = ((.54 * X) + (-.18 * X^2) + (.01 * X^3) + (.93)$, $R2 = 0.20$, $P = 0.0252$

Table 4-4. Means summary for Experiment Two where sweet potatos were grown in a glasshouse for 4 wk.

Fungal Trt.	First Leaf P ($mg\ g^{-1}$)	Vine Dry Mass (mg)	Vine P Conc. ($\mu g\ g^{-1}$)	Total Vine P (g)	Root Length (cm)	P Inflow ($\mu g\ d^{-1}\ cm^{-1}$)	Colonized Root Length cm	%Colonized Root Length (%)
				wk 2				
Control	1.36	442	720	333	541	-0.05		
A. rugosa	1.39	477	761	370	304	-0.08	4	0.58
G. etunicatum	1.66	377	689	266	260	-0.09	6	2.22
CV(%)	23	28	18	41	13	100	118	36
				wk 4				
Control	1.38	797	589	465	2,519	0.002		
A. rugosa	2.41	952	1,058***	1,042**	4,246	0.010***	635	13
G. etunicatum	3.03	1,127	1,520***	1,731	4,875	0.022**	1,481	29*
CV(%)	37	31	26	47	7	55	12	23

Asterisks (***, ** and *) indicate the mean is significantly different from the control at $P \leq 0.01$, $P \leq 0.05$ and $P \leq 0.10$, respectively, within each harvest date excluding colonization data where controls were excluded from the analysis and comparisons were made between the two remaining means.

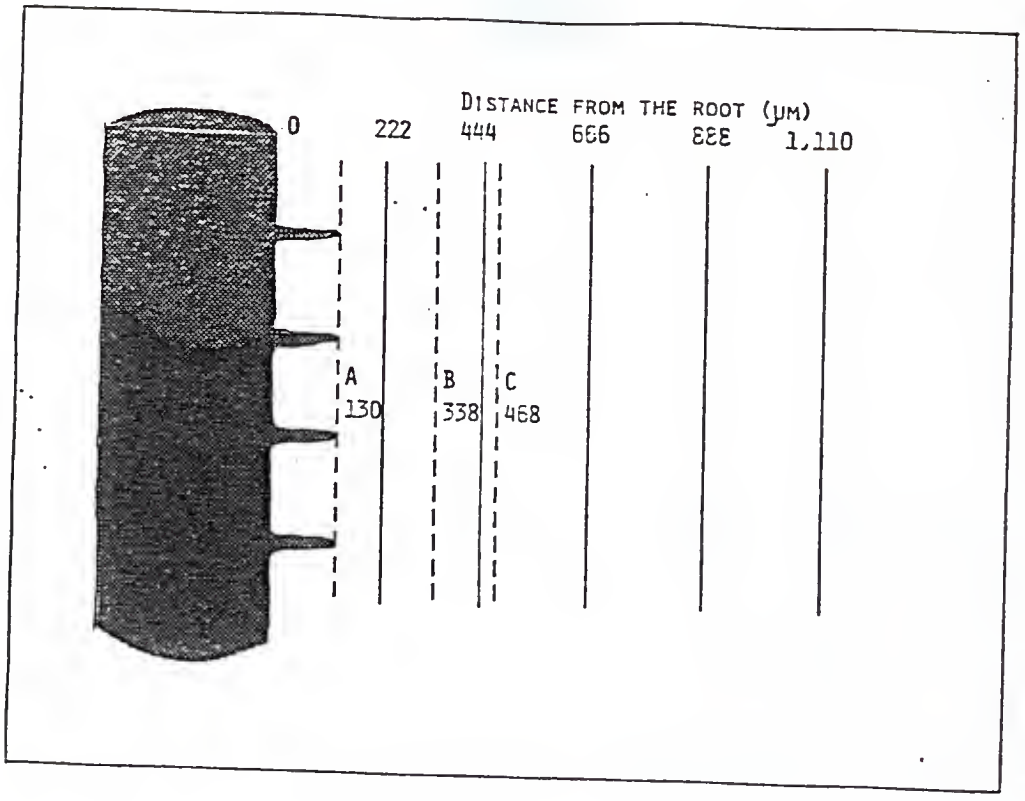
Table 4-5. Hyphal density in soil inoculated with VAM fungi at various distances from a root in cores taken over the plant stem (Core A) and midway between the center and the edge of the pot (Core B).

Fungal Trt.	Hyphal Density (cm cm^{-2})	
	Core A	Core B
	Distance From the Root (μm)	
	0-222	
Control	0.28	0.45
A. rugosa	0.53	0.12
G. etunicatum	0.36	0.46
	222-444	
Control	0.32	0.32
A. rugosa	0.27	0.80
G. etunicatum	0.17	0.34
	444-666	
Control	0.21	0.01
A. rugosa	0.49	0.36*
G. etunicatum	0.62	0.07
	666-888	
Control	0.18	0.11
A. rugosa	1.21	0.05
G. etunicatum	0.24	0.26
	888-1,111	
Control	0.06	0.10
A. rugosa	0.03	0.30
G. etunicatum	0.75*	0.30
CV(%)	65	133

* Within a core and zone, differences between means control and plants inoculated with VAM fungus at $P \leq 0.05$.

Figure 4-1. Spatial relationship between calculated P-depletion zones at 7 days, roots, root hairs, and zones used to determine hyphal distribution. The three broken lines represent the following: extent of the root hair cylinder (A); the extent of the 1-wk P depletion zone excluding the effect of root hairs (B); and the extent of the 1-wk P depletion zone with the effect of root hairs (C).

Figure 4-2. Vine length of sweet potato grown in a glasshouse for 8 wk.. Differences among treatments ($P \leq 0.05$) at a given date, between the inoculated treatments and the control are indicated by *. Symbols represent means of five replicates.


DISTANCE FROM THE ROOT (µM)
0
222
444
666
888
1,110
A
130
B
338
C
468

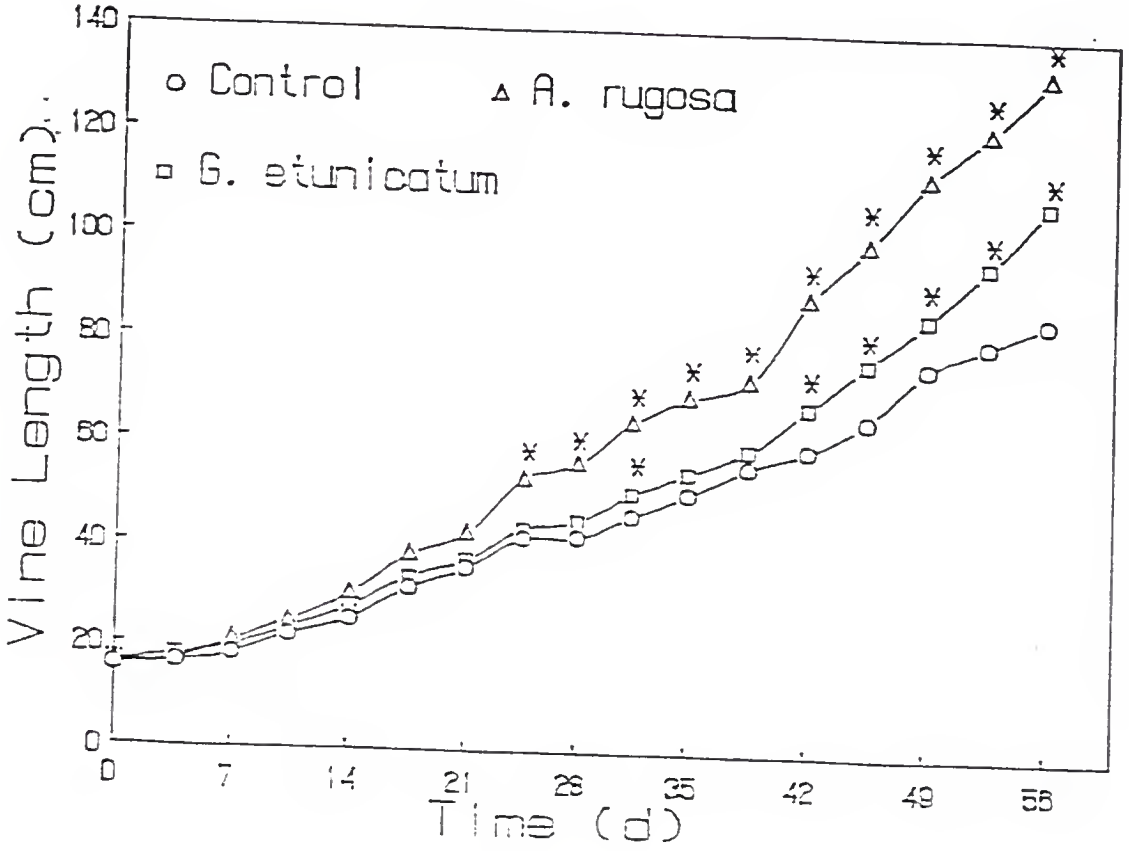

o Control
Δ A. rugosa
□ G. etunicatum
Vine Length (cm)
Time (d)
0
20
40
60
80
100
120
140
0
7
14
21
28
35
42
49
56

Figure 4-3. Harvest effects on sweet potato grown in a glasshouse for 8 wk. Symbols represent means of five replications except for the first harvest where VAM fungus inculated treatments had three replications and the second harvest where *A rugosa* treatments had four replications. Regression equations and R^2 values are:
(a) total root P of sweet potato
Control $Y = (0.26 * X) + (0.26)$, $R^2 = 0.62$
A. rugosa $Y = (0.65 * X) - (0.71)$ $R^2 = 0.89$
G. etunicatum $Y = (0.42 * X) + (-0.04)$ $R^2 = 0.58$
(b) Total vine P of sweet potato.
Control $Y = (0.152 * X) + (1.52)$ $R^2 = 0.20$
A. rugosa $Y = (0.66 * X) + (0.39)$ $R^2 = 0.56$
G. etunicatum $Y = (1.38 * X) + (-2.48)$ $R^2 = 0.69$
(c) Vine P concentration of sweet potato.
Control $Y = (-0.13 * X) + (1.61)$ $R^2 = 0.68$
A. rugosa $Y = (-0.07 * X) + (1.46)$ $R^2 = 0.17$
G. etunicatum $Y = (0.09 * X) + (0.61)$ $R^2 = 0.54$
(d) P concentration in the first fully expanded leaf of sweet potato.
Control $Y = (-0.21 * X) + (2.93)$ $R^2 = 0.45$
A. rugosa $Y = (-0.30 * X) + (3.78)$ $R^2 = 0.48$
G. etunicatum $Y = (-0.15 * X) + (3.13)$ $R^2 = 0.15$
(e) Log root length of sweet potato growing in a glasshouse for 8 wk.
Control $Y = (0.31 * X) + 8.2$ $R^2 = 0.88$
A. rugosa $Y = (0.38 * X) + 8.1$ $R^2 = 0.85$
G. etunicatum $Y = (0.25 * X) + 8.7$ $R^2 = 0.78$
(f) P-inflow of sweet potato roots.
Control $Y = (-0.3 * X) + (0.026 * X^2) + 1.0$ $R^2 = 0.46$
A. rugosa $Y = (0.6 * X) + (-0.05 * X^2) + (-0.8)$ $R^2 = 0.74$
G. etunicatum $Y = (0.47 * X) + (-0.039 * X^2) + (-0.7)$ $R^2 = 0.76$

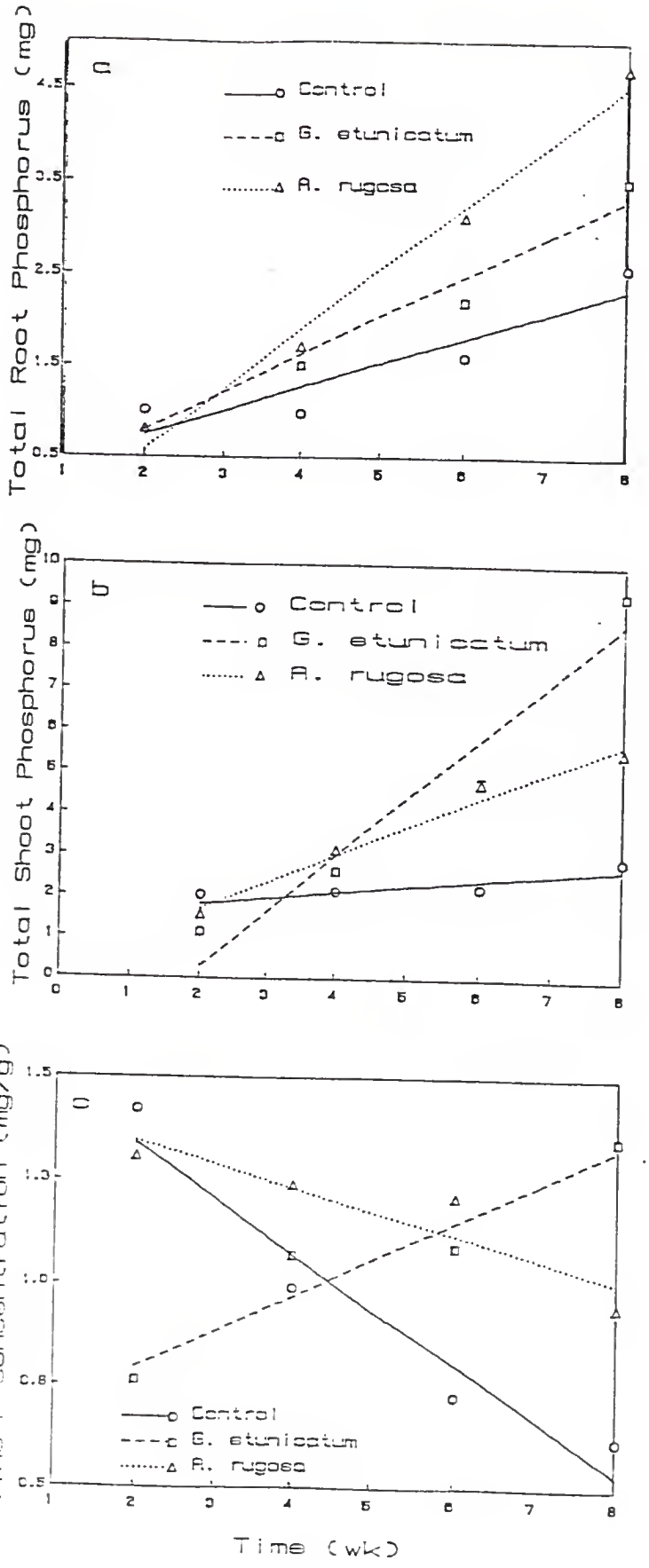

Total Root Phosphorus (mg)
Control
G. etunicatum
A. rugosa
Total Shoot Phosphorus (mg)
Control
G. etunicatum
A. rugosa
Vine P Concentration (mg/g)
Control
G. etunicatum
A. rugosa
Time (wk)

Figure 4-3 (Continued)

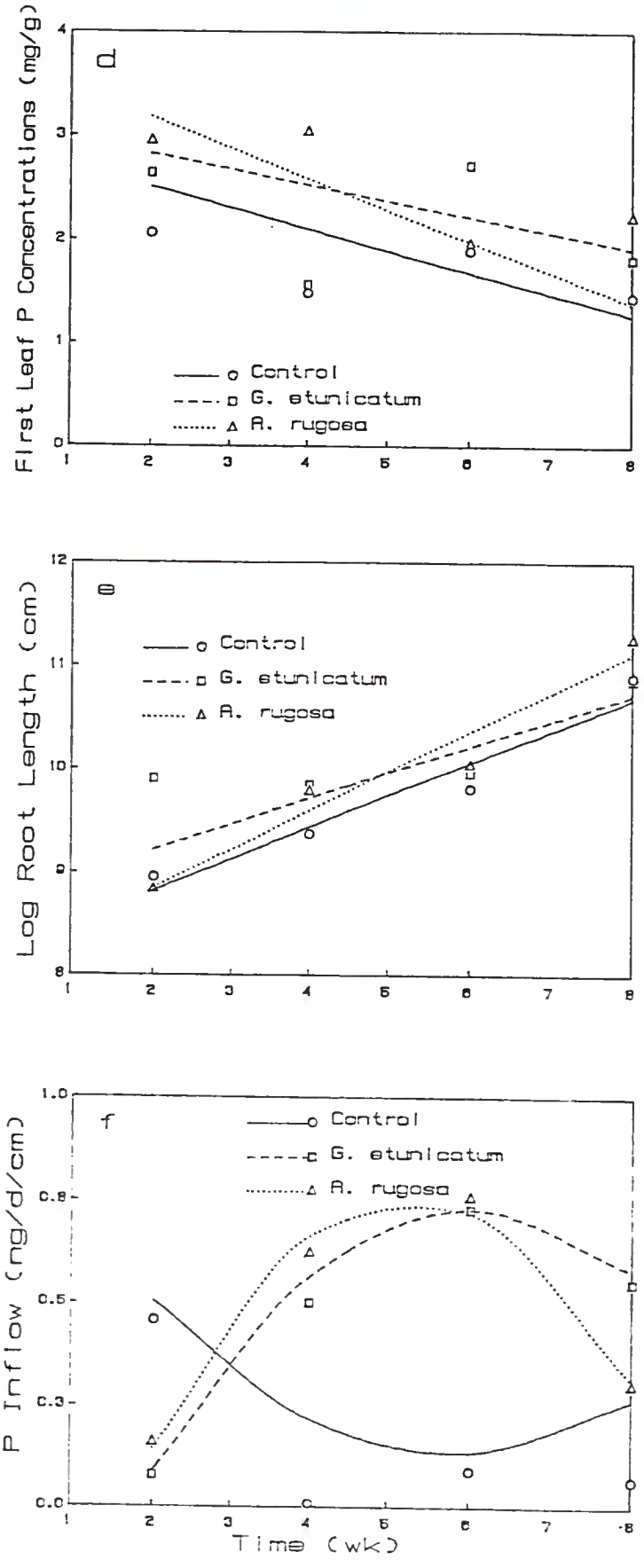
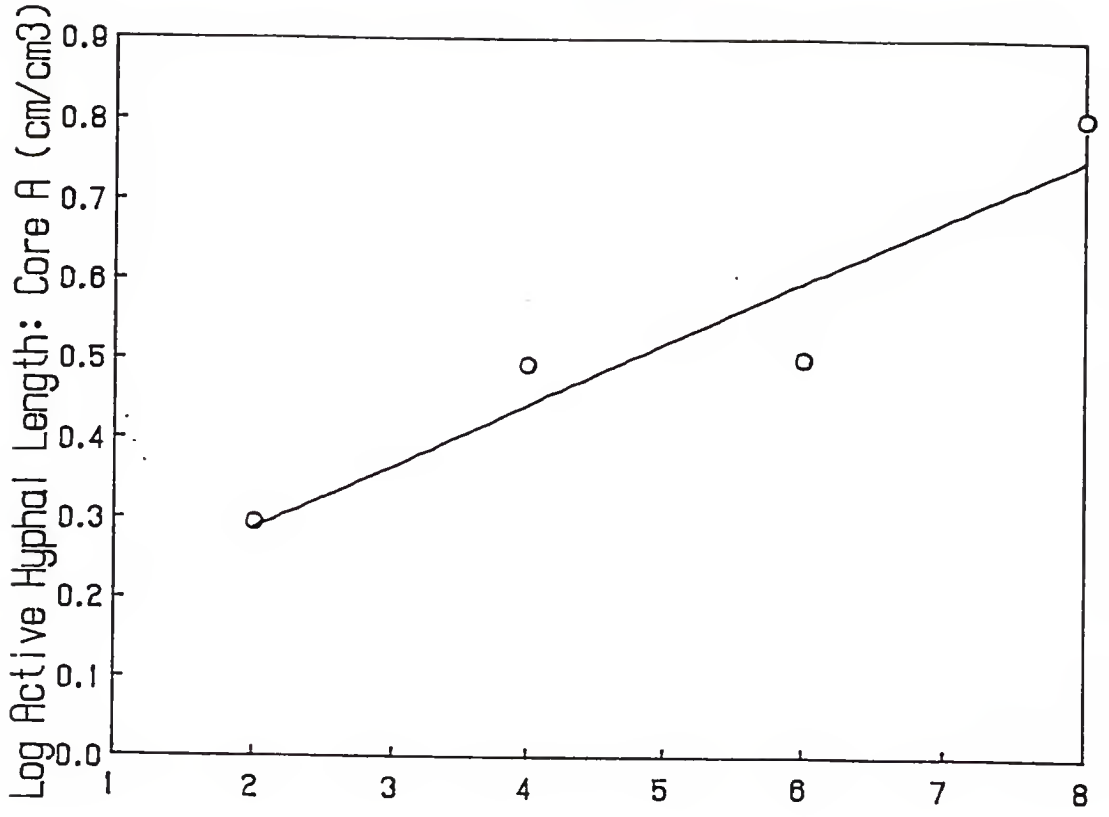
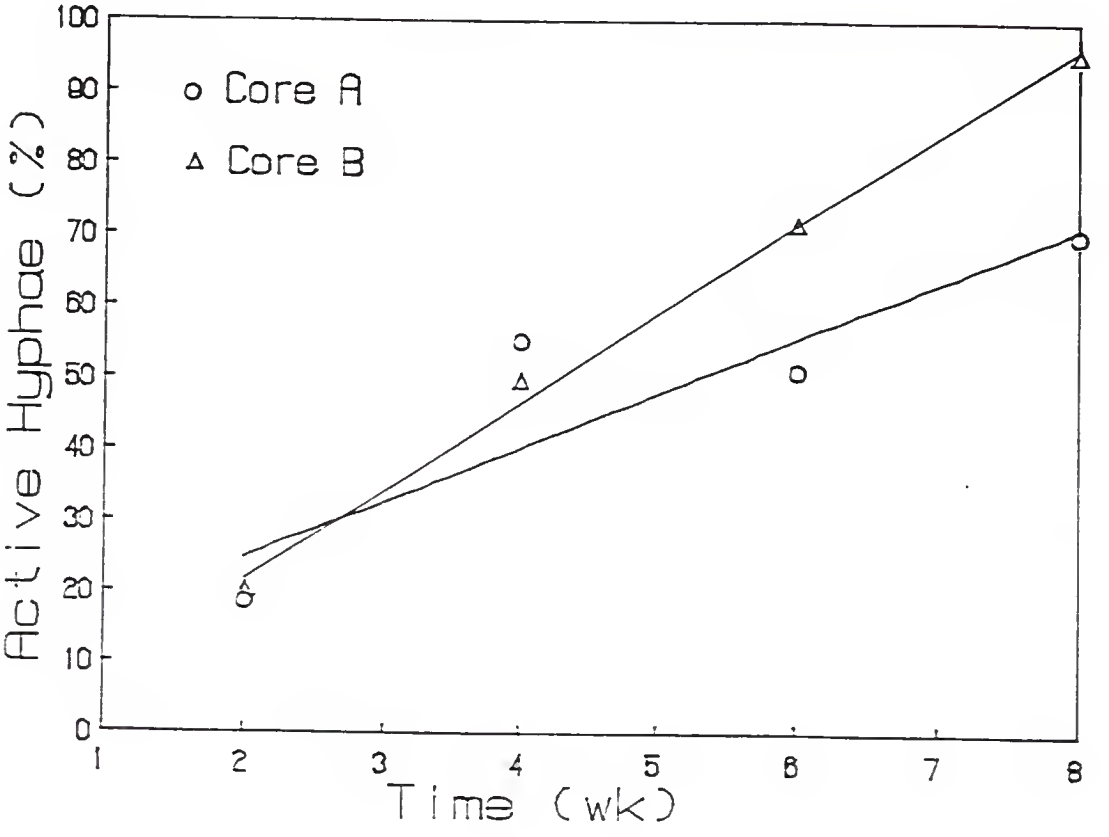


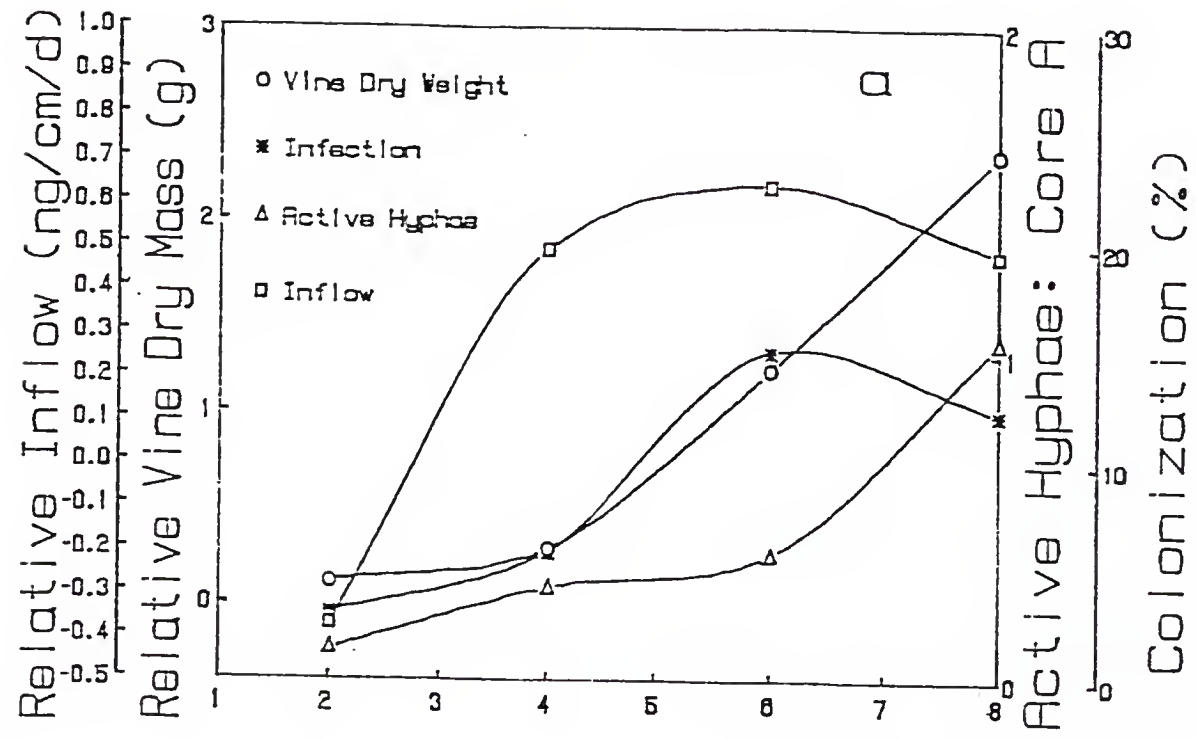
Figure 4-4a. Log of active hyphal length in core A from pots containing sweet potato growing in a glasshouse for 8 wk. Symbols represent means of 15 replications except for the first harvest where there were 11 replications and the second harvest where there were 14 replications. The regression equation and R^2 value are:
$Y = (0.0773 * X) + (0.1337)$ $R^2 = 0.13$

Figure 4-4b. Percent of hyphae that are active in cores A and B from pots containing sweet potato growing in a glasshouse for 8 wk. Symbols represent means of 15 replications except for the first harvest where there were 11 replications and the second harvest where there were 14 replications. Regression equations and R^2 values are:
Core A $Y = (7.67 * X) + (9.55)$ $R^2 = 0.19$
Core B $Y = (12.45 * X) + (-3.3029)$ $R^2 = 0.41$


Log Active Hyphal Length: Core A (cm/cm3)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1
2
3
4
5
6
7
8


Active Hyphae (%)
Core A
Core B
0
10
20
30
40
50
60
70
80
90
100
1
2
3
4
5
6
7
8
Time (wk)

Figures 4-5. Chronology of the VAM plant growth response for sweet potato inoculated with *G. etunicatium* (a) or *A. rugosa* (b) and growing in a glasshouse for 8 wk. Phoshorus inflow and vine dry mass differences are between the control plants and plants inoculated with VAM fungi.


o Vine Dry Weight
* Infection
Δ Active Hyphae
□ Inflow
a
Relative Inflow (ng/cm/d)
Relative Vine Dry Mass (g)
Active Hyphae: Core A
Colonization (%)

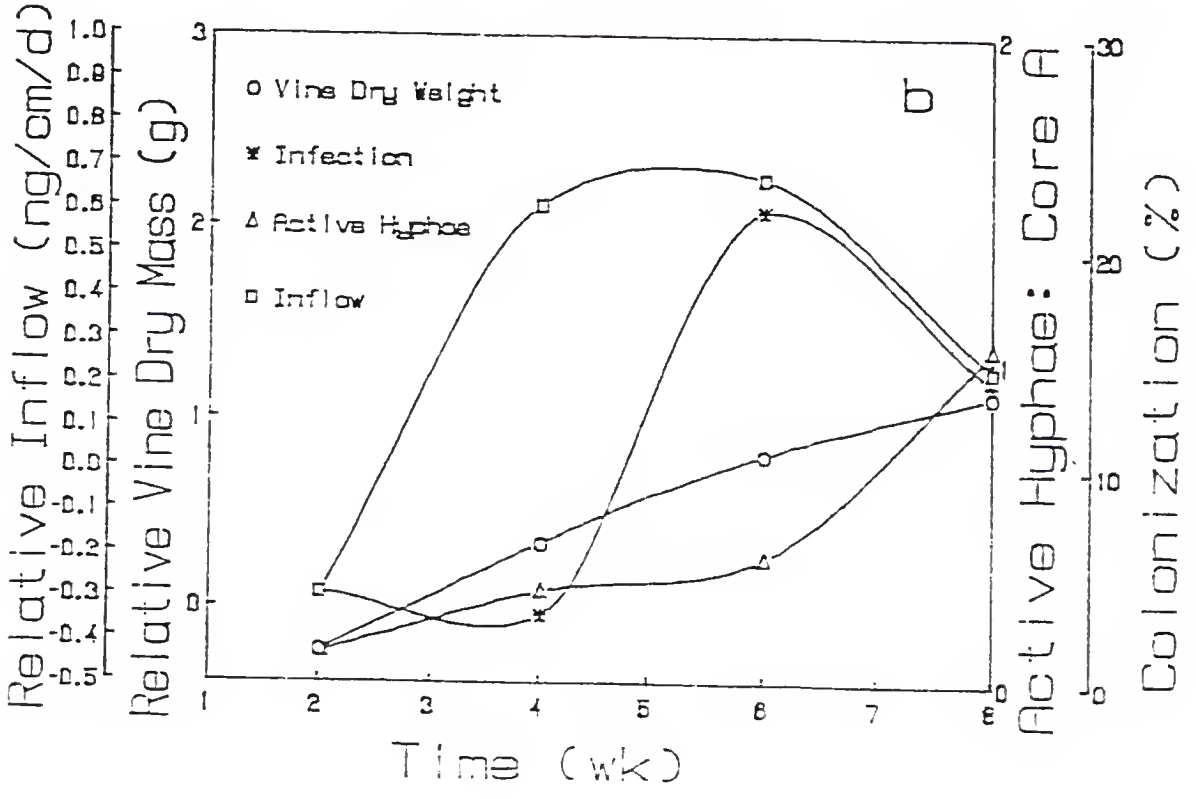
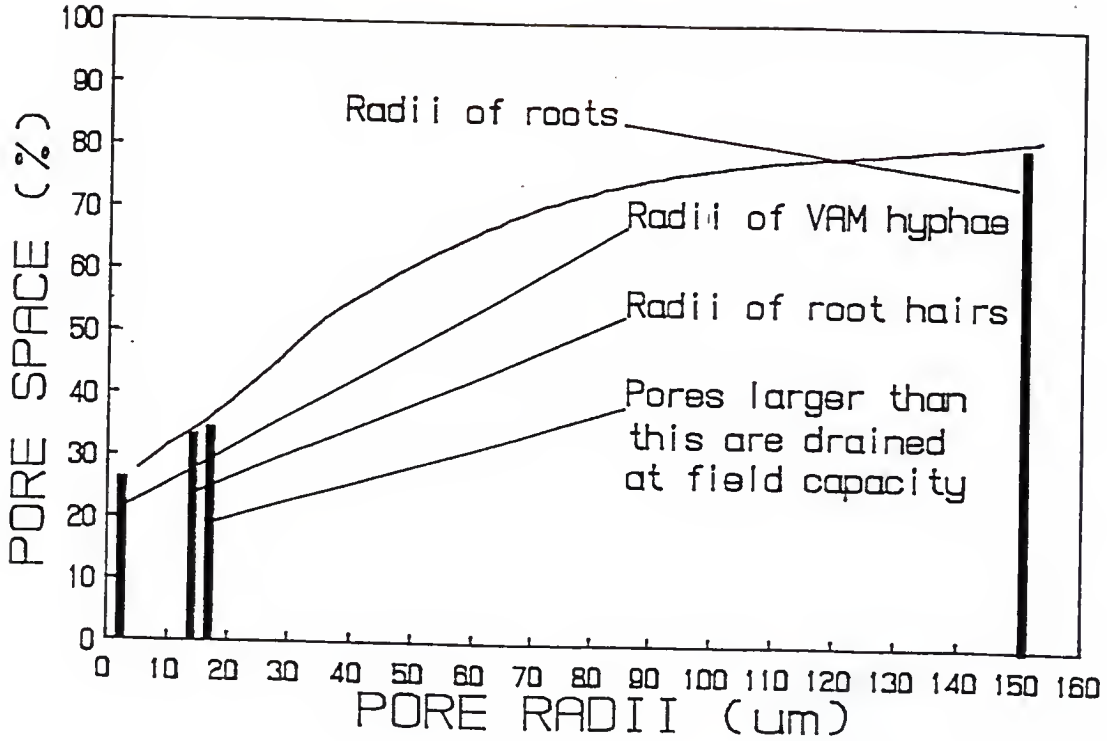

o Vine Dry Weight
* Infection
Δ Active Hyphae
□ Inflow
b
Relative Inflow (ng/cm/d)
Relative Vine Dry Mass (g)
Active Hyphae: Core A
Colonization (%)
Time (wk)

Figure 4-6. The relationship of average root, root hair, and VAM hyphal radii to pore size distribution and the proportion of pores drained when soil water is at field capacity.


PORE SPACE (%)
100
90
80
70
60
50
40
30
20
10
0
Radii of roots
Radii of VAM hyphae
Radii of root hairs
Pores larger than
this are drained
at field capacity
0 10 20 30 40 50 60 70 80 90 100 110 120 130 140 150 160
PORE RADII (um)

CHAPTER FIVE
SEASONAL VARIATION IN GROWTH, NUTRIENT CONTENT, ROOT HAIRS, AND COLONIZATION BY VAM FUNGI OF SWEET POTATO

Introduction

To understand the function of VAM fungi in plant-growth responses it is necessary to document the symbiosis over the life of the plant. Plant nutrient and water demand, allocation of carbohydrates and mineral nutrients, and benefits of colonization by VAM fungi can vary considerably through the growing season (Douds and Chaney, 1986; Fitter, 1986; Dunne and Fitter, 1989). It is generally believed that yield increases attributable to inoculation with VAM fungi at planting are due to colonization by the introduced fungus followed by increased uptake of P or other diffusion-limited nutrients such as Zn (Abbott and Robson, 1984). However, this relationship may be obscured in field experiments. There may be little difference in root colonization among plants inoculated with different VAM

fungi and noninoculated controls due to the presence of indigenous VAM fungi (Mosse, 1977; Medina, et al., 1988). This would be true particularly in nonfumigated soils, late in the growing season, and further away from the point of inoculum placement. Furthermore, the P concentrations of plants among various inoculation treatments may be different only during a portion of the growing season (Fitter, 1986; Dunne and Fitter, 1989). As plant-growth rate increases dilution effects may obscure treatment differences in initial-P concentrations while differences in total-P content may be carry-over effects of early growth. Plant partitioning of P may also change during different plant-growth stages (Dunne and Fitter, 1989).

It is important to know the distribution of the root system over time as there may be interactions of mycorrhizal effects with soil water content and proximity to the point of inocula placement. In addition root hairs, which may be functionally analogous to external hyphae of VAM fungi in terms of water and P uptake, may be suppressed by mycorrhizae (Chapter Three), and are sensitive to soil water content and P level (Mackay and Barber, 1984). If the full extent of the root system, along with root hairs and mycorrhizae, can be determined, root measurements can be

made with more confidence that the sampling is spatially and temporally representative.

Greenhouse screening of VAM fungi for selection of effective isolates for biomass sweet potato has been conducted (Hung et al., submitted). To better understand mechanisms of effectivity, the growth dynamics of sweet potato mycorrhizae over the growing season, and the relationship of the mycorrhizae to the overall growth of the plant, was studied. The specific objectives of this research were to: (i) determine the changes in distribution of roots, root hairs, and mycorrhizae of sweet potato over a growing season and (ii) evaluate the relationships among seasonal variation in root length, colonization by VAM fungi, P and Zn uptake, vine growth, and yield.

Materials and Methods

The experiment was a three by six factorial with a randomized complete block design. There were three VAM fungus inoculation treatments (*G. etunicatum* INVAM LETC216, *A. rugosa* INVAM 981, and a noninoculated control), six harvests (2-, 4-, 8-, 12-, 18-, and 24-wk), and three replications per treatment. Roots were sampled at each harvest at three depths (0 to 30, 30 to 60, and 60 to 90 cm)

and at four distances from the base of the vine (0, 23, 83, and 116 cm).

Cultural Practices

The field soil was a Pomona sand (sandy, siliceous, hyperthermic, Ultic Haplaquod) intensively managed for vegetable production. One month prior to bed preparation the field was fumigated with Telone II (1,3-dichloropropene) at 21 L ha^{-1}. Routine pesticide applications (Appendix B) and irrigation were applied as needed. A preplant assessment of the native VAM fungi was done using the MPN assay. The VAM fungus propagule densities were 0.0, 1.3, and 0.45 propagules g^{-1} at the 0 to 30, 30 to 60 and 60 to 90-cm depths, respectively.

Initial soil characteristics are presented in Table 5-1. A 15-0-14 analysis fertilizer was incorporated at a rate of 224 kg ha^{-1} prior to bed preparation. Additional fertilizer applications were side dressed at 36 kg ha^{-1} at planting, and 8 and 12 wk after planting.

Vine cuttings of sweet potato were planted 1.5 m apart on 1.5 m centers directly into the bed, in the case of noninoculated plants, or over a 5-g pad of inoculum from pot cultures of either *G. etunicatum* or *A. rugosa*. The

propagule densities of the inocula, as determined by the MPN test, were 4.2 and 4.4 propagules g^{-1} soil for *G. etunicatum* and *A. rugosa*, respectively. A slurry consisting of 20 g of pot culture material from both isolates suspended in 2 L H_2O was sieved three times through a 1-mm-mesh screen and five times through a 0.22-µm-mesh screen. This was applied in the planting hole at a rate of 10 mL per plant to all plants in an attempt to standardize bacterial populations among treatments.

Plant Sampling and Tissue Analysis

At each harvest, vine length, vine dry mass, P and Zn concentration of the vine, and storage root yield were determined. Dry mass, P, and Zn concentrations were determined on entire plants at the first three harvests. At the last three harvests, fresh mass was determined in the field and subsamples (approximately 1 kg) were taken randomly for determination of dry mass and nutrient content. These values were then adjusted to a whole plant basis. Tissue samples were dried at 70°C and ground to pass though a 2-mm sieve. A 0.2 g subsample was dry ashed at 500°C for 3 h. The sample was then digested in sequential solutions of HCl (Chapter Two), brought to a 20 mL volume in, and 0.1

N HCl and analyzed using atomic adsorption spectrophotometry and P colorimetricly (Murphy and Riley, 1962) for Zn and P, respectively.

Root Sampling

At the 2- and 4-wk harvest, entire root systems were excavated. At the remainder of the harvests root systems were sampled with a sharp, 7-cm-diam. bucket corer to a depth of 90 cm. At the 8-wk harvest roots were not sampled at the 116-cm distance. Roots were removed from the cores in the field by wet sieving and decanting on 2.0- and 0.199-mm mesh screens. Two cores were taken and pooled from each distance/depth combination, except for the samples taken directly over the base of the main vine where the sample consisted of one core. Total root length, percent of the root with root hairs, and percent VAM colonization were determined by the gridline-intersect method (Giovannetti and Mosse, 1980).

Statistical Analysis

Data was subjected to analysis of variance using the General Linear Models Procedure (SAS, 1985). Parameters where $P \leq 0.10$ were further analyzed using regression

analysis for quantitative parameters or for VAM fungus inoculation effects by single degree of freedom contrasts comparing the two inoculated treatments to the control. Means of root density, percent root length colonized with VAM fungi, and percent of the root with root hairs were correlated positively with the variance so these data were power transformed following the method of Glenn et al. (1987) before statistical analysis.

Results

Vine Growth and Yield

Vine growth and nutrient content were affected by harvest date, but not VAM-fungal inoculation (Table 5-2). Vine dry mass increased rapidly between 4 and 12 wk, and then leveled off (Figure 5-1a). Vine length increased throughout the growing season (Figure 5-1b). Storage root yield was analyzed at each harvest. At the 18-wk harvest, plants inoculated with *G. etunicatum* (2.3 kg / plant, $P \leq 0.05$) and *A. rugosa* (1.7 kg / plant, $P \leq 0.10$) had higher yields than the noninoculated control (0.2 kg / plant). At the 24-wk harvest, only plants inoculated with *A. rugosa* (2.9 kg / plant, $P \leq 0.10$) had higher yields than the

control (1.2 kg /plant) while plants inoculated with *G. etunicatum* had a mean yield of 1.7 kg/plant.

Phosphorus and Zn content of the vines was affected by harvest date, but not VAM-fungus inoculation (Table 5-2 and Figures 5-2c and 5-2d). Vine-P concentration was affected by inoculation and harvest, and there was no interaction (Table 5-2). Phosphorus concentration of vines inoculated with *A. rugosa* at the 2- and 4-wk harvest was significantly higher than the control (Figure 5-2). Vine-Zn concentration decreased linearly with harvest date (Figure 5-1e).

Root Response

Few roots were found in the 60-90 cm depth so this depth was excluded from further analysis. Significant ($P \leq .10$) interactions of depth with the other parameters led to separate analysis of the two remaining depths.

In the 0 to 30 cm samples, root-length density was higher near the base of the vine at all harvests except the last (Figure 5-3a). At the 30- to 60-cm depth the only significant regression of root-length density against distance from the main stem was at the 4-wk harvest (Figure 5-3b). At all other harvests there were no discernible root distribution patterns. The only effects due to inoculation

were at the 18-wk harvest and 30- to 60-cm depth. There *G. etunicatum* (2.49 cm cm^{-3}) and *A. rugosa* (2.35 cm cm^{-3}) had higher ($P \leq 0.05$) root-length densities than the control (1.05 cm cm^{-3}).

Colonization by VAM Fungi

Root colonization by VAM fungi followed a pattern similar to root density at the 0- to 30-cm depth. There was a significant distance by harvest interaction ($P = 0.0333$, see Appendix A) so regression analysis was done on distance at each harvest date. There was a negative, linear relationship between distance and percent colonization, except for the first and last harvest dates where the relationship was cubic (Figure 5-3c). At the 30- to 60-cm depth there was no interaction between harvest and distance and no effect of distance on percent colonization. The effect of harvest date at this depth was quadratic and parallelled vine dry mass (Figure 5-4).

Differences between treatments in colonization of roots was equivocal until the 12-wk harvest when plants inoculated with *A. rugosa* had less ($P \leq 0.0001$) colonization at both depths , (24 and 22% at 0- to 30-cm depth and 30- to 60-cm depth, respectively) than the controls (64 and 55% at the

respective depths). At the 18-wk harvest, colonization was higher ($P \leq 0.01$) in the 0- to 30-cm depth in both *G. etunicatum* (48%) and *A. rugosa* (59%) inculated plants than the control (33%).

Root Hairs

At the 0- to 30-cm depth the percent of the total root length with root hairs decreased with increased distance from the plant at the first two harvests (Figure 5-3d). At the 30-to 60-cm depth distance effects occurred at the 12-, 18-, and 24-wk harvests; the percent of the root with root hairs was highest at the two middle distances (Figure 5-3e). At the 0- to 30-cm depth the percent of the root with root hairs was increased by inoculation with *A. rugosa* ($P \leq 0.10$, 31%) and *G. etunicatum* ($P \leq 0.05$, 32%) over the control (22%) at the 18-wk harvest. This same parameter was increased by inoculation with *A rugosa* ($P \leq 0.05$, 27%) over the control (18%) at the 24-wk harvest. At the 30- to 60-cm depth the percent of the root with root hairs was increased by inoculation with *A. rugosa* ($P \leq 0.10$, 8%) over the control (4%) at the 12-wk harvest.

Discussion

Plant Response

The only measurable difference between inoculated and noninoculated sweet potato vines was their P concentration early in the growing season. Had harvests only been done at crop maturity I would not have been able to relate the increase in yield due to VAM inoculation to an early increase in P concentration. The decrease in vine-P concentration across all inoculation treatments occurred during the rapid growth phase from 8 to 18 wk and was likely caused by a dilution effect. The drop in vine-P concentration late in the growing season when vine mass was decreasing suggests a net movement of P out of the vine, perhaps to the fibrous or storage roots. Dunne and Fitter (1989) found that strawberry rhizomes are "recharged" with P towards the end of the growing season. Sweet potato may have a similar strategy for storing P until the next season through reallocation of P from the vines to the roots. The reduction in yield for plants inoculated with *G. etunicatum* from 18 to 24 wk may have been due to storage root rot in

the field. Since the plants inoculated with *G. etunicatum* had the greatest yield at the 18-wk harvest it is possible that they had passed their physiological prime by the final harvest and were more susceptible to rot.

Root Density

Changes in root densities were most pronounced in the upper 30 cm of the bed and closest to the plant over the growing season. The major difference in root density between the two depths was greater proliferation of roots proximal to the vine base in the 0- to 30-cm depth.

It is surprising that inoculation effects on root-length density appeared only after the 18-wk harvest. Some environmental condition, such as water stress, may have accentuated the effectiveness of the introduced isolates. An intermittent benefit of VAM colonization corresponding to water stress has been reported by Fitter (1986) in grasslands.

Colonization by VAM Fungi

As with root density, VAM-fungal colonization was most dynamic in the roots closest to the base of the vine. There was only one harvest and depth combination where there was a

measurable increase in colonization due to inoculation. This points to the need to sample roots throughout the growing season when evaluating effectivity of isolates in the field. In addition, while effectivity has often been attributed to rapid and early colonization (Abbott et al., 1983; Medina et al., 1988), another component of effectivity may be the isolates long-term relationship with the host. For instance, the ability to maintain a hyphal network under environmental extremes such as temperature or water supply may play a role in symbiotic effectivity. Also, introduced isolates must be able to compete with indigenous VAM fungus populations (Abbott et al., 1983; Menge, 1982)

The low level of colonization of plants inoculated with *A. rugosa* at the 12-wk harvest was unexpected, particularly as colonization in this treatment increased dramatically by 18 wk. A reasonable explanation for this is that the *A. rugosa* isolate used in this experiment may have been particularly sensitive to Bravo (tetrachloroisophthalonitrile) which had been applied between the 8- and 12-wk harvests. While Bravo has been shown to suppress VAM fungi and isolates may vary in sensitivity to pesticides (Trappe et al., 1984), this explanation assumes that a majority of the root system was

colonized by the introduced fungus. This was impossible to confirm with existing methods.

Root Hairs

Changes in the amount of root hairs were most pronounced proximal to the vine base, following the pattern seen with root density and VAM colonization. This was primarily the result of the high root hair density near the base of the plant at the first two harvests.

The increase in root hairs in the plants inoculated with VAM fungi at the 18- and 24-wk harvests was small and therefore of uncertain biological significance. Colonization by VAM fungi suppressed root hair formation in a pot study (Chapter Three). Further studies are required to clarify this relationship.

Chronology

The temporal relationship of four parameters important to understanding the performance of VAM fungi in the field; i.e. root-length density, VAM-fungal colonization, plant-P concentration, and yield are described below. Plant-P concentration peaked at 8 wk and declined thereafter. VAM-fungal colonization and root density peaked at 12- and 8-wk

respectively and declined until the final harvest. The production of storage-root yield lagged behind the period of most rapid root growth by 4 wk and continued to increase after both VAM-fungal colonization and root-length density began to decrease. The importance of delineating the chronology is twofold: Firstly, it points out that the parameters traditionally measured in experiments dealing with mycorrhizae are dynamic and values measured at the time of final harvest may not be indicative of levels during other times in the growing season (Abbott and Robson, 1984; Fitter, 1986). Secondly, it indicates when in the growing season the measurement of a given parameter may be most predictive of crop response. For example, tissue P and VAM-fungal colonization were higher when the plants first began to produce a yield than at the final harvest. In this study it would have been more useful to measure VAM colonization intensively during the 12- to 18-wk period and tissue-P levels during the 0- to 8-wk period to understand why a particular VAM fungus was more effective than the indigenous populations in enhancing yield. Intensity of colonization began to decline at the same time that yield began to increase, suggesting a shift in carbohydrate allocation from the symbiont to the storage root. There is evidence that

seasonal variation in root carbohydrates is related to seasonal variability in colonization by VAM fungi (Douds and Chaney, 1986).

Table 5-1 Soil characteristics at three depths collected prior to fertilization. Values represent nine pooled samples.

Depth	Mehlich I Extractable			pH	
	K	P	Zn	In H_2O	Organic Matter
	------- (mg kg^{-1}) ------				(mg g^{-1})
(0 - 30 cm)	12	24	2.8	5.5	13
(30 - 60 cm)	13	159	1.5	5.3	16
(60 - 90 cm)	12	60	0.2	5.5	5

Table 5-2 Summary of *P* values from ANOVA of plant data from a 24 wk field study with sweet potato.

Source of Variation	Vine		Phosphorus		Zinc	
	Dry Mass	Vine Length	Concentration	Total	Concentration	Total
Inoculation	0.4390	0.4027	0.0125	0.6059	0.6899	0.1485
Harvest	0.0001	0.0001	0.0001	0.0001	0.0001	0.0021
Inoculation X Harvest	0.4463	0.1206	0.1754	0.7385	0.7683	0.1788
CV(%)	79	69	30	101	27	79

Figure 5-1. Vine dry mass (a), vine length (b), vine P content (c), vine Zn content (d), vine Zn concentration (e), of sweet potato over the 24-wk growing season. Symbols represent the mean of nine replicates. Regression equations and R^2 are as follows:

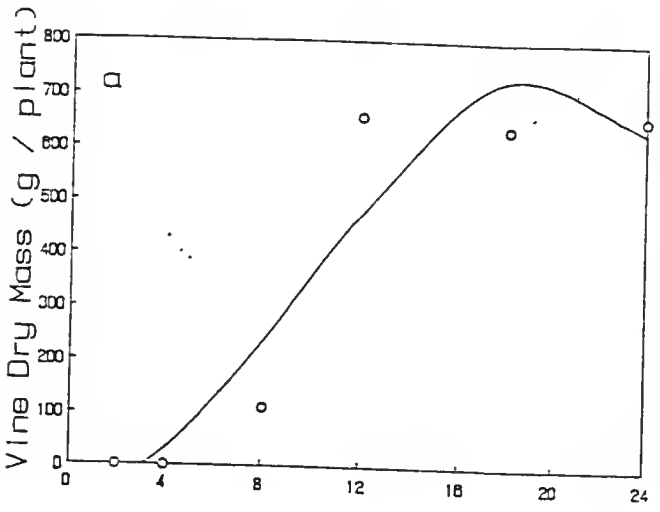
(a) $Y = (83 * X) + (-2 * X^2) + (-241)$, $(R^2 = 0.46)$

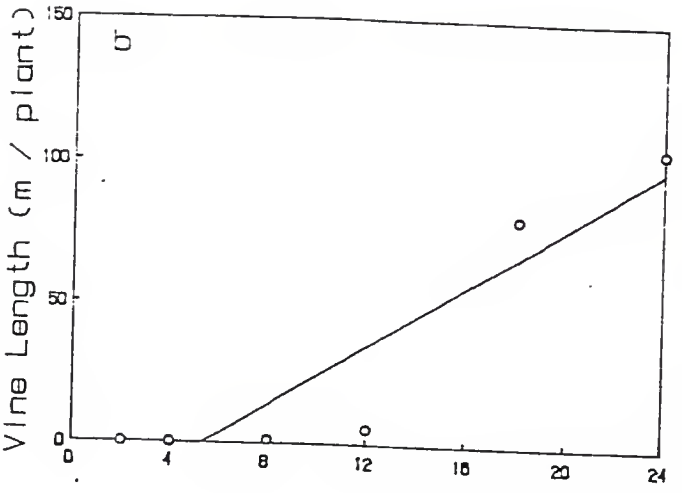
(b) $Y = (-527 * X) + (-2,812)$, $(R^2 = 0.66)$

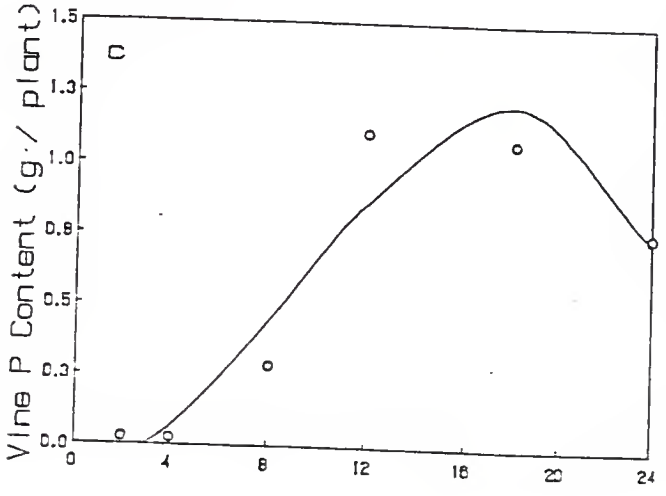
(c) $Y = (0.174 * X) + (-0.005 * X^2) + (-0.486)$, $(R^2 = 0.43)$

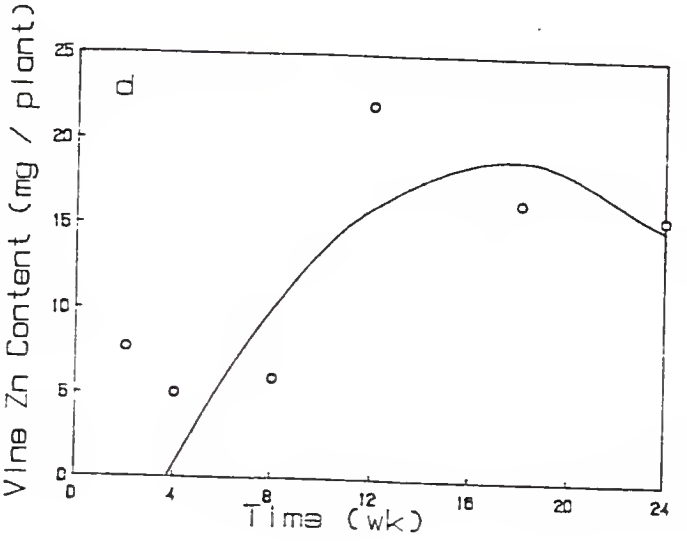
(d) $Y = (3.503 * X) + (-0.099 * X^2) - (11)$, $(R^2 = 0.33)$

(e) $Y = (-1.22 * X) + (51.4)$, $(R^2 = 0.42)$


a
Vine Dry Mass (g / plant)
0
100
200
300
400
500
600
700
800
0
4
8
12
16
20
24


b
Vine Length (m / plant)
0
50
100
150
0
4
8
12
16
20
24


c
Vine P Content (g / plant)
0.0
0.3
0.5
0.8
1.0
1.3
1.5
0
4
8
12
16
20
24


d
Vine Zn Content (mg / plant)
0
5
10
15
20
25
0
4
8
12
16
20
24
Time (wk)

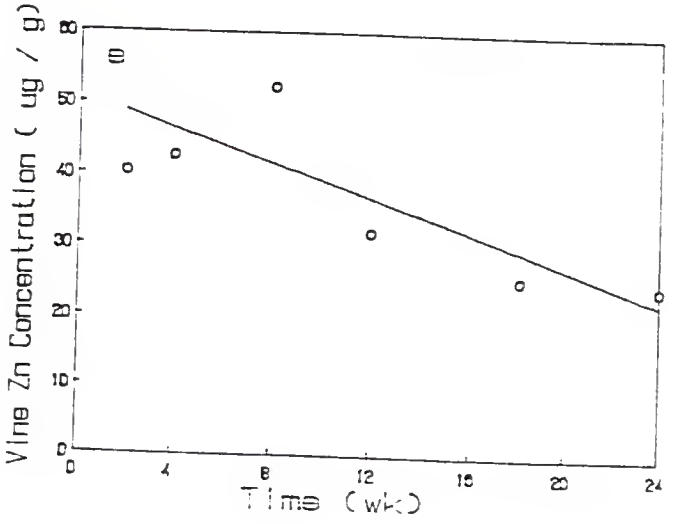

e
Vine Zn Concentration (ug / g)
0
10
20
30
40
50
60
0
4
8
12
16
20
24
Time (wk)

Figure 5-2. Vine-P concentration of sweet potato over the 24-wk growing season. Symbols represent the mean of 3 replicates. Asterisks (*=$P \leq 0.10$, **=$P \leq 0.05$) indicate differences within a harvest date between the control and the inoculated treatments using single degree of freedom contrasts.

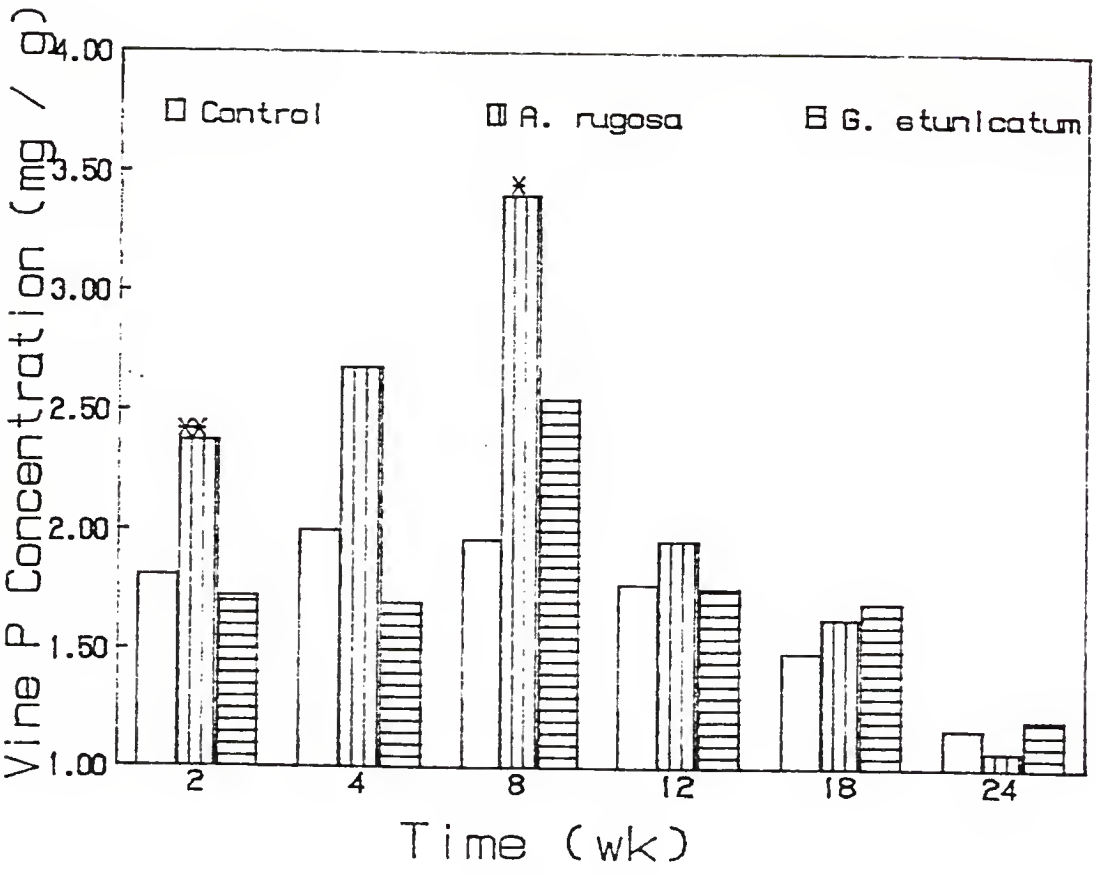

Vine P Concentration (mg / g)
4.00
3.50
3.00
2.50
2.00
1.50
1.00
Control
A. rugosa
G. etunicatum
2
4
8
12
18
24
Time (wk)

Figure 5-3. The effect of distance from the vine base at each harvest date and depth on power transformed root density (a and b), root length colonized by VAM fungi (c) and roots with root hairs (d and e) at the 0- to 30-cm and 30- to 60-cm depth. Lines represent transformed means. Exponents for transformations were 0.225, 0.35, and 0.40 for root density, percent of the root with root hairs, and percent of the root colonized by VAM fungus respectively. If means had a significant distance effect they are described by the regression equations below. Regression equations and R^2 values for data showing a significant ($P \leq 0.10$) distance response at each harvest date are as follows:

(a)

wk	
2	$Y = (-.0109 * X) + (0.0005 * X^2) + (.57)$, $R^2 = 0.70$
4	$Y = (0.0278 * X) + (-0.0007 * X^2) + (3.40E-06 * X^3) + (.5473)$, $R^2 = 0.94$
8	$Y = (-0.0130 * X) + (0.0001 * X^2) + (.7416)$, $R^2 = 0.38$
12	$Y = (-0.0322 * X) + (0.0005 * X^2) + (-2.0E-06 * X^3) + (1.46)$, $R^2 = 0.66$
18	$Y = (-0.0370 * X) + (0.0007 * X^2) + (-3.5E-06 * X^3) + (1.70)$, $R^2 = 0.65$
24	Not Significant

(b)

wk	
2	Not significant
4	$Y = (0.0409 * X) + (-0.0008 * X^2) + (4.23E-06 * X^3) + (0.0506)$, $R^2 = 0.55$
8	Not significant
12	Not significant
18	Not significant
24	Not Significant

(c)

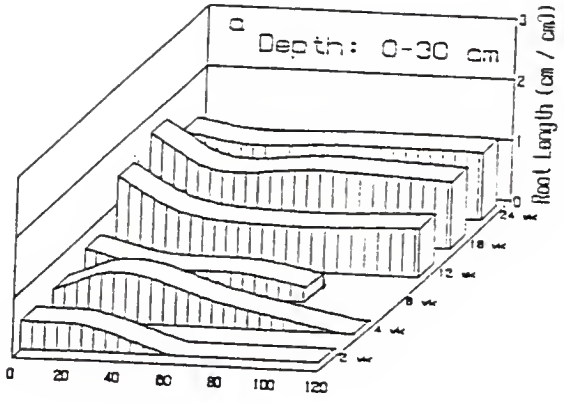
wk	
2	$Y = (0.0458 * X) + (-0.0012 * X^2) + (6.87E-06 * X^3) + (.2115)$, $R^2 = 0.32$
4	$Y = (-0.0153 * X) + (1.69)$, $R^2 = 0.30$
8	$Y = (-0.0400 * X) + (4.08)$, $R^2 = 0.34$
12	$Y = (-0.0123 * X) + (5.07)$, $R^2 = 0.14$
18	$Y = (-0.0082 * X) + (4.89)$, $R^2 = 0.14$
24	$Y = (0.0619 * X) + (-0.0015 * X^2) + (7.80E-06 * X^3) + (3.81)$, $R^2 = 0.38$

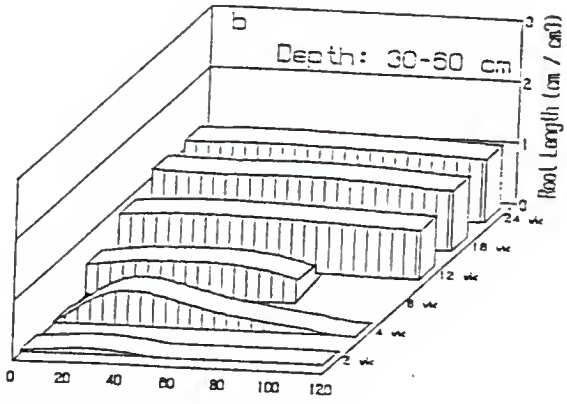
(d)

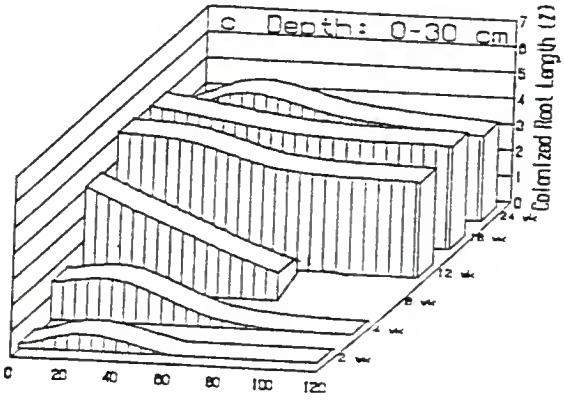
wk	
2	$Y = (-0.09 * X) + (0.001 * X^2) + (3.48)$, $R^2 = 0.92$
4	$Y = (-0.07 * X) + (0.0003 * X^2) + (3.71)$, $R^2 = 0.90$
8	Not significant
12	Not significant
18	Not significant
24	Not significant

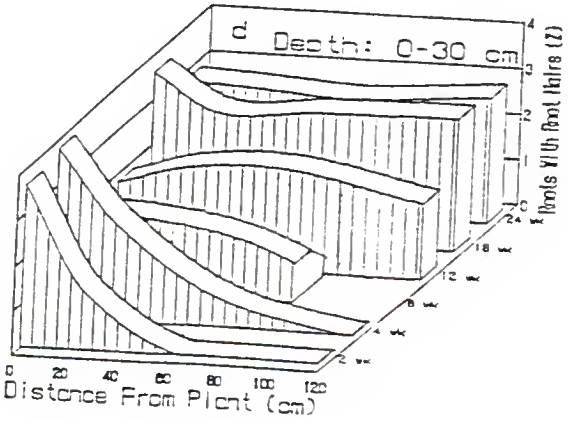
(e)

wk	
2	Not significant
4	Not significant
8	Not significant
12	$Y = (0.029 * X) + (-0.0002 * X^2) + (1.12)$, $R^2 = 0.21$
18	$Y = (0.03 * X) + (-0.0003 * X^2) + (1.35)$, $R^2 = 0.13$
24	$Y = (0.03 * X) + (-0.0003 * X^2) + (1.33)$, $R^2 = 0.20$


a
Depth: 0-30 cm
Root Length (cm / cm3)
0 20 40 60 80 100 120
2 wk
4 wk
8 wk
12 wk
18 wk
24 wk


b
Depth: 30-60 cm
Root Length (cm / cm3)
0 20 40 60 80 100 120
2 wk
4 wk
8 wk
12 wk
18 wk
24 wk


c
Depth: 0-30 cm
Colonized Root Length (I)
0 20 40 60 80 100 120
2 wk
4 wk
8 wk
12 wk
18 wk
24 wk


d
Depth: 0-30 cm
Roots With Root Hairs (I)
0 20 40 60 80 100 120
Distance From Plant (cm)
2 wk
4 wk
8 wk
12 wk
18 wk
24 wk

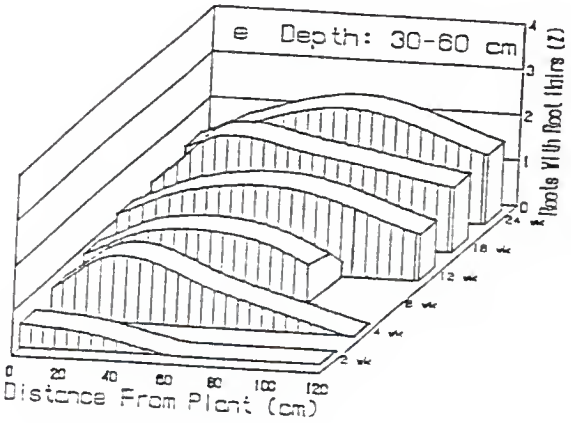
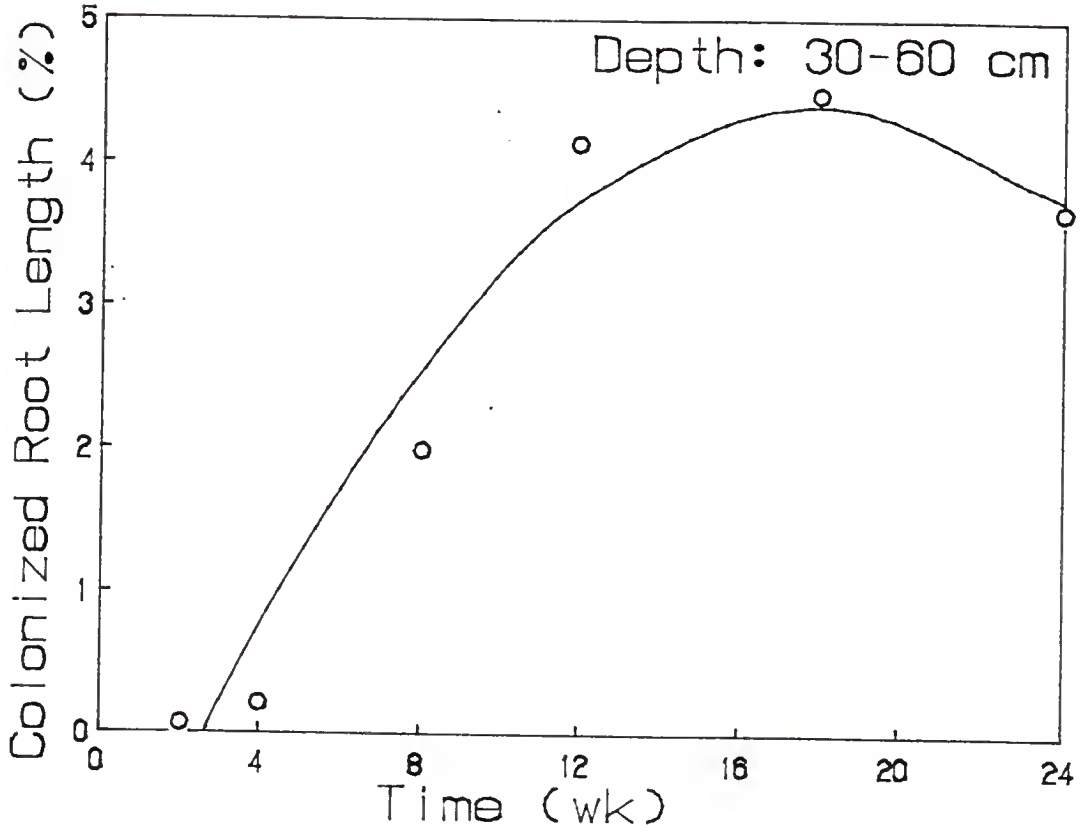

e
Depth: 30-60 cm
Roots With Root Hairs (I)
0 20 40 60 80 100 120
Distance From Plant (cm)
2 wk
4 wk
8 wk
12 wk
18 wk
24 wk

Figure 5-4. The effect of harvest date on root length colonized by VAM fungi 30 to 60 cm depth. Lines represent transformed means. Exponent for the transformation was 0.35. Symbols represent the mean of 9 replicates. The regression equation and R2 value for the curve is as follows:

$Y = (.6671 * X) + (-0.0185 * X^2) + (-1.60)$, $R^2 = 0.71$


Depth: 30-60 cm
Colonized Root Length (%)
Time (wk)
0
1
2
3
4
5
0
4
8
12
16
20
24

CHAPTER 6
SUMMARY AND CONCLUSIONS

The process of P uptake by sweet potatoes is directly influenced by VAM fungi and root hairs. While it is evident that root hairs and VAM fungi enable the roots to overcome limitations in P uptake caused by low diffusion coefficients and soil-solution levels of P, an understanding of the processes involved is far from complete. Interactions among root hairs, VAM fungi, and soil P exist, which make the system very complex. My research was undertaken to address some of the more straight-forward hypotheses concerning the processes and interactions, and to identify areas for further work.

Results of this study support the theory that VAM fungi promote P uptake by plants by increasing P inflow to roots under soil conditions that limit P availability. In addition, the results of this research support the well established ideas that colonization by VAM fungi may be inversely related to soil-P level and that the degree of

suppression varies with VAM-fungal isolate (Schubert and Hayman, 1986). In this study root colonization by *A. rugosa* was more sensitive to P then *G. etunicatum*. The effect of soil-solution-P level on root hairs was less clear. Root hair length and percent of the root with root hairs increased within a range of P levels, yet root hair density was not affected by P level. Colonization by VAM fungi suppressed the percent of roots with root hairs in one pot experiment, and increased this parameter in the field study. In the glasshouse experiment the degree of this suppression did not vary with soil-P level.

The chronology of the VAM plant-growth response in sweet potato does not fit the pattern seen in the data of Gueye et al. (1987). With sweet potato there was an increase in colonization and P inflow by VAM fungi before there was a substantial increase in external hyphae. This suggests that, in addition to the ability of VAM-fungal hyphae to affect quantitative changes in root systems, such as increases in the surface area or radial extent of the absorbing unit, they may qualitatively change the absorbing system. This may occurr by increasing the degree of contact between the absorbing system and the soil solution. In addition, because of their smaller radii, hyphae of VAM

fungi should be able to penetrate pores that hold water at water contents less than field capacity while roots, and to a lesser extent root hairs, are excluded from these pores. I found no evidence that sweet potato roots, whether or not they were colonized by VAM fungi, could alter the organic acid composition of the rhizosphere. While there is at present no conclusive means of distinguishing between VAM and other fungal hyphae in the soil, VAM-fungus colonized roots did maintain a higher proportion of metabolically active hyphae in soil than did noncolonized roots. There was little difference in the hyphal distribution around roots colonized by VAM fungi and controls. However, in two instances, hyphal densities around the roots of plants colonized by VAM fungus were higher than the control at distances beyond calculated P-depletion zones. This was the expected result, but the high variability inherent with *in situ* measurements of soil microorganisms precluded the detection of any discernible patterns.

In a field experiment, tissue-P concentration, root-length density, root colonization by VAM fungi, and yield reached their maximal levels at different times in the growing season. This points to the need to sequentially assess relevant parameters when evaluating the effectivity

of VAM fungi in the field. In addition, VAM-fungus colonization began to decrease as yield increased, suggesting a reallocation of carbohydrates from the symbiont to the storage root.

The dynamics of the amounts of roots, root hairs, and mycorrhizal colonization varied with depth and distance from the plant. Nonetheless, the most dynamic portion of the root system was the part proximal to the main stem. Consequently, though it may be desirable to sample the root system extensively, without the luxury of unlimited time or manpower, it is justifiable to limit sampling to the upper 30 cm, close to the plant. Nonetheless, it may be useful to sample by soil horizon, not depth, as mycorrhizal colonization and percent of the root with root hairs can vary with the horizon sampled (Appendix D). Frequent sampling is required for representative sampling, as root hair development and VAM-fungus colonization levels fluctuate over the growing season. Root monitoring during periods of water stress or after pesticide applications may be particularly instructive when attempting to understand the function of root hairs and VAM fungi in the field.

Before a complete understanding of the function of root hairs and VAM fungi is obtained, it will be necessary to

know more about how these structures interact physically with the soil. To do this will require additional ultrastructural analysis of intact soil and the ability to distinguish between hyphae of VAM and other fungi. In addition, it may be fruitful to investigate if the increased P-diffusion coefficients in small pores accessible only to root hairs and VAM-fungus hyphae are partly responsible for increased P uptake. It is reasonable to expect that the VAM plant-growth response is as much due to the way the hyphae contact the soil surface as to the distance they extend from the root.

APPENDIX A
ANOVA SUMMARY FOR ROOT DATA FROM FIELD STUDY

Summary of the combined ANOVA results and analysis by depth of the root data from a 24 wk field experiment.

Effect[a]	Root Density	% Root With Root Hairs	% Root Colonized By VAM Fungi
		Combined Analysis	
B	.6892	.4064	.3518
F	.3567	.7608	.1483
B*F	.1386	.0014	.0116
Z	.0001	.0001	.0001
D	.0001	.0001	.0001
Z*D	.0001	.0001	.0133
F*Z	.2970	.4267	.9064
F*D	.0623	.0080	.5454
F*Z*D	.7745	.4636	.6752
B*F*Z*D	.9995	.9553	.0116
H	.0001	.0001	.0001
H*F	.0533	.1093	.0001
H*Z	.0001	.0001	.0378
H*D	.0003	.0227	.1399
H*Z*D	.0219	.0072	.7509
H*F*Z	.8075	.3440	.6292
H*F*D	.1804	.0477	.9325
H*F*Z*D	.7225	.0954	.9820
CV(%)	24	52	35
		Analysis by Depth	
----------0- to 30-cm Depth----------			
B	.7013	.3859	.1055
F	.3952	.8854	.1519
B*F	.1657	.1059	.1711
Z	.0001	.0001	.0001
H	.0001	.0001	.0001
H*Z	.0001	.0001	.0333
F*H	.2002	.0486	.0001
F*Z	.9489	.8771	.9459
F*Z*H	.5111	.9186	.8526
CV(%)	20	42	31
----------30- to 60-cm Depth----------			
B	.8702	.4786	.8331
F	.2350	.3469	.2167
B*F	.4468	.0058	.0279
Z	.0001	.0001	.0228
H	.0001	.0001	.0001
H*Z	.0174	.2474	.6052
F*H	.0292	.0782	.0001
F*Z	.7473	.3570	.7758
F*Z*H	.7958	.0060	.7771
CV(%)	26	65	36

[a]Letters represent the following effects: B, block; F, VAM inoculation; D, depth; Z, horizontal distance from the stem; H, harvest.

APPENDIX B
PESTICIDE APPLICATION SUMMARY

Pesticides applied to sweet potato crop in field study from Chapter Five.

Date	Target organism	Brand name	Rate
5/5/87	Nematodes	Telone II	126.23 L ha^{-1}
6/5/87	Fungi	Manzate	2.34 L ha^{-1}
6/5/87	Insects	Diaznon	2.34 L ha^{-1}
6/12/87	Fungi	Kocide 101	2.24 kg ha^{-1}
6/12/87	Insects	Lannate	2.34 L ha^{-1}
6/16/87	Fungi	Thiodane	2.24 kg ha^{-1}
6/16/87	Insects	Pydrin	2.24 kg ha^{-1}
6/20/87	Insects	Thodane	2.24 kg ha^{-1}
6/20/87	Fungi	Bravo	1.17 L ha^{-1}
6/26/87	Insects	Pydrin	0.58 L ha^{-1}
6/26/87	Fungi	Bravo	1.17 L ha^{-1}
6/31/87	Insects	Nailed	0.58 L ha^{-1}
7/6/87	Insects	Ambush	6.70 L ha^{-1}
7/13/87	Weeds	Post	2.34 L ha^{-1}
7/14/87	Weeds	Post	2.24 kg ha^{-1}
7/31/87	Insects	Thiodane	1.12 kg ha^{-1}
7/31/87	Fungi	Bravo	2.34 L ha^{-1}
8/10/87	Weeds	Post	2.34 L ha^{-1}

APPENDIX C
THE EFFECT OF SOIL HORIZON ON ROOT HAIRS AND COLONIZATION BY VAM FUNGI

Means of sweet potato roots from three soil horizons from the field study described in Chapter Four.

Horizon	% Root Length Colonized By VAM Fungi	% Root Length With Root Hairs
Ap	32a	29a
Bh	56ab	18ab
E2	74b	3b

Letters within columns represent differences at $P \leq 0.05$ using Duncan's multiple range test. Values represent the mean of nine replicates.

APPENDIX D
MEANS SUMMARY FROM CHAPTER THREE, EXPERIMENT ONE

Summary of means from a 9-wk glasshouse study described in Chapter Three. Statistical analysis is summarized in Table 3-1 in text.

P Level ($\mu m\ mL^{-1}$)	VDM* (g)	TRL (cm)	%CRL	%RLRH	CRL (cm)	RLRH (cm)	VPC (%)
----------Control----------							
0.01	0.18	2971	0.00	65.40	0	1952	0.09
0.20	0.55	5519	0.00	47.93	0	2657	0.03
0.30	0.83	7038	0.00	58.47	0	4122	0.04
1.00	1.26	9228	0.00	50.87	0	4646	0.04
----------*A. rugosa*----------							
0.01	0.56	3674	13.84	61.14	508	2553	0.05
0.20	1.03	6854	16.73	76.30	1146	5280	0.05
0.30	1.02	6517	13.87	54.16	904	3444	0.05
1.00	1.31	7512	17.61	68.48	1323	5004	0.06
----------*G. etunicatum*----------							
0.01	0.29	2767	20.77	53.91	574	1488	0.05
0.20	0.74	6676	13.41	64.62	895	4244	0.03
0.30	1.06	7332	12.44	41.34	911	3038	0.10
1.00	1.23	8617	2.27	70.15	195	5809	0.07

*Abbreviations for parameters are as follows: VDW=vine dry mass; TRL=total root length; %CRL=percent colonized root length; %RLRH= percent to the root length with root hairs; CRL=colonized root length; RLRH=root length with root hairs; VPC=vine P concentration.

REFERENCES

Abbott, L.K. and A.D. Robson. 1984. The effect of mycorrhizae on plant growth. p.113-130. In: Powell, C.L. and Bagyaraj, D.J. (ed.) VA mycorrhiza. CRC Press, Boca Raton, FL. p. 113-130.

Abbott L.K., and A.D. Robson. 1985. Formation of external hyphae in soil by four species of vesicular-arbuscular mycorrhizal fungi. New Phytol. 99:245-255.

Abbott L.K., A.D. Robson, and G. De Boer. 1984. The effect of phosphorus on the formation of hyphae in soil by the vesicular-arbuscular mycorrhizal fungus, *Glomus fasciculatum*. New Phytol. 97:437-446.

Abbott L.K., A.D. Robson, and I.R. Hall. 1983. Introduction of vesicular-arbuscular mycorrhizal fungi into agricultural soils. Aust. J. Agric. Res. 33:389-408.

Alexander C., I.J. Alexander, and G. Hadley. 1984. Phosphate uptake by *Goodyera repens* in relation to mycorrhizal infection. New Phytol. 97:401-411.

Allen, M.F.. 1982. Influence of vesicular-arbuscular mycorrhizae on water movement through *Bouteloua gracilis* (H.B.K.) Lag Ex Steud. New Phytol. 91:191-196.

Ames R.N., C.P.P. Reid, and E.R. Ingham. 1984. Rhizosphere bacterial populations responses to root colonization by a vesicular-arbuscular mycorrhizal fungus. New Phytol. 96:555-564.

Anderson E.L., P.D. Millner, and H.M. Kunishi. 1987. Maize root length density and mycorrhizal infection as influenced by tillage and soil phosphorus. J. Plant Nutr. 10:1349-1356.

Azcon, R., F. Borie, and J.M. Barea. 1982. Extracellular acid phosphatase activity of lavender and wheat roots as effected by phytase and mycorrhizal inoculation. p. 83-85. In: Gianinazzi, S., Gianinazzi-Pearson, V. and Trouvelot, A. (ed.) Les mycorhizes: biologie et utilization. Les collogues de I'INRA, vol. 13. INRA, Paris.

Azcon, R., Borie, F. and Barea, J.M., and D.S. Hayman. 1976. Utilization of rock phosphate in alkaline soils by plants inoculated with mycorrhizal fungi and phospate solubilizing bacteria. Soil Biol. Biochem. 8:135-138.

Azcon-Aguilar C., V. Gianinazzi-Pearson, J.C. Fardeau, and S. Gianinazzi. 1986. Effect of vesicular-arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria on growth and nutrition of soybean in a neutral-calcareous soil amended with 32P-45Ca-tricalcium phosphate. Plant and Soil 96:3-15.

Baldwin J.P., P.B.H. Tinker, and P.H. Nye. 1972. Uptake of solutes by multiple root systems from soil. II. The theoretical effects of rooting density and pattern of uptake of nutrients from soil. Plant and Soil 36:693-708.

Barber, S.A. 1984. Soil nutrient bioavailability. John Wiley and Sons, Inc., New York.

Baylis, G.T.S. 1972. Minimum levels of available phosphorus for non-mycorrhizal plants. Plant and Soil 36:233-234.

Baylis, G.T.S. 1975. The magnolioid mycorrhiza and mycotrophy in root systems derived from it. p. 373-389. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (ed.) Endomycorrhizas. Academic Press, New York, NY.

Beever R.E., and D.J.W. Burns. 1980. Phosphorus uptake, storage and utilization by fungi. Adv. Bot. Res. 8:127-219.

Bhat K.K.S., and P.H. Nye. 1973. Diffusion of phosphate to plant roots in soil. I. Quantitative autoradiography of the depletion zone. Plant and Soil 38:161-175.

Blake, G.R., and K.H. Hartge. 1986. Bulk density. p..363-375. In: A. Klute (ed.) Methods of soil analysis: Part 1, Physical and mineralogical methods. Second Edition. American Society of Agronomy, Inc. Madison, Wisconsin.

Bolan N.S., A.D. Robson, N.J. Barrow, and L.A.G. Aylmore. 1984. Specific activity of P in mycorrhizal and nonmycorrhizal plants in relation to the availability of P to plants. Soil Biol. Biochem. 16:299-304.

Bolan N.S., A.D. Robson, and N.J. Barrow. 1987. Effects of vesicular-arbuscular mycorrhiza on the availability of iron phosphates to plants. Plant and Soil 99:401-410.

Bowen, G.D. 1973. Mineral nutrition of ectomycorrhizae. p. 151-205. In: Marks, G.C. and Kozlowski, T.T. (ed.) Ectomycorrhiza: Their ecology and physiology. Academic Press, New York and London.

Bowen G.D., and A.D. Rovira. 1976. Microbial colonization of roots. Annu. Rev. Phytopathol. 14:121.

Bowen, G.D. and C. Theodoru. 1967. Studies on phosphate uptake by mycorrhizas. Proc. Int. Union Forest Res. Organ. 14th. p. 5-116.

Chilvers M.T., and M.F.J. Daft. 1981. Mycorrhizae of the Liliflore. II. Mycorrhizae formation and incidence of root hairs in field grown *Narcissus L., Tulipa L.* and *Crocus L. cultivars.* New Phytol. 89:247-262.

Cooper K.M., and P.B. Tinker. 1978. Translocation and transfer of nutrients in vesicular-arbuscular mycorrhizae. II. Uptake and translocation of phosphorus, zinc. and sulfur. New Phytol. 81:43-52.

Cress W.A., G.V. Johnson, and L.L. Barton. 1986. The role of endomycorrhizal fungi in iron uptake by Hilaria jamesii. J.Plant Nutr. 9:547-556.

Cress W.A., G.O. Throneberry, and D.L. Lindsey. 1979. Kinetics of phosphorus adsorption by mycorrhizal and nonmycorrhizal tomato roots. Plant Physiol. 64:484-487.

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

Diem, H.G., Gueye, M. and Y.R. Dommergues. 1986. The development of extraradical hyphae in relation to the response of cowpea to VA mycorrhizal infection. p. 227-232. In: Gianinazzi-Pearson, V. and Gianinazzi (ed.). S. Physiological and Genetical Aspects of Mycorrhizae. Proceedings of the 1st European Symposium on Mycorrhizae. INRA, Paris.

Dodd J.C., C.C. Burton, R.C. Burns, and P. Jeffries. 1987. Phosphatase activity associated with roots and rhizosphere of plants infected with vesicular-arbuscular mycorrhizal fungi. New Phytol. 107:163-172.

Douds D.D., and W.R. Chaney. 1986. The effect of high nutrient addition upon seasonal patterns of mycorrhizal development, host growth, and root phosphorus and carbohydrate content in *Fraxinus pennsylvanica* Marsh. New Phytol. 103:91-106.

Draper, N.R., and H. Smith. 1981. An introduction to nonlinear estimation. p. 458-517. In: Applied Regression Analysis. John Wiley and Sons. New York.

Drew M.C., and P.H. Nye. 1970. The supply of nutrient ions by diffusion to plant roots in soil III. Uptake of phosphate by roots of onion, leek, and rye-grass. Plant and Soil 33:545-563.

Dunne M.J., and A.H. Fitter. 1989. The phosphorus budget of a field-grown strawberry (*Fragaria x ananassa* cv. Hapil) crop: evidence for a mycorrhizal contribution. Ann. Appl. Biol. 114:185-193.

Fitter A.H. 1986. Effect of benomyl on leaf phosphorus concentration in alpine grasslands: a test of mycorrhizal benefit. New Phytol. 103:767-776.

Foster, R.C., A.D. Rovira, and T.W. Cook. 1983. Ultrastructure of the root-soil interface. The American Phytopathological Society, St. Paul Minnesota.

Fox, R.L., and E.J. Kamprath. 1970. Phosphate sorption isotherms for evaluating the phosphate requirements of soils. Soil Sci. Soc. Am. Proc. 34:902-907.

Gianinazzi-Pearson, V. and S. Gianinazzi. 1986. The physiology of improved phosphorus nutrition in mycorrhizal plants. p. 101-109. In: Gianinazzi-Pearson, V. and Gianinazzi S. (ed.) Physiological and genetical aspects of mycorrhizae. First European symposium on mycorrhizae. INRA, Paris.

Giovannetti, M., and B. Mosse. 1980. An evaluation of techniques for measuring VA mycorrhizal infections in roots. New Phytol. 84:489-500.

Glenn, D.M., M.W. Brown, and F. Takeda. 1987. Statistical analysis of root count data from minirhizotrons. p. 81-87. In: H.M. Taylor (ed.) Minirhizotron Observation Tubes: Methods and Applications for Measuring and Applications for Measuring Rhizopshere Dynamics. ASA Spec. Publ. 50. American Society of Agronomy Inc., Madison, Wisconsin.

Gomez, K.A. and A.A. Gomez, 1976. Statistical procedures for agricultural research with emphasis on rice. p. 193-199. The International Rice Research Institute., Los Banos, Laguna, Philippines.

Graham, J.H. (1987) Non-nutritional benefits of VAM fungi--do they exist?. p. 237-239. In: Sylvia, D.M., Hung, L.L. and Graham, J.H. (ed.) Mycorrhizae in the Next Decade: Practical Applications and Research Priorities. Proceedings of the 7th North American Conference on Mycorrhizae, Institute of Food and Agricultural Sciences, University of Florida, Gainesville, Florida.

Gueye M., H.G. Diem, and Y.R. Dommergues. 1987. Variation in N2 fixation, N and P content of mycorrhizal Vigna unguiculata in relation to the progressive development of extraradical hyphae of Glomus mosseae. MIRCEN J. 3:75-86.

Harley, J.L. 1969. The biology of mycorrhizae. Leonard Hill, London.

Hattingh M.J., L.E. Gray, and J.W. Gerdemann. 1973. Uptake and translocation of 32P-labled phophate to onion roots by endomycorrhizal fungi. Soil Sci. 16:383-387.

Hayman D.D., and B. Mosse. 1972. Plant growth responses to vesicular-arbuscular mycorrhiza.III. Increased uptake of labile P from soil. New Phytol. 71:41-47.

Hoagland D.R., and D.I. Arnon. 1950. The water-culture method for growing plants without soil. Cal.Agric.Exp.Stat.,Berkeley, California.

Hung, L.L., D.M. Sylvia, and D.M. O'Keefe. (Submitted). Isolate selection and phosphorus interaction of vesicular-arbuscular mycorrhizal fungi of biomass crops. Soil Sci. Soc. Am. J..

Itoh S., and S.A. Barber. 1983. A numerical solution of whole plant nutrient uptake for woil-root systems with root hairs. Plant and Soil 70:403-413.

Jungk A. 1987. Soil-root interaction in the rhizosphere affecting plant available phosphorus. J. Plant Nutr. 10:1197.

Karunaratne R.S., J.H. Baker, and A.V. Barker. 1986. Phosphorus uptake by mycorrhizal and nonmycorrhizal roots of soybean. J. Plant Nutr. 9:1303-1313.

Kucey R.M.N., and H.H. Janzen. 1987. Effects of VAM and reduced nutrient availability on growth and phophorus and micronutrient uptake of wheat and field beans under greenhouse ocnditions. Plant and Soil 104:71-78.

Lambert D.H., D.E. Baker, and H. Cole. 1979. The role of mycorrhizae in the interactions of phosphorus with zinc, copper and other elements. Soil Sci. Soc. Am. J. 43:976-980.

Larsen, S. 1967. Soil phosphorus. Adv.Agron. 19:151-210.

Lee, D.P., and A.D. Lord. 1987. A high performance phase for the organic acids. L.C.-G.C. 5:261-265.

Lopez-Aguillon R., and B. Mosse. 1987. Experiments on competitiveness of three endomycorrhizal fungi. Plant and Soil 97:155-170.

MacDonald R.M., and M. Lewis. 1978. The occurrence of some acid phosphatases and dehydrogenases in vesicular-arbuscular fungus Glomus mosseae. New Phytol. 80:135-142.

Mackay A.D., and S.A. Barber. 1984. Comparison of root and root hair growth in solution and soil culture. J. Plant Nutr. 7:1745-1757.

Manjunath A., and M. Habte. 1988. Development of vesicular-arbuscular mycorrhizal infection and the uptake of immobile nutrients in *Leucaena leucocephala*. Plant and Soil 106:97-103.

Marschner H., V. Romheld, and I. Cukmak. 1987. Root-induced changes of nutrient availabililty in the rhizosphere. J. Plant Nutr. 10:1175-1184.

Marshall, T.J. 1979. Soil Physics. p. 194-195. Cambridge University Press, Cambridge.

Medina O.A., D.M. Sylvia, and A.E. Kretschmer. 1988. Response of Siratro to vesicular-arbuscular mycorrhizal fungi: I. Selection of effective vesicular-arbuscular fungi in amended soil. Soil Sci. Soc. Am. J. 52:416-419.

Menge, J.A., W.M. Jarrell, C.K. Labanauskas, J.C. Ojala, C. Huszar, E.L.V. Johnson, and D. Sibert. 1982. Predicting myorrhizal dependency of Troyer citrange on *Glomus fasciculatum* in California citrus soils and nursery mixes. Soil Sci. Soc. Am. J. 46:762-768.

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

Meyer J.R., and R.G. Linderman. 1986b. Response of subterranean clover to dual inoculation with vesicular-arbuscular mycorrhizal fungi and a plant growth-promoting bacterium, *Pseudomonus putida*. Soil Biol. Biochem. 18:185-190.

Miller, D.D., M. Bodmer, and H. Schuepp. 1989. Spread of endomycorrhizal colonization and effects on growth of apple seedlings. New Phytol. 111:51-59.

Mosse B. 1973. Advances in the study of vesicular-arbuscular mycorrhizae. Annu. Rev. Phytopathol. 11:171-196.

Mosse, B. 1977. Plant growth responses to vesicular-arbuscular mycorrhiza X. Responses of *Stylosanthes* and *Maize* to inoculation in unsterile soils. New Phytol. 78:277-288.

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

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

Nye P.H. 1973. The relation between the radius of a root and its nutrient-absorbing power (α). J. Exp. Bot. 24:783-786.

Nye P.H., and G.J.D. Kirk. 1987. The mechanism of rock phosphate solubilization in the rhizosphere. Plant and Soil 100:127-134.

Owusu-Bennoah E., and A. Wild. 1979. Autoradiography of the depletion zone of phosphate around onion roots in the presence of vesicular-arbuscular mycorrhiza. New Phytol. 82:133-140.

Owusu-Bennoah E., and A. Wild. 1980. Effects of vesicular-arbuscular mycorrhiza on the size of the labile pool of soil phosphate. Plant and Soil 54:233-242.

Pacovsky R.S. 1986. Micronutrient uptake and distribution in mycorrhizal of phosphorus-fertilized soybeans. Plant and Soil 95:379.

Pang P.C., and E.A. Paul. 1982. Effects of vesicular-arbuscular mycorrhiza on 14C and 15N distribution in nodulated Faba beans. Can. J. Soil Sci. 60:241.

Parsons, R. 1959. Handbook of electrochemical constants. Academic Press, New York.

Pearson, V., and P.B. Tinker. 1975. Measurement of phosphorus fluxes on the external hyphae of endomycorrhizas. p. 277-289. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (ed.) Endomycorrhizas. Academic Press, London.

Powell C.L. 1975. Plant growth responses to vesicular-arbuscular mycorrhiza. VII. Uptake of P by onion and clover infected with different Endogone spore types in 32P labelled soils. New Phytol. 75:563-566.

Raj J., D.J. Bagyaraj, and A. Manjunath. 1981. Influence of soil inoculation with vesicular-arbuscular mycorrhizae *Glomus fasciculatum* and a phosphate-dissolving bacterium *Bacillus circulans* on plant growth and 32P-uptake. Soil Biol. Biochem. 13:105-108.

Raju P.S., R.B. Clarck, J.R. Ellis, and J.W. Maranville. 1987. Vesicular-arbuscular mycorrhizal infection effects on sorghum growth, phosphorus efficiency, and mineral element uptake. J. Plant Nutr. 10:1331-1339.

Rhodes L.H., and J.W. Gerdemann. 1975. Phosphate uptake zones of mycorrhizal and non-mycorrhizal onions. New Phytol. 75:555-561.

Rovira A.D. 1965. Interactions between plant roots and soil microorganisms. Annu. Rev. Microbiol. 19:241-266.

Rozycki H., and E. Strzelczyk. 1986. Organic acids production by Strepomyces spp. isolated from soil, rhizosphere and mycorrhizosphere of pine (*Pinus sylvestris* L.). Plant and Soil 96:334-345.

Same B.I., A.D. Robson, and L.K. Abbott. 1983. Phosphorus, soluble carbohydrates and endomycorrhizal infection. Soil Biol. Biochem. 15:593-597.

Sanders F.E., and P.B. Tinker. 1971. Mechanism of absorption of phosphate from soil by endogone mycorrhizas. Nature 233:278-279.

Sanders F.E., and P.B. Tinker. 1973. Phosphate flow into mycorrhizal roots. Pestic. Sci. 4:385-395.

Sanders F.E., P.B. Tinker, R.L.B. Black, and S.M. Palmerley. 1977. The development of endomycorrhizal root systems: I. Spread of infection and growth-promoting effects with four species of vesicular-arbuscular endophyte. New Phytol. 78:257-268.

SAS Institute Inc. 1985. SAS User's Guide: Statistics. Version 5. SAS Institute, Cary, N.C. USA.

Schenk, M.K., and S.A. Barber. 1979. Phosphate uptake by corn as affected by soil characteristics and root morphology. Soil Sci. Soc. Am. J. 43:880-883.

Schubert A., and D.S. Hayman. 1986. Plant growth responses to vesicular-arbuscular mycorrhizae XVI. Effectiveness of different endophytes at different levels of soil phosphate. New Phytol. 103:79-90.

Schubert A., C. Marzachi, M. Mazzitelli, M.C. Cravero, and P. Bonfante-Fasolo. 1987. Development of total and viable extraradical mycelium in the vesicular-arbuscular mycorrhizal fungus *Glomus clarum* Nicol. & Schenck. New Phytol. 107:183-190.

Silberbush M., and S.A. Barber. 1983. Sensitivity of simulated phophorus uptake to parameters used by a mechanistic-mathematical model. Plant and Soil 74:93-100.

Singh J.P., R.E. Karamanos, and J.W.B. Stewart. 1986. Phosphorus-induced zinc deficiency in wheat on residual phosphorus plots. Agron. J. 78:668-675.

Snellgrove R.C., W.E. Splittstoesser, D.P. Stribley, and P.B. Tinker. 1982. The distribution of carbon and the demand of the fungal symbiont in leek plants with vesicular-arbuscular mycorrhizas. New Phytol. 92:75-87.

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

St. John T.V., D.C. Coleman, and C.P.P. Reid. 1983. Growth and spatial distribution of nutrient-absorbing organs: selective exploitation of soil heterogeneity. Plant and Soil 71:487-493.

Swaminathan K., and B.C. Verna. 1979. Responses of three crop species to vesicular-arbuscular mycorrhizal infection on zinc-deficient Indian soils. New Phytol. 82:481-488.

Swanson, C.L.W., and J.B. Peterson. 1941. The use of the macrometric and other methods for the evaluation of soil structure. Soil Science 53:173-185.

Sylvia D.M. In Press. Distribution, structure, and function of external hyphae of vesicular-arbuscular mycorrhizal fungi. In: Box, J.E. and L.C. Hannond (ed.) Rhizosphere Dynamics. Westview Press. Boulder, CO.

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

Sylvia D.M. 1988. Activity of external hyphae of vesicular-arbuscular mycorrhizal fungi. Soil Biol. Biochem. 20:39-43.

Tien, T.M., M.H. Gaskins, D.H. Hubbell. 1979. Plant growth substances produced by *Azospirillum brasilense* and thier effect on the growth of pearl millet (*Pennisetum americanum L.*). Appl. Environ. Microbiol. 37:1016-1024.

Timmer L.W., and R.F. Leyden. 1980. The relationship of mycorrhizal infection to phosphorus - induced upper deficiency in sour-orange seedlings. New Phytol. 85:15-24.

Tinker P.B. 1984. The role of microorganisms in mediating the uptake of plant nutrients from soil. Plant and Soil 76:77-92.

Tinker, P.B. 1975a. Soil chemistry of phophorus and mycorrhizal effects on plant growth. p. 353-371. In: Sanders, F.E., Mosse, B. and Tinker, P.B. (ed.) Enocmycorrhizas. Academic Press, London.

Tinker P.B. 1975b. Effects of vesicular-arbuscular mycorrhizas on higher plants. Symp. Soc. Exp. Biol. No. 29:325-350.

Tinker, P.B. and Gildon, A. 1983. Mycorrhizal fungi and ion uptake. p. 21-32. In: Robb, D.A. and Pierpoint, W.S. (ed.) Metals and Micronutrients, Uptake and Utilization by Plants. Academic Press, New York, 1983.

Tisdall J.M., and J.M. Oades. 1979. Stabilization of soil aggregates by the root segments of ryegrass. Aust. J. Soil Res. 17:429-441.

Trappe J.M., R. Molina, and M.A. Castellano. 1984. Reactions of mycorrhizal fungi and mycorrhizal formation to pesticides. Annu. Rev. Phytopathol. 22:331-359.

Warncke, D.D., and S.A. Barber. 1972. Diffusion of zinc in soil: I. The influence of soil moisture. Soil Sci. Soc. Am. Proc. 36:39-42.

Warner A., and B. Mosse. 1983. Spread of vesicular-arbuscular mycorrhizal fungi between separate root systems. Trans. Brit. Mycol. Soc. 80:353-354.

Wilkinson, H.F. 1972. Movement of micronutrients to plant roots. p. 139-169. In: Dinauer, R.C. (ed.) Micronutrients in agriculture. Soil Sci. Soc. of Am., Inc., Madison, Wisconsin.

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