

INFLUENCE OF VESICULAR-ARBUSCULAR MYCORRHIZAE
ON THE GROWTH AND WATER RELATIONS
OF VEGETABLE CROPS

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THIS WORK DEDICATED TO:

BILL WESELAKE

AND

THE FOLKS AT JUDD STREET, HONOLULU AND SADDLE CITY, WAIMANALO.

ABSTRACT

Onion, leek, pepper, lettuce and tomato transplants grown in a soilless medium inoculated with the VAMF Glomus aggregatum (Schenck and Smith emend. Koske) were larger and had higher tissue phosphorus (P) concentrations than noninoculated plants if P levels in the medium were low. At higher P concentrations, inoculation had little or a slightly negative effect on transplant growth. Increasing P concentrations in the medium increased transplant growth, but decreased root infection by the VAMF. Increasing VAMF inoculum concentrations did not affect growth or P uptake but increased VAMF infection of the transplants. Daily application of low P fertilizer solutions produced larger transplants with more extensively infected root systems than did similar amounts of P supplied less frequently but at higher concentrations. Different crops required different combinations of P concentration and application interval to produce vigorous mycorrhizal transplants. The controlled-release fertilizer Osmocote (Sierra Chemical Co., Milptas Calif.) produced predictable and stable solution P concentrations in the soilless medium used for transplant production. Growth and VAMF infection of the transplants could be manipulated by altering Osmocote P concentrations in the transplant medium.

Pre-transplant inoculation of peppers subsequently planted into P deficient soil improved early P uptake, vegetative growth and total fruit yields relative to plants inoculated at transplanting. In P deficient soils, maximum pre-transplant VAMF infection of peppers increased subsequent growth and fruit yields more than maximum pre-transplant growth. Extensive pre-transplanting infection improved post-transplant P uptake earlier than in less heavily infected plants. In contrast, pre-transplant growth of lettuce was more important than mycorrhizal infection in determining subsequent growth, at all soil P levels.

In pots, G. aggregatum increased total dry matter yields, promoted early fruit set and improved fruit yields of peppers at solution P concentrations below 0.3 to 0.4 mg/liter. At higher P concentrations, VAMF infection had no beneficial or harmful effects. Tissue P requirements for dry matter production by mycorrhizal plants were lower than in nonmycorrhizal plants, suggesting that mycorrhizae may influence the efficiency of utilization of absorbed P in addition to increasing P uptake efficiency.

In the field, inoculation of peppers increased tissue P concentrations, growth and fruit yields by 28, 120 and 350% respectively relative to nonmycorrhizal plants in a fumigated P-fixing soil with 0.03 mg/liter solution P. Inoculation had no significant effect at 0.30 mg P/liter. Under similar

conditions, VAMF infection increased yields of lettuce by 16%. Although nonmycorrhizal lettuce and peppers had similar solution P requirements for maximum growth, lettuce was more tolerant of sub-optimal solution P concentrations and was correspondingly less responsive to infection by the VAMF.

Moderate water stress increased mycorrhizal responsiveness of peppers growing in P deficient soil. VAMF colonization of pepper seedlings growing in P deficient media increased the hydraulic conductivity of their roots, possibly by improving seedlings tissue P status. Mature mycorrhizal peppers had higher rates of transpiration per unit leaf area than similar size nonmycorrhizal plants. At wilting, mycorrhizal transplants and mature peppers had higher leaf water potentials at lower soil water potentials than nonmycorrhizal plants. The influence of mycorrhizae on the water relations of mature peppers was apparently related to the mycorrhizae improving P uptake by their host but the change in water relations of inoculated seedlings was generally independent of host P-status.

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Preface

This thesis is presented in a series of six papers written in the format required by the Journal of the American Society for Horticultural Science.

The word processing program Multimate was used for the text of this thesis. The figures were constructed using the graphics programs Graphwriter, Freelance, Surfer and Lotus 1-2-3. The text and figures were printed by a Hewlett-Packard LaserJet printer.

CHAPTER I
INTRODUCTION

Many plants form symbiotic associations with root-infecting vesicular-arbuscular mycorrhizal fungi (VAMF). The VAMF association may improve plant disease tolerance (Dehne 1982), and drought tolerance (Nelsen and Safir 1982), and increase the uptake of immobile nutrients from the soil (Mosse 1973). In return, the plant provides the fungus with carbohydrates and a protected niche for development (Hayman 1983).

In pot studies, the growth of many vegetable crops is improved following root infection with a VAMF (Daft and Nicolson 1969, Plenchette et al. 1982). Vegetables have relatively high phosphorus (P) requirements for optimal yields, yet their roots are often poorly adapted for P uptake (ie; low root/shoot ratios, coarse, shallow roots with few root hairs). The mycorrhizae appear to increase plant growth by increasing the P uptake capability of infected roots. The fungal hyphae grow into the soil, absorb P and transport it back to the root, thereby increasing the absorptive surface area of the roots (Rhodes and Gerdemann 1975).

Although the potential contribution of mycorrhizae to the growth of many vegetable crops has been demonstrated in many pot

studies, the existing or potential contribution of VAMF in the field less well known. Comparisons of the soil P requirements of mycorrhizal and nonmycorrhizal crops would be valuable as indicators of the potential impact of mycorrhizae on P-use efficiency.

The management techniques employed in intensive vegetable crop production may impair colonization by mycorrhizal fungi. Colonization is inhibited in plants amply supplied with P (Buwalda et al 1982) and commercially grown vegetables are heavily fertilized with P. Present cultivation and pest control practices may also adversely affect the levels of VAMF inoculum in the soil (Miller et al. 1986). Together, these factors probably limit the contribution of VAMF in most crops grown under intensive management. Some highly mycorrhizae-dependant crops (onion, cowpea and cassava) may derive benefits from the VAMF association even under intensive management.

As fertilizer-use efficiency and pollution by run-off from heavily fertilized lands become more important, the use of mycorrhizae as a means of increasing P-use efficiency may have greater significance in intensive vegetable production. Also, as the impact of mycorrhizae on non-nutritional factors such as plant water relations, disease resistance and stress tolerance are determined, mycorrhizae may be usefully employed in

situations where their contribution to mineral uptake is not required.

Mycorrhizae make a definite contribution in low-input vegetable crop production. Mycorrhizae are particularly important in developing tropical countries, where limited P inputs and P fixing soils restrict the availability of P to the crop. Efficient employment of the symbiosis may substantially increase yields and/or reduce the need for costly fertilizers.

At present, the production of large amounts of mycorrhizae inoculum is difficult, yet inoculation may be necessary when indigenous VAMF are rare or of low symbiotic effectiveness. Efficiency of inoculum use is therefore important. In transplanted crops, inoculation of the seedlings prior to transplanting may be more efficient and effective than adding the inoculum to the field. However, the fertility and management requirements for the production of horticulturally acceptable mycorrhizal transplants have not been established. Phosphorus nutrition is a particular concern, as the P applied to produce vigorous growth of the transplants may inhibit root colonization. Phosphorus fertilizer levels must be adjusted to compromise between maximum growth or maximum mycorrhizal colonization. The amounts, form and placement of mycorrhizal inoculum for maximum colonization are not known. There may be significant interactions between P fertility and inoculum factors, in terms of the balance between growth and

colonization. The optimum balance of mycorrhizal colonization and growth of transplants relative to their subsequent performance in the field also need to be determined. Soil fertility levels may have a significant effect on the optimum balance of growth and colonization.

It appears that mycorrhizae may also influence the water relations of their plant hosts and that the mycorrhizal symbiosis may, in turn, be influenced by moisture availability. Uptake of P and other immobile nutrients are adversely affected by low soil water potentials (Viets 1972). Hyphae from mycorrhizal roots may extend into regions of the rhizosphere where soil water has not been reduced by root uptake. Nutrient uptake from these zones results in improved plant nutrient status relative to nonmycorrhizal plants. The potential for mycorrhizae to improve nutrient uptake in water stressed plants has not been adequately investigated.

There are indications that mycorrhizal plants may survive the shock of transplanting better than nonmycorrhizal plants (Barrows and Roncadori 1974). Water stress is an important component of transplant shock. Whether mycorrhizae reduce transplant shock by some favorable change in plant water relations has yet to be determined.

The objectives of the studies presented here were:

- 1) Determine inoculum and P fertility regimes for the production of horticulturally acceptable mycorrhizal vegetable transplants.
- 2) Examine how mycorrhizae influence the soil P requirements of vegetable crops.
- 3) Examine the performance of pre-inoculated transplants relative to plants inoculated in the field.
- 4) Examine the relationship between growth and mycorrhizal colonization of transplants and their subsequent performance in the field.
- 5) Determine the effect of water stress on P uptake and mycorrhizal responsiveness.
- 6) Assess the impact of mycorrhizae on water relations of water stressed and non-stressed vegetable crops.

CHAPTER II
REVIEW OF LITERATURE

The term mycorrhizae (Frank 1885) describes the symbiotic relationship between certain fungi (Greek mukes) and the roots (Greek rhiza) of many higher plants. With many plants, health and vigor are associated with the mycorrhizal state, suggesting a mutually beneficial, rather than pathogenic relationship. The fungus appears to be obligately dependent on the plant, with the plant supplying the fungus with carbohydrates and a niche where it is protected from the microbial antagonists of the soil. In return, the fungus enhances the uptake and supply of immobile nutrients from the soil. Mycorrhizal associations may also influence plant hormonal relations, disease, drought and salinity tolerance, water relations and nitrogen fixation.

The mycorrhizal association represents a complex balance between host, fungus and environmental variables. Variation in any of these components alters the nature of the association. This complexity explains the observed unpredictability of field responses to mycorrhizae and represents a challenge to those attempting to utilize the symbiosis to achieve gains in agricultural crops.

Mycorrhizae are generally grouped into ecto-, endo- and ecto-endomycorrhizae based upon the type of fungus-root structures formed, and whether the fungi penetrate the host

cells (endophytic) or remain strictly intercellular (ectophytic) (Lewis 1973). Ectomycorrhizae are common in temperate forest and ornamental tree species. The fungal partners are usually Basidiomycetes, although some Ascomycetes may also form ectomycorrhizae. Ecto-endomycorrhizae form in Ericaceae species; little is known about the fungus specie(s) involved. This review will focus on the endomycorrhizae, as this association is of greatest importance to agricultural crops.

THE MYCORRHIZAL SYMBIOSIS

Morphology and Taxonomy

There are no visually discernible modifications in the external structure of endomycorrhizal roots (Rhodes and Gerdemann 1975), although in some hosts, freshly harvested roots may exhibit a distinctive yellow pigmentation (Daft and Nicolson 1966). A fungal mantle is not present, but the root is instead surrounded by a loose network of hyphae which may extent up to 10 cm into the adjacent soil (Rhodes and Gerdemann 1975). Infection commences with penetration of the root by hyphae arising from soil-borne fungal propagules. The hyphae spread inter- and intracellularly within the root cortex. Intracellular arbuscles are formed by repeated branching of penetrating hyphae. Sac-like swellings or vesicles form inter

or intracellularly at the tips of hyphae. The presence of these distinctive structures has led this type of root-fungus association being designated as a vesicular-arbuscular mycorrhizae (VAM).

The endomycorrhizal fungi are aseptate members of the order Phycomyceteae, family Endogonales, genera Glomus, Sclerocystis, Endogone, Glaziella, Gigaspora and Acaulospora (Mosse et al. 1981). Taxonomic differentiations between species and genera are primarily based upon spore and sporocarp morphology (Gerdemann and Trappe 1975). The taxonomy of the entire group is unstable due to the frequency of intergradations between taxa.

Distribution

Endomycorrhizal fungi are amongst the most common and widely distributed of the soil fungi. Virtually all soils contain some VAMF, but inoculum density and the fungal species are variable (Mosse et al. 1981). Most endomycorrhizal species are distributed worldwide, but localized distribution patterns are unpredictable (Walker and McNabb 1979). The VAMF will form associations with most herbaceous, shrub and tree species, including the majority of crop species (Mosse et al. 1981). Infections are rare in aquatic plants, but bog species are frequently colonized. Species belonging to the Cruciferae,

Chenopodiaceae and Proteaceae are the only plants of agronomic importance which do not commonly form endomycorrhizal associations. Sporadic infections may occur in normally nonmycorrhizal species (Hirrel et al 1978).

Development and anatomy of infection

The VAMF appear to be obligate symbiotes; despite extensive research they have not been successfully cultured on synthetic media (Mosse 1959). Infections are initiated by hyphae emanating from sporocarps, chlamydospores or azygospores residing in the soil or from hyphae arising from internal vesicles or other viable components found in the residue of previously infected roots (Carling and Brown 1982, Hayman 1983). The range of potential infective propagules produced by the fungus makes quantification or comparison of inoculum potential in soils difficult.

Spore germination and the growth of infective hyphae are influenced by soil temperature (Schenck et al. 1975), soil pH (Greenwood et al. 1976) and soil nutrient status (Mosse 1959) and by the presence of fungicides or other inhibitors (Hepper 1979). The development of infective hyphae may be enhanced by the presence of exudates from the host's roots (Hepper and Mosse 1975). However, germ tubes do not exhibit any trophic orientation towards the host roots (Hayman 1983). Contact

between the germ tubes and roots is largely a chance occurrence and is strongly influenced by the density of roots and germ tubes in the soil.

Once the hyphae contacts the root, an appressorium may be formed, followed by penetration of the root or root hair (Hayman 1983). Penetration and subsequent colonization generally occur in the regions of differentiation and elongation of active feeder roots (Carling and Brown 1982). The hyphae spread inter- and intracellularly within the cortex but their growth does not appear to impair the physiological functions of the cells, nor does it elicit any conspicuous defense reactions (Carling and Brown 1982). Simultaneously, external hyphae branch out from the point of root entry, spreading around the root and into the adjacent soil.

Arbuscles develop a short distance behind the advancing internal hyphae (Carling and Brown 1982). The initial hyphae to penetrate a cell bifurcates to form a fine, haustoria-like, net which eventually occupies a substantial portion of the host cell lumen (Brown and King 1984). Arbuscle development appears to have little impact on cell function, although the amount of cytoplasm and concentration of organelles tends to increase (Carling and Brown 1982). Arbuscles survive for 1-3 weeks, after which they collapse and deteriorate (Cox and Sanders 1974). It is not known whether this process is autocatalytic or results from the physiological activity of the host cell.

Vesicles usually form after arbuscles, becoming more numerous as the plant matures (Brown and King 1982). The vesicles may be inter- or intracellular, terminal or intercalary, depending upon the fungus and host species (Brown and King 1982). Vesicles may become so abundant in the older cortex that the root is distorted and the cortical tissue partially destroyed (Mosse et al. 1981). Vesicles appear to function as food storage organs; during development most of their volume is occupied by triglyceride and glycogen globules (Cooper and Losel 1978). Some VAM fungi produce thick walled vesicles which resemble in size and structure the chlamyospores produced by the species (Brown and King 1982). Biermann and Lindermann (1983) demonstrated that these specialized vesicles can germinate, producing germ tubes capable of initiating new infections. Vesicles may, therefore, also function as reproductive structures. Gigaspora sp. do not produce vesicles, suggesting that they are not vital to the survival of the fungus (Hayman 1983).

The VAMF genera produce two types of spores. Acaulospora, Enterospora and Gigaspora produce azygospore-like spores (Mosse et al. 1981, Trappe and Schenck 1982). Whether their formation involves sexual or asexual processes is not known (Trappe and Schenck 1982). Glomus, Glaziella and Sclerocystis produce specialized asexual chlamyospores. Azygospores are usually borne singly, arising as terminal swellings of external hyphae

(Mosse et al. 1981). In chlamydosporic genera, the spores may be single or they may be aggregated into distinct sporocarps (Trappe and Schenck 1982). Like the vesicles, spores contain globules of oil which serve as energy reserves (Brown and King 1982). Spore formation generally commences once the internal infection has become well established. The stimuli directing spore formation are not known. In annual hosts, spore formation peaks late in the season (Mosse et al 1981). Whether this reflects the maturation of the host or is determined by fungal processes is not known. The number of spores produced varies greatly with the fungus, the host and the prevailing environmental conditions. In general, factors promoting good growth of the host and substantial root colonization by the fungus tend to increase spore production (Ferguson and Woodhead 1982).

Information on the lifespan of spores and the factors influencing viability is limited. Spores stored in soil under dry, cool conditions may remain viable for years (Mosse and Hayman 1980). Survival under normal soil conditions appears to be more restricted. High soil temperatures and moisture content reduce spore lifespan (Ferguson and Woodhead 1982), as does the presence of microbes capable of parasitizing the spores (Ross and Daniels 1982). Dissemination of mycorrhizal fungi is limited to spread of hyphae between living roots and by movement of spores and other propagules in water and by wind and animal vectors (Mosse et al. 1981).

Spread of infection in roots

Infections generally begin as a series of discrete infection units, each originating from an individual point of penetration and expanding about 5-10 mm on either side of the entry point (Cox and Sanders 1974). With time, the individual infections may coalesce to form a continuous infection. The infection may also spread down the root via external, stolon-like runners which penetrate the root at frequent intervals (Nicolson 1959).

Infection of the root system generally follows a sigmoidal curve (Sutton 1973, Saif 1977). The initial lag phase reflects the slow establishment of the infection. The subsequent exponential increase in infection reflects spread within previously infected roots and from infected roots to those previously unaffected. The infection finally plateaus. Total infection is rare. The rate and final extent of infection varies with the fungus and host species and the prevailing environmental conditions. The dominant factors influencing the rate and degree of root colonization will be discussed in detail later. It should be noted that symbiotic effectiveness is not always correlated with the greatest degree of colonization of the host root system (Mosse 1972, Powell 1977, Sanders et al. 1977) or the most extensive growth of external hyphae (Sanders and Tinker 1973).

Frequently, two or more species of VAMF may be found co-existing within the roots of a single host plant (Tinker 1976). There is little apparent antagonism, and multiple infection may actually increase the extent of root colonization (Mosse et al. 1981). Bowen (1980) has discussed the factors influencing the relative development of co-existing fungi.

EFFECT ON THE PLANT

Growth increases

The ubiquitous distribution of VAMF has made it difficult to determine the effect of the symbiosis on plant growth under field conditions. Attempts to quantify the effect usually involve treating the soil to eliminate the indigenous VAM fungi. Plant growth in this soil is compared with plants growing in untreated soil or in sterilized soil where the mycorrhizal fungi have been reintroduced. This procedure has been used to assess the impact of the mycorrhizal symbiosis under an incredibly diverse range of host, endophyte, soil and environmental conditions (see reviews by Mosse 1973, Gerdemann 1968, 1975, Hayman 1983, Maronek et al. 1981, Miller et al. 1986). Where beneficial mycorrhizal responses are observed, the improvement in growth of the host can usually be traced to an improvement in mineral nutrition. In the majority of

experiments, phosphorus was the nutrient most influenced by the mycorrhizal symbiosis. The fact that the mycorrhizal effect can usually be duplicated by increasing the supply of P to the plant lends credence to the mycorrhizae-P uptake relationship.

Mechanism of P uptake

The supply of P to roots is primarily determined by diffusion (Barber et al. 1963). Uptake by rapidly growing plants frequently exceeds the rate of P diffusion into the root zone (Bhat and Nye 1974) resulting in P depletion zones surrounding the root, extending 1-2 mm into the adjacent soil (Baldwin et al. 1971). The potential mechanisms by which mycorrhizae may increase P have been extensively studied and reviewed (Tinker 1976, Hayman 1983). Presently, the dominant hypothesis is that the extension of the fungal hyphae into the soil increases the absorptive area of the roots by exploring a greater volume of the soil than is accessible to nonmycorrhizal roots. The fungal hyphae extend up to 10 cm from the root, well beyond the zone of depletion created by root uptake (Rhodes and Gerdemann 1975). Radiotracers have been utilized to demonstrate P uptake and transport by these distant hyphae. Rhodes and Gerdemann (1975) and Hattingh et al. (1973) found that mycorrhizal plants could absorb ^{32}P applied several centimeters away from the root, well beyond the distance the P can travel by diffusion. When

hyphae growing from the mycorrhizae into the distant zone of P application were cut, P uptake from these points ceased.

Nonmycorrhizal plants only responded to the added P when it was applied very close to the root, ie; within the 1-2 mm diffusion radius of P (Hattingh et al. 1973).

Sanders and Tinker (1971 and 1973) calculated that the mean rate of P uptake per unit length of mycorrhizal onions was four times greater than nonmycorrhizal plants. Calculations of diffusive of P ions to single cylindrical roots indicated that the uptake value for the nonmycorrhizal roots ($4 \times 10^4 \text{ } \mu\text{mol cm}^{-1} \text{ s}^{-1}$) was about equal to the theoretical maximum. Any additional inflow must have occurred via the hyphae. Based on data for the concentration of P observed in the hyphae and their calculated flux characteristics, Sanders and Tinker (1973) determined that the number of connections (entry points) between the fungus inside and outside the root was sufficient for hyphal transfer to account for the increased P uptake of mycorrhizal onion roots.

Baylis (1970 and 1975) and Tinker (1975) have discussed the structural and functional analogies between mycorrhizal hyphae and root hairs. The longer hyphae may be more efficient than root hairs, as the uptake zones of the shorter root hairs may overlap. The development of mycorrhizae may compensate for the lack of soil contact observed in plant species with coarse fibrous root systems or few root hairs. Frequently, species

with low root-soil contact root systems obtain the greatest benefit from the extra absorbing surface provided by the VAMF hyphae.

Although the increased absorptive area provided by the mycorrhiza hyphae appears to be the primary reason for increased P uptake, additional factors may be involved. Cress et al. (1979) and Splitstoesser (1982) found that mycorrhizal roots had higher affinities for P (lower k_m for absorption than nonmycorrhizal roots. This difference was most apparent when solution P concentrations were high (>0.2 ppm P). They were not able to differentiate between any direct effect of the hyphae on P affinity and any effects related to altered P nutrition of the mycorrhizal roots. Bowen et al. (1975) found little difference in P uptake kinetics of mycorrhizal and nonmycorrhizal onion roots growing with 5 μ mol solution P. They concluded that the hyphae function by virtue of their position rather than any unique uptake property. Mosse et al. (1973) found that in some extremely P deficient soils, mycorrhizal plants grew well, while nonmycorrhizal plants were unable to obtain any P from the soil. The data suggests that mycorrhizal roots may be able to absorb P at concentrations lower than the thresholds for nonmycorrhizal roots.

Several researchers have noted increased P uptake and superior growth of mycorrhizal plants when P sources of low availability, such as rock phosphate, are added to the soil

(Daft and Nicolson 1966, Murdoch et al. 1967, Mosse 1973). The mycorrhizal effect could be due to the solubilization of the normally unavailable P compounds by phosphatases on the surface on the hyphae (Bartlett and Lewis 1973) or by changes in the rhizosphere pH or chemical composition mediated by the mycorrhizae. Sanders and Tinker (1971), Powell (1975), Tinker (1975) and Owusu-Bennoah and Wild (1980) found that specific activities of mycorrhizal and nonmycorrhizal roots grown in low P soils labeled with ^{32}P were not significantly different. These results indicate that the mycorrhizae do not expand the size of the labile pool of P; instead they make more efficient use of the available P. Mycorrhizal infection does increase the potential for utilizing low-solubility P sources in agricultural fertilization practices. Finally, roots may stay functional longer when mycorrhizal (Bowen 1975), thereby further increasing the absorptive area of the root system.

Physiology and chemistry of P transfer

The process by which mycorrhizae supply P to the plant has several stages: 1) P uptake by the hyphae from the soil, 2) translocation from external to internal hyphae, 3) release to the plant. Like roots, the hyphae draw on the supply of orthophosphate ions in the labile pool of the soil. Absorbed orthophosphate is rapidly converted to polyphosphates granules

which are stored in the fungal vacuoles (Cox et al. 1980). Sanders and Tinker (1973) traced the movement of ^{32}P applied to the soil near P stressed mycorrhizal onions. The P first appeared in the external hyphae, but with time was also found in the internal mycelium of infected roots. Direct and theoretical measurements of P transfer through endomycorrhizal hyphae indicate translocation rates of 0.1 to $3.8 \times 10^{-9} \text{ mol cm}^{-2} \text{ s}^{-1}$ (Sanders and Tinker 1971, 1973, Pearson and Tinker 1975). Phosphorus moves through the hyphae at 10 cm/h (Littlefield 1966) which is $10 \times$ the rate observed in roots (Crosset and Loughmann 1966). Cytoplasmic streaming, coupled with some contribution by bulk flow, could account for the observed rates of movement (Tinker 1976). Fungitoxicants which inhibit cyclosis decrease P uptake and movement in mycorrhizal roots (Tinker 1976).

The polyphosphate granules disappear from the fungal vacuoles in the fine branches of the arbuscles. It is thought that arbuscles are the site of P transfer between the fungus and the host. The arbuscles possess the phosphatases required for polyphosphate degradation (Cox et al. 1975). Traditionally, the transfer of P from the fungus to the plant was thought to occur during the deterioration of the short-lived arbuscles (Hayman, 1973). The exchange process, however, requires energy and the presence of functional membranes, indicating that the transfer may be an active rather than passive process.

Other elements.

Mycorrhizae may facilitate increased or selective uptake of elements in addition to P, however, investigations of these reactions are complicated by interactions with increased P uptake. A VAMF-related increase in P uptake may improve root growth or increase the ion uptake capacity of the root, increasing the uptake of other minerals. Alternatively, increased plant growth due to the alleviation of P stress in mycorrhizal plants may result in a reduction in the tissue concentrations of other elements due to dilution.

The increased absorptive area provided by the fungal hyphae should increase the uptake of immobile ions more than those supplied by mass flow. Uptake of Cu and Zn, which are relatively immobile, are frequently increased in mycorrhizal plants (Ross and Harper 1970, Gilmore 1971). Translocation through the external hyphae to the root cortex has been demonstrated with Zn and Cu (Rhodes and Gerdemann 1980). Elevated tissue concentrations of relatively mobile elements such as K and S likely reflect the effects of mycorrhizae on growth rather than any specific uptake of these elements (Gray and Gerdemann 1973, Powell 1975).

When large amounts of fertilizer P are added to the soil, the uptake of Zn and Cu may be reduced due to precipitation with PO_4 or by reduced root affinity (Timmer and Leyton 1978). By reducing the P requirements of the crop, VAMF may aid in the avoidance of mineral deficiencies related to excess soil P.

In nitrogen fixing species, infection with VAMF and Rhizobium frequently increases nodulation, N fixation, tissue N and plant growth relative to inoculation with only Rhizobium (Daft and El-Giahmi 1974, Mosse et al. 1976, Manjunath and Bagyaraj 1984). The beneficial effect of VAMF on N fixation appears to operate through its influence on P nutrition; increasing the efficiency of the Rhizobium symbiosis rather than by an direct fixation of N by the fungus. Nodule formation and N fixation are very sensitive to tissue P deficiencies.

Benefits to the fungus

All VAMF are believed to be obligate symbiotes, association with a host is vital to their survival. The fungus receives almost all of its nutrients from the plant, primarily as carbohydrates. Radiotracer techniques have been used extensively to study C distribution and use in mycorrhizal plants (Ho and Trappe 1973, Snellgrove et al. 1982). The carbohydrate requirements of the fungus are clearly met by the host plant, with 7-20% more fixed carbon being translocated to

the roots of mycorrhizal plants as compared to similar size nonmycorrhizal plants (Pang and Paul 1980, Snellgrove et al. 1982). The additional translocated C can be accounted for by increased respiration and an increase in the total C content of the mycorrhizal roots (Bevege et al. 1975, Cox et al. 1975). Endomycorrhizal fungi transform plant photosynthates into lipids, which act as an energy store for the fungus (Cooper and Losel 1978). Oil droplets occur in the external and internal hyphae, in the vesicles and chlamydospores. Autoradiographic studies have traced the movement of photosynthates as they pass from internal fungal structures to the external hyphae via cytoplasmic streaming of lipid globules (Cooper and Losel 1978).

The carbohydrate requirements of mycorrhizal fungi are usually small relative to the photosynthetic capacity of the host. Plants experiencing increased energy demands may compensate by increasing the efficiency of their photosynthetic process (Kucey and Paul 1982). This results in little competition for energy between the fungus and the roots of its host. If, however, the photosynthetic capacity of the host is substantially reduced by shading or by loss of leaves, the metabolic costs of the mycorrhizae may represent a substantial drain on the plants' energy supply. This may reduce the growth of mycorrhizal plants relative to nonmycorrhizal plants (Stribley et al. 1980). Nitrogen fixation requires substantial amounts of energy and photosynthates. Any competition between

mycorrhizae and Rhizobium for carbohydrates may reduce N fixation and ultimately yields (Bethlenfalvay et al. 1982). Normally, however, the nutritional benefits derived from the mycorrhizal association outweigh the associated energy costs.

NON-NUTRITIONAL EFFECTS ON PLANT GROWTH

Water relations

Whether mycorrhizae influence the water relations of their plant hosts is of considerable academic and practical interest. It was proposed that the aseptate hyphae might act as a low resistance channel for the transport of water from the soil to the host roots. However, axial resistances in the hyphae appear to be too high to allow for significant flux relative to the plants' transpirational requirements (Sanders and Tinker 1973). Reid (1979) suggests that the hyphae may directly contribute to water uptake by helping maintain soil-root contact during drying of the soil. Safir et al. (1972) found that mycorrhizal soybeans growing in low P soil had higher root hydraulic conductivities than nonmycorrhizal plants. The mycorrhizal effect could be duplicated by adding P to the nonmycorrhizal plants, indicating that the lower resistance in mycorrhizal plants was due to a mycorrhizae-mediated improvement in plant nutrition rather than any direct contribution by the fungus in water movement.

Atkinson and Davison (1973) showed that axial resistances in the xylem of P stressed plants was much greater than in plants grown with adequate P.

When onions growing in low P soil were subjected to water stress, mycorrhizal plants had higher tissue P concentrations and greater growth than nonmycorrhizal plants (Bolgiano et al. 1983). The difference in growth due to mycorrhizae was greater in water stressed treatments than in unstressed treatments. Olsen et al. (1961) demonstrated that P uptake is greatly reduced in plants subjected to water stress. Reductions of plant P uptake by 50-70% may occur once the soil water potential (SWP) drops below -2 bars (Viets 1972). Low soil water potential reduces nutrient availability by slowing diffusion, while increasing the concentration of potentially toxic mobile ions in the rhizosphere. Water stress in the plant impairs root uptake capacity and reduces nutrient interception by slowing root growth. By increasing P uptake in areas away from the drying influence of the root, the mycorrhizae may aid in the maintenance of a steady P supply under stress conditions (Bolgiano et al. 1983).

Disease resistance

Colonization of plant roots by VAMF may protect roots from subsequent attack by certain fungi (Schenck et al. 1977, Dehne and Schonbeck 1979) and nematodes (Sikora and Schonbeck 1975, Kellam and Schenck 1978) or may increase host's tolerance to other pathogens (Schonbeck and Dehne 1977). In other situations, colonization may have no effect or may actually increase disease severity (Ross 1971, Menge et al. 1977, Baath and Hayman 1983). Viral diseases are particularly enhanced in mycorrhizal plants (Daft and Okusanya 1973). The interactions between host/mycorrhizae/parasite are complex, and vary greatly with the combination. Factors which retard infection by one pathogen may stimulate another. Reactions between host/mycorrhizae/parasite have been reviewed by Dehne (1982).

FACTORS INFLUENCING COLONIZATION AND PLANT GROWTH RESPONSES

The processes involved in the formation and maintenance of the mycorrhizal symbiosis, as well as any resulting plant growth response, are complex. Each step involves interactions amongst the host, the fungus, and the soil. The availability and movement of P is of prime importance in this interaction. These components of the VAMF symbiosis and the effects of

environmental variables on their interaction will be considered in this section.

Host variability

Under a given set of environmental conditions, the response to a particular mycorrhizal fungus may vary considerably from host to host. The host clearly plays an important role in determining the extent and effectiveness of the infection. The concept of "mycorrhizal dependency" (Gerdemann 1975), is useful in comparing the responses of plants to VAMF under varying conditions. Dependency is defined as the degree to which any plant is dependent on the mycorrhizal condition to produce maximum growth or yield at a given level of soil fertility.

Some plants do not form endomycorrhizal associations under any conditions and therefore have no mycorrhizal dependency. Colonization may be inhibited by specific chemicals in the root epidermis and cortex (Iqbal and Qureshi 1976). Frequently, these nonmycorrhizal species possess root systems which are highly efficient at P uptake, enabling the plants to maintain adequate tissue P concentrations in soils with limited available P (Baylis 1975). Other species appear to be facultatively mycorrhizal; the development and significance of the symbiosis in these plants is determined by the prevailing environmental conditions. The mycorrhizal dependency of some species has

progressed to where they are virtually obligately mycorrhizal (ie; onion, cassava, carrot); their growth severely restricted by the absence of mycorrhizae, even when nutrients are abundant.

Baylis (1972), Crush (1974) and St John (1980) have discussed the correlation between root hair development, root system fineness and size, relative to the plants mycorrhizal dependency. Each of these features increase the contact area between the root and the soil, increasing the potential surface area for nutrient absorption and thereby reducing the relative usefulness of the mycorrhizae hyphae. Obligately mycorrhizal species frequently possess limited root systems with thick unbranched roots and few root hairs. These plants have come to depend on the absorptive surface provided by the fungal hyphae.

The inherent characteristics of plant growth rate and the minimum tissue P concentration required for growth may also affect mycotrophy (Hayman 1983). Slow growing species and those with low tissue P requirements tend to be relatively unresponsive to mycorrhizae.

Responses of different plant cultivars to a particular mycorrhizal fungus may be variable. The speed and extent of colonization can be influenced by the cultivar (Azcon and Ocampo 1981, Rajapakse and Miller 1984). Similarly, cultivars may differ in their responses to colonization, in terms of any changes in their growth or nutrient status (Powell et al. 1982). Individual cultivars may also respond differently to

different species of mycorrhizal fungi (Powell et al. 1982, Rajapske and Miller 1984).

The interaction between host and mycorrhizal fungi is physiologically complex, involving the inter-play of specific gene products from both partners in the symbiosis. Differences in the genetic make-up of host plants and the different fungal endophytes contribute to the observed variability in root colonization and plant response to infection.

Endophyte variability

Numerous screening trials indicate that the VAM fungi differ greatly in their symbiotic effectiveness (Mosse and Hayman 1971, Owusu-Bennoah and Mosse 1979). Effectiveness is a function of the fungi's adaptation to particular soils and host plants as well as their ability to stimulate plant growth, compete with indigenous microflora and effectively colonize the rhizosphere (Hayman 1983). The associations between VAMF and their plant hosts does not exhibit the specificity commonly found between plants and obligately pathogenic fungi. Virtually any VAM fungal species can infect any VAMF plant host, but the extent of infection and its effect upon the host will vary with the host/fungus combination. Each combination will also respond differently to changing environmental conditions. Studies comparing the effectiveness of introduced fungi with those

indigenous to the soil or environment are illustrative. In some trials, the introduced fungus had higher colonization rates and improved plant growth relative to the indigenous population (Khan 1972, Mosse 1977, Plenchette et al. 1982). The introduced isolate appeared to be better adapted to the existing conditions than the native mycorrhizae. However, due to the uncertainties involved in establishing equal inoculum densities of introduced versus indigenous fungi, comparisons of relative effectiveness are difficult. Other researchers have observed that introduced isolates completely fail to colonize the crop. The introduced species may have been unable to compete with indigenous species or they may have been unable to tolerate some unfavorable factor in the environment. Clearly, no single VAM fungus is most effective under all environmental conditions or with every host.

Many factors influence the relative effectiveness of any VAMF isolate. The speed at which an endophyte establishes a reasonable level of infection and begins to function is of critical importance, particularly if the host is a short-lived species (Hayman 1983). Although the fungi differ substantially in the amount of external hyphae produced, there is no evidence of a direct correlation between mycelium production and effectiveness (Sanders and Tinker 1973). The same applies to the extent of root colonization (Mosse 1972, Powell 1977, Sanders et al. 1977). Isolates have been shown to differ in their absorption and exchange of nutrients with the host and in

their energy demands upon the host (Hayman 1983). Each of these factors may help determine the relative effectiveness of a particular fungus. The variability apparent in the relative performance of VAM fungi under different conditions suggests that efforts to select the most effective endophyte for the existing crop and soil conditions may be very worthwhile.

Light

Shading generally reduces colonization of roots (Hayman 1974), increasing arbuscle formation but decreasing vesicle and spore development (Daft and El-Giahmi 1978). The plants' responsiveness to mycorrhizae also declines under reduced light, often before the plants cease to respond positively to added P (Hayman 1983). The restricted development and effectiveness of mycorrhizal associations under reduced light is likely linked to decreased photosynthetic activity of the host. As photosynthesis slows, translocation of carbohydrates to the roots is restricted, reducing the energy available to the fungus for growth and P uptake (Hayman 1983, Miller et al. 1986). Furlan and Fortin (1977), however, found more rapid and extensive colonization of onions to occur at low light intensities, suggesting that the effect of light on the symbiosis may vary with the species.

Temperature

The VAM fungi have distinct optimum temperatures for both establishment of the symbiosis and for survival of the fungus (Schenck et al. 1975). These optima vary with the species and isolate and are usually correlated with prevailing conditions in the environment. Colonization tends to increase with temperature (Furlan and Fortin 1973). The optimal temperature for arbuscle and mycelial development is lower than that for spore and vesicle production (Furlan and Fortin 1973). Furlan and Fortin (1973) observed that mycorrhizal colonization reduced the growth of onions held at 11-16 C but increased growth at higher temperatures.

Soil factors

Soil fertility and particularly the availability of soil P are critical factors governing the extent of root infection by mycorrhizal fungi. They also determine whether the infection has any beneficial effect upon plant growth. The classic studies of Daft and Nicholson (1969) illustrate the effect of cumulative P application on growth and mycorrhizal colonization of corn and tomato. When P applications were low, root colonization was high and mycorrhizae had a beneficial effect on plant growth. As fertilizer P was increased, the growth

benefits due to inoculation were lost and colonization declined. Many other researchers using different host-endophyte-soil P combinations have reported an inverse relationship between the supply of labile P in the soil and the extent and effect of mycorrhizal infection (Hayman and Mosse 1971, Stribley et al. 1980, Buwalda et al. 1982). Some plants (onion, citrus and cassava) continue to show strong colonization and positive growth responses even when soil P concentrations are very high (Mosse and Hayman 1971, Kleinschmidt and Gerdemann 1972, Vander Zaag et al. 1979).

Sanders (1975) demonstrated that establishment and spread of infection in plant roots is inhibited by high tissue P levels rather than by any direct inhibition by high soil P. Ratnayake et al. (1978) noted that plants under P stress experience membrane disfunction, resulting in leakage of metabolites into the rhizosphere. They proposed that mycorrhizal fungi may respond to this chemical stimuli in a manner which ultimately leads to increased colonization in P stressed plants. Gianinazzi-Pearson and Gianinazzi (1976) found that high internal P concentrations in roots may specifically inhibit certain fungal enzymes. Whether this inhibition restricts colonization has not been determined. Regardless of the mechanisms involved, the symbiosis appears to be largely self regulatory. Colonization progresses when the plant will benefit from the resulting increased P uptake. When the supply of P

from the soil is adequate, colonization is suppressed, thereby avoiding the costs of the symbiosis when the added nutrients would be superfluous.

Nitrogen fertilizers may exert a negative effect upon the infection process (Hayman 1970, Krucklemann 1975, Hepper 1983). It is not known whether nitrate or ammonium ions are more inhibitory and whether the ions influence the infection through the plant or through the soil (Hayman 1983). Bjorkman (1970) suggested that the increased N supply would stimulate protein synthesis in the roots, thereby reducing the availability of the sugars which may stimulate infection. Hepper (1983) found that if supplied N increased plant growth, the resulting dilution of tissue P concentrations led to increased mycorrhizal colonization.

Soil pH may influence mycorrhizal colonization and the efficiency of the resulting symbiosis (Mosse 1972). The various species and strains of VAMF differ in their tolerance to pH extremes, as well as their pH optima (Lambert and Cole 1980). Attempts to assess the effects of pH on the symbiosis are complicated by the changes in soil nutrient availability and plant nutrition which occur with changes in pH.

Soil water has both direct and indirect effects upon mycorrhizal infection and host growth promotion. Waterlogging of soils inhibits infection, probably due to oxygen depletion (Mosse et al. 1981). The processes of infection and hyphal

growth are much more tolerant of water stress than root growth, although drought does reduce both infection and spore production (Read and Bowen 1979).

PHOSPHORUS NUTRITION OF VEGETABLE CROPS

Mycorrhizal colonization generally influences growth by improving the hosts' tissue P status. The extent and effectiveness of the symbiosis is strongly influenced by the tissue P status of the host. In studying the potential effects of mycorrhizae, the factors in addition to mycorrhizae which may influence the P status of the host plant must be considered. This section will review the plant, environmental and management variables which determine the P status of vegetable crops.

Plant factors

The mineral nutrition of vegetable crops has been extensively studied and reviewed (Lorenz and Tyler 1971, Geraldson et al. 1973, Lorenz and Vittum 1980). Vegetables tend to be more sensitive to soil nutrient supply than are most field crops. Their rapid growth, short life cycle, and intolerance of nutrient stress contribute to their sensitivity to the nutrient supply. Additionally, many vegetables have poorly developed,

shallow root systems which restricts their ability to exploit the available nutrient supply.

Tissue analysis has been used extensively in the determination of the nutrient status, fertilizer needs and responses of many vegetable crops. Deficiency and sufficiency levels of tissue P for most vegetables have been determined from field trials and compilation reports (Lorenz and Tyler 1971, Geraldson et al. 1973). The P requirements tend to decrease with plant maturity (Peck and MacDonald 1972 and 1975). Samples taken early in the plants' growth are usually more reliable for estimating requirements for P (Tremblay and Bauer 1952). This reflects the importance of adequate P during early growth (Hayman 1983). During the later part of the growing season, correlations between tissue P concentrations and growth are frequently poor. Standardization of the tissues sampled is also important. Total tissue P concentrations of 0.25 to 0.35 % (dry weight basis) produce yields within 10% of the maximum in many vegetables (Lorenz and Vittum 1980). The cole crops have somewhat higher tissue P requirements, while the P requirements of sweet corn and beans are lower. For maximum yields, tissue P concentrations of 0.5% may be required. The tissue P concentrations required for optimal growth of vegetable crops are generally higher than that of field crops.

P uptake patterns

Many vegetables have a short growth period or are harvested prior to maturity. In these crops, the majority of P is absorbed during the last quarter of the growth period. However, the rate of P uptake/unit root length is greatest early in the plants' development. In crops grown to maturity, P accumulation rates decrease once the plant switches from vegetative to reproductive growth. Plants with indeterminate growth patterns continue to accumulate P throughout the fruiting period (Lorenz and Vittum 1980). For virtually all vegetable crops, the greatest need for P and consequently the highest response to fertilization occurs during the seedling stage.

P deficiency

Phosphorus deficiencies in vegetable crops are usually difficult to detect visually. Poor growth and retarded development are often the only signs of deficiency. When stress is severe, leaves and stems may become dark green or purplish due to decreased growth and/or anthocyanin accumulation. The delay in maturity due to P deficiency may be especially important in short season vegetables. In lettuce, P deficiency will greatly reduce early yields without affecting total yields (Lorenz and Vittum 1980).

Crop quality

Market quality determinants, such as size and grade are of greater significance in vegetables than in most field crops. Phosphorus nutrition may influence both the size and grade characteristics of the crop. Inadequate P results in stunted, poor quality produce, while excessive fertilizer may cause uneven growth. P fertilization may hasten maturity, leading to changes in quality and price. Information on the effect of P on the nutritional quality of vegetable crops is limited. Environmental and management factors have a greater effect on the nutritive value of vegetables than do fertilizers (Greenwood et al. 1974).

Soil factors

Vegetable crop production in developed regions usually involves intensive management, with optimal inputs provided for plant growth. It is rare for commercial growers to raise vegetables without added P fertilizer. Due to the high fertilization rates and low crop removal (<10 kg/ha/crop) levels of both available and total P are generally high in soils devoted to vegetable crop production (Lorenz and Vittum 1980).

Crop responses to soil P levels

There is great variation in the levels of available P recognized as either deficient or adequate for any given vegetable crop (Lorenz and Bartz 1968). The crop, the cultivar, the season, cultural conditions, the soil and the soil test used to estimate the available P all influence the relationship between estimated available P and crop responses. Critical concentrations of available soil P for various vegetable crops have been determined or compiled by Reisenauer (1976), Lorenz and Vittum (1980), Nishimoto et al. (1977), Yost and Fox (1979), and Lorenz and Thompson (1980). The leafy vegetables (spinach, lettuce, celery) require relatively high levels of available P for maximum growth. Members of the onion family also require high soil P. Beans, sweet potato, cole crops, sweetcorn and melons are relatively unresponsive to P. The mycorrhizal status of plants has rarely been determined in studies of either critical tissue P or critical soil P concentrations for vegetable crops. Considering the potential impact of mycorrhizae on plant P relations, data on the mycorrhizal status of the plants is vital in interpretation and comparison of results for crop growth versus available P.

P fertilization

The rates of P fertilizer applied to vegetable crops varies greatly depending upon the crop, the region and existing soil fertility levels (Maynard and Thompson 1970, Lorenz and Maynard 1980). Compared to field crops, the rates of P applied to vegetables are usually high. According to Lorenz and Vittum (1980) 45 kg/ha P is the average application in vegetables, but rates in excess of 200 kg/ha may be used in high value or high demand crops. General recommendations for vegetable crops have been compiled by Greenwood et al. (1974) and Lorenz and Thompson (1980).

Placement and method of application

No single method of application of P fertilizers is appropriate for all vegetable crops. Factors such as rooting characteristics, length of growing season, soil structure, temperature and moisture, soil P supply, rate of fertilizer P application and source of P must all be considered. It is recommended that the bulk of planting fertilizer be placed in bands below and to the side of the seed row (Lucas and Vittum 1976). The limited mobility of P restricts problems of burning even when applications are heavy.

In theory, banding should be more efficient than broadcasting

the P, since placement of concentrated P near the seed should promote early growth while reducing P fixation. However, experiments comparing broadcast versus banding in vegetables have produced conflicting results (Lorenz and Vittum 1974). Frequently the amount of P applied to the crop is so large that any potential benefit of banding is obscured. This is particularly evident in soils which have already received repeated applications of fertilizer P. When low rates of P are applied to soils with sub-optimal amounts of available P, banding has been shown to be superior to broadcasting (Salinas and Sanchez 1981). Banding may also be superior in temperate regions where cool soils early in the season limit root growth and P uptake (Lorenz et al. 1964). Highly soluble forms of P are generally preferred in the production of vegetable crops. Immediate availability of P during the crucial early growth stages is imperative for optimal yields.

Attempts at utilizing low solubility P sources have met with limited success (Lorenz and Vittum 1980). Despite very high application rates, the available P supplied by these fertilizers is generally inadequate to meet the demands of a rapidly growing crop. Starter solutions of soluble P are generally applied to transplanted crops at the time of field setting to aid in rapid establishment.

Foliar applications of P is usually uneconomical, as several applications/week are required to meet the demands of most

vegetable crops (Silberstein and Wittwer 1951). Research on most vegetable crops indicates that all of the P fertilizer should be applied at seeding or transplanting. Responses to later applications are rare. This reflects the immobility of the element in the soil as well as the critical need for adequate P during early growth.

P fertilization and micronutrients

Excessive levels of P fertilizer have been shown to reduce the availability and uptake of various micronutrients (Adams 1980). This problem is of considerable importance in vegetable crops due to the prevailing practices of applying P to soils already high in available P. The effect of P fertilization on micronutrient concentrations in various vegetables are summarized by Lorenz and Vittum (1980). Zinc deficiencies are the most common result of excessive P applications, particularly on high pH soils (Peck and MacDonald 1972).

MYCORRHIZAE AND VEGETABLE CROPS

Researchers interested in the physiology of the mycorrhizal symbiosis frequently employ horticultural crops as the test host. Vegetables are particularly popular as experimental hosts; their small size and rapid maturation makes them amenable

to growth in pots under greenhouse conditions. Most importantly, many of the vegetable crops are very responsive to mycorrhizae under pot conditions. This reflects the high P demand, rapid growth and limited root system factors discussed in the previous section. Miller et al. (1986) reviewed the extensive literature pertaining to pot studies of the mycorrhizal symbiosis in vegetables. Many of the host, endophyte and environmental variables which influence the establishment and effectiveness of any VAMF association in the field cannot be adequately tested or controlled in greenhouse pot studies. Pot studies are, therefore, of limited value as indicators of the existing or potential contributions of VAMF to the growth of vegetable crops in the field. This section will review; 1) the existing data pertaining to the contribution of VAMF to the growth and nutrition of field-grown vegetables, 2) speculations as to the potential contributions by VAMF, and 3) important concepts in the management of the VAMF symbiosis under field conditions.

The benefits of mycorrhizal associations on plant growth are greatest and most frequently observed when crops are grown with low inputs on soils with low nutrient availability. Conversely, vegetable crop production in developed countries usually involves intensive cultivation and management, with inputs optimized in order to produce maximum plant growth. Many of the management procedures employed in commercial vegetable crop

production are potentially detrimental to the survival and establishment of mycorrhizal fungi.

Vegetable fields are extensively cultivated prior to seeding and during crop growth. Krucklemann (1975) and Schenck and Kinlock (1976) found that the size and diversity of the VAMF inoculum supply declined as soil disturbance by cultivation increased. Vegetable crops are frequently grown as monocrops or in very limited rotations with other vegetables. Prolonged monocropping reduces the diversity of mycorrhizal species (Krucklemann 1975), although total inoculum levels may increase or decline depending upon the host species and the fertility conditions (Hayman 1975). Inclusion of nonmycorrhizal crops or a fallow period in the crop rotation results in a drastic reduction in inoculum density (Black and Tinker 1977 and 1979).

The high value of vegetable crops, coupled with their sensitivity to disease and insect damage has led to an extensive reliance on pesticides in modern operations. Many of the compounds used for pest control are either directly toxic to the VAM fungi or are inhibitory to colonization of plant roots (Barrows and Roncadori 1977, Nemeč 1980). The systemic nature of the VAMF infection makes the fungi very susceptible to the increasingly popular systemic fungicides (Bailey and Safir 1978). Several of the commonly applied nematocides are also toxic to mycorrhizae (Nemeč 1980). There is an urgent need for more research on the impact of pesticides on VAMF.

Due to tightening restrictions on the application of traditional nematicides such as EDB, vegetable growers are increasingly opting for soil fumigation for control of nematodes and persistent soil-borne diseases such as Verticillium wilt. The VAM fungi are very sensitive to the methylbromide chloropicrin formula used in soil fumigation (Menge et al. 1978). Fumigation generally eliminates the VAM fungi in the top meter of soil, which is the region of greatest inoculum potential and root development in most soils (Menge et al. 1978).

In commercial vegetable crop production, P fertilizers are regularly applied to soils already high in available P. As VAMF colonization is frequently inhibited when the host plant has ample P, the levels of VAMF in these intensively fertilized fields is likely to be low. Hayman (1982), however, found abundant VAMF in heavily fertilized fields of corn in the mid-western United States. Certain isolates of the VAMF may be able to survive and colonize plants even under high soil fertility conditions.

Soilless mixes are becoming increasingly popular in the production of vegetable transplants and in the culture of vegetables in greenhouses. These mixes are devoid of indigenous mycorrhizae. In transplanted crops, the field residence time after transplanting may be insufficient to allow for the

development of a functional infection or for the reproduction of the VAM fungus.

These data suggest that the mycorrhizal colonization of commercial fields is probably relatively rare. It would seem unlikely that the VAMF are making a significant contribution to the crop. There are several methods for testing this hypothesis. One approach is to monitor crop performance following elimination of the mycorrhizal fungi. Maronek et al. (1981) reported that large acreages of strawberry and tomato are fumigated in the United States without any apparent ill effects on subsequent crop growth. The VAMF association appears to be of little consequence under the prevailing production conditions in those crops. In onion (Owusu-Bennoah and Mosse 1979), cassava (Vander Zaag et al. 1979, Kang et al. 1980), and cowpea (Islam 1977) fumigation may result in substantial growth and yield reductions. The effect of the loss of mycorrhizae is greatest when soil P levels are low, indicating that the mycorrhizae are improving the plants' nutritional status. Onions and cassava, because of their high degree of mycorrhizal dependancy, may be difficult to grow on fumigated lands, irrespective of the amount of P available to the plants (Owusu-Bennoah and Mosse 1979, Vander Zaag et al. 1979).

Another approach for assessing the potential role of VAMF in vegetable crops is to monitor the effect of adding inoculum to the plants. This addresses the question of whether the lack of

observed VAMF response in a given situation means that the symbiosis is of no consequence under the prevailing conditions or whether the lack of response is due to some shortcoming in the supply or effectiveness of the indigenous fungi. Adding inoculum to the soil increases the total inoculum density and may also increase the diversity and effectiveness of the VAMF population. Mosse and Hayman (1980) reviewed the published field experiments on inoculation with VAMF. Vegetable crops generally responded positively to added inoculum, particularly when soil P was low. When the supplemental inoculum consisted of VAMF species indigenous to the soil tested, the observed growth responses likely represented a response to increased inoculum density (Khan 1972, Islam 1977, Black and Tinker 1977, Kang et al. 1980). This supports the hypothesis that a lack of inoculum may limit the potential for VAMF associations in intensively managed vegetable crops. Where pure inoculum of introduced VAMF strains were applied, the improvements in growth may have been due to increased inoculum density or increased infectivity or effectiveness of the introduced VAMF relative to the indigenous species (Owusu-Bennoah and Mosse 1979). Negative (Ross and Harper 1970) or absent responses (Bagyaraj et al. 1979) indicate that either the changes in host nutrition or physiology provided by the symbiosis were not limiting the growth of the crop under the prevailing conditions or that the introduced inoculum did not successfully colonize the plant

roots. In all of the field studies, the beneficial effect of mycorrhizae could be duplicated by adding fertilizer P to the soil. Improved P nutrition appears to be the basis of the observed mycorrhizal response.

In developed countries, vegetable crop production presently hinges on the utilization of intensive inputs to maximize plant growth. If the mycorrhizal effect can be duplicated by adding fertilizer, habit, simplicity and dependability will likely cause growers to opt for that approach rather than attempting to manipulate and manage the mycorrhizal symbiosis. In that case, the role of VAMF in commercial production of most vegetable crops is likely to be minimal. Changing conditions in the foreseeable future may alter this situation. The public and governments are increasingly concerned about pollution stemming from run-off or seepage from excessively fertilized agricultural lands. As intensive users of P fertilizer, vegetable growers are also faced with rising costs and dwindling supplies of high analysis P rock. These developments are causing growers to seek means of reducing fertilizer inputs without excessive yield losses. The proven ability of mycorrhizae to improve P use efficiency may allow growers to cut-back their P applications or to increase their utilization of the more inexpensive and abundant low solubility P fertilizers. The potential for using VAMF to increase fertilizer use efficiency is a promising field for investigation.

There is also emerging evidence that mycorrhizae may also influence a range of physiological processes in addition to the mineral nutrition of the host ie; water relations, stress and disease tolerance and hormonal balances. Whether the VAMF symbiosis may be manipulated in order to produce a consistent, desirable effect on any of these additional variables has yet to be adequately determined.

Although mycorrhizae are, at present, of questionable significance in high input vegetable production, the symbiosis is of great importance under the low input situations characteristic of developing countries. Prohibitive costs of P fertilizers and problems of supply, limit the amounts of soluble P available to the growers. In tropical countries the dominant Oxisols and Ultisols are commonly deficient in P and exhibit high P fixation capacities (Salinas and Sanchez 1981). Consequently, crops are grown with relatively low levels of available P, with low solubility rock P forming the bulk of the added P fertilizer. Mycorrhizae improve the crops' tolerance of low soil P conditions while making more efficient use of the P released by the dissolving rock P. The role of mycorrhizae in cassava production is illustrative. The crop is of great significance in many developing regions because of its substantial yields of starch on soils too deficient in P to allow adequate growth of less tolerant crops. Although the crop is tolerant of low soil P, the cassava root system is not

particularly efficient at P uptake (Edwards et al. 1977). Very high levels of available P are required for growth if VAMF colonization of the plants is inhibited (Vander Zaag et al. 1977). The mycorrhizal symbiosis is imperative for growth of cassava under low P conditions.

Another largely overlooked low input situation for vegetable crop production is the home garden. The soil is frequently the low fertility sub-soil excavated during the construction of adjacent buildings. Fertilizer inputs rarely match crop requirements. Indigenous mycorrhizae are likely present at low densities under these conditions and the addition of inoculum may represent a long-lasting dependable improvement in crop nutrition. The potential for mycorrhizae to improve stress and disease tolerance may also be useful in the average home garden.

INOCULATION OF VEGETABLE CROPS

The ubiquitous occurrence of mycorrhizae in most soils makes the inoculation of crops a questionable process. If the fungi are already there, is the crop not already receiving the maximum potential benefits of the VAMF symbiosis? Some of the reasons for supplemental inoculation of vegetable crops were discussed in the previous section. Indigenous fungi may be rare or absent due to the adverse cultural or fertility conditions or they may have been eliminated by pesticides. As a consequence,

colonization is slow and the crop does not receive the benefits of the symbiosis in a timely fashion. Alternatively, the indigenous fungi may be ineffective. Indigenous species are usually adapted to the prevailing host and soil conditions. If these are changed by cultivation, fertilization or by the planting of a different host species, infection by the indigenous fungi may fail (Mosse 1977, Powell and Daniel 1978). Finally, supplemental inoculation may be used to introduce fungi selected for their effectiveness under the new host and cultural conditions.

The need for inoculation arises from the desire to either increase the inoculum level in the field or to introduce new strains of fungi. If the goal is to increase the levels of resident fungi, the most expeditious approach is to grow one or two crops of a compatible host species, insuring that cultural and fertility conditions favorable to the symbiosis are maintained (Mosse and Hayman 1980). The resulting accumulation of spores and infective root residues should alleviate any problems related to insufficient inoculum densities. This soil may also be used as an inoculum supplement in other fields (Black and Tinker 1977).

When there are no indigenous mycorrhizae or when a new selected fungi is to be introduced, large amounts of inoculum must be added to the field. VAM fungi cannot be cultured axenically, a host plant is required for multiplication of the

fungus. Inoculum production involves growing an appropriate host in sterilized media inoculated with the desired fungus (Ferguson and Woodhead 1982). Although the basic requirements for inoculum production are known, efforts at increasing efficiency and yields have just begun (Coltman et al. 1988). At present, the difficulties involved in producing sufficient inoculum is the main obstacle limiting the broad use of VAM fungi.

Presently, two main types of inoculum are feasible; VAMF colonized roots and VAMF infested soil carrying spores and infected root fragments. In VAMF species which produce large numbers of spores at the soil surface (*G. epigeous*) or in large discrete sporocarps (*G. aggregatum*) it may also be possible to recover sufficient quantities of spores to allow for their use as inoculum. The relative merits of the various inoculum types remains to be adequately determined. Production factors such as relative yields and ease of storage and handling require investigation. Studies on the relative effectiveness of the various inoculum types are inconclusive. Hall (1979) found that the relative effectiveness of spore versus root inoculum varied with the fungal species. Manjunath and Bagyaraj (1981) found that spores were more effective than roots in sterile soil, but were of equal effectiveness in unsterile soil.

Inoculum application

Several different methods of inoculum application may be employed with vegetable crops, depending on whether the crop is direct seeded or transplanted. Pre-inoculation of transplants appears to be the most efficient means of inoculation. The inoculum only needs to be mixed into the relatively small area of the nursery beds or trays. Competition between strains of the fungi may be easily managed under these conditions. Since the mycorrhizae association would be well established at transplanting, there should be little delay in the commencement of the symbiotic effect. Any corresponding benefits would occur early, when the plants are most sensitive to nutrient stress. Pre-inoculation should, therefore, be more effective than field inoculation in rapid turnover crops such as lettuce. Pre-inoculation with VAMF may increase survival and speed of establishment of transplanted crops (Barrows and Roncadori 1977, Johnson and Crews 1979) while reducing the fertilizer P required for transplant production (Maronek et al. 1983).

Specific infection of transplants should not necessitate excessive departures from the current production systems (Maronek et al. 1981). However, management techniques aimed at optimizing the establishment of the VAM fungi without adversely affecting the growth of the transplants need to be developed. The heavy fertilizer rates presently employed during transplant

production would likely inhibit mycorrhizal colonization of the plants. The commonly used pesticides need to be evaluated for their impact on the establishment of the fungi.

For seeded crops, placement of the inoculum adjacent to the seed represents a more efficient use of limited inoculum than broadcast applications. Placement of the inoculum near the seedling promotes rapid development of the infection, while favoring the selected endophyte over the more dispersed indigenous fungi (Jackson et al. 1972, Mosse and Hayman 1980). All three types of inoculum may be mixed with a flowable carrier to aid in even distribution (Menge and Timmer 1982). Inoculum may also be applied directly to the seed using a sticker (Crush and Pattison 1975), by pelleting or in a fluid drilling program (Jung et al. 1981). Seed pelleting and fluid drilling are already popular in vegetable crop production as aids to precision planting and rapid uniform emergence.

At present, the economics of applying mycorrhizal inoculum to vegetable crops are difficult to determine. Its value, in terms of increased growth, improved transplant survival and reduced fertilizer requirements must be balanced against the costs and effort required in inoculum production and application and any growth or management restrictions necessary to insure establishment. Persistence of the inoculum and spread in the field will effect the cost/benefit calculations. If each crop must be inoculated, the short growing season and resultant rapid

turnover of many vegetable crops would require the production and distribution of restrictively large amounts of inoculum. If inoculation proves to be too costly, efforts to stimulate symbiotic associations with naturally occurring endophytes may be more productive.

CONCLUSION

Knowledge of the VAMF symbiosis has increased enormously since the first comprehensive research on host-endophyte interactions began in the 1960's. The VAMF association has been shown to improve the growth of many plant species under widely divergent growing conditions. Growth improvements are most dramatic when soil P is deficient and the host root system is poorly adapted to low soil P availability. The mycorrhizae increase the P uptake of infected roots, thereby alleviating any depression in growth related to low soil P. The extent and effectiveness of mycorrhizal development is determined by interacting host-endophyte-environmental factors. The association is largely autoregulatory, developing to the required degree without placing excessive demands on either the host or endophyte. The VAMF association may also influence micronutrient uptake, stress tolerance and the hormonal balance of the plant.

The mycorrhizal status of the plant material utilized in most studies of plant nutrition and physiology is usually unknown or not reported. Considering the range of physiological processes potentially influenced by VAMF, the absence of information on the mycorrhizal status raises questions about the applicability and reproducibility of the data from these experiments. Differences in mycorrhizal status may help explain why pot studies are of limited use as predictors of plant responses under field conditions.

The majority of information on the VAMF symbiosis has been derived from pot studies conducted under controlled greenhouse conditions. The role of mycorrhizae under field conditions requires further investigation. The need is particularly great in horticultural crops. Many of these crops are extremely responsive to mycorrhizae in pot trials. At present, the existing or potential significance of the VAMF to horticultural crops in the field is virtually unknown.

Additional research into the following aspects of the VAMF association in horticultural crops is needed;

- 1) Mechanisms and processes ultimately leading to increased vigor and growth of mycorrhizal plants.

- 2) Factors determining infectivity and effectiveness of VAM fungi under differing host and environmental conditions.

- 3) Selection of fungi for maximum symbiotic effectiveness, competitiveness, tolerance to environmental stress, tolerance to mineral deficiency or toxicity and resistance to pesticides.
- 4) Prior knowledge of the potential responsiveness of the crop or cultivar under the prevailing conditions.
- 5) Management of inputs, cultural practices and pesticides to maximize potential benefits from the mycorrhizae.
- 6) Inoculum production and application. Determination of whether a single species or isolate should be used as inoculum rather than a mixture of fungal species. For a given host, site and climatic combination, one species or isolate may be best, but this determination may be difficult. The alternative is to use a mixed inoculum source, with the hope that at least one species will be suitable under the existing conditions.
- 7) Determination of the cost/benefit/risk of increasing reliance on mycorrhizae as an alternative or amendment to fertilizer or other management inputs. Regional differences in the practices and economics of production must be considered when evaluating the significance of the mycorrhizal symbiosis.

Chapter III

Phosphorus Concentration and Application Interval Influence
Growth and Mycorrhizal Infection of Tomato and
Onion Transplants¹

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Introduction

There is increasing interest in using vesicular-arbuscular mycorrhizal fungi (VAMF) in horticultural crop production since VAMF may improve plant disease resistance (Dehne 1982) and drought tolerance (Nelsen and Safir 1982), and increase uptake of immobile nutrients, such as P and Zn (Ames et al. 1983, Mosse 1973). The benefits of VAMF may be of particular importance in the infertile soils of the tropics. Inoculation of seedlings prior to transplanting may increase crop uniformity, reduce transplant mortality and improve growth (Barrows and Roncadori 1977, Biermann and Linderman 1983, Menge et al. 1978). Infection of plants within the confines of transplant flats may require less VAMF inoculum than field inoculation. Transplants can be inoculated with pure strains of highly effective VAMF, giving the introduced strains a competitive advantage over strains encountered in the field.

Information on the production of VAMF-infected transplants is limited. Transplants generally are given ample fertilizer P to encourage vigorous early growth. However, high rhizosphere P levels promote high tissue P concentrations that inhibit the development and spread of the mycorrhizae within the root system (Menge et al. 1978). This study examined the growth, shoot P concentration, and mycorrhizal infection of tomato and onion transplants when the concentrations and intervals between

applications of P fertilizer were varied. The goal was to develop a P fertilization regime that would yield vigorous, horticulturally acceptable transplants with a significant degree of mycorrhizal colonization.

Materials and Methods

The treatments were factorial combinations of P concentration, intervals between P solution application, and mycorrhizal inoculation (inoculated or noninoculated). The treatments were randomly arranged on a greenhouse bench with four replicates per treatment and four to six plants per replicate. Tomato and onion transplants were grown simultaneously but in independent experiments. The experiments were conducted without supplemental lighting during November and December 1985. The photoperiod was about 11 hr. Average day/night temperatures were 31/23°C.

Seeds of 'Healani' tomato and 'Yellow Granex' onion were surface sterilized in 90% ethanol for 1 min followed by 0.5% sodium hypochlorite for 10 min. The sterilized seeds were sown into transplant flats containing a mixture of 1 sphagnum peat : 1 vermiculite (by volume) that had been adjusted to pH 6.5 with 3.0 kg dolomitic limestone per m³. Micronutrients were supplied as 0.5 kg Micromax (Sierra Chemical Co., Milipitas, Calif.) per m³. Transplant cell volume was 100 ml. In the

mycorrhizal treatments, each cell received 500 10-mm-long, dried, sweetcorn (Zea mays L. 'Hawaiian Supersweet #9') root fragments heavily infected with the VAMF Glomus aggregatum. The inoculum and the transplant cell medium were mixed thoroughly before seeding. Aliquots of mycorrhizae-free washings from the root fragments were added to the noninoculated treatments to insure equivalent background populations of microorganisms. The seeds were then covered with a thin layer of the medium.

Phosphorus was supplied by adding 25 ml per plant of solutions containing 4, 16, 64, or 256 mg P/liter from NaH_2PO_4 . These P solutions were applied daily, every 4 days or every 8 days. Every 8 days, each seedling received macro-nutrients as 25 ml of one-half strength phosphorus-free Long Ashton solution (Hewitt 1966). The appropriate concentration of P for each treatment was included in this solution. On days when the plants were not scheduled to receive P fertilizer or macro-nutrients, they were given 25 ml of distilled water.

Plants fertilized daily received the same cumulative amount of P over each 8 day fertilization cycle as plants fertilized twice per cycle with the next higher concentration of fertilizer. This arrangement of treatment levels allowed for comparison of the relative effect of delivering a given amount of fertilizer as a diluted solution with short intervals between

applications versus applying a more concentrated form less frequently.

Fresh weights of shoots and roots were determined 40 days after planting. Roots were cut into 10-mm lengths and a random sample from each replicate was cleared and stained (Phillips and Hayman 1970). The degree of mycorrhizal infection of 60 root segments was visually rated as follows: 0= no infection, 1= entry points only present, 2= small areas of hyphae present, 3, 4 and 5 = hyphae present over 50, 75 and 100% of the root, respectively. Phosphorus concentrations in the shoots were determined colorimetrically (Murphy and Riley 1962) after dry ashing at 550°C.

Results and Discussion

The total fresh weights (TFW) of the tomato and onion transplants were influenced similarly by the varying P fertilizer regimes. The main effects of, and interactions between, P concentration and application intervals on TFW were all significant (Table 3.1). At low P concentrations, decreasing the interval between solution applications increased TFW (Fig. 3.1). At higher concentrations, shorter application intervals had little effect on TFW.

The main effect of inoculation on TFW was not significant for either species, but the interaction between inoculation and

Table 3.1. F-test significances for total fresh weight, shoot P concentration, and mycorrhizal infection of tomato and onion transplants inoculated or not inoculated with Glomus aggregatum and grown with differing concentrations and intervals between application of P fertilizer.

Source of variation	Total fresh weight		Shoot P concn		Root infection intensity	
	Tomato	Onion	Tomato	Onion	Tomato	Onion
Inoculation (I)	0.28	0.41	0.16	<0.01		
P concn (C)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Interval (V)	<0.01	0.03	<0.01	<0.01	<0.01	<0.01
I x C	<0.01	<0.01	0.21	0.03		
I x V	0.63	0.29	0.46	0.52		
C x V	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
I x C x V	<0.01	0.17	0.21	0.63		

I - VAMF inoculated vs. noninoculated.

C - solution P concentrations of 4, 16, 64 or 256 mg/liter.

V - P solutions applied daily, every 4 days or every 8 days.

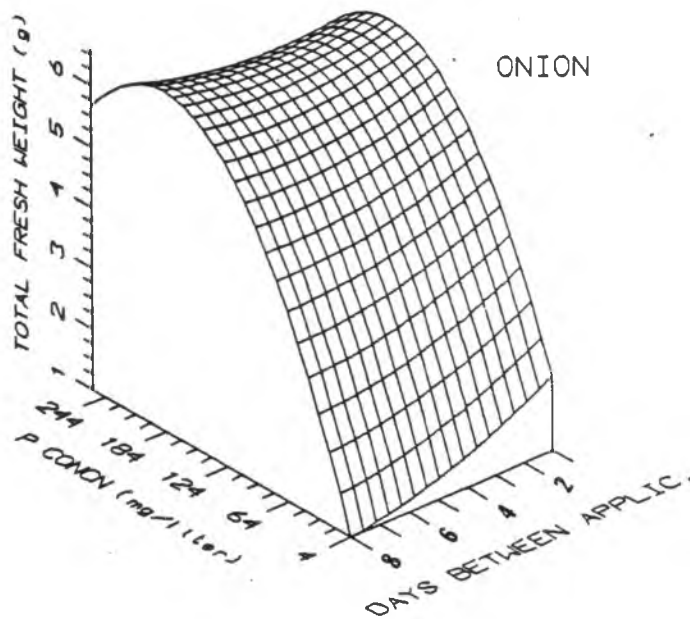
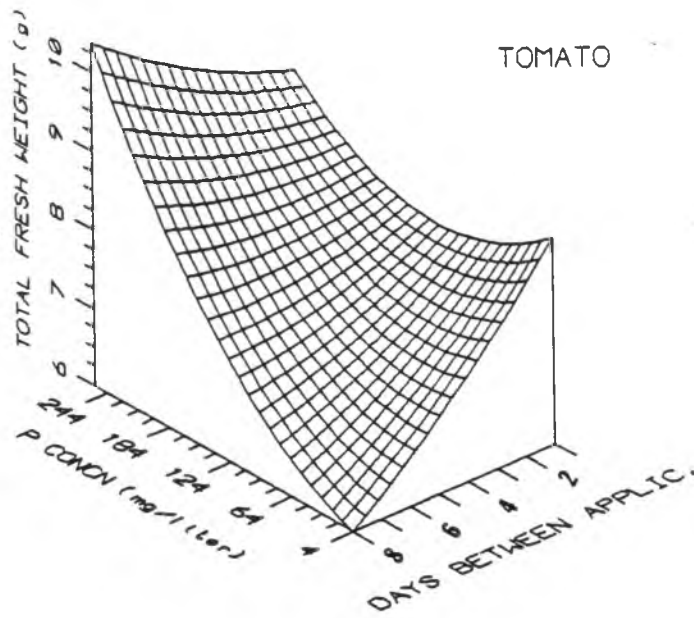


Fig. 3.1. Total fresh weights (TFW) of tomato and onion transplants grown with various concentrations (C) and application intervals (V) of P fertilizer. Response surfaces generated from the following equations: Tomato TFW = $9.144 - 0.014 C - 0.537 V + 0.001 C^2 + 0.002 CV + 0.001 V^2$. $R^2 = 0.47^{**}$. Onion TFW = $2.799 + 0.056 C - 0.349 V - 0.001 C^2 - 0.001 CV$. $R^2 = 0.53^{**}$. Equation variables significant at the 5% level.

P concentration was significant for both tomato and onion (Table 3.1). At the lowest P concentration, inoculated transplants were larger than noninoculated plants but at higher P concentrations inoculation did not increase growth (Appendix Fig. 3A).

There was a significant three-way interaction between inoculation, P concentration and application interval on tomato TFW (Table 3.1). With low P concentrations, inoculation increased TFW to a greater degree with daily P applications than with less frequent applications (Fig. 3.2). With high P concentrations, inoculated transplants were smaller than noninoculated plants and inoculation depressed plant growth to a greater degree when the fertilizer was applied daily than when it was applied less often.

The main effect of inoculation on shoot P concentrations was significant for onion, but not for tomato transplants (Table 3.1). With 4 or 16 mg P·liter⁻¹, inoculation significantly increased onion shoot P concentration, but at higher P concentrations, inoculated and noninoculated onions had similar shoot P concentrations (Appendix Fig. 3B).

Decreasing the interval between P applications or increasing the concentration of P solutions increased the average shoot P concentrations of both species (Fig. 3.3). Decreasing the application interval did not increase the shoot P

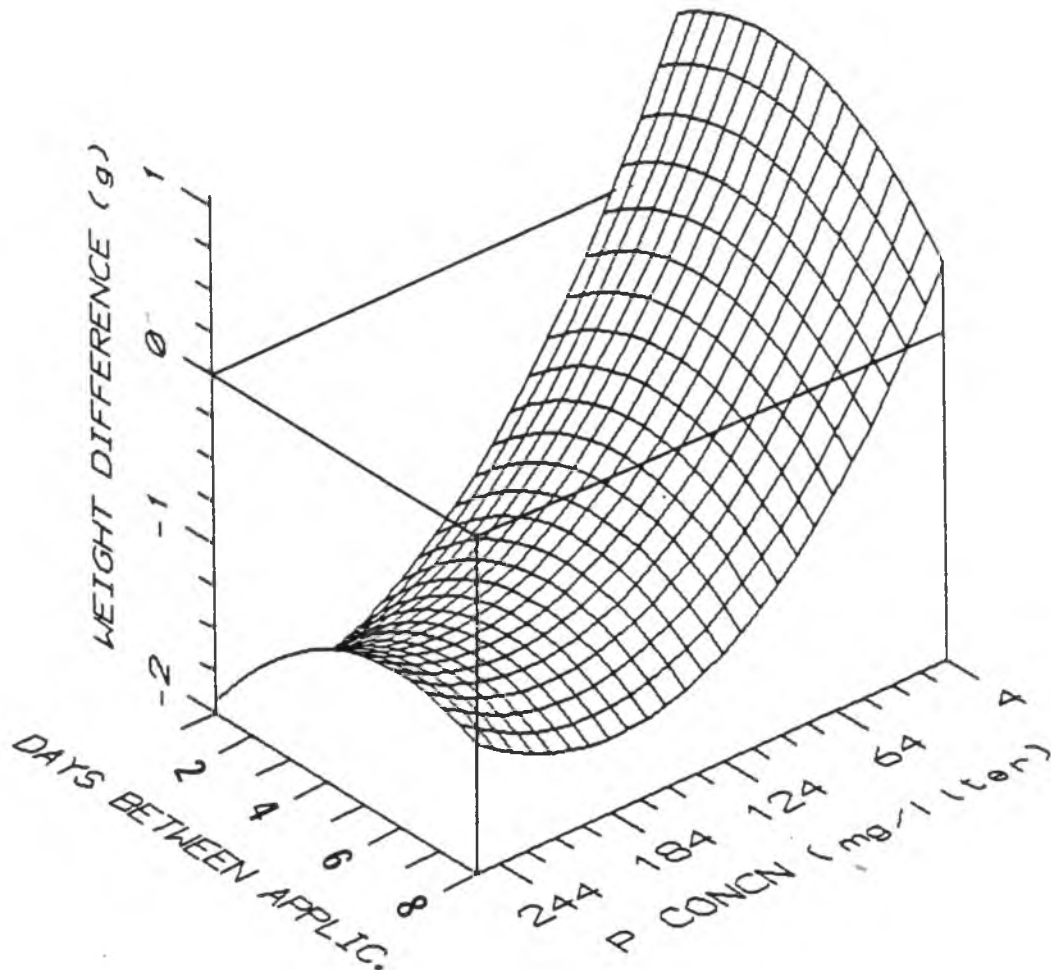


Fig. 3.2. Influence of mycorrhizal inoculation on total fresh weights (TFW) of tomato transplants grown with various concentrations (C) and application intervals (V) of P fertilizer. Response surface represents the difference between TFW of inoculated (I) and noninoculated (NI) plants with each treatment combination, and was generated by subtracting the following equations (I - NI), where $TFW_I = 9.638 - 0.028 C - 0.398 V + 0.001 C^2 + 0.001 CV - 0.001 V^2$. $R^2 = 0.46^*$, and $TFW_{NI} = 8.651 - 0.001 C - 0.641 V + 0.001 C^2 - 0.001 CV$. $R^2 = 0.55^{**}$. Equation variables significant at the 5% level.

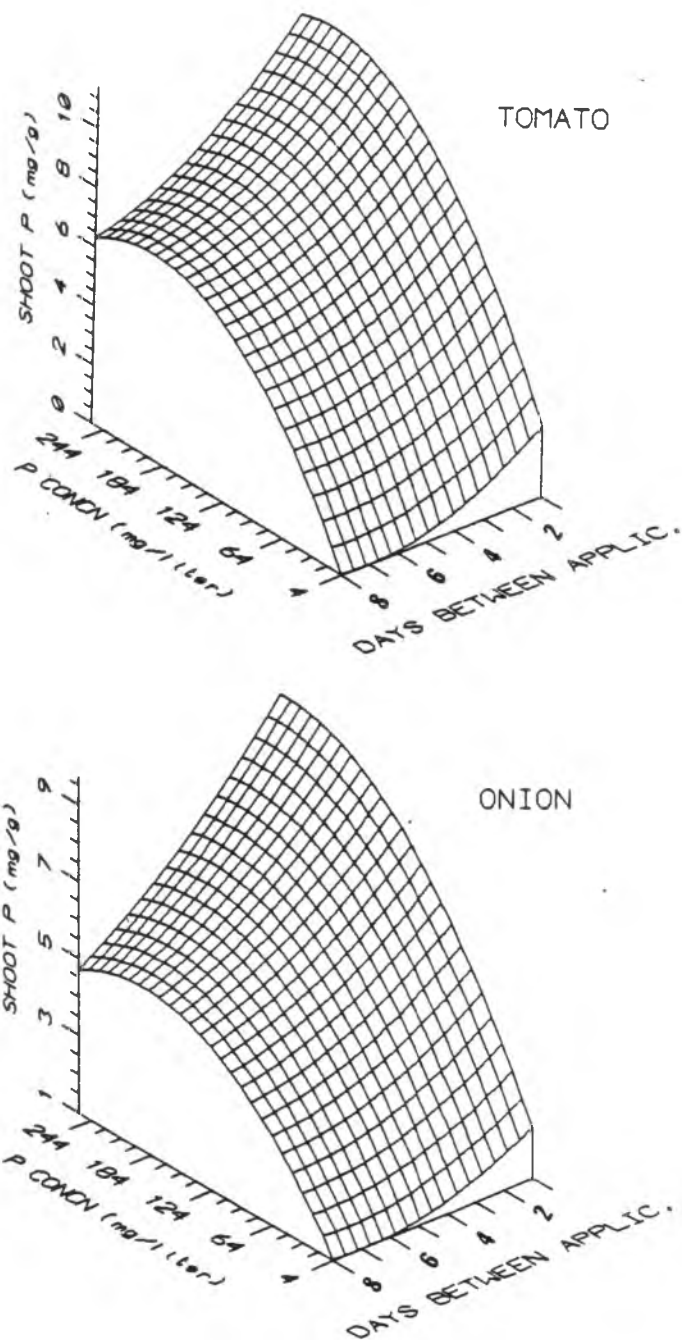


Fig. 3.3. Shoot P concentrations of tomato and onion transplants grown with various concentrations (C) and application intervals (V) of P fertilizer. Response surfaces generated from the following equations: Tomato mg P/g = $3.322 + 0.086 C - 0.041 V - 0.001 C^2 - 0.001 CV + 0.001 V^2$. $R^2 = 0.96^{**}$. Onion mg P/g = $2.857 + 0.063 C - 0.547 V - 0.001 C^2 - 0.001 CV + 0.001 V^2$. $R^2 = 0.94^{**}$. Equation variables significant at the 5% level.

concentrations as dramatically with low applied P concentrations as when more concentrated fertilizer solutions were applied.

The intensity of mycorrhizal infection of the tomato and onion root systems was influenced significantly by both P concentration and application interval (Table 3.1). Decreasing the interval between applications reduced mycorrhizal infection intensity more with low than with high P concentrations (Fig. 3.4).

When a total of 3.2 mg P was applied per 8 day cycle, dilute solutions applied daily increased tomato and onion TFW more than concentrated solutions applied at greater intervals (Table 3.2). When 51.2 mg of P was applied per cycle, less frequent applications of more concentrated solution resulted in larger plants than recurrent applications of dilute solutions. Daily application of dilute P solutions consistently resulted in lower shoot P concentrations than greater intervals between applications of more concentrated solutions.

Infection intensity did not differ between the two P application strategies when 3.2 or 12.8 mg of P were applied during the 8 day cycle. When 51.2 mg of P was added per cycle, the daily application of the more dilute solution gave higher levels infection than the less frequent application of more concentrated solutions.

Vegetable transplants require ample P to promote vigorous early growth. In this study, as the total amount of P applied

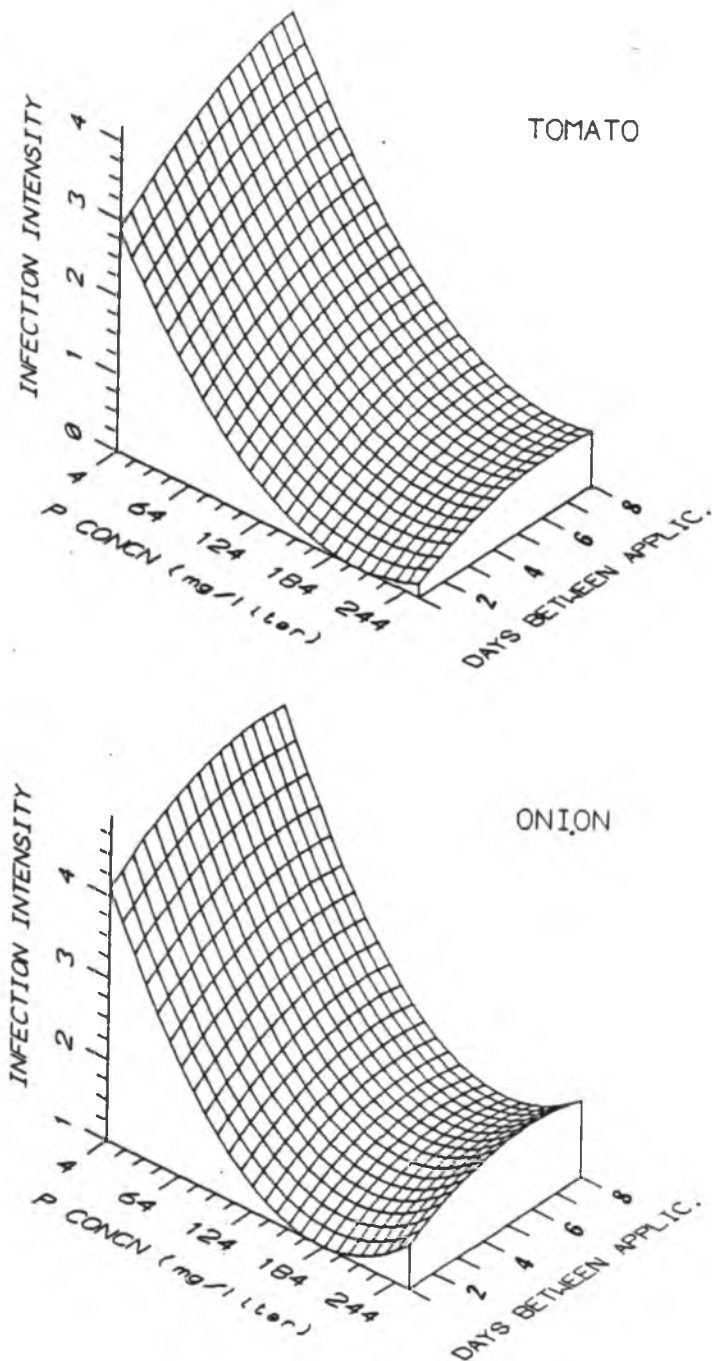


Fig. 3.4. Intensity of mycorrhizal infection (MI) of tomato and onion transplants grown with various concentrations (C) and application intervals (V) of P fertilizer. Rating of 0 - no roots infected, 5 - all roots heavily infected. Response generated from the following equations: Tomato MI = $2.633 - 0.029 C + 0.386 V + 0.001 C^2 - 0.001 CV$. $R^2 = 0.87^{**}$. Onion MI = $3.432 - 0.036 C + 0.352 V + 0.001 C^2 - 0.001 CV - 0.001 V^2$. $R^2 = 0.84^{**}$. Equation variables significant at the 5% level.

Table 3.2. Total fresh weights, shoot P concentrations and intensity of mycorrhizal infection of tomato and onion transplants inoculated with *Glomus aggregatum* and supplied with equal amounts of P delivered at different concentrations and application intervals.

Total P applied per cycle (mg/plant)	P treatment (concn x interval)	Total wt (g/plant)		Shoot P (mg/g)		Infection intensity ^Z	
		Tomato	Onion	Tomato	Onion	Tomato	Onion
3.2	4 x 1 ^Y	9.6	2.8	1.6	2.0	2.8	3.5
	16 x 4	7.4	2.6	1.8	2.2	2.8	4.1
Contrast ^X		**	*	NS	NS	NS	NS
12.8	16 x 1	9.2	3.4	4.2	3.7	2.2	2.7
	64 x 4	6.8	3.4	5.1	4.4	2.2	2.5
Contrast		**	NS	**	*	NS	NS
51.2	64 x 1	6.7	3.7	7.8	5.8	1.8	2.5
	256 x 4	10.3	6.8	8.2	7.0	0.6	2.0
Contrast		**	**	*	**	**	*

^Z Rating of 0 = no roots infected, 5 = all roots heavily infected.

^Y Concentration = mg P liter⁻¹. Interval = days between P applications.

^X Contrasts nonsignificant (NS) or significant at 5% (*) and 1% (**) levels.

to the transplants was increased, the shoot P concentration and growth of the transplants increased, but the intensity of mycorrhizal infection of the roots of the transplants declined. Interactions between P availability, tissue P content, plant growth, and VAMF infection have been well documented (Biermann and Linderman 1983, Graham et al. 1981). Mycorrhizal infections can reduce growth by utilizing carbohydrates from the host without providing any beneficial contribution to the plant's mineral nutrient status (Stribley et al. 1980). Low P availability in the medium results in low tissue P, reducing growth, but enhancing mycorrhizal infection. By increasing the absorptive area of infected roots the VAMF may improve the plant's P foraging ability, alleviating the nutrient stress on the plant.

Daft and Nicolson (1969) reported that the infection of corn roots by Endogone macrocarpa was affected more adversely by a single application of concentrated P solutions than by recurring applications of more dilute solutions. We found a similar trend for G. aggregatum infection of tomato and onion. For the same total P application, shoot P concentrations were lower in the plants given P at low concentrations with short intervals between applications. This may have facilitated more rapid mycorrhizae establishment and spread in those treatments.

The production of horticulturally acceptable, yet mycorrhizal, tomato and onion transplants requires a compromise

between supplying enough P to support vigorous host growth and maintaining tissue P levels low enough to promote the establishment and spread of the mycorrhizal symbiosis. Daily applications of solutions that contained $4 \text{ mg P} \cdot \text{liter}^{-1}$ produced vigorous growth and strong mycorrhizal infection of the tomato transplants (Figs. 3.1 and 3.4). However, the response surfaces for the onion transplants indicated that the best combined growth and mycorrhizal infection would be obtained using solutions of about $150 \text{ mg P} \cdot \text{liter}^{-1}$ applied every four days (Figs. 3.1 and 3.4). The difference in recommendations for the two crops reflects the stronger growth response of onions to P concentrations and their weaker responsive to application intervals. Appropriate P fertilization rates and intervals between applications for mycorrhizal vegetable transplants of other species also may vary.

Chapter IV

Effects of Controlled-Release Phosphorus and Mycorrhizae
Inoculum Density on Leek and Pepper Transplants¹

¹Submitted to; HortScience.

Introduction

Many horticultural plants are capable of forming symbiotic associations with vesicular-arbuscular mycorrhizal fungi (VAMF) (Barrows and Roncadori 1977, Miller et al. 1986). Inoculation of plants with VAMF may increase the efficiency of nutrient and H₂O uptake (Maronek et al. 1981), improve plant uniformity (Barrows and Roncadori 1977), and increase disease resistance (Dehne 1982). Mycorrhizal infection can improve the survival and regrowth of transplanted crops (Barrows and Roncadori 1977, Biermann and Linderman 1983b).

Soilless media frequently are used in the production of nursery stock, bedding plants, and vegetable transplants. These media usually lack indigenous VAMF, and attempts to produce plants heavily infected with VAMF through the introduction of inoculum have not been very successful (Biermann and Linderman 1983, Menge et al. 1982). The high rates of soluble P typically used with soilless media inhibit establishment of, and host growth response to VAMF (Biermann and Linderman 1983). Moderate levels of VAMF infection have been obtained in soilless media when available P levels have been kept low by limiting the addition of soluble P, or by the addition of P-sorbing amendments to the medium (Biermann and Linderman 1983).

Controlled release sources of P may provide a convenient means of avoiding problems of excess P availability. Nutrient

release characteristics of plastic-coated fertilizers such as Osmocote tend to be more predictable than those of low-solubility P sources, such as rock phosphate (Oertli and Lunt 1962). Verkade and Hamilton (1983) and Maronek et al. (1980) used Osmocote to produce mycorrhizal seedlings of various ornamental tree species on soilless media. However, information on the potential for using Osmocote in the production of high quality mycorrhizal vegetable transplants in soilless media is lacking.

The objectives of this study were to: 1) examine the growth, shoot P concentrations, and mycorrhizal colonization of pepper (Capsicum annum L. 'Emerald Giant') and leek (Allium ampeloprasum L. 'Catalina') transplants under a range of Osmocote P rates, and 2) test the effect of varying mycorrhizae inoculum density at each P level. We sought to determine if high inoculum levels could be used to produce strong mycorrhizal infections in peppers and leeks receiving sufficient P fertilization to produce vigorous, horticulturally acceptable transplants.

Materials and Methods

Experiments with peppers and leeks were conducted on adjacent greenhouse benches during June and July 1986. Each experiment included nine levels of P from Osmocote 19N-2.6P-10K

(0, 43, 60, 90, 120, 180, 240, 360, and 480 g P per m³ of transplant medium) and five levels of inoculation with spores of G. aggregatum (0, 100, 2500, and 12,500 spores per plant).

Treatments were factorial combinations of the P and inoculation levels, with 4 replicates per treatment, and 7 to 10 plants per replicate. Replicates for each crop were completely randomized on the greenhouse benches.

A transplant medium of 1 sphagnum peat: 1 vermiculite (by volume) was adjusted to pH 6.5 by incorporating 3 kg dolomitic limestone per m³. Micronutrients were added as 0.5 kg Micromax per m³ of medium. Osmocote 40N-0P-0K and 0N-0P-38.2K were used to provide equal nitrogen and potassium in all treatments.

Plastic transplant flats with 100 ml cells were filled with the fertilized medium and arranged on greenhouse benches. The flats were sprinkle-irrigated, to excess, twice daily for 6 days to leach excess salts released by damaged Osmocote granules, and to allow the nutrient levels to stabilize. Extra planted flats were included so that solution P concentrations in the fertilized medium could be monitored during transplant development.

Six days after the start of medium leaching, sporocarps of G. aggregatum, sieved from a sweetcorn (Zea mays L. 'Hawaiian Supersweet No. 9') nurse culture, were mixed with water in a high speed blender at 4° C to separate the individual spores.

The spore suspension was diluted to produce 5-ml aliquots containing 100, 500, 2500, or 12,500 spores. Aliquots of the appropriate concentration of inoculum were then pipetted onto the surface of the medium in each cell of the transplant flats. Noninoculated treatments received 5 ml of spore-free washings from the mycorrhizae nurse culture to insure equivalent background populations of microorganisms (Menge and Timmer 1982). Seeds of pepper and leek which had been surface-sterilized by soaking in 90% ethanol for one min followed by 0.5% sodium hypochlorite for 10 min were placed on top of the medium. The seeds were then covered with a thin layer of unfertilized medium.

The transplant flats were sprinkle-irrigated twice daily. Average day/night temperatures were approximately 34°/25°. The photoperiod was about 13.5 hours. Every 2 or 3 days, samples of the solution in the medium of the extra flats were obtained by applying suction to the bottom of the cells. These samples were filtered to remove particulates, and the solution P concentrations were measured colorimetrically (Murphy and Riley 1962).

Fresh weights of shoots and roots were determined 34 days after sowing. Roots were cut into 1 cm lengths and a random sample from each treatment was cleared and stained (Phillips and Hayman 1970). The degree of mycorrhizal infection of 60 root segments was visually rated as follows: 0 - no infection, 1 -

entry points only present, 2 = small areas of hyphae present, 3, 4 and 5 = hyphae present in over 50, 75 and 100% of the root, respectively. Phosphorus concentrations in shoots were determined colorimetrically after dry ashing at 550° C.

Results and Discussion

Solution P concentration was linearly related to the rate of P applied to the medium in the Osmocote by the equation $y = -0.132 + 0.035x$, where y is solution P in $\text{mg}\cdot\text{liter}^{-1}$ and x is Osmocote rate in $\text{kg}\cdot\text{m}^3$ ($r^2 = 0.94$). The type of crop growing in the medium did not affect the solution P concentrations. There was little fluctuation in the solution P concentration associated with each level of Osmocote level P during the experiment (Appendix Fig. 4A). Consequently, the mean solution P concentrations at each Osmocote P application rate were used in the subsequent analyses of transplant growth and mycorrhizal infection. The ability to control growth medium P concentrations is important in the study and management of the symbiosis between VAMF and host plants because solution P concentrations influence the tissue P concentrations, growth, and mycorrhizal infection of the host (Menge et al. 1978). The predictability and stability of solution P concentrations in media fertilized with Osmocote suggest this product may be useful for these purposes.

Total fresh weights (TFW) of the pepper transplants increased with increasing solution P concentrations up to the highest P level obtained (Fig. 4.1). Growth responses to increments of P were greater at the lower solution P concentrations. Total fresh weights of the leek transplants were less responsive to solution P concentration (Fig. 4.1) and were unaffected by inoculation (Table 4.1).

Total fresh weights of peppers were influenced by the interaction of VAMF inoculation with the concentration of P in the medium. With 6 mg P·liter⁻¹ or less in solution, inoculated peppers were larger than noninoculated peppers (Fig. 4.1). At higher P levels, inoculated peppers were smaller than noninoculated plants.

Shoot P concentrations of both leeks and peppers were influenced by the P x I interaction (Table 4.1). Shoot P concentrations of both species increased with increasing P in the medium (Fig. 4.1). At low solution P concentrations, the concentration of P in the shoots of the inoculated leeks was lower than in the noninoculated plants. At higher solution P concentrations, shoot P concentrations were similar in inoculated and noninoculated plants. In contrast, inoculated peppers had higher tissue P levels than noninoculated plants when solution P concentrations were low. Again this difference disappeared as the solution P concentrations increased.

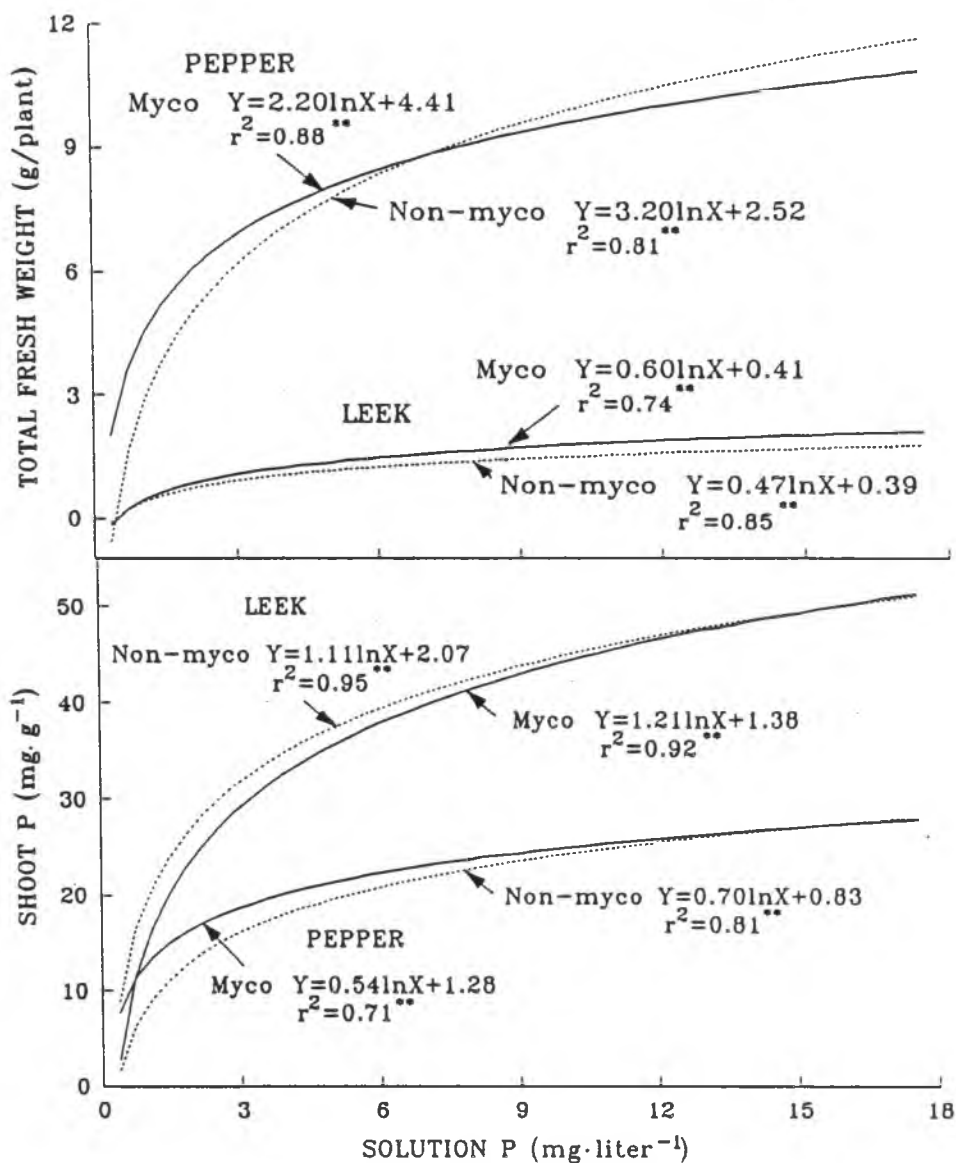


Fig. 4.1. Total fresh weights and shoot P concentrations of mycorrhizal and nonmycorrhizal pepper and leek transplants grown with various solution P concentrations in a soilless medium. Plotted values for mycorrhizal treatments represent means of all inoculum densities.

Table 4.1. F-test significances for total fresh weight, shoot P concentration, and mycorrhizal infection of pepper and leek transplants as influenced by growth medium solution P concentrations and mycorrhizal inoculum density.

Source of variation	Total fresh weight		Shoot P concentration		Root infection intensity	
	Pepper	Leek	Pepper	Leek	Pepper	Leek
Solution P concn (P)	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
Inoculation (I)	<0.01	0.12	0.09	<0.01		
Inoculum density (D)	0.22	0.63	0.46	0.74	<0.01	<0.01
P x I	<0.01	0.20	<0.01	<0.01		
P x D	0.27	0.68	0.53	0.87	<0.01	<0.01

P = solution P concentrations from 0.6 to 17.5 mg/liter.

I = Noninoculated vs 100 to 12,500 spores/plant.

D = 100, 500, 2500, and 12,500 spores/plant.

Inoculum density did not significantly affect the tissue P concentration of either species (Table 4.1).

The intensity of mycorrhizal infection of both crops was significantly affected by the interaction of solution P concentration with inoculum density (Table 4.1). Lower intensities of infection occurred at higher solution P levels (Fig. 4.2). Increasing the numbers of spores in the inoculum increased the intensity of root infection in both crops. At solution P levels from 0.6 to approximately 10 mg·liter⁻¹, higher inoculum densities produced much more intense infections than lower inoculum densities (Fig. 4.2). At solution P concentrations above 10 mg·liter⁻¹, increasing the inoculum density had relatively less effect upon the final intensity of root infection, indicating that the inhibitory effect of P on the establishment and spread of the VAM infection could not be overcome by increased inoculum densities at these higher solution P concentrations. Noninoculated plants showed no sign of mycorrhizal infection.

The interactions between P availability, tissue P, plant growth, and VAMF infection observed in these experiments have been previously explained (Menge et al. 1978). An infection developing under conditions of high P availability may function parasitically, without making any beneficial contribution to a plant's nutrient supply (Stribley et al. 1980). Low P availability reduces growth but enhances mycorrhizal infection

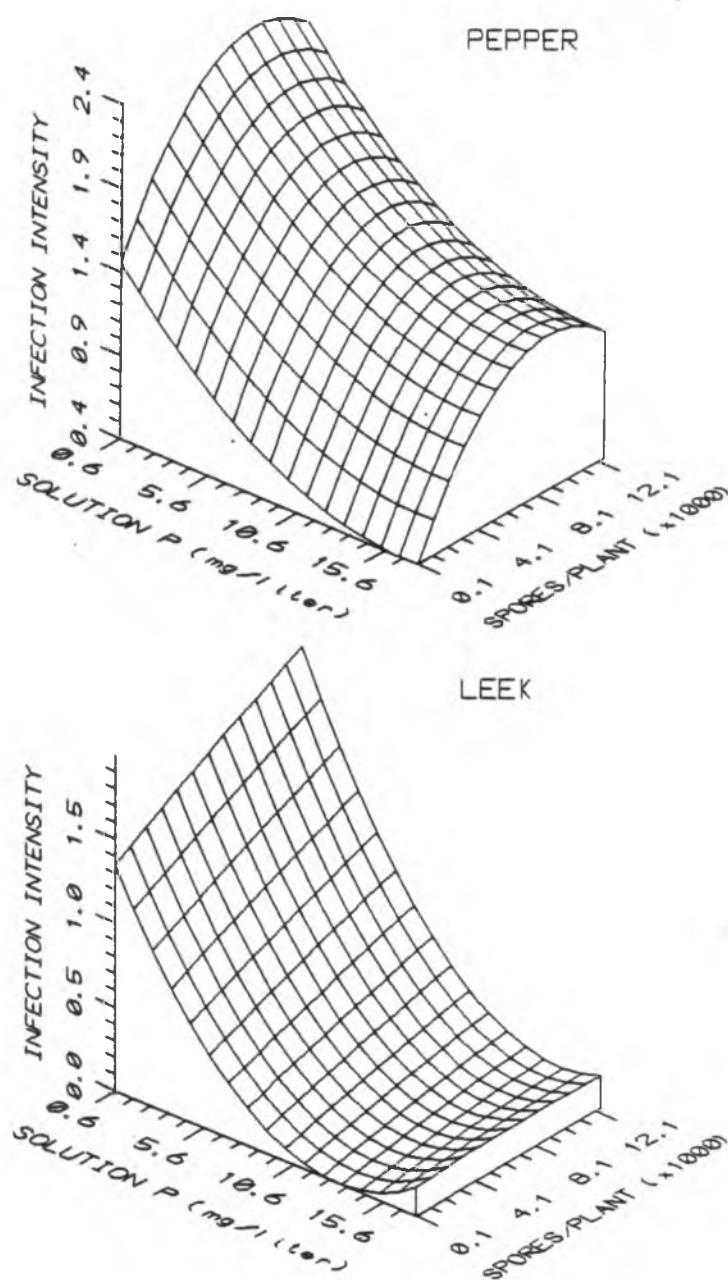


Fig. 4.2. Intensity of mycorrhizal infection in roots of pepper and leek transplants grown in a soilless medium with various solution P concentrations and inoculum intensities. Intensity rating of 0 = no roots infected, 5 = all roots heavily infected. Response surfaces generated from the equations: Pepper $Z = 1.561 - 0.165X + 0.168Y + 0.006X^2 - 0.001XY - 0.008Y^2$, $R^2 = 0.83^{**}$. Leek $Z = 1.505 - 0.240X + 0.062Y + 0.001X^2 - 0.003XY - 0.001Y^2$, $R^2 = 0.77^{**}$. All factors significant at the 5% level.

because mycorrhizal establishment is inversely related to tissue P concentrations in the roots (Sanders 1975). Once functional, the VAMF infection improves the P foraging ability of the plant, often completely alleviating the P deficiency.

By varying the medium P concentration and mycorrhizae inoculum densities, it was possible to produce transplants of pepper and leek of various sizes, shoot P concentrations, and degrees of mycorrhizal infection. Extensive mycorrhizal infections were obtained within 5 weeks from seeding. Although the intensity of root infection may not be an accurate estimator of the functional efficiency of the association (Daft and Nicolson 1972), these results demonstrate that it is possible to produce significant mycorrhizal infections in transplants given enough P to be horticulturally acceptable. Further work is needed to ascertain the field performance of VAMF infected transplants.

Chapter V

Response of Field and Greenhouse-Grown Mycorrhizal Bell Peppers
to Inoculation Timing, Phosphorus and Water Stress.

Introduction

Infection of plant roots by vesicular-arbuscular mycorrhizal fungi (VAMF) often increases plant growth through increased uptake of soil phosphorus (P) and other relatively immobile nutrients (Hayman and Mosse 1971, Menge et al. 1982). Infection may also increase drought tolerance of the host (Gianninazzi-Pearson and Gianninazzi 1983). Water stress may increase the external P requirements of plants because diffusion of soil P to the plant roots and root uptake capacity are reduced when soil moisture tension increases (Olsen et al. 1961). The decreased availability of P to roots in dry soils may increase the plants responsiveness or dependancy on mycorrhizae for P uptake (Bolgiano et al. 1983).

Early colonization of the plant roots is important if VAMF are to benefit annual crops, otherwise any growth stimulation would be expressed too late in the season to improve yields. In transplanted crops, inoculation with VAMF at seeding should promote rapid colonization relative to inoculating in the field at transplanting (Biermann and Linderman 1983). Preinoculated plants also may be more tolerant of transplanting shock than nonmycorrhizal plants or plants inoculated at transplanting (Menge et al. 1978).

Peppers (Capsicum sp.) are highly responsive to VAMF, with improvements in growth and yields of infected plants noted even

in relatively P rich soils (Bagyaraj and Sreeramulu 1982, Plenchette et al. 1983, Haas et al. 1986). In this study, the effects of VAMF on the growth and yields of bell pepper grown at two concentrations of soil solution P were examined under greenhouse and field conditions. The performance of plants inoculated at seeding was compared with plants inoculated at transplanting. We also examined the effect of soil moisture on growth and mycorrhizal responsiveness.

Materials and Methods

Transplant production. Transplants of bell pepper (cv. Emerald Green Giant) were grown in a 1 sphagnum peat : 1 vermiculite (by volume) medium, adjusted to pH 6.3 with dolomitic lime. Micronutrients were supplied as 0.5 kg/m³ Micromax (Sierra Chemical Co., Milipitas Calif.). Osmocote (18N-2.6P-10K Sierra Chemical Co., Milipitas Calif.) at 10 kg/m³ served as the macronutrient source. Levels of P in the medium resulting from this level of fertilization had previously been found to produce adequate growth of pepper transplants, while allowing for substantial mycorrhizal colonization of inoculated plants (Waterer and Coltman 1988).

In the greenhouse study, inoculated plants received 250, 1-cm long, dried corn root fragments (Zea mays L. 'Hawaiian Super Sweet No. 9') heavily colonized by the VAMF Glomus

aggregatum. In the field study, 2500 spores of G. aggregatum were suspended in water and applied to each plant. The mycorrhizae treatments in both the greenhouse and field studies were: a) preinoculated, in which the inoculum was mixed into the transplant medium at the time of seeding, b) inoculated, in which the inoculum was placed directly below the plant at transplanting, and c) noninoculated. To establish equivalent background microbial populations in all treatments, mycorrhizae-free filtrates from the inoculum sources were applied to all noninoculated plants. The seedlings were ready for transplanting after five weeks growth in a greenhouse.

Greenhouse study. A factorial experiment was designed with three types of transplants (preinoculated, inoculated and noninoculated), two soil solution P levels (0.03 and 0.30 mg P/liter) and two moisture regimes ("no-stress" and "stressed"). Soil solution P concentrations were determined by desorption in 0.01 M CaCl_2 (Fox and Kamprath 1970) of a 1:1 mixture (by weight) of Waialua clay soil (Vertic Haplustoll) and basaltic sand. The soil/sand mix was fumigated with methyl-bromide/chloropicrin and fertilized with the 0.6 g KNO_3 /kg three weeks prior to transplanting. Pots lined with plastic to prevent water loss were filled with 4.1 kg (dry weight) of the soil/sand mix.

The moisture regimes were established after transplanting. In the "no-stress" regime, the pots were rewatered to field capacity whenever 50% of the available water had been utilized. This corresponded to a soil water potential (SWP) of approximately -10 kPa, as determined by tensiometers maintained in the pots. In the "stressed" regime, rewatering was delayed until 75% of the available water had been utilized, corresponding to a SWP of -80 kPa.

At transplanting, pots were arranged randomly on adjacent greenhouse benches. Average maximum and minimum temperatures were 33 and 24° C. The daylength was approximately 13h and no supplemental light was supplied. After anthesis, 0.1 g KNO₃/kg soil was added to each pot at three week intervals. There were five replicates of each treatment.

Plant phosphorus status and the development of VAMF activity were monitored at seven day intervals by removing 15 mm diameter punches from the most recently fully expanded leaves. Phosphorus concentrations in the leaf punches were determined colorimetrically (Murphy and Riley 1962) following dry-ashing at 550°C. Plant heights were measured weekly. Fruit were harvested and weighed as they matured. Accumulated fruit fresh weights and shoot dry weights were determined 15 weeks after transplanting. Plant water use was determined by subtracting the amount of water lost from pots without plants from the total amount of water supplied to each treatment.

Samples from the root system of each plant taken at the final harvest were cleared and stained (Phillips and Hayman, 1970) and the degree of mycorrhizal infection in 60, 1-cm root segments visually rated as follows: 0 - no infections, 1 - entry points only present, 2 - small areas of mycorrhizal structures (vesicles or arbuscles) present, 3, 4 and 5 - structures present over 50, 75 and 100% of the root, respectively.

Field experiment. An experiment with nearly identical treatments to the greenhouse experiment was conducted in the field. The experiment was a 3 X 2 X 2 factorial (mycorrhizal treatments X soil P levels X water regimes) designed as a split-split plot, with soil P levels as the main plots, water regimes as the sub-plots and mycorrhizal treatments as the sub-sub plots. There were four replicates of each treatment.

The field had been fertilized two years previously with two rates of treble super phosphate, and soil solution P concentrations in the high and low P whole plots at transplanting were 0.03 and 0.30 mg P/liter, respectively. Prior to transplanting, the field was disked and rotovated, then fumigated with methyl bromide/chloropicrin (1 kg/25 m²). After fumigation, 180 kg N/ha as urea and 150 kg K/ha as K₂SO₄ were incorporated during the formation of raised beds on 1.2 m centers. At 10 weeks after transplanting, 80 kg N/ha

as KNO_3 was applied at the bases on the plants and incorporated by thorough irrigation.

Preinoculated, inoculated or noninoculated transplants were set at 23-cm intervals along a drip irrigation tube in the center of the beds. Each plot consisted of four rows with 12 plants per row. Mycorrhizal and nonmycorrhizal plots on each bed were separated by 1 m. The moisture regimes established in the field experiment differed only slightly from the regimes tested in the greenhouse experiment. Tensiometers 10 cm from the drip tubes were used to monitor soil water potentials (SWP) at 23 cm depth. Irrigation of "no-stress" treatments commenced when the SWP reached -10 kPa. This corresponded to the loss of approximately 11% of the water available in the top 23 cm of the soil profile. Watering continued until the SWP at 23 cm had risen above -10 kPa. "Stressed" treatments were not irrigated until the SWP at 23 cm had reached -80 kPa, corresponding to the loss of 27% of the available soil water. Watering ceased once the SWP at 23 cm had risen above -80 kPa.

Leaf P concentrations and heights of plants from the center two rows of each four row plot were determined weekly using the procedures described in the greenhouse experiment. Fruit were harvested and weighed as they matured. Seventeen weeks after transplanting, the plants were harvested and the fresh weights of the tops determined. Samples of the root systems were examined for mycorrhizae infection as previously described.

Orthogonal contrasts were used to partition interactions between treatments (Appendix Tables 5A and 5B). Mycorrhizal responsiveness for the growth parameters was calculated as: $(\text{mycorrhizal} - \text{nonmycorrhizal}/\text{nonmycorrhizal}) \times 100$ (Daniels-Hetrick et al. 1983).

Results

Transplants. Preinoculation did not effect the size or shoot P concentrations of the peppers at transplanting in either the greenhouse or field trials (Appendix Table 5C). Roots of the preinoculated plants were heavily colonized by the VAMF at transplanting.

Mycorrhizae and soil P. Within two weeks of transplanting, leaf P concentrations in plants growing in the high P soil (0.30 mg/liter solution P) were higher than in the low P soil (0.03 mg/liter solution P) in both the greenhouse and the field (Fig. 5.1). Within 3 to 4 weeks of transplanting, leaf P concentrations in inoculated plants in the low P soil began to increase relative to noninoculated controls. Six weeks after transplanting, leaf P concentrations in the mycorrhizal plants in the low P soil were similar to leaf P concentrations in all treatments in the high P soil. The pattern and timing of the response to soil P and inoculation variables were similar in the

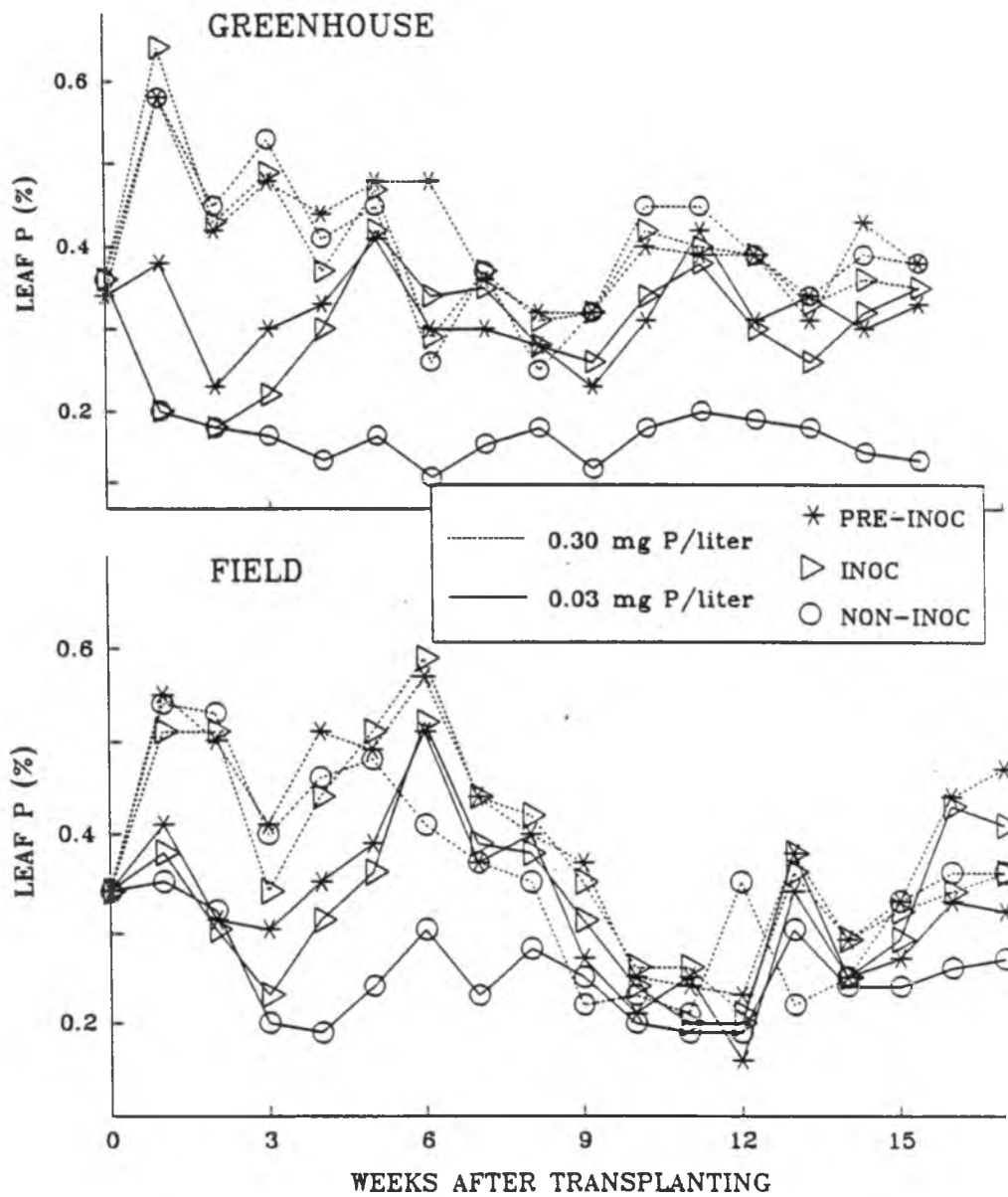


Fig. 5.1. Influence of soil solution phosphorus and mycorrhizae on leaf P concentrations of peppers under greenhouse and field conditions.

greenhouse and field trials. Plant heights showed a similar response to soil P and the onset of mycorrhizal function (Fig 5.2).

Six or seven weeks after transplanting, leaf P concentrations declined in all treatments except for the noninoculated plants in the low P soil (Fig. 5.1). The decline was correlated with fruit set in all affected treatments and was more pronounced in the field, possibly due to the heavier fruit set relative to the greenhouse.

Average leaf P concentrations were significantly higher in the high P soil than at the lower P level (Table 5.1, $P < 0.01$). Inoculation with the VAMF increased leaf P concentrations in the low P soil by an average of 93% in the greenhouse ($P < 0.01$) and 28% in the field ($P < 0.01$). Inoculation did not effect average leaf P concentrations in the high P soil.

Inoculation increased average plant heights in the low P soil by 21% in the greenhouse ($P < 0.01$) and 28% in the field ($P < 0.01$) but had no significant effect in the high P soil (Table 5.1). Under greenhouse conditions, inoculated plants in the low P soil were as tall as plants in the high P soil (Table 5.1). In the field, the high soil P plants were significantly taller than the low P-inoculated plants ($P < 0.01$).

In the greenhouse, mycorrhizal inoculation of the low soil P treatments increased fruit number by 150%, fruit weight by 350% and the final dry weights of the plants by 120% relative to

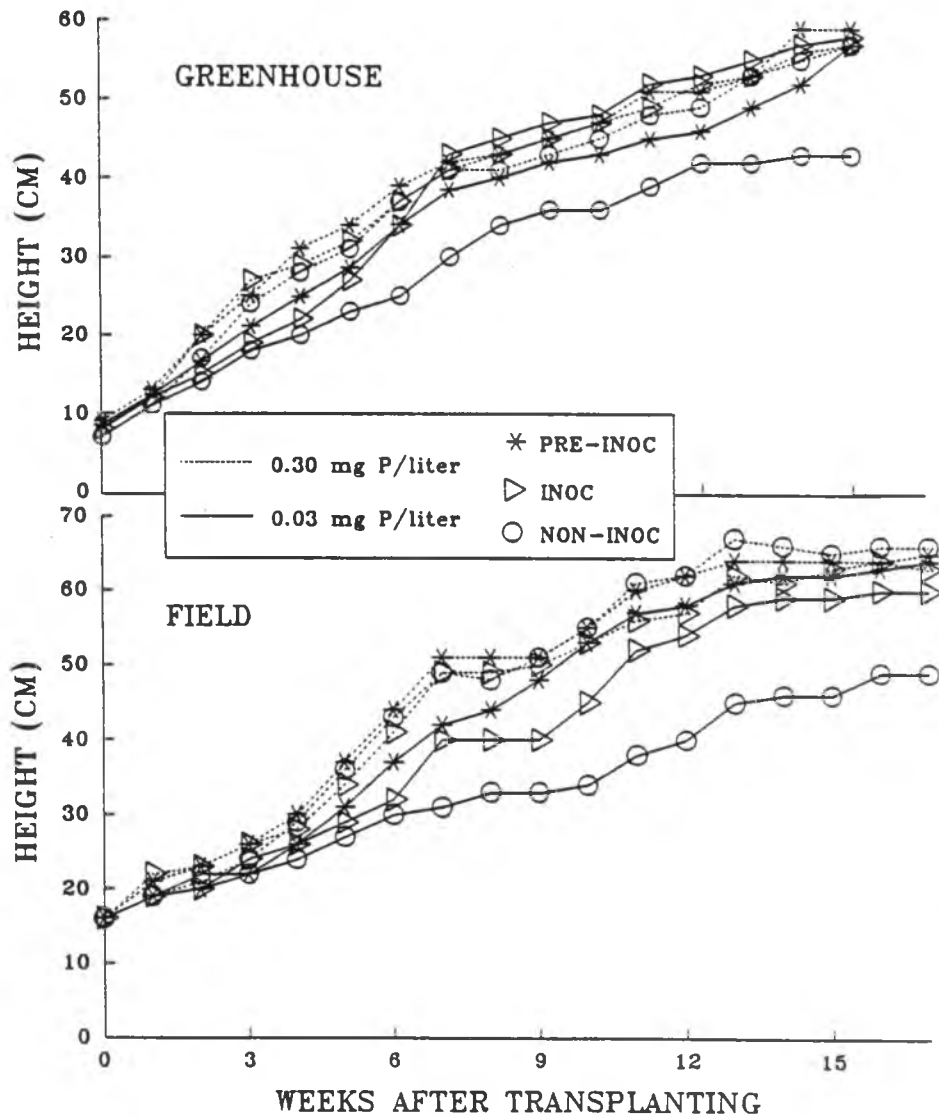


Fig. 5.2. Influence of soil solution phosphorus and mycorrhizae on the heights of peppers under greenhouse and field conditions.

equivalent noninoculated plants (Table 5.1). In the field, inoculation increased fruit number, fruit yields and the final fresh weights of the plant tops by 128, 183 and 189% respectively. Inoculation had no significant effect on fruit number, yields or plant weights at the higher solution P level. In the greenhouse, fruit numbers and yields of the low P inoculated plants were statistically equivalent to the high P treatments (Table 5.1, $P=0.05$). Otherwise, fruit set, yields and plant weights were significantly greater in the high soil P treatments.

At the final harvests, root infection by the VAMF was more intense in the low P soil than in the high P soil (Table 5.1). There was little evidence of mycorrhizal infection of noninoculated plants in either experiment.

Timing of inoculation. In the low P soil, leaf P concentrations and plant heights increased earlier in preinoculated plants than when inoculum was added at transplanting (Figs. 5.1 and 5.2). This difference disappeared within six weeks of transplanting, and the average leaf P concentrations and heights for preinoculated and inoculated treatments were not significantly different (Appendix Table 5B).

In the greenhouse, yields of plants inoculated at seeding or at transplanting did not differ (Appendix Table 5B). However,

Table 5.1. Average leaf phosphorus concentrations, plant heights, total fruit number, fruit yields and final plant weights for peppers grown with different mycorrhiza inoculation and soil solution P treatments under greenhouse and field conditions.

Treatment (Soil P/ Myco)	Leaf P concn (%)		Height (cm)		Fruit no.		Fruit yield (g/plant)		Plant wt. ^z (g/plant)		Infect- ion ^y	
	G ^x	F	G	F	G	F	G	F	G	F	G	F
H/Pre ^w	0.41	0.40	39	50	5.2	19.7	173	1150	38	437	2.4	2.0
H/Plnt	0.41	0.38	38	47	5.3	18.9	148	1100	37	377	2.2	1.7
H/Non	0.40	0.37	36	49	4.4	18.0	155	1085	38	383	0	0
L/Pre	0.32	0.32	35	43	4.7	14.5	149	887	33	288	3.2	2.5
L/Plnt	0.30	0.32	37	41	5.3	11.1	160	644	33	250	2.9	2.7
L/Non	0.16	0.25	29	33	1.8	5.6	43	269	15	94	0	0
LSD 0.05	0.04	0.05	5	6	1.1	2.8	28	68	7	78	0.3	0.4

^zDry weights for greenhouse, fresh weights for field.

^yRating of 0 - no roots infected, 5 - all roots heavily infected.

^xG - greenhouse, F - field.

^wHigh P = 0.30 mg P/liter soil solution, Low = 0.03 mg/liter. Pre = preinoculated, Plnt = inoculated at transplanting, Non = noninoculated.

in the field, preinoculation of plants subsequently grown in the low P soil increased fruit numbers and yields by 34 and 43%, respectively, relative to plants inoculated at transplanting (Table 5.1). Preinoculation also increased final plant weights in both the high and low P soil.

Timing of inoculation did not affect mycorrhizal colonization of the roots at the final harvest (Table 5.1)

Water availability. Water availability did not significantly affect leaf P concentrations, plant heights or final plant weights in the greenhouse or field (Appendix Table 5A). In the greenhouse, average fruit yields were reduced by 35% when irrigation was delayed until SWP reached -80 kPa (Table 5.2). In the field, fruit yields were not affected by the water stress treatment. In both experiments, the interaction between soil P, mycorrhizal inoculation and water availability significantly affected fruit yield. In the high P soil, differences between the yields of mycorrhizal and nonmycorrhizal plants (mycorrhizal responsiveness) were small, but fruit yields benefitted more from inoculation when the peppers were watered at the -10 kPa irrigation set point (Table 5.2). In the low P soil, fruit yields were significantly increased by inoculation but the response to inoculation was much greater with the -80 kPa than with the -10 kPa irrigation set point. Fruit set

Table 5.2. Influence of soil phosphorus, soil water availability and mycorrhizae on fruit yields of greenhouse and field grown peppers.

	Fruit yield (g/plant)			
	Low P soil ^Z		High P soil	
	-10 kPa	-80 kPa	-10 kPa	-80 KPa
<u>Greenhouse</u>				
Inoculated	172a ^Y	137b	201a	126b
Noninoculated	48c	23d	183a	127b
Response ^X	+255%	+495%	+10%	-1%
<u>Field</u>				
Inoculated	679b	840a	1143a	1042a
Noninoculated	291c	247c	931a	1242a
Response	+133%	+240%	+23%	-11%

^ZLow P = 0.03 mg P/liter soil solution, high = 0.30 mg/liter.

^YMeans within soil P levels followed by the same letter are not significantly different (P=0.05).

^XResponse = (wt. inoc - wt. noninoc/wt. noninoc) x 100.

showed a similar interaction between soil P, inoculation and water availability (Appendix Table 5D).

In the greenhouse experiment, the water use of individual treatments was monitored, enabling calculation of water-use efficiencies (WUE) for growth and yield. Watering once SWP reached -80kPa increased WUE for vegetative growth by 10% but decreased WUE for fruit production by 28% compared to watering at -10 kPa SWP (Table 5.3). Inoculation increased WUE of fruit production in the low solution P treatments by 140% ($P < 0.01$), but had no effect at the higher soil P level (Table 5.3).

Discussion

Peppers grown in the field were larger, with considerably higher yields than plants of similar age grown in the greenhouse. Otherwise, responses to the soil P, mycorrhizae inoculation, and watering variables were quite similar in the greenhouse and field.

Soil solution P concentrations of 0.03 mg/liter were insufficient for optimal growth of nonmycorrhizal peppers under either greenhouse or field conditions. Inoculation with a VAMF dramatically improved growth and yields of the peppers probably due to enhanced phosphorus uptake by infected roots (Gerdemann 1968). Tissue P concentrations in noninoculated plants were

Table 5.3. Water-use efficiencies (WUE) for vegetative top growth and fruit yield of greenhouse grown peppers as influenced by water availability, soil phosphorus and mycorrhizae.

Variable	<u>WUE - Vegetative growth</u> (g dry weight/liter)	<u>WUE - Fruit Yield</u> (g fresh weight/liter)
a) <u>Water</u>		
-10 kPa	1.7	7.2
-80 kPa	1.9	5.2
	*	**
b) <u>P/Myco</u>		
H/I	1.9	6.7
H/N	2.0	6.7
L/I	1.8	6.9
L/N	1.7	2.8
LSD 0.05	0.2	0.4

*,** t tests significant P = 0.05 and P = 0.01, respectively.

H and L = soil solution P concentrations of 0.30 and 0.03 mg/liter, respectively.

I and N = inoculated and noninoculated.

generally deficient (0.19 - 0.23%), while inoculated plants had adequate leaf P (0.30 - 0.34%) (Lorenz and Tyler 1976).

Responses to inoculation were generally greater in the pot trial than in the field, suggesting the increased P uptake potential provided by the mycorrhizae may be more important when soil or rooting volumes are limited. In the pot trial, growth and yields of the low soil P-inoculated plants were statistically equivalent to plants growing with 10 times greater solution P concentrations, while in the field, the high P plants were larger and had greater yields than low P-inoculated plants. In the greenhouse study, pot size may have constrained growth of the high soil P treatments.

With 0.30 mg/liter solution P, the P requirements of peppers could be met by a nonmycorrhizal root system. Average leaf P concentrations were above the levels considered adequate for maximum growth of peppers (Lorenz and Tyler 1976). Although mycorrhizal colonization was not beneficial in the high P soil, yields were not adversely affected by the VAMF. Bethlenfalvay (et al. 1983) and others found mycorrhizae in P rich soils may reduce growth by absorbing carbohydrates from the root without contributing significantly to the mineral nutrition of the host.

In the field, withholding irrigation until soil water potentials reached -80 kPa did not alter growth or yields but in the greenhouse, fruit yields were significantly reduced when irrigation was delayed until SWP in the pots reached -80 kPa.

The greater sensitivity to soil moisture deficits in the greenhouse is likely due to the restricted soil volume available to the potted plants. In the field, roots in moist soil below the tensiometers may have kept the plants supplied with water.

Withholding water reduced fruit yields of nonmycorrhizal plants in P deficient soil more than the yields of mycorrhizal plants. At higher soil P levels, the mycorrhizal had little affect on the response of yield to water stress, suggesting the mycorrhizal effect in the P-deficient soil was related to increased P uptake by infected plants. Bolgiano et al. (1983) found moderate water stress increased the difference between yields of mycorrhizal and nonmycorrhizal onions in soil with moderate available P. The mycorrhizal effect on moisture stress tolerance also was suppressed in P rich soil. The ability of mycorrhizae to increase P uptake might become progressively more important as increasing water deficits reduce the diffusion of P to the roots (Viets 1972) and inhibit root uptake (Olsen et al. 1961). However, Hetrick et al. (1984) found that mycorrhizal responsiveness of corn declined as moisture stress increased. They suggested that beyond certain soil moisture deficits mycorrhizae were no longer able to increase nutrient uptake.

Withholding water increased the WUE of vegetative growth, but decreased the efficiency of fruit set and yield. This may be due to the particular sensitivity of fruit set and early development to water stress. Inoculation increased the WUE of

fruit production when soil P was limited by increasing fruit yield more than vegetative growth, thereby reducing the transpiring leaf area per unit fruit weight.

Preinoculation improved P nutrition and growth of peppers planted into P deficient soil during the first weeks after transplanting, prior to the establishment of infections initiated at planting. In the field, this early advantage increased final fruit yields and plant weights. In quick maturing crops or plants with high post-transplant P requirements, the apparent advantage provided by preinoculation might be of even greater importance.

In summary, mycorrhizal inoculation of peppers was highly beneficial when soil P was limited and had no discernible negative effects when soil P was more abundant. Inoculation would appear to be a good management strategy, particularly when indigenous mycorrhizae are scarce or inefficient. Inoculation could potentially substitute for a significant portion of the P fertilizers commonly applied to peppers. In the soil used in this experiment, over 1400 kg P_2O_5 /ha would be needed to raise soil solution P concentrations from 0.03 mg/liter to the 0.30 mg/liter level required for optimum growth of nonmycorrhizal peppers (Waterer and Coltman, unpublished data).

Chapter VI

**Mycorrhizal Infection of Bell Pepper Transplants Influences
Subsequent Responses to Soil Solution Phosphorus**

Introduction

The symbiotic association between vesicular-arbuscular mycorrhizal fungi (VAMF) and plant roots frequently increases growth of the host due to enhanced absorption of phosphorus (P) and other relatively immobile mineral nutrients (Mosse 1973). The significance of the symbiosis is greatest when plants with high internal or external P requirements are grown in P deficient or P fixing soils (Gianinazzi-Pearson and Diem 1982, Habte and Manjunath 1987). Many horticultural crops form effective associations with mycorrhizal fungi. Peppers (Capsicum sp.) are responsive to mycorrhizae, even when soil P is relatively abundant (Bagyaraj and Sreeramulu 1982, Dodd et al. 1983, Plenchette et al. 1983, Haas et al. 1986 and 1987). Promotion of VAMF infection of peppers could improve the plants' mineral nutrition while potentially reducing requirements for P fertilizers.

For VAMF to improve growth of annual crops, such as peppers, the host must be rapidly colonized by the fungus, otherwise the resulting growth stimulation will come too late in the season to affect yields (Haas et al. 1986). Rapid colonization depends on the presence of adequate infective inoculum. When soil inoculum levels have been reduced by fumigation, fallow or monocropping with a nonmycorrhizal host (Hetrick and Bloom 1983), inoculum densities may be insufficient

to insure adequate early infection. The difficulty of producing large amounts of inoculum presently limits the potential for field-scale amendments of the VAMF population (Black and Tinker 1977, Khan 1975), although techniques for producing sufficient amounts of Glomus aggregatum inoculum for small acreages have been developed (Coltman et al. 1988). Inoculation of seedlings growing in nurseries, followed by transplanting of the mycorrhizal plants into the field may represent a more efficient means of utilizing available inoculum (Maronek et al. 1981, Bagyaraj and Sreeramulu 1982, Biermann and Linderman 1983). However, the high rates of fertilization generally employed in the production of horticultural transplants can inhibit VAMF infection (Biermann and Linderman, 1983, Waterer and Coltman 1988). Production of mycorrhizal transplants may therefore involve a compromise between maximum growth prior to transplanting and maximum VAMF infection, based upon the amount of P supplied to the transplants. The relative importance of infection versus transplant size and tissue P status as determinants of subsequent growth of transplanted crops is unknown.

The objectives of this experiment were to: 1) evaluate the effects of mycorrhizae on the response of bell peppers to differing levels of soil solution P; and 2) to determine the relative effects of transplant size, P status and extent of

pretransplant mycorrhizal infection on subsequent growth in soil with differing levels of available P.

Materials and Methods

Transplant production. Transplants of bell pepper (cv. Emerald Green Giant) were grown in plastic flats containing a 1 sphagnum peat : 1 vermiculite (by volume) medium, adjusted to pH 6.3 with dolomitic lime. Micronutrients were supplied as 0.5 kg/m³ Micromax (Sierra Chemical Co., Milpitas Calif). At seeding, half the flats were inoculated with dried corn roots (Zea mays L. 'Hawaiian Supersweet No. 9') heavily colonized by the VAMF Glomus aggregatum. The medium surrounding each seed contained about 250, 1cm-long root fragments. To establish equivalent background microbial populations in all treatments, mycorrhizae-free filtrates from VAMF-infected corn roots were applied to all noninoculated flats.

To produce a range in transplant size, tissue P status and mycorrhizal colonization, the medium was fertilized with 60, 180 or 540 g P/m³, with 19N-2.6P-10K Osmocote as the P source (Sierra Chemical Co., Milpitas Calif.). Appropriate amounts of 39-0-0 and 0-0-40 Osmocote were added to provide equivalent N and K to all treatments. In an earlier experiment (Waterer and Coltman 1988), we found pepper transplants grown with 60 g Osmocote P per m³ were somewhat stunted and P deficient, but

development of VAMF infections had been extensive in inoculated plants. The 180 g/m³ rate produced larger plants with moderately well infected roots. The highest rate of Osmocote P further increased growth while depressing infection of inoculated plants.

Transplants were grown under 15% shade in a greenhouse in Honolulu, Hawaii (21°51' N and 156°22' W). Peak light intensity under the shade cloth was about 1200 $\mu\text{mol/s/m}^2$ and the daylength about 13.5 hr. Average maximum and minimum temperatures were 35 and 26°C.

Five weeks after seeding, the medium was carefully washed from the seedling roots in preparation for transplanting. Total dry weights, tissue P concentrations and mycorrhizal infection ratings were determined for twenty inoculated and noninoculated plants from each pretransplant P level. Tissue P concentrations were determined by the molybdenum blue method (Murphy and Riley 1962), following dry-ashing at 550°C. Samples from the root system of each plant were cleared and stained (Phillips and Hayman 1970) and 60, 1-cm long root fragments examined to determine the percentage of roots colonized by the VAMF and the intensity of the infection. Infection intensities were rated as follows; 0 = no infection, 1 = entry points only present, 2 = small areas of hyphae present, 3, 4 and 5 = hyphae present over 50, 75 and 100% of the root, respectively.

Plant responses. The peppers were transplanted into a Waialua clay soil (Vertic Haplustoll), pH 6.1, mixed 1:1 (by volume) with basaltic sand. Four weeks prior to transplanting, sufficient KH_2PO_4 was added to the soil/sand mix to adjust the equilibrium solution P concentrations (0.01 M CaCl_2 extraction) to 0.01, 0.03, 0.10, 0.30 or 1.0 mg/liter (Fox 1981). The equivalent of 200 kg N/ha as KNO_3 was added with the P. The mixture was placed into plastic-lined pots (4.1 kg dry weight/pot) and fumigated with methyl-bromide/chloropicrin. The pots were allowed to stand for 10 days following fumigation to dissipate the fumigants. The pots were then watered to approximate field capacity and planted with the bare-rooted transplants.

The experimental design was a complete factorial with 5 solution P levels, three pretransplant P levels, two mycorrhizae treatments (inoculated and noninoculated) and five replicates. The pots were arranged in a completely randomized design on five adjacent greenhouse benches. Average maximum and minimum temperatures over the eight weeks of the experiment were 37 and 26°C. Peak light intensity was about 1300 $\mu\text{mol/s/m}^2$ and the daylength about 13 hr. Pots were watered daily.

Phosphorus concentrations in 1.5 cm diam. leaf disks taken from the most recently fully expanded leaves were monitored weekly to track the development of VAMF activity (Aziz and Habte 1987). Weekly plant heights and the number of days to anthesis

also were recorded. Eight weeks after transplanting, dry weights of the tops, fruit and roots were determined. Samples of the root system from each plant were examined for mycorrhizal infection, as previously described, but using a different intensity rating scale: 0 - no infection, 1 - entry points only present, 2 - small areas of mycorrhizal structures present (vesicles or arbuscles), 3, 4 and 5 - structures present over 50, 75 and 100% of the root segment, respectively.

Results

Growth and VAMF infection of transplants. Total dry weights and tissue P concentrations of the transplants increased as the amount of Osmocote P in the medium increased (Fig. 6.1). Transplants inoculated with the VAMF weighed slightly less than noninoculated plants at all pretransplant P levels. Tissue P concentrations were similar in mycorrhizal and nonmycorrhizal transplants. The percentage of roots colonized by the VAMF and the intensity of infections decreased steadily as the P available to the transplants increased (Fig. 6.1). Noninoculated plants were not infected.

Plant responses to mycorrhizae and soil phosphorus.

Phosphorus concentrations in the most recently expanded leaves increased with increasing P levels in the soil, with differences

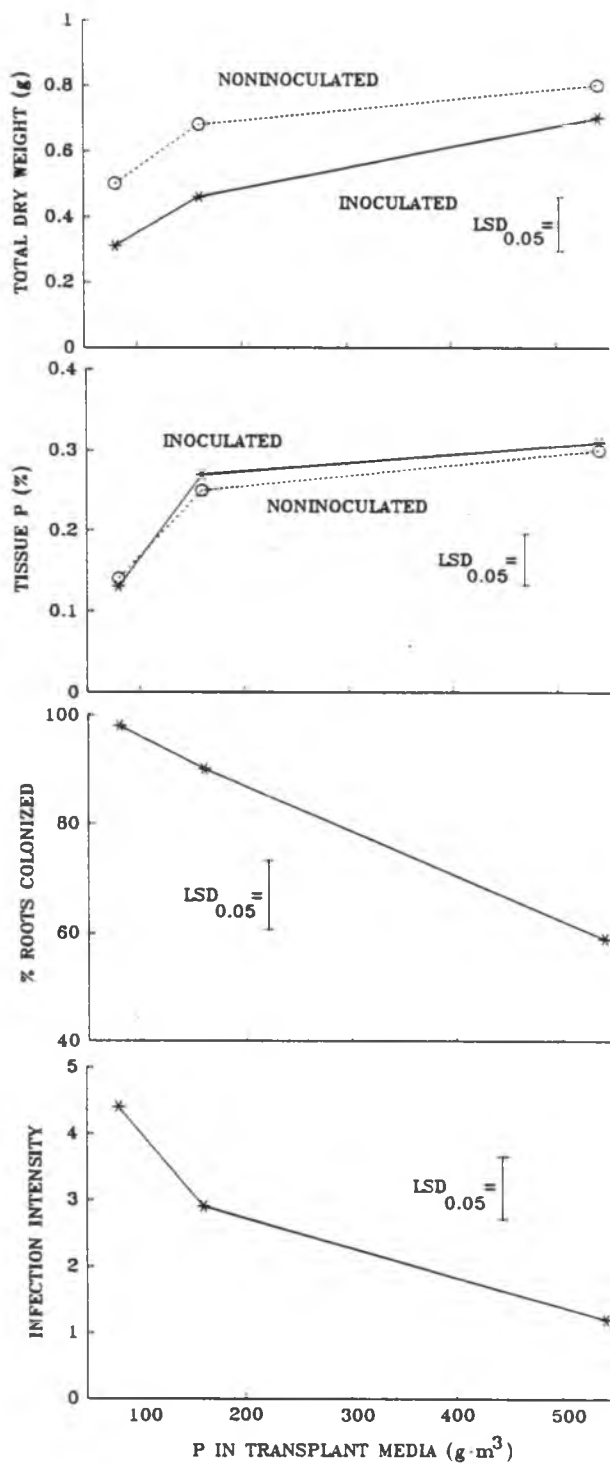


Fig. 6.1. Influence of pretransplant phosphorus on growth, tissue phosphorus concentration, extent and intensity of mycorrhizal infection of roots of mycorrhizal and nonmycorrhizal pepper transplants (n=20). Infection intensity of 0 = no infection, 5 = all roots heavily infected.

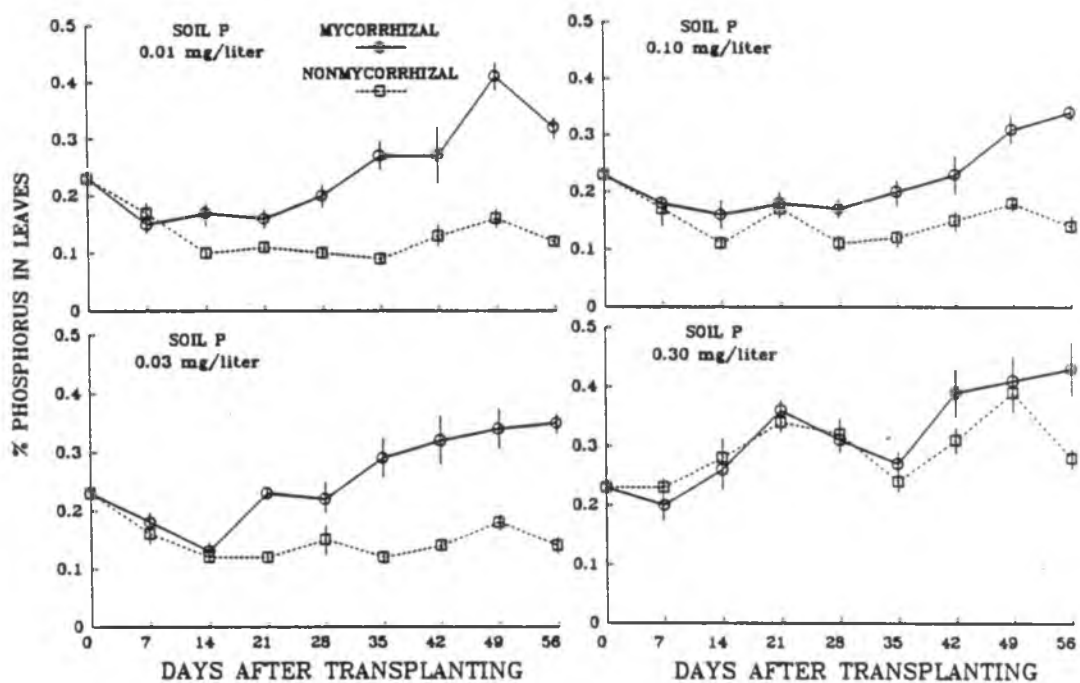


Fig. 6.2. Influence of soil solution phosphorus on weekly concentrations of phosphorus in the newest leaves of mycorrhizal and nonmycorrhizal peppers. Average of three pretransplant P levels ($n=15$, means \pm SE). Data for 1.0 mg P/liter similar to 0.30 mg/liter.

between soil P levels evident within one week of transplanting (Fig. 6.2). At soil P concentrations from 0.01 to 0.10 mg/liter, peppers inoculated with the VAMF had higher weekly leaf P levels than noninoculated plants. The mycorrhizal effect on P uptake was apparent two to three weeks after transplanting and increased as the plants matured. At solution P concentrations above 0.10 mg/liter, weekly leaf P concentrations in mycorrhizal and nonmycorrhizal plants were similar (Appendix Fig. 6A). Plant heights were similarly influenced by soil P and mycorrhizae (Appendix Fig. 6B), suggesting a good correlation between concentrations of P in leaf punches and plant P nutrition.

Total dry weights (TDW) of both mycorrhizal and nonmycorrhizal peppers responded asymptotically to increasing solution P concentrations (Fig. 6.3). At the plateau, TDW were similar in mycorrhizal and nonmycorrhizal plants, but mycorrhizal plants reached the plateau at a much lower solution P level (0.03 versus 0.4 mg/liter).

Increasing soil solution P levels reduced the time to anthesis for both mycorrhizal and nonmycorrhizal peppers (Fig. 6.3). At solution P concentrations below about 0.2 mg/liter, flowering of nonmycorrhizal plants was significantly delayed relative to inoculated plants.

Fruit yields of both mycorrhizal and nonmycorrhizal plants increased with increasing solution P concentration, through to

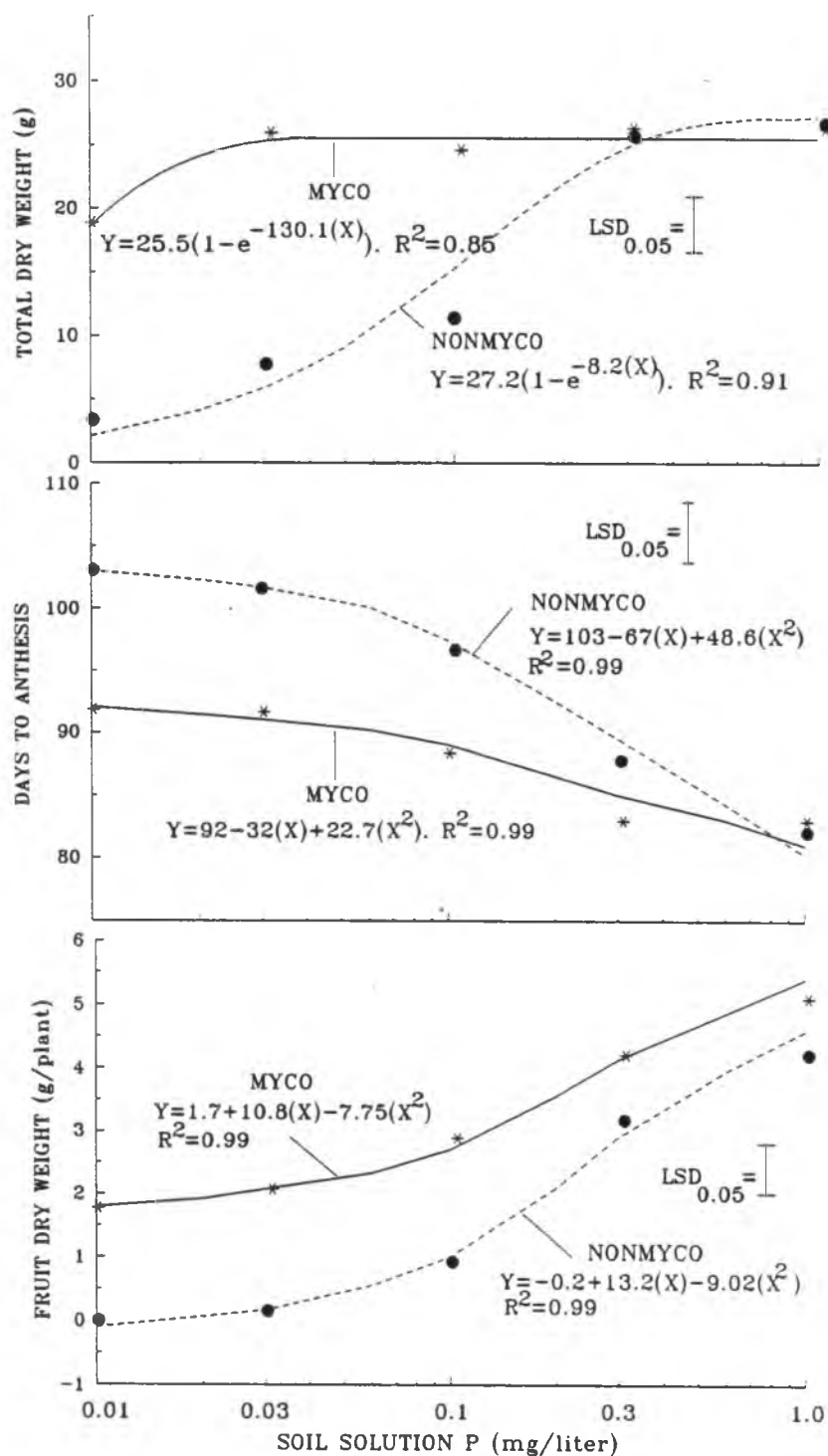


Fig. 6.3. Influence of soil solution phosphorus on total dry weights, time to anthesis and fruit yields of mycorrhizal and nonmycorrhizal peppers.

the highest level tested (Fig. 6.3). Yields of mycorrhizal plants were consistently higher than yields of nonmycorrhizal plants, although the difference only was significant at solution P concentrations below 0.2 mg/liter.

At the final harvest, the percentage of roots infected in the inoculated treatments was uniformly high (mean 84%). Soil P levels had no significant effect on the degree of colonization (Table 6.1). Infection intensities ratings declined from 2.5 of a possible 5 in the most P deficient soil to 1.7 in the highest P soil. There was no evidence of mycorrhizal colonization of any noninoculated plants.

Using the data for average leaf P concentrations as an indicator of plant P status, we found that dry matter production by mycorrhizal peppers was less affected by declining tissue P concentrations than dry matter production of nonmycorrhizal plants (Fig. 6.4).

Responses to pretransplant P. The amount of phosphorus in the transplant medium did not significantly effect the final TDW, fruit yields or P status of peppers (Table 6.1). Responses to mycorrhizal inoculation were significantly interacted with soil P levels. Therefore, the data were divided to examine responses at soil P levels where nonmycorrhizal peppers were P deficient (0.01 to 0.10 mg/liter solution P) relative to responses at higher solution P levels (0.30 and 1.0 mg/liter). In the low P

Table 6.1. F-test probabilities for tissue phosphorus concentrations, growth, yield and mycorrhizal infection of mycorrhizal and nonmycorrhizal peppers grown at different levels of soil solution phosphorus from transplants given different amounts of phosphorus.

Source of Variation	Average Leaf P	Plant Height	Total Weight	Fruit Weight	Days to Anthesis	<u>Root Infection</u> % Intensity	
Solution P (P)	<0.01	<0.01	<0.01	<0.01	<0.01	0.72	<0.01
Transplant P (T)	0.06	0.17	0.26	0.21	0.06	<0.01	<0.01
Mycorrhizae (M)	<0.01	<0.01	<0.01	<0.01	<0.01	-	-
P x T	0.15	0.34	0.42	0.53	0.43	0.54	0.63
P x M	<0.01	<0.01	<0.01	0.03	0.02	-	-
T x M	0.13	0.44	0.06	0.17	0.12	-	-
P X T x M	0.26	0.61	0.15	0.42	0.17	-	-

P = soil solution P concentrations ranging from 0.01 to 1.0 mg/liter.

T = pretransplant Osmocote P rates of 60, 180 or 540 g/m³ transplant medium.

M = mycorrhizal or nonmycorrhizal.

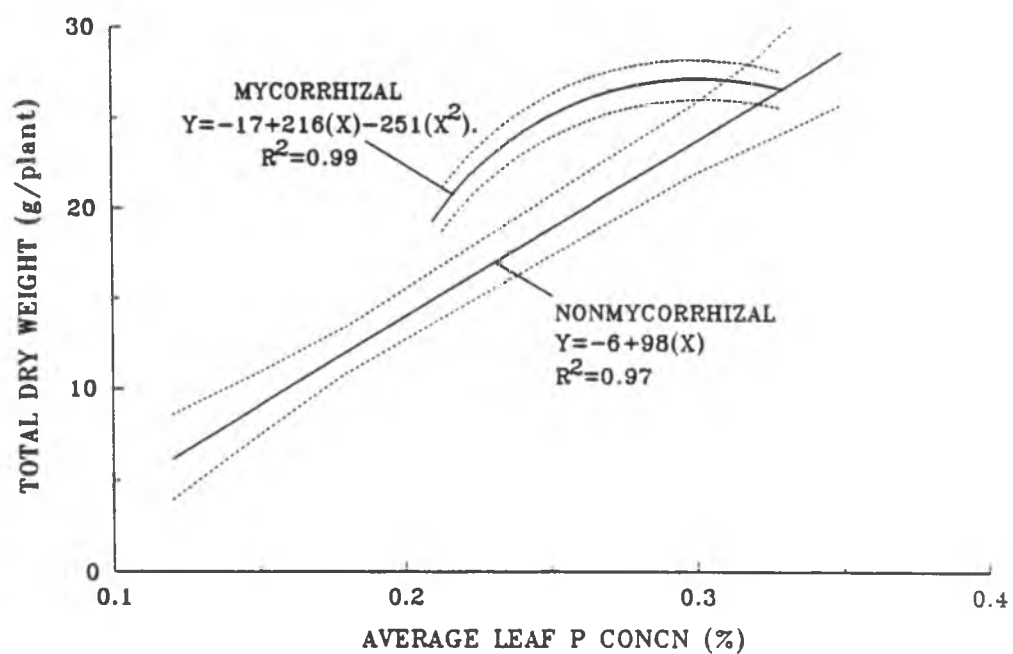


Fig. 6.4. Influence of mycorrhizae on relationship between tissue phosphorus concentrations and dry matter production by peppers. Broken lines represent 95% confidence intervals.

soils, the interaction between transplant P and mycorrhizal inoculation was significant for tissue P concentrations and total dry weights, and nearly significant for fruit yield (Table 6.2). The TDW data illustrates the interaction. In low P soils, TDW of nonmycorrhizal plants at the final harvest were not affected by pretransplant P (Fig. 6.5). By contrast, TDW of inoculated plants decreased markedly as the P supplied during transplant production increased. In soils with higher levels of available P, the amount of P supplied during the transplant stage had no significant effect on subsequent growth, fruit yields or leaf P concentrations (Table 6.2).

Following transplanting into P deficient soils, leaf P concentrations in mycorrhizal plants increased most rapidly when relatively little P was supplied during transplant production (Fig. 6.6), probably because of the presence of well established mycorrhizal associations in the roots at transplanting (Fig. 6.1). Early improvement of P uptake may explain the TDW of plants extensively colonized by the VAMF at transplanting.

At all soil P levels, the inhibitory effect of pretransplant P on VAMF infection of the transplants resulted in suppressed root colonization and infection intensities through to the final harvest (Table 6.3).

Table 6.2. F-test probabilities for tissue phosphorus concentrations, growth and yields of mycorrhizal and nonmycorrhizal peppers grown with different levels of solution phosphorus from transplants given different amounts of phosphorus.

Source of Variation	(0.01-0.10 mg P/liter)			(0.30-1.0 mg P/liter)		
	Average Leaf P	Total Weight	Fruit Weight	Average Leaf P	Total Weight	Fruit Weight
Solution P (P)	0.08	<0.01	0.09	0.61	0.27	0.34
Transplant P (T)	0.14	0.32	0.08	0.46	0.43	0.76
Mycorrhizae (M)	<0.01	<0.01	<0.01	0.91	0.61	0.12
P x T	0.54	0.20	0.89	0.63	0.37	0.52
P x M	0.18	0.91	0.63	0.27	0.61	0.61
T x M	0.02	<0.01	0.06	0.41	0.68	0.46
P x T x M	0.27	0.37	0.80	0.92	0.71	0.88

T - pretransplant P rates of 60, 180 or 540 g/m³.

M - mycorrhizal or nonmycorrhizal.

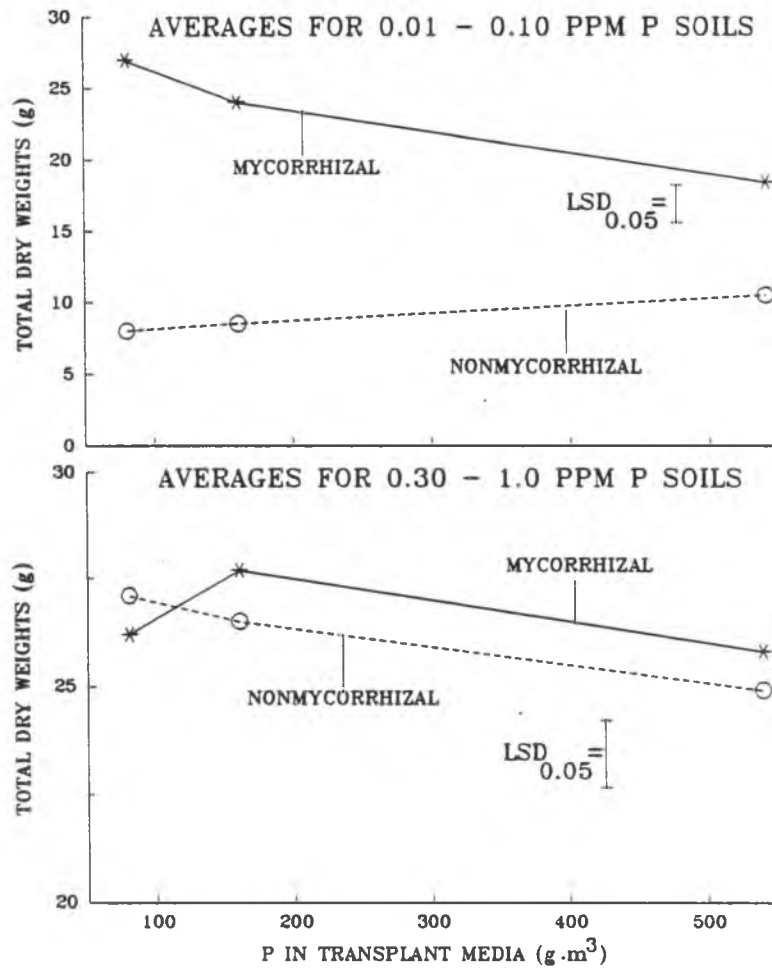


Fig. 6.5. Influence of pretransplant phosphorus on dry matter yields of mycorrhizal and nonmycorrhizal peppers following transplanting into soils with differing concentrations of soil solution phosphorus (n=15 for 0.01-0.10 mg P/liter, n=10 for 0.30-1.0 mg P/liter)

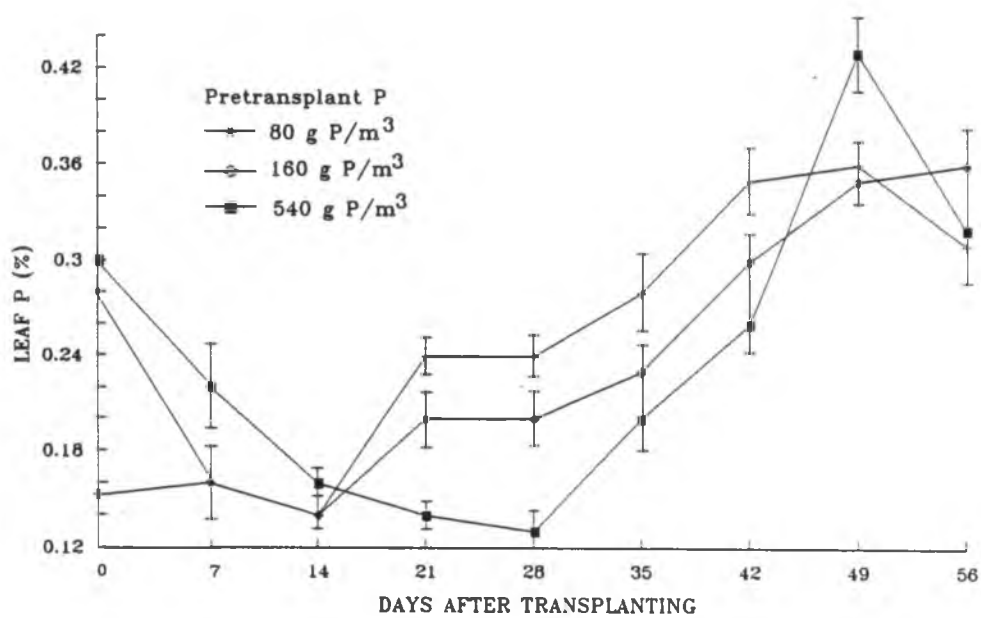


Fig. 6.6. Influence of pretransplant phosphorus on concentrations of phosphorus in the newest leaves of mycorrhizal peppers following transplanting into phosphorus deficient soil (n=15, means \pm SE).

Table 6.3. Percent and intensities of root infection by mycorrhizae, 56 days after transplanting of peppers grown with three levels of pretransplant phosphorus.

Pretransplant P	Colonization (%)	Infection Intensity ^Z
80 g/m ³	90	2.6
160	83	2.1
540	84	2.0
LSD 0.05	4	0.2

^ZIntensity rating of 0 = no infection, 5 = all roots heavily infected.

Discussion

Inoculation with a mycorrhizal fungus reduced the soil solution P requirements of bell peppers by an order of magnitude in a P-fixing soil. Mycorrhizae reduce plant P requirements by increasing the absorptive surface of the host roots. External mycorrhizal hyphae absorb P from beyond the zones depleted by the root (Rhodes and Gerdemann 1975), and transport the P back to the root in exchange for the carbohydrates required for fungal metabolism (Rhodes and Gerdemann 1975).

Flowering and fruit development were promoted by solution P levels in excess of those required for maximum TDW production. Optimum solution P concentrations are known to vary between growth stages (Fox et al. 1974). Higher P requirements for flowering and fruit production may explain why inoculation with the mycorrhizae improved fruit production at solution P levels sufficient for maximum TDW production. Promotion of flowering and fruit development by VAMF has been noted in peppers (Haas et al. 1986, Bagyaraj and Sreeramulu 1983) and other flowering species (Daft and Okusanyo 1973). By improving P uptake, VAMF may speed host development (Loehwing 1951). The fungi may also influence flowering by altering the hormonal balance of the host (Allen et al. 1980). Improved fruit yields, early market

considerations and the avoidance of late season frosts make early fruit set in peppers highly desirable.

Bolan et al. (1983) found the relationship between internal P content and shoot yields of subterranean clover did not change following inoculation with a VAMF. Stribley et al. (1980) suggest the higher energy demands of the VAMF roots might reduce yields of mycorrhizal plants relative to nonmycorrhizal plants with similar tissue P concentrations or total absorbed P. Our data, however, suggests that VAMF may increase the efficiency of utilization of absorbed P, allowing the plants to better tolerate the low tissue P levels encountered in P deficient soils. The effects of mycorrhizae on internal P-use efficiency requires further investigation.

Infection by VAMF is inhibited by high P levels in the host roots (Menge et al. 1978). External P concentration affect colonization through their influence on internal P levels. We found the solution P levels had little affect on VAMF infection of mature peppers, perhaps because the external P levels had little influence on the internal P concentrations of mycorrhizal plants. When P is not limiting growth, extensive VAMF colonization may reduce plant growth as the fungi drain carbohydrates from the host without contributing beneficially to the plants' nutrient supply (Bethlenfalvay et al. 1983). Mycorrhizae did not significantly reduce total yields at the highest soil P level tested in this study.

By manipulating the P supply to the transplants, it was possible to compare the relative importance of growth prior to transplanting, tissue P content and mycorrhizal infection as determinants of subsequent growth. When VAMF infection was required to overcome deficiencies in soil P, extensive colonization of the transplants was more important than transplant size or tissue P content in determining subsequent performance. As P requirements are greatest in young plants (Loehwing 1951), rapid post-transplant development of a functional VAMF symbiosis could be particularly beneficial. Restricting P during transplant production may promote rapid establishment of the VAMF symbiosis following transplanting by allowing extensive development of the root infection prior to transplanting. Extensive VAMF colonization has been shown to improve post-transplant performance of mycorrhizal seedlings (Bagyaraj and Sreeramulu 1983, Biermann and Linderman 1983b), although Haas et al. (1986) found increasing seedling stage fertility enhanced subsequent growth of mycorrhizal peppers in a low P soil, despite the negative effect on VAMF colonization.

In this study, 60% root infection at transplanting was not sufficient for maximum mycorrhizal response during subsequent growth. Early infection levels may strongly influence the extent of subsequent infection, particularly in soils devoid of secondary inoculum. Other researchers have found lower rates of transplant colonization to be adequate (Haas et al 1986,

Bagyaraj and Sreeramulu 1983, Biermann and Linderman 1983b). More research is needed to determine the optimum balance of growth versus infection in transplants intended for soils of different P fertility and mycorrhizal status.

By increasing P uptake, VAMF substantially reduced the external P requirements of bell peppers in this pot study. Differences in root volume and yield potentials, and the barrier effects produced by pot walls, make it risky to extrapolate fertility recommendations from pot studies to the field. The solution P requirements for mycorrhizal and nonmycorrhizal bell peppers in this pot study were substantially lower than in the field study by Haas et al. (1987) but were similar to the responses observed in the field study by Waterer and Coltman (unpublished). Crop P requirements may vary with the growing conditions and P buffer capacity of the soil. The soil P requirements of mycorrhizal plants may also be influenced by the efficiency of the VAMF symbiosis. The potential for VAMF to improve the mineral nutrition of peppers appears promising, particularly in the P deficient and P-fixing soils of the tropics.

Chapter VII

Response of Lettuce to Pre- and Post-transplant Phosphorus and
Pretransplant Inoculation with a VA-Mycorrhizal Fungus¹.

¹Submitted: Plant and Soil.

Introduction

Many plants form symbiotic associations with root infecting vesicular-arbuscular mycorrhizal fungi (VAMF) (Maronek et al. 1981). By increasing the efficiency of absorption of relatively immobile mineral nutrients such as phosphorus (P), mycorrhizae may improve the mineral nutrient status and growth of the host (Daft and Nicolson 1966). Benefits of the symbiosis are greatest when a rapidly growing host with high internal P requirements is grown on P deficient or fixing soils (Yost and Fox 1979, Menge 1983, Habte and Manjunath 1987). In increasingly P rich soils, the significance of the symbiosis declines and frequently there is a corresponding decrease in the development of the VAMF/root association (Hayman 1983).

Abundant phosphorus is critical for the rapid, uniform growth required of most horticultural crops. Promotion of mycorrhizal infection of these plants might insure an adequate supply of nutrients, while potentially reducing the fertilizer requirements of the crop. The importance of adequate P during early crop development suggests that rapid establishment of a functional VAMF symbiosis would be desirable, particularly in rapidly maturing crops. In transplanted crops, inoculation with the VAMF prior to transplanting should speed infection relative to infections instigated upon transplanting into the field (Bagyaraj and Sreeramulu 1982, Biermann and Linderman 1983). Pretransplant

inoculation also may allow more efficient use of limited VAM inoculum than inoculation at transplanting (Waterer and Coltman unpublished).

Generous applications of soluble fertilizers are generally employed in the production of transplants. However, excessive P in the roots of the host may inhibit VAMF colonization (Menge et al. 1978). The P supply to the transplants must balance the apparently contradictory goals of maximum growth and infection (Waterer and Coltman 1988a and b).

The purposes of this study were 1) to determine the effects of mycorrhizae on the growth and P uptake of lettuce transplanted into P-rich and P-deficient soils and 2) to assess the relative importance of size versus VAMF infection status of lettuce transplants as determinants of subsequent performance in the field.

Materials and Methods

Transplant production. Transplants of a semi-head lettuce (cv. Green Mignonette) were grown in plastic flats using a 1 sphagnum peat : 1 vermiculite (by volume) medium, adjusted to pH 6.1 with dolomitic lime. Micronutrients were supplied as 0.5 kg/m³ Micromax (Sierra Chemical Co., Milipitas, Calif). At seeding, half the flats were inoculated with dried corn roots (Zea mays L. 'Hawaiian Supersweet No. 9') heavily infected with

the mycorrhizal fungus Glomus aggregatum. The media surrounding each seedling contained about 250, 10-mm-long root fragments. To establish equivalent background microbial populations in all treatments, mycorrhizae-free filtrates from VAMF-infected root fragments were applied to all noninoculated flats.

To produce transplants that varied in size, tissue P status and mycorrhizal colonization, the medium was fertilized with 80, 210 or 600 g P/m³, using 19N-2.6P-10K Osmocote as the P source (Sierra Chemical Co., Milpitas, Calif). Appropriate amounts of 39-0-0 and 0-0-40 Osmocote were added to provide equal N and K to all treatments. In a preliminary experiment, lettuce transplants produced with 80 g Osmocote P/m³ were stunted and P deficient, but extensively colonized by VAMF. The 210 g P/m³ rate produced larger plants with reasonably well-colonized roots. The highest P rate produced very large transplants, but the resulting high levels of tissue P significantly reduced mycorrhizal colonization of inoculated plants.

Lettuce transplants were raised in a greenhouse in Honolulu, Hawaii (21°51' N and 156°22' W) during October 1986. Peak light intensity was about 1400 $\mu\text{mol/s/m}^2$ and the daylength about 12 hr. Average maximum and minimum air temperatures were 36 and 26°C.

Five weeks after seeding, dry weights, tissue P concentrations and mycorrhizal colonization ratings were determined on 20 plants from each transplant production-P level

and mycorrhizal inoculation treatment. The molybdenum blue method (Murphy and Riley 1962) was used for tissue P concentration determinations following dry-ashing at 550°C. Samples from the root system of each plant were cleared and stained (Phillips and Hayman 1970) and 30, 1-cm-long fragments examined to determine the extent and intensity of infection in each segment (rating of 0 - no infection, 1 - entry points only present, 2 - small areas of hyphae present, 3, 4 and 5 - hyphae present over 50%, 75% and 100% of the root, respectively).

Plant responses. Lettuce was transplanted into a Waialua clay (Vertic Haplustoll), pH 6.1, on the Waimanalo Research Station of the University of Hawaii. Two years previously, the field had been divided into eight subplots and fertilized with two rates of treble superphosphate. At transplanting, the solution P concentrations of the subplots had equilibrated at 0.02 and 0.30 mg/liter. Prior to transplanting, the field was disked, rotovated and fumigated with 1kg/25m² methyl-bromide/chloropicrin. The equivalent of 150 kg N/ha as urea and 150 kg K₂O/ha as KCl were broadcast and incorporated during rotovation.

Five week old transplants were set at 23 cm intervals, with 10 plants/row, 30 cm between rows, and 8 rows/plot. Adjacent plots were 1 m apart. A trickle irrigation system was used to insure

that soil water potentials (SWP) at 23 cm below the soil surface never went below -10kPa throughout the experiment.

Eight adjacent plants were harvested from each plot every seven days after transplanting. Groups of plants were selected at random from within each plot and a row of plants was maintained between each group of plants in order to minimize edge effects. The final harvest was five weeks after transplanting. Weights of the tops were obtained following oven drying at 60°C. The concentration of P in the dried tops was determined and the total P accumulated in the tops calculated from the dry weight and P concentration data. At the final harvest, roots outside the transplant media ball were collected and examined for mycorrhizal infection. The intensity of infection was rated using a somewhat different scale than previously described for the transplants: 0 = no infection, 1 = entry points only present, 2 = small areas of mycorrhizal structures (vesicles or arbuscles) present, 3, 4 and 5 = structures present over 50%, 75% and 100% of the root respectively.

The experiment was a 2 X 3 X 2 factorial (soil solution P x pretransplant P x mycorrhizal inoculation), with four replicates of each treatment combination. The field design was a split plot, using soil solution P as the main plots. Pretransplant P and mycorrhizal inoculation treatments were randomly assigned within each main plot. Mean separation tests were used to

examine the effects of transplant medium P, as there was insufficient data for analysis by regression.

Results and Discussion

Transplants. Increasing the Osmocote supplied during transplant production increased total dry weights and tissue P concentrations in the lettuce seedlings at transplanting (Fig. 7.1). At the lowest rate of pretransplant P, inoculated plants were heavier and had higher tissue P concentrations than noninoculated plants. Mycorrhizae probably enhanced plant growth in the P deficient medium by increasing the P uptake efficiency of the hosts' roots (Rhodes and Gerdemann 1975).

At transplanting, 80 to 90% of the roots of inoculated plants were colonized by the VAMF (Fig. 7.1). Percent colonization and infection intensities were depressed at the highest level of pretransplant P. High P concentrations in the roots at the highest P level apparently inhibited mycorrhizal colonization, as previously noted (Menge et al. 1978). There were no indications of mycorrhizal colonization of any noninoculated plants.

Response to soil P. Shoot P concentrations in lettuce transplanted into the P-rich plots (0.30 mg/liter solution P) were significantly greater than in the low P plots (0.02 mg/liter P) within a week after transplanting (Fig. 7.2). Greater shoot

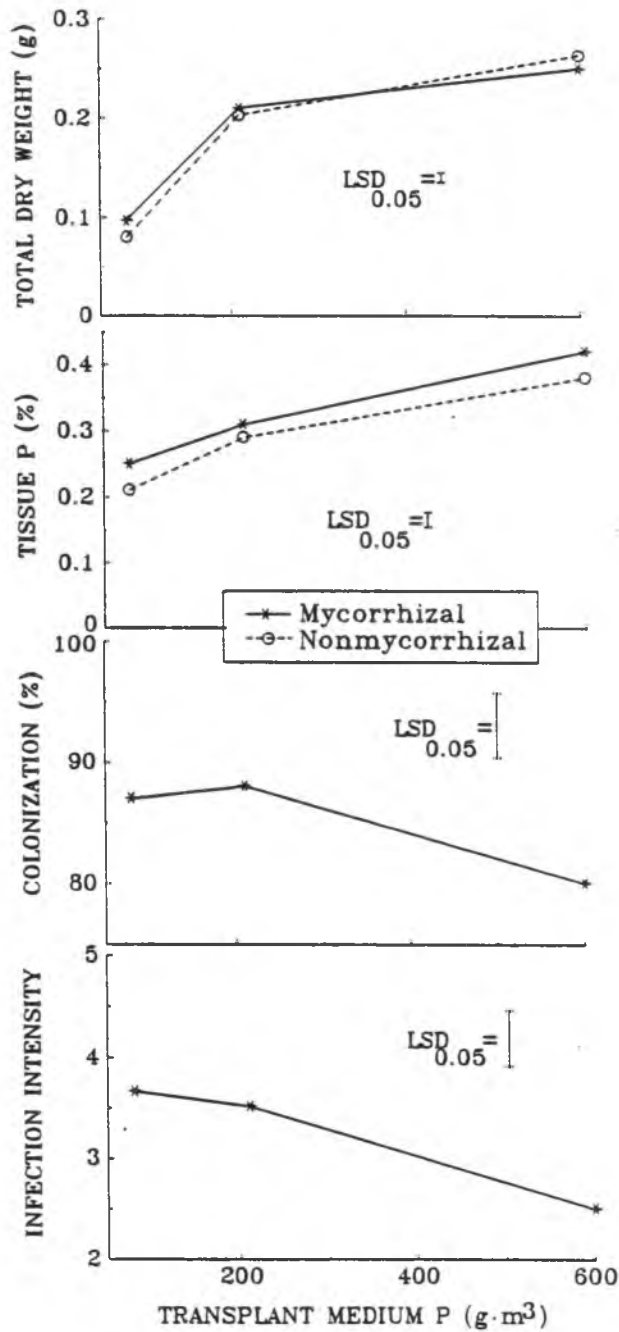


Fig. 7.1. Total dry weights, tissue phosphorus concentrations, percentage of roots colonized by mycorrhizal fungi and the intensity colonization at transplanting of lettuce transplants grown with three rates of P ($n=20$). Infection intensity of 0 = no infection, 5 = all roots heavily infected.

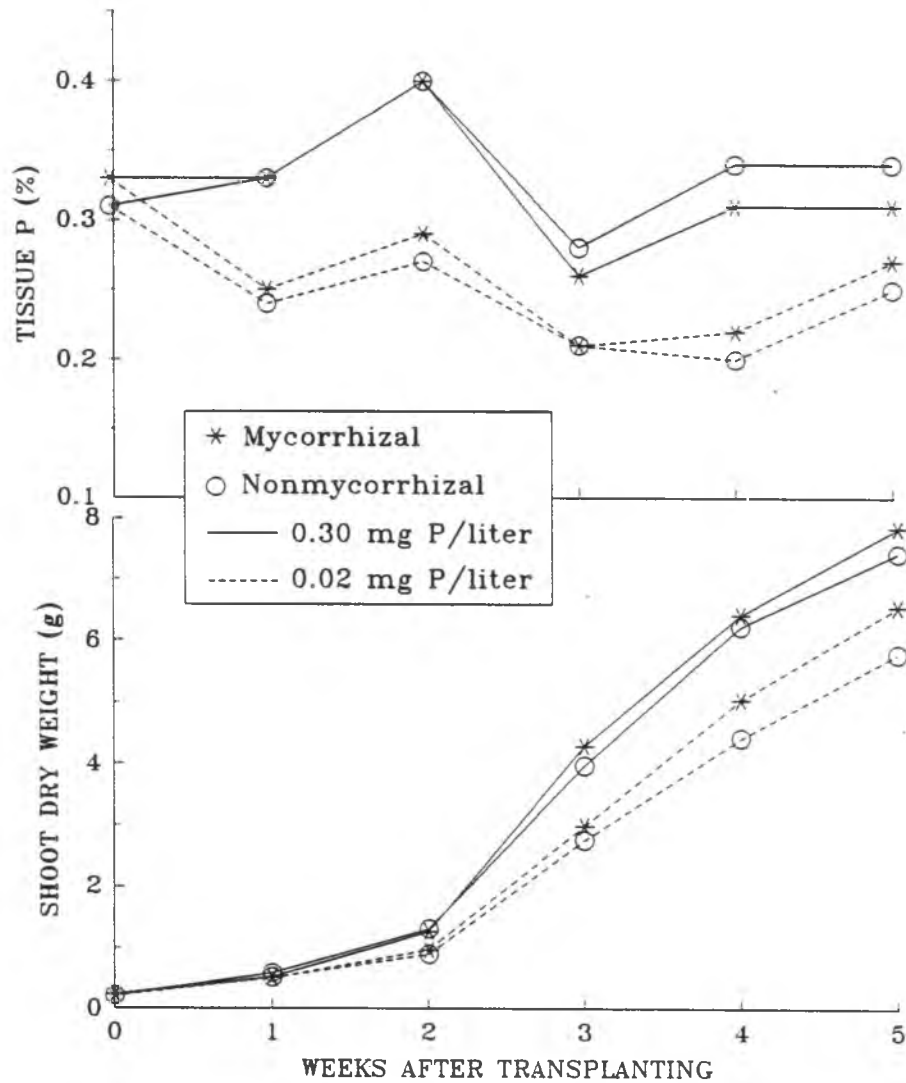


Fig. 7.2. Influence of soil solution phosphorus on tissue phosphorus concentrations and growth of mycorrhizal and nonmycorrhizal lettuce at varying intervals after transplanting (n=12).

dry weights followed from the third week after transplanting through to the final harvest. Average shoot P concentrations and shoot dry weights of plants growing in the high P plots were respectively, 25% and 26% greater than in the low P plots. Plants in the high P plots reached marketable size within four weeks of transplanting; a full week ahead of the lettuce in the low-P plots. The difference between dry matter yields in the high and low P plots was relatively small at the final harvest, as growth in the high P plots had slowed with maturation (Fig. 7.2). Phosphorus deficiencies generally slow the growth and maturity of lettuce rather than reducing final yields (Loewing 1951, Lorenz and Vittum 1980).

The soil P level did not significantly influence either the percentage of roots colonized by the VAMF or the intensity of root infection in inoculated plants at the final harvest (Table 7.1). Although tissue P concentrations were significantly elevated in the high P soils, development of the VAMF infection was apparently not inhibited. After five weeks in the field, mycorrhizal colonization of noninoculated plants was rare and considered insignificant.

Response to mycorrhizae. Inoculation increased the average shoot dry weights by 16% in lettuce transplanted into soil with 0.02 mg/liter solution P without affecting tissue P concentrations (Table 7.2). The beneficial effect of the

Table 7.1. F-test probabilities for growth, tissue phosphorus concentrations and mycorrhizal infection of mycorrhizal and nonmycorrhizal lettuce grown with different levels of pre- and post-transplant phosphorus and harvested at weekly intervals after transplanting.

Source of Variation	Weight Tops	Tissue P %	<u>Root Infection</u> % Intensity ²	
Solution P (P)	<0.01	<0.01	0.31	0.22
Transplant P (T)	<0.01	<0.01	0.28	0.02
P x T	0.08	0.95	0.75	0.67
Mycorrhizae (M)	0.02	0.89		
P x M	0.42	0.03		
T x M	0.01	0.48		
P x T x M	0.96	0.42		
Harvests (H)	<0.01	<0.01		
P x H	0.02	0.03		
T x H	0.01	0.13		
M x H	0.30	0.32		
P x T x H	0.55	0.91		
P x M x H	0.97	0.78		
T x M x H	0.89	0.79		
P X T X M X H	0.72	0.98		

²Infection assessed at the final harvest, five weeks after transplanting.

P = soil solution P concentrations of 0.02 or 0.30 mg/liter.

T = 80, 210 or 600 g Osmocote P/m³ transplant medium.

M = mycorrhizal or nonmycorrhizal.

H = weekly harvests, for weeks 1 to 5 after transplanting.

Table 7.2. Tissue phosphorus concentrations and shoot dry weights averaged over three pretransplant P levels and five post-transplant harvests for mycorrhizal and nonmycorrhizal lettuce grown with two levels of soil solution phosphorus.

Soil P	Tissue P (%)		Shoot dry weight (g)	
	Myco	Non	Myco	Non
Low ^z	0.25b ^y	0.23b	3.3b	2.8c
High	0.31a	0.33a	4.1a	3.9a

^zLow soil P=0.02 mg/liter, high=0.30 mg /liter soil solution.

^yMean separations for parameters by Duncan's test at 5% level.

mycorrhizae on growth became apparent two weeks after transplanting and gradually increased through to the final harvest (Fig. 7.2). With 0.30 mg P/liter soil solution, mycorrhizae did not significantly effect P uptake or growth of the lettuce. Bethlenfalvay et al. (1983) found that mycorrhizae decreased the growth of soybeans at high soil P levels. Lettuce, however, was not adversely affected by mycorrhizae at high soil P levels.

Response to pretransplant P. Greater amounts of P supplied during transplant production generally increased the average tissue P concentrations and shoot dry weights after transplanting (Fig. 7.3). The benefits of abundant pretransplant P decreased as the time from transplanting increased (Appendix Fig. 7A), reflecting the greater importance of P in promoting rapid early growth of lettuce than in affecting final P uptake or yields. Because the Osmocote in the transplant medium continued to supply P after transplanting, it was not possible to completely discriminate between the effects of pre- and post-transplant P. However, by the final harvest, lettuce given the least pretransplant P had accumulated several times as much P as was available in the Osmocote (Appendix Fig. 7A), indicating that the soil was the dominant source of P after transplanting.

Pretransplant P had relatively less effect on the subsequent growth of inoculated plants than on noninoculated plants (Fig.

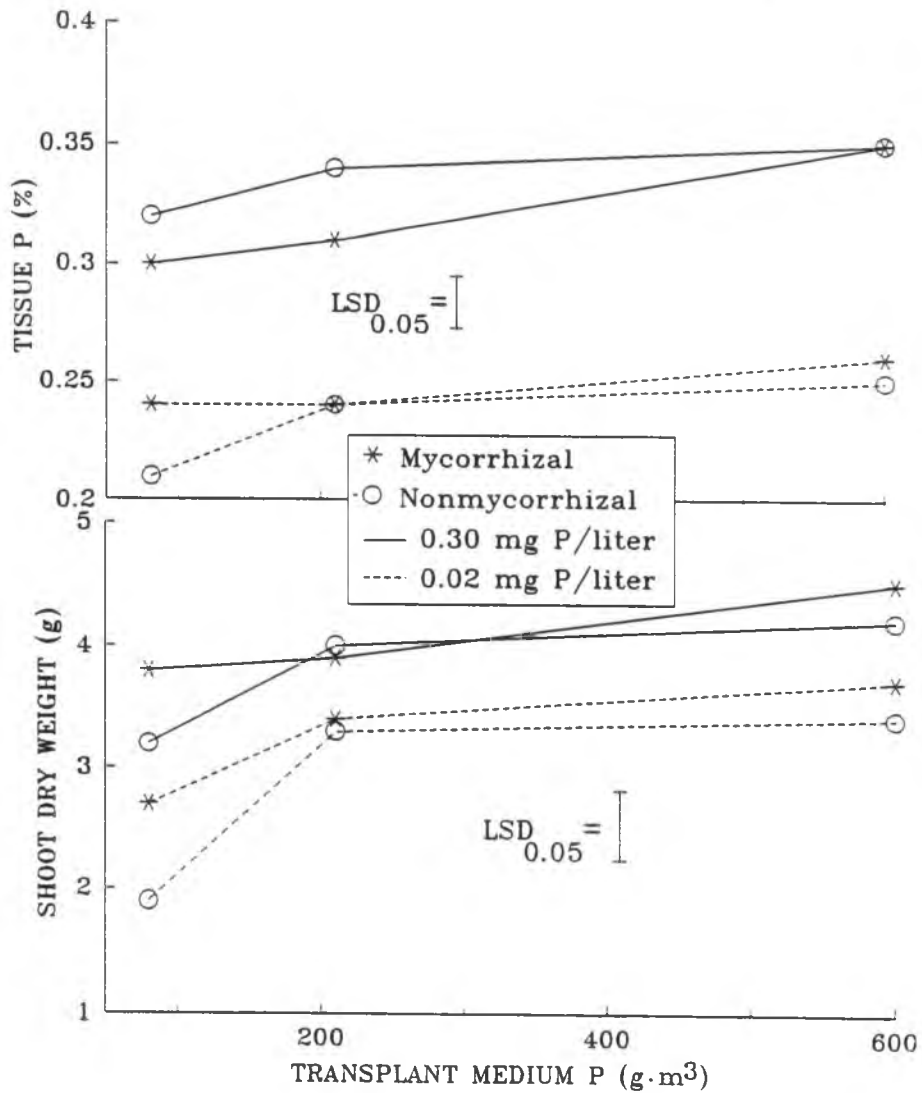


Fig. 7.3. Influence of pretransplant phosphorus on the average post-transplant tissue P concentrations and shoot dry weights of mycorrhizal and nonmycorrhizal lettuce at two levels of soil phosphorus ($n=4$).

7.3). Mycorrhizal transplants seemed better able to tolerate and recover from stress imposed by limited P during transplant production. The greater P uptake efficiency of mycorrhizal roots likely explains the improved performance of inoculated plants. Pretransplant P did not significantly influence the percentage of new roots infected by the VAMF at the final harvest (Fig. 7.4). However, the lower infection intensity observed in transplants produced with the highest rate of Osmocote P (Fig. 7.1) carried through to the final harvest (Fig. 7.4). The importance of vigorous early infection as a determinant of final infection has been noted in other studies (Haas et al. 1986, Waterer and Coltman unpublished).

The absence of a significant positive response to mycorrhizae at 0.30 mg P /liter suggests that this level of available P is sufficient for maximum yields of lettuce, regardless of their mycorrhizae status. Nishimoto et al. (1977) found a similar P response threshold in direct-seeded leaf lettuce. In our study, with 0.02 mg/liter solution P, average yields of mycorrhizal and nonmycorrhizal plants were 85 and 75% of the yields obtained at 0.30 mg P/liter. In Nishimoto's study, plant fresh weights at 0.02 mg P /liter were only 40% of maximum. Crops responsiveness to solution P is relatively uniform in different soils (Fox et al. 1974), however, climatic and cultural variables conceivably could influence plant P requirements (Viets 1972, Nishimoto et al. 1977). The greater sensitivity of lettuce to suboptimal soil

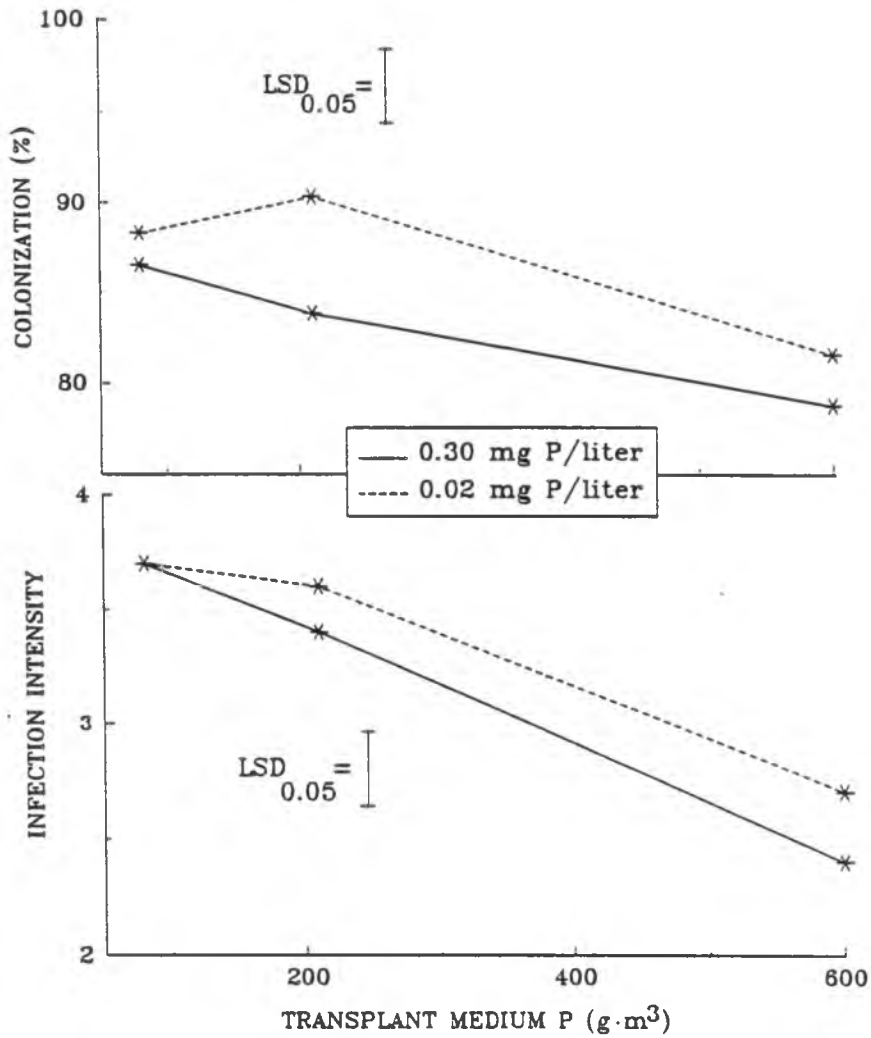


Fig. 7.4. Influence of pretransplant phosphorus and soil solution P levels on the extent and intensity of mycorrhizal colonization of lettuce five weeks after transplanting (n=8). Intensity rating of 0 = no infection, 5 = all roots heavily infected.

P levels observed by Nishimoto et al. may be related the direct-seeding of their crop while we used transplants. Early growth of lettuce requires more P than later stages of development.

Preinoculation of transplanted crops represents an efficient means of producing mycorrhizal infections early in the development of the crop. However, maximizing infection requires the restriction of tissue P concentrations, which may compromise growth. To produce dry matter yields of a rapidly maturing crop such as lettuce, we found that transplants had to be given sufficient P to optimize pretransplant growth. Restricting pretransplant P to maximize infection inappropriate since the overall response to infection was relatively small. Further, the difference in the degree or intensity of infection of the transplants produced with the differing concentrations of pretransplant P was not large. Maximizing the infection of transplants might be of greater consequence in crops more responsive to the mycorrhizae or in more P deficient soils.

Chapter VIII

Influence of Vesicular-Arbuscular Mycorrhizae on Water Relations
of Seedling and Mature Bell Pepper

Introduction

By enhancing the absorption of immobile mineral nutrients such as phosphorus (P), symbiotic vesicular-arbuscular mycorrhizal fungi (VAMF) may increase growth of their plant hosts (Gerdemann 1968). In addition to increasing nutrient uptake, the mycorrhizal symbiosis may influence the hosts' tolerance to disease (Davis and Menge 1980), salinity (Hirrel and Gerdemann 1980) and excess heavy metals (Hayman 1980). Inoculation of tree seedlings with ectomycorrhizal fungi prior to transplanting is used to increase survival and subsequent recovery from transplant shock (Theodorou and Bowen 1970, Dixon et al. 1981). Similar responses to pre-transplanting inoculation with VAMF have been reported in avocado (Menge et al. 1978), poinsettia (Barrows and Roncadori 1977) onion (Mosse and Hayman 1971) and tomato (Yost et al. unpublished). The increased survival of mycorrhizal transplants is generally attributed to a greater ability to tolerate water stress (Menge et al. 1978, Dixon et al. 1983). Limited root volumes, low root to shoot ratios and poor initial contact between the roots and the soil would appear to leave transplants particularly prone to water stress.

Several mechanisms have been suggested to explain how mycorrhizae increase water uptake and/or moisture stress tolerance: 1) the fungal hyphae act as a low resistance pathway

for water flow from the soil into the roots (Hardie and Leyton 1981), 2) the hyphae increase the absorptive surface area of the root (Safir et al. 1972), 3) hyphae penetrate pore spaces smaller than the root hairs, increasing the available water supply (Reid 1979), and 4) indirect benefits resulting from improved host nutrition (Safir et al. 1972, Levy and Krikun 1980), because P availability influences membrane permeability (Nelsen and Safir 1982) and vascular development (Daft and Okusanya 1973). Mycorrhizae also may influence the hormonal regulation of stomatal aperture (Levy and Krikun 1980). Each of these factors may, in turn, influence plant water relations.

The purpose of this study was to determine the effects of VAMF on the water relations of seedling and mature peppers under well-watered and water-stress conditions. To segregate the direct effects of mycorrhizae from the effects related to improved host nutrition, responses of plants grown with differing amounts of available phosphorus were compared.

Materials and Methods

Seedling production. Inoculated and noninoculated pepper seedlings (cv. Emerald Green Giant) were grown in plastic transplant flats with 75 ml cells filled with a 1:1 mixture of sphagnum peat and vermiculite (pH 6.0). Micronutrients were supplied as Micromax (Sierra Chemical Co., Milpitas Calif.)

incorporated at 0.3 kg/m^3 . To produce variation in size, P status and mycorrhizal infection of the seedlings, sufficient 19-3-10 Osmocote (Sierra Chemical Co.) was added to supply 80, 210 or 600 g P per m^3 of medium. Appropriate amounts of 39-0-0 and 0-0-40 Osmocote were added to provide equivalent N and K to all treatments.

Each inoculated seedling received approximately 250, 1-cm long dried corn root fragments heavily infected with the VAMF Glomus aggregatum. Mycorrhizae-free washings from the inoculum were applied to the noninoculated plants to insure equivalent background microbial populations in all treatments.

The seedlings were grown in a greenhouse in Honolulu, Hawaii. Maximum light intensity was about $1200 \text{ umol s}^{-1} \text{ m}^{-2}$, the daylength about 12 hr and the average maximum and minimum temperatures 34 and $27 \text{ }^\circ\text{C}$. Five weeks after seeding, roots in all treatments had extended throughout the available medium but the plants were not root-bound.

Seedling water relations. Whole plant transpiration rates were determined for five uniform plants from each seedling production treatment. The medium surrounding each seedling was watered to field capacity, then the roots and associated medium were carefully transferred into a polyethylene bag which was sealed around the base of the stem to prevent evaporation. The bags were placed on a greenhouse bench and the rate of water

loss determined by weighing at 4 h intervals. At dusk, each bag was opened and rewatered to prevent water stress or oxygen deficits. Transpiration (E) was calculated as gravimetric water loss per plant or per unit leaf area per second averaged over three successive 12 hr photoperiods.

Root hydraulic conductivity of the same seedlings was evaluated using the pressure bomb method described by Hardie and Leyton (1981) and modified by Graham and Syvertsen (1984). Before measurement, the medium was watered to field capacity, and the plants were equilibrated for 24 h at 23°C and low light intensities ($400 \text{ } \mu\text{mol s}^{-1} \text{ m}^{-2}$) in the laboratory. The stem was cut 8 cm above the roots and the intact roots and associated medium were placed in the pressure bomb, with the cut stump exposed through the rubber stopper. The pressure within the chamber was gradually increased to 0.25 MPa and after 5 min of equilibration, the weight of xylem fluid extruded from the cut stem was measured during three, 3 min collection periods.

The medium was washed from the roots and the total length of the root system (excluding tap root) was estimated by the line-intersect method (Tennant 1975). The hydraulic conductivity of the roots (K_{root}) was expressed as the weight of xylem exudate per length of root per unit time and pressure ($\mu\text{g m}^{-1} \text{ s}^{-1} \text{ MPa}^{-1}$). The roots were dried (60°C) and weighed, then cleared, stained and examined for mycorrhizal colonization (Phillips and Hayman 1970). Sixty, 1 cm long root

fragments from each plant were rated for infection as: 0 = no infection, 1 = entry points only present, 2 = small areas of hyphae present, 3, 4 and 5 = hyphae present over 50, 75 and 100% of the root segment, respectively.

Leaf areas were determined with a leaf area meter (Licor model 3050A). Tissue P concentrations in the combined leaves and stems were then determined by the molybdenum blue method (Murphy and Riley 1962) after dry-ashing at 550°C.

Seedling water relations under water stress. Seedlings were raised as previously described. Five weeks after seeding, uniform plants from each P rate and mycorrhizal treatment combination were set, one to a pot, into plastic-lined pots filled with 5.1 kg (dry weight) of a 1:1 mixture of Waialua clay and basaltic sand (pH 5.9). Solution P concentrations (0.01 M CaCl_2 extraction, Fox and Kamprath 1970) in the soil/sand mix were 0.01, 0.03, 0.10, 0.30 or 1.0 mg/liter. The integrity of the plug surrounding the roots was carefully maintained. Soil was firmed around the root ball and the pots were watered, for the first and only time, to field capacity (approx. 27% by weight).

The experiment was conducted in a greenhouse, with maximum light intensities of about $1200 \text{ umol s}^{-1} \text{ m}^{-2}$, daylengths about 12 hr, and average maximum and minimum temperatures of 36 and 26 °C. The turgor status of the plants was checked each

day prior to sunrise. The plants were considered wilted if turgor was not recovered by dawn. Wilting occurred within 3 to 10 days of transplanting, depending on the size of the plants. The gravimetric water content of the soil at wilting was determined by weighing the pots. Water potentials at wilting of three leaves from each plant were estimated using a pressure bomb.

The experiment was conducted as a 2 X 3 X 5 factorial (mycorrhizal inoculation x seedling P rate x soil P) with five replicates. Discriminant function analysis methods were used to determine if the mycorrhizae or P rates influenced the relationship between soil water content and plant water potentials at wilting. Multivariate functions incorporating the soil water content and LWP data were derived for each treatment combination. Treatments were classified according to how closely the derived functions resembled one another, based on Hotelling's T^2 test for multivariate functions (Snedecor and Cochran 1937).

Mature plant water relations. Plants were grown to maturity from seed in pots containing 2.8 kg (dry weight) of a 1:1 mixture of a fumigated Waialua clay soil and basaltic sand (pH 5.8). Solution P concentrations in the soil/sand mix were 0.03, 0.1 or 0.30 mg/liter. The equivalent of 200 kg/ha KNO_3 was mixed into the soil prior to planting. Inoculated treatments

received 1000 spores per pot of *G. aggregatum* placed just below the seeds. Noninoculated pots received mycorrhizae-free washings from inoculum cultures.

The pots were maintained in a greenhouse with maximum light intensities of about $1200 \text{ umol s}^{-1} \text{ m}^{-2}$, daylengths about 13.5 hr and average maximum and minimum temperatures of 37 and 26°C. Pots were watered to approximately field capacity every day. To monitor the onset and effectiveness of the mycorrhizal infection, the concentration of phosphorus in the most recently fully expanded leaves was determined at 7 day intervals using disks removed from the leaf blades (Aziz and Habte 1987).

Sixty-five days after planting, the pots were watered to field capacity a final time, then enclosed in plastic bags which were sealed around the stem to prevent evaporation from the soil. Prior to sunrise each day, the turgor status of the plants was visually checked and the pots were weighed to determine water use and soil moisture content. Transpiration rates were calculated as gravimetric water loss per unit leaf area per second for the daylight hours from the final watering to wilting. Plants were considered wilted when the most recently fully expanded leaf remained wilted at dawn. Plant water potentials at wilting were determined for three leaves from each plant using a pressure bomb. Once wilted, the plants were harvested and leaf areas, and root, shoot and fruit dry weights determined. Root samples were examined for mycorrhizal

infection as previously described, but using a different intensity rating scale due to the more advanced stage of infection: 0 = no infection, 1 = entry points only present, 2 = small areas of mycorrhizal structures (vesicles or arbuscles) present, 3, 4 and 5 = mycorrhizal structures present over 50, 75 and 100% of the root segment, respectively.

The experiment was a 2 X 3 factorial (mycorrhizal infection X soil P) with five replicates. Discriminant function analyses were again used to determine if mycorrhiza or soil P levels influenced the relationship between soil water content and leaf water potentials at wilting.

Results

Water relations in well-watered transplants. Increasing phosphorus in the transplant medium increased the total dry weights (TDW), leaf areas, root lengths and tissue P concentrations in the pepper seedlings (Table 8.1). Inoculation did not significantly effect any of these parameters. The mycorrhizae increased total P uptake at the lowest P rate, but decreased P uptake at higher P rates (Appendix Table 8a). The percentage of roots colonized in the VAMF inoculated treatments and the intensity of the infection both decreased as more P was applied (Table 8.1). Mycorrhizae had no effect on the

Table 8.1. F-test probabilities and mean values for the growth characteristics, P concentrations and mycorrhizal infection of mycorrhizal and nonmycorrhizal pepper seedlings raised with three rates of phosphorus.

Effect	Total Dry Weight (g)	Leaf Area ($m^2 \times 10^{-3}$)	Root Length (m)	P (%)	Root Infection % Intensity ^y	
Statistics						
Inoc-ulation	0.90	0.51	0.89	0.96	-	-
P Level	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
I x P	0.01	0.11	0.37	0.14	-	-
Means						
<u>Inoc-ulation</u>						
+	0.69a	10.7a	10.0a	0.24a	-	-
-	0.64a	10.6a	9.8a	0.23a	-	-
<u>P Rate^x</u>						
Low (L)	0.37c	5.8c	6.1b	0.14c	-	-
Medium (M)	0.64b	10.0b	8.7b	0.26b	-	-
High (H)	1.05a	16.5a	15.2a	0.33a	-	-
<u>Treatment</u>						
L/+	0.47cd	6.7cd	7.4c	0.13c	98a	4.4a
L/-	0.27d	4.8d	4.6c	0.14c	-	-
M/+	0.53bc	9.0bc	7.7c	0.27b	90a	2.9b
M/-	0.74b	11.4b	9.9bc	0.25b	-	-
H/+	1.03a	15.8a	14.5b	0.32a	58b	1.1c
H/-	1.10a	17.8a	16.5a	0.33a	-	-

^zValues within blocks followed by the same letter are not significantly different (Duncan's or t-test, $p=0.05$).

^yIntensity scale, 0 = no roots infected, 5 = all roots heavily infected.

^xLow = 80 g P/m³ media, medium = 210 g/m³ and high = 600 g/m³.

root/shoot weight ratio, although the ratio declined as more P was made available to the plants (Appendix Table 8A). Mycorrhizal roots were significantly heavier per unit length than nonmycorrhizal roots (Appendix Table 8A).

Total transpiration increased with leaf area ($r^2=0.85^*$) and was unaffected by mycorrhizae inoculation (Appendix Table 8B). Transpiration per unit leaf area was significantly lower for seedlings raised with the highest P rate than for seedlings given less P (Fig. 8.1) and also was unaffected by inoculation. Transpiration per unit root length was not affected by inoculation or the varying rates of P (Appendix Table 8B). Inoculation increased root hydraulic conductivity by three fold at the lowest P rate but did not effect conductivity at higher P rates (Fig. 8.1).

Post-transplant water stress. Soil P concentrations did not influence any of the measured water relation parameters of the pepper seedlings and the data reported are averages over soil P levels. Seedlings raised with the most pretransplant P wilted significantly earlier than the smaller transplants produced with the lower P rates (Appendix Table 8B). Mycorrhizal and nonmycorrhizal transplants wilted at similar soil moisture contents (Fig. 8.2). Although plant weights and tissue P concentrations were similar for mycorrhizal and nonmycorrhizal plants from each pretransplant P level (Table 8.1), wilted mycorrhizal plants consistently had higher leaf water potentials

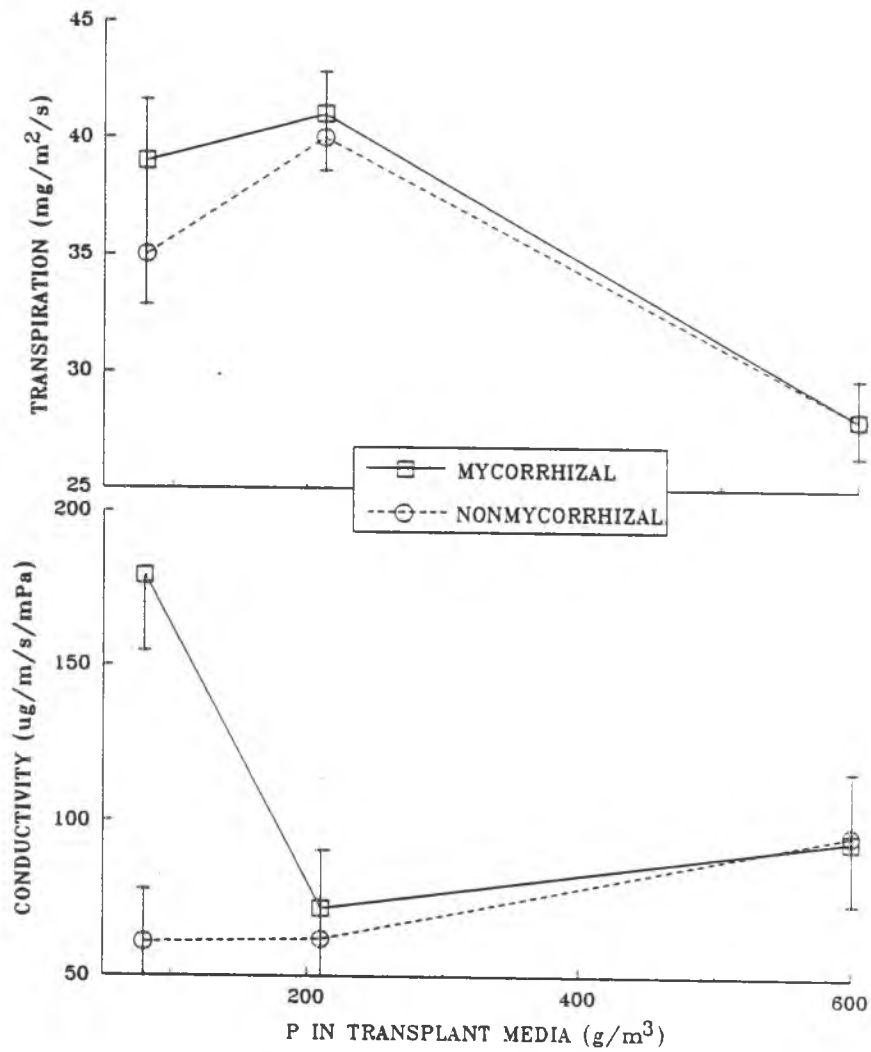


Fig. 8.1. Transpiration and root hydraulic conductivities of mycorrhizal and nonmycorrhizal pepper seedlings raised with three rates of phosphorus.

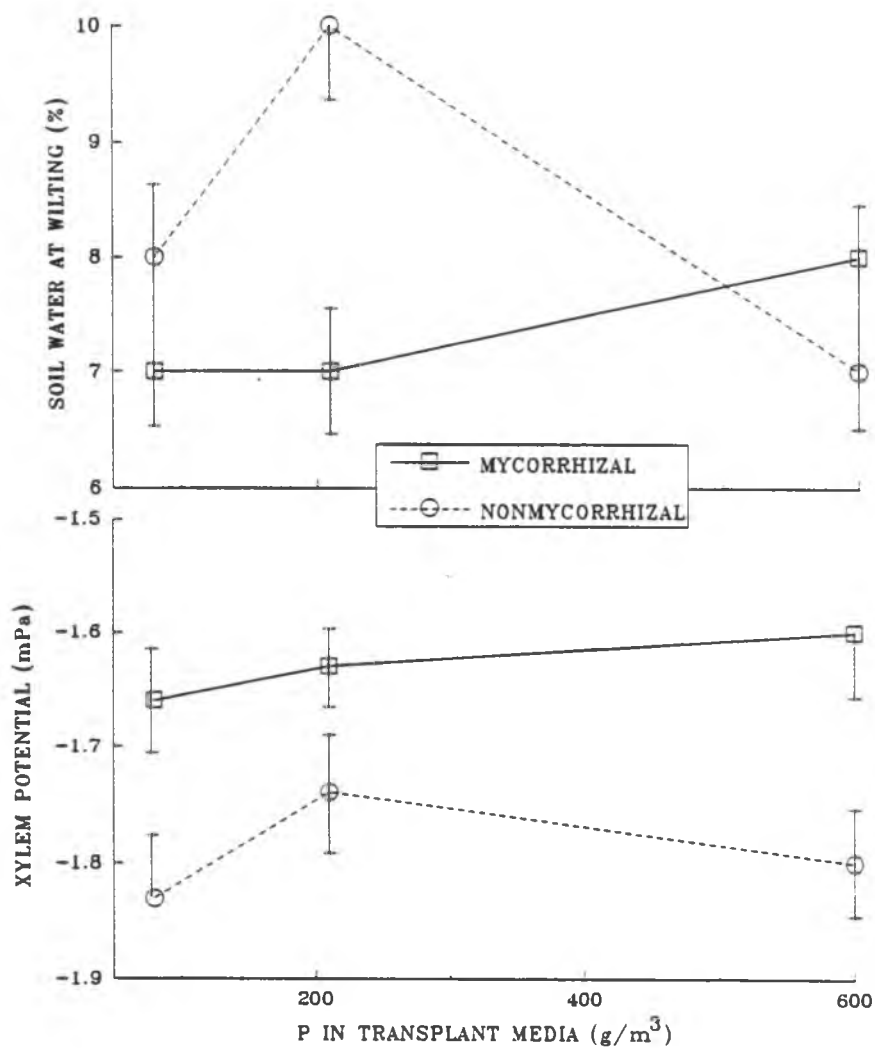


Fig. 8.2. Soil water content and xylem water potentials at wilting for transplanted mycorrhizal and nonmycorrhizal pepper seedlings raised with three rates of phosphorus.

(LWP) than nonmycorrhizal plants (Fig. 8.2). The mycorrhizal status of the transplants could be predicted with about 80% accuracy based on discriminant function analyses of the relationship between LWP and soil moisture at wilting (Appendix Table 8C).

Mature plant water relations. Average TDW and leaf areas increased as soil solution P increased (Table 8.2). Inoculation with the VAMF increased TDW, leaf areas and leaf P concentrations at 0.03 mg/liter solution P, but had little effect at higher solution P concentrations. The extent and intensity of mycorrhizal infection of the roots was not significantly influenced by soil solution P concentrations.

In contrast to the results obtained with the well-watered seedlings, where transpiration per unit leaf area was unaffected by inoculation, the average rate of transpiration per unit leaf area for droughted mature plants was 30% greater for inoculated as compared to noninoculated plants (Table 8.2). Higher transpiration rates for inoculated plants were consistent across the soil P levels. Mycorrhizal plants grown with 0.10 or 0.30 mg P/liter extracted significantly more water from the surrounding soil before wilting than comparable nonmycorrhizal plants (Fig 8.3). At wilting, the LWPs of mycorrhizal plants were significantly higher than the LWPs of nonmycorrhizal plants when soil P was low. However, the LWPs of nonmycorrhizal plants increased to levels comparable to values in mycorrhizal plants

Table 8.2. F-test probabilities and mean values for the growth characteristics, P concentrations, mycorrhizal infection and transpiration (E) of mycorrhizal and nonmycorrhizal peppers grown to maturity with three levels of soil solution phosphorus.

Effect (mg/m ² /s)	Total Dry Weight (g)	Leaf Area (m ²)	P (%)	<u>Root Infection</u> % Intensity ^y		E
Statistics						
Inoc- ulation	0.08	0.01	0.01	-	-	0.01
Soil P	<0.01	<0.01	<0.01	0.12	0.80	0.92
I x P	<0.01	0.02	0.15	-	-	0.84
Means						
<u>Inoc- ulation</u>						
+	12.0a ^z	7.3a	0.27a	-	-	67.9a
-	10.5a	5.4b	0.22b	-	-	51.5b
<u>Soil P^x</u>						
Low (L)	5.8c	4.2c	0.21b	-	-	61.1a
Medium (M)	8.4b	6.1b	0.22b	-	-	60.2a
High (H)	19.0a	8.8a	0.31a	-	-	60.2a
<u>Treatment</u>						
L/+	8.9c	6.4ab	0.26ab	87a	2.8a	66.6a
L/-	1.9d	1.4c	0.17c	-	-	51.4b
M/+	9.9c	6.7ab	0.22bc	90a	3.1a	70.3a
M/-	6.9c	5.5b	0.21bc	-	-	49.9b
H/+	17.1b	8.9a	0.33a	88a	2.9a	66.3a
H/-	20.8a	8.7a	0.29a	-	-	53.7b

^zValues within blocks followed by the same letter are not significantly different (Duncan's or t-test, p=0.05).

^yIntensity scale, 0 = no roots infected, 5 = all roots heavily infected.

^xLow = 0.03 mg P/liter soil solution, medium = 0.10 mg/liter, high = 0.30 mg/liter.

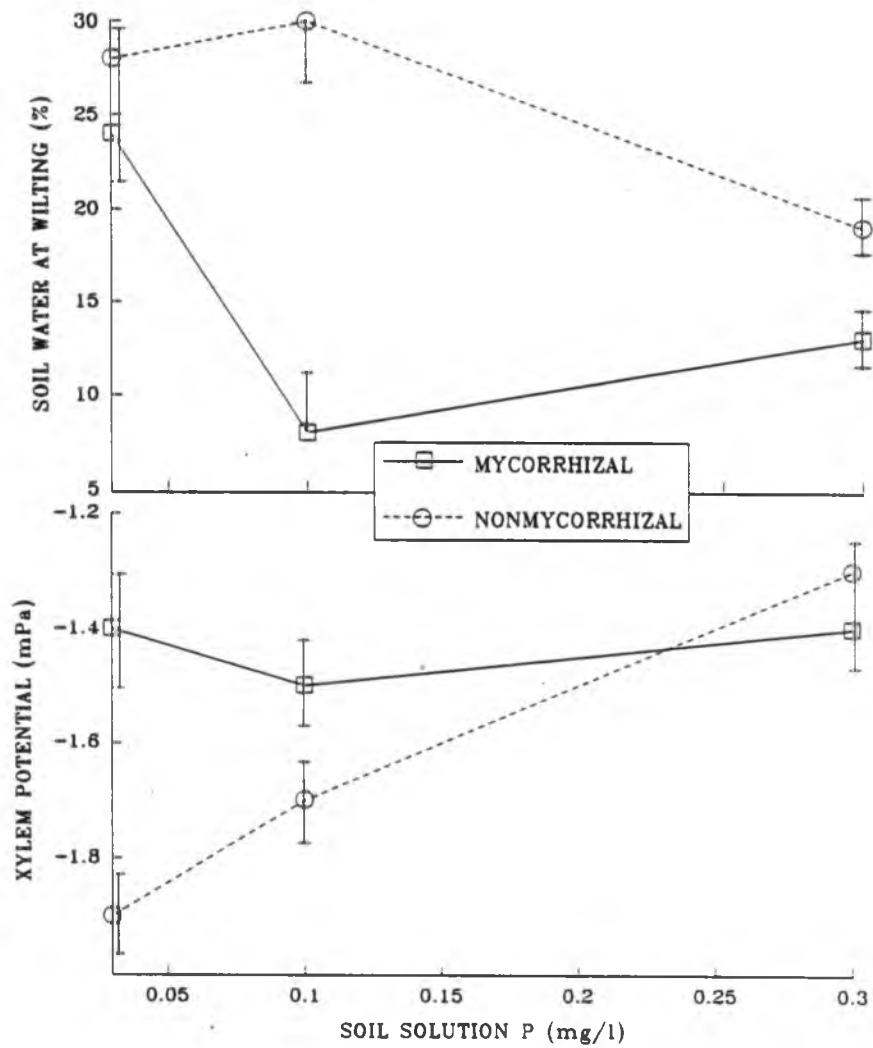


Fig 8.3. Soil water content and xylem water potentials at wilting for mature mycorrhizal and nonmycorrhizal peppers grown with three levels of soil solution phosphorus.

as soil P concentrations increased. Treatments could be divided into three groups based on discriminant function analysis of the relationship between leaf water potentials and soil moisture levels at wilting (Table 8.3). Inoculated plants in the high and moderate P soils had high xylem water potentials at low soil water contents. Noninoculated plants in the low and medium P soils had low xylem water potentials with relatively large amounts of water remaining in the soil. The low P, inoculated and high P, noninoculated treatments fit between the two extremes.

Discussion

Inoculation of pepper seedlings with the VAMF *G. aggregatum* increased root hydraulic conductivity when little P was available, but had no effect at higher P concentrations. Improvements in root hydraulic conductivity following VAMF infection have been observed in P-stressed soybean (Safir et al. 1972), citrus (Levy et al. 1983, Graham and Syvertsen 1984) and the range grass *Bouteloua gracilis* (Allen 1982). The increased conductivity of mycorrhizal roots is generally attributed to improved P status of the infected plants (Safir et al. 1972, Graham and Syvertsen 1984) rather than any direct contribution by the fungal hyphae to moisture flow (Hardie and Leyton 1981) as the mycorrhizae effect usually can be duplicated by

Table 8.3. Classification of soil P and mycorrhizae treatments based on discriminant function analysis of the relationship between soil moisture content and xylem water potentials at wilting of mature mycorrhizal and nonmycorrhizal peppers grown with three levels of soil phosphorus.

Treatment P/Inoculation	Characteristic of Class ^z
Low ^y / Medium/-	Low Xylem Ψ /High Soil \ominus
Low/+ High/-	Intermediate Xylem Ψ and Soil \ominus
High/+ Medium/+	High Xylem Ψ /Low Soil \ominus

^zDiscriminant functions for members of each class not significantly different ($p=0.05$) by Hotelling's T^2 test.

^yLow = 0.03 mg P/liter soil solution, medium = 0.10 mg/liter, high = 0.30 mg/liter.

supplying P to nonmycorrhizal plants (Safir et al. 1971, 1972, Levy et al. 1980). The hydraulic conductivity of roots under P stress may be restricted due to impairment of vascular development (Daft and Okusanya 1973) or the trans-membrane movement of water (Nelsen and Safir 1982). In this study, inoculation with the VAMF dramatically increased root hydraulic conductivity of the most P-stressed seedlings, although the mycorrhizae did not significantly improve the P status of the host. This suggests a non-nutritional mycorrhizal effect on root conductivity. Root infection by the VAMF was very intense in the most P stressed plants. At higher P levels in medium, conductivity of mycorrhizal roots fell to levels comparable to nonmycorrhizal roots. This decline might be due to the inhibitory effect of P on the intensity of root infection by the VAMF.

Inoculation with VAMF has increased transpiration under both well-watered (Nelsen and Safir 1982, Graham and Syvertsen 1984) and water-stress conditions (Levy and Krikun 1980, Hardie and Leyton 1981). This increased transpiration has been attributed to the mycorrhizae increasing root hydraulic conductivity, altering hormonal regulation of stomatal aperture or improving nutrient uptake and subsequent growth. In this study, mycorrhizae did not influence transpiration of well-watered pepper seedlings, but inoculation did increase the transpiration of full-size peppers as soil moisture declined from field

capacity to the wilting point. The effect of mycorrhizae on transpiration previously was found to vary with moisture availability, host species and fertility conditions (Graham et al. 1987).

Graham and Syvertsen (1984) found mycorrhizal plants had lower root/shoot ratios but higher transpiration rates than smaller nonmycorrhizal plants, resulting in higher transpiration rates/unit root length. We observed little effect of mycorrhizae on root/shoot ratios or the water uptake efficiency of the seedlings' root system. Mycorrhizal roots were heavier per unit length than nonmycorrhizal roots, possibly due the added internal and external biomass of the fungus.

Davison and Atkinson (1973) found the xylem water potentials at wilting of plants adequately supplied with P were consistently higher than for P deficient plants. Osmotic adjustments, protoplasmic viscosity or changes in cell dimension caused by P deficiency may change the rigidity of tissues, delaying collapse. By improving P uptake under P stress conditions, mycorrhizae might be expected to increase plant water potentials at wilting. In this study, both newly transplanted and mature moisture-stressed mycorrhizal peppers had higher average LWPs than comparable nonmycorrhizal plants growing at similar soil moisture levels. Leaf water potentials of the mature mycorrhizal and nonmycorrhizal plants only differed significantly when soil P concentrations were low,

suggesting the difference was related to improved P nutrition. On the other hand, the higher water potentials of transplanted and droughted mycorrhizal seedlings persisted at high soil and tissue P concentrations, indicating the difference in water potentials was not entirely due to mycorrhizal effects on P nutrition. Reid (1979) suggests that the hyphae of VAMF may help maintain contact between the root and the soil as the soil dries, thereby increasing moisture absorption at low soil water potentials. The maintenance of higher water potentials at wilting may explain why some mycorrhizal plants are better able to tolerate and recover from moisture stress than nonmycorrhizal plants (Levy and Krikun 1980, Safir et al. 1972). Because water stress is the primary cause of transplant shock, mycorrhizal inoculation may improve the performance of transplanted crops.

CHAPTER IX

SUMMARY

Objective 1. Phosphorus and inoculum requirements for production of mycorrhizal vegetable transplants in soilless media.

We were able to produce substantially colonized mycorrhizal seedlings of some of the most important transplanted vegetable crops (pepper, tomato, onion, leek and lettuce) using production techniques similar to those employed by commercial growers. The peat/vermiculite medium favored by many growers was not excessively inhibitory to establishment of the mycorrhizal symbiosis. Inoculation with the VAMF *G. aggregatum* had relatively little effect on the growth or mineral nutrition of the transplants. At very low P levels, inoculation occasionally increased seedling growth by increasing P uptake. When P levels in the medium were adequate for optimum growth of nonmycorrhizal seedlings, inoculated plants were often somewhat smaller than noninoculated seedlings. The additional energy demands of the VAMF infection may have caused this decline in transplant size.

Phosphorus levels in the growth medium strongly influenced growth and mycorrhizal infection of the transplants. High levels of P in the medium promoted growth of the seedlings, but the associated high P concentrations in the roots inhibited

colonization by the fungus. The goal of producing highly mycorrhizal, yet vigorous, non-P stressed transplants was complicated by the antagonistic effects of P on growth and infection.

We used up to 12,500 *G. aggregatum* spores or between 250 and 500, 1 cm long, infected root fragments as inoculum for each transplant. These inoculum rates were high relative to the levels employed by most researchers, but considering the positive correlation between inoculum density and infection, generous inoculum applications would appear to be desirable in the production of mycorrhizal transplants. Given the relative ease of producing inoculum of *G. aggregatum* (Coltman et al. 1988) sufficient inoculum for substantial acreages of horticultural crops could be produced with little difficulty. We found that increasing inoculum concentrations increased the degree and intensity of mycorrhizal colonization of transplants, but was only partially effective in overcoming the inhibitory effects of excess tissue P.

In assessments of different means of supplying P to the transplants we found that constant low levels of P (4 mg P/liter, applied daily) more closely met the goal of good growth and infection than when more concentrated P solutions were supplied at greater intervals (ie; 256 mg/liter, applied every eight days). The optimum balance between fertilizer solution

concentration and application interval varied between host species.

The positive effects of consistent low concentrations of P suggested that controlled release sources of P such as Osmocote might prove useful in the production of mycorrhizal transplants. Osmocote produced stable, predictable and reproducible solution P concentrations in the transplant medium. These characteristics allowed us to manipulate growth and infection of transplants simply by altering the Osmocote P supply. In all subsequent studies, Osmocote was used as the P source for production of mycorrhizal transplants. Controlled release P sources may also be useful tools in the study and management of other aspects of the mycorrhizal symbiosis ie; infection and inoculum production (Coltman et al. 1988).

In summary, by manipulating the P supply and inoculum concentrations, mycorrhizal vegetable transplants varying in vigor and degree of infection could be produced in soilless media. This study raised several questions for further research: 1) Considering the problems and constraints encountered in producing mycorrhizal transplants, is pretransplant inoculation cost, labor and input effective relative to inoculation at transplanting? 2) Given that a grower wishes to use mycorrhizal transplants, the production procedure must reflect some knowledge of the relative importance

of seedling size versus infection as determinants of subsequent growth.

Objective 2. Determination of the relative benefits of pretransplant inoculation with mycorrhizae versus inoculation at transplanting.

By speeding the establishment of a functional symbiosis following transplanting, we expected that pretransplant inoculation with the VAMF would more be effective than adding the inoculum at transplanting. We found that pretransplant inoculation of peppers with G. aggregatum increased early growth of peppers in low P soils, relative to plants inoculated at setting. The improved growth of the pretransplant inoculated treatments was due to a more rapid enhancement of P uptake by the pre-established fungal infection. With time, vegetative growth of field inoculated plants equalled the preinoculated plants. However, the early benefits of preinoculation had a lasting positive effect on fruit yields, perhaps due to the promotion of early fruit set and development by adequate early P.

Pretransplant inoculation of vegetable crops might be most beneficial in rapid maturing crops with high early season P demands (ie; lettuce). Preinoculation might also have, as yet undetermined, advantages over field inoculation in terms of

inoculum use efficiency and efficacy. The influence of preinoculation with VAMF on the tolerance of seedlings to transplanting stress should also be investigated.

Objective 3. Determination of the relative importance of size versus infection of mycorrhizal transplants as determinants of subsequent growth in soil with different levels of available phosphorus.

As P promotes growth and but inhibits mycorrhizal colonization of vegetable transplants a compromise between maximum growth and infection is necessary. We expected that host P requirements and the post-transplant soil P levels would influence the optimum balance of growth versus infection by determining the relative benefits of inoculation. We examined the effects of pretransplant growth versus infection on bell peppers and lettuce. In soils with sufficient available P for maximum post-transplanting growth of nonmycorrhizal peppers, we found that maximum transplant size had a greater beneficial effect on final yields than extensive mycorrhizal infection. At lower soil P levels, extensive colonization of the transplants became more important than maximum pretransplant growth. We found that extensive pretransplant colonization by the VAMF reduced the time required for the establishment of a functional symbiosis following transplanting. In P deficient soils, timely

improvement in P uptake was more important than pretransplant size.

In lettuce, maximum pretransplant growth of seedlings increased subsequent yields at all soil P levels tested. The difference in the relative importance of pretransplant infection versus growth in peppers and lettuce can be traced to a number of factors. Peppers are relatively slow maturing, have a high soil P requirement, particularly for fruit growth, and are highly responsive to mycorrhizae at sub-optimal soil P levels. Consequently, some pretransplant growth can be sacrificed in order to maximize development of the beneficial VAMF infection. By contrast, lettuce is vegetative and quick to mature, relatively tolerant of low soil P and unresponsive to mycorrhizae. In this type of crop, the early growth promoted by abundant pretransplant P cannot be compromised in order to promote a marginally important mycorrhizal infection.

These results indicate the P levels used in the production of mycorrhizal vegetable transplants should be based on prior knowledge of the relative importance of early growth versus infection as determinants of final yields. The balance will vary with the P requirements and mycorrhizal responsiveness of the crop and P levels in the soil.

Objective 4. Influence of mycorrhizae on the soil solution P requirements of pepper and lettuce.

In a series of greenhouse and field studies we found that the critical external P requirement of peppers inoculated with G. aggregatum was an order of magnitude lower than nonmycorrhizal plants (0.03 mg P/liter versus 0.40 mg/liter). In P deficient and P-fixing soils, this difference in external P requirement could substantially reduce the fertilizer P requirements for pepper production. Yields of mycorrhizal and nonmycorrhizal plants at non-limiting soil P levels were similar, indicating that mycorrhizal infection represented a minimal cost to the plants. Inoculation improved fruit yields more than vegetative growth, suggesting that flowering and fruit production in peppers is more sensitive to P stress than vegetative growth. The critical internal P requirement for total dry matter production by mycorrhizal peppers was lower than for noninoculated plants. This suggests that in addition to increasing the efficiency of P uptake, mycorrhizae may also influence the efficiency of utilization of absorbed P. To our knowledge, this is the first report of mycorrhizae increasing internal P-use efficiency.

The external P requirements for maximum yield of nonmycorrhizal lettuce were similar to that of pepper (approx. 0.30 mg P/liter). However, nonmycorrhizal lettuce was much more

tolerant of lower P levels than nonmycorrhizal peppers. Sub-optimal P levels slowed lettuce growth, but had little effect on final yields. Mycorrhizae had relatively little effect on the P status or growth. Tissue P concentrations in lettuce were relatively unaffected by the soil P level, possibly because of the corresponding slowing of growth. Stable tissue P concentrations in the low P soils may have hindered development or function of the mycorrhizal infection, resulting in a minimal mycorrhizal effect.

In summary, mycorrhizae substantially reduced the external P requirements of pepper but had relatively little effect on lettuce over the P levels studied. There were no discernible negative effects of inoculation in either crop, allowing us to recommend the inoculation of peppers and lettuce as a means of reducing P fertilizer requirements in P deficient soils and as a low-cost/low-risk insurance measure in soils thought to contain adequate P.

Objective 5. Influence of water stress on P uptake and mycorrhizal responsiveness of peppers.

Moisture stress reduces P uptake by plant roots by slowing the diffusion of P, disrupting contact between roots and the soil and by reducing root growth. As P availability declines with moisture deficit, the greater P uptake efficiency of

mycorrhizal root systems should become increasingly advantageous.

We subjected peppers to moderate water stress under greenhouse and field conditions in order to determine how moisture stress would effect growth, P uptake and responsiveness to mycorrhizae. Moisture stress had little effect on growth, P uptake or mycorrhizal responsiveness in P rich soil, suggesting that increasing solution P concentrations can compensate for the inhibitory effect of moisture stress on P availability and uptake. In P-deficient soils, growth and P uptake by the peppers were adversely affected by moisture stress. In both the greenhouse and the field, benefits of inoculation with the VAMF in P-deficient soil were greater under moisture stress conditions than when moisture was not limited. Mycorrhizae may increase P uptake in low moisture soils by: 1) the hyphae preventing shrinkage of the soil away from the roots during drying, 2) hyphae continuing to grow and absorb P at moisture levels inhibitory to plant roots, 3) hyphae penetrating and absorbing P in pore spaces smaller than the diameter of root hairs. These pores would remain hydrated at low soil moisture levels.

This study indicates that mycorrhizae may be useful in the maintenance of plant P uptake as declining soil moisture levels reduce P availability. In P deficient soils, the mycorrhizal effect would be apparent at relatively modest soil moisture

deficits, and might come into effect during more severe stress in high P soil. This mycorrhizal effect might be of considerable significance to horticultural crops which otherwise show little response to mycorrhizae due to heavy P fertilization.

Objective 6. Influence of mycorrhizae on the water relations of seedling and mature bell peppers.

In a series of studies we measured the effects of mycorrhizae on water relations of seedling and mature peppers under well-watered and water-stress conditions. To segregate the direct effects of the mycorrhizae from effects related to enhanced P uptake, responses of plants grown with optimal and sub-optimal levels of soil P were compared. Under well-watered conditions, mycorrhizae had no effect on transpiration but inoculation significantly increased the root hydraulic conductivities of pepper seedlings growing in P-deficient medium. The mycorrhizae effect on root conductivity was not duplicated by adding P, indicating that the results were not entirely due to the mycorrhizae improving P uptake.

Under water-stress conditions, transplanted mycorrhizal seedlings and mature plants growing in P-deficient soil had higher leaf water potentials at lower soil moisture potentials than nonmycorrhizal plants. The difference in water relations

of mature mycorrhizal and nonmycorrhizal plants was eliminated by adding P to the soil, suggesting the difference was related to the mycorrhizae improving the P-status of their host. However, in the transplanted seedlings, the difference in leaf water potentials of mycorrhizal and nonmycorrhizal seedlings persisted at soil and tissue P concentrations adequate for optimal growth. The mycorrhizae appear to exert a non-nutritional effect on seedling water relations. The mechanisms whereby mycorrhizae might influence plant water status at low soil moisture levels have not been adequately examined. The maintenance of high plant water potentials at low soil water potentials may increase the host plant's tolerance and recovery from water stress. This suggests a potential role for mycorrhizae in increasing the growth and survival of horticultural crops exposed to stresses such as drought, salinity, transplanting shock or any other conditions reducing moisture availability or uptake.

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APPENDIX TABLES

Appendix Table 5A. ANOVA for growth and mycorrhizal infection of peppers grown with varying moisture regimes, mycorrhizal inoculation and soil P treatments under greenhouse and field conditions.

Source of Variation	Leaf P		Height		Fruit no.		Fruit wt.		Plant wt.		Infection intensity		WUE Plant wt.		WUE Fruit wt.	
	G	F	G	F	G	F	G	F	G	F	G	F	G	F	G	F
	Solution P (P)	**	**	**	**	*	**	**	*	**	*	**	*	NS	.	NS
Inoculation (I)	**	**	**	*	**	**	**	**	**	**	NS	NS	NS	.	NS	.
Water(W)	NS	NS	NS	NS	**	*	**	NS	NS	NS	NS	NS	*	.	**	.
P x I	**	**	**	**	**	**	**	**	**	**	NS	NS	NS	.	*	.
P x W	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	.	NS	.
I x W	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	.	NS	.
P x I x W	NS	NS	NS	NS	**	*	**	*	NS	NS	NS	NS	NS	.	NS	.

F tests significant at P = 0.05 (*) and P = 0.01 (**) or non-significant (NS).

P = soil solution P concentrations of 0.03 or 0.30 mg/liter.

I = pretransplant inoculated, inoculated at transplanting or noninoculated.

W = rewatered at -10 or -80 kPa soil water potential.

Appendix Table 5B. Orthogonal contrasts for growth, yield and water use parameters of peppers grown with varying mycorrhizal inoculation and soil solution P treatments under greenhouse and field conditions.

Contrast	Leaf P concn.		Height		Fruit no.		Fruit wt.		Plant wt.		Fruit WUE	
	G	F	G	F	G	F	G	F	G	F	G	F
I vs N	**	**	**	**	**	**	**	**	**	**	*	.
P vs T	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	.
(P vs T)*HL	NS	NS	NS	NS	NS	*	NS	*	NS	NS	NS	.
(I vs N)*HL	**	**	**	**	**	*	*	**	**	**	*	.

Contrasts significant at P=0.01 (**), P=0.05 (*), or nonsignificant (NS).

H and L - soil solution P levels of 0.30 and 0.03 mg/liter, respectively.

I and N - inoculated and noninoculated.

P and T - preinoculated and inoculated at transplanting.

Appendix Table 5C. Total dry weights and shoot phosphorus concentrations of VAMF inoculated and noninoculated pepper transplants, 35 days after seeding.

	Greenhouse Expt.		Field Expt.	
	Weight (g)	P (%)	Weight (g)	P (%)
Inoculated	0.44	0.28	0.61	0.33
Noninoculated	0.58	0.31	0.66	0.30
t-test	NS	NS	NS	NS

NS Nonsignificant (P=0.05).

Appendix Table 5D. Influence of soil phosphorus, soil water potential and mycorrhiza on fruit set of greenhouse grown peppers.

	Fruit/plant			
	Low P soil ^z		High P soil	
	-10 kPa	-80 kPa	-10 kPa	-80 kPa
<u>Greenhouse</u>				
Inoculated	5.4a ^y	4.5a	6.0a	4.1b
Noninoculated	2.3b	1.4b	5.2b	4.7b
Response ^x	+135%	+221%	+15%	-14%
<u>Field</u>				
Inoculated	11.5a	14.0a	19.4a	19.1a
Noninoculated	5.9b	5.6b	16.9a	19.4a
Response	+95%	+150%	+14%	-2%

^zLow P = 0.03 mg P/liter soil solution, high = 0.30 mg/liter.

^yMeans within soil P levels followed by the same letter are not significantly different (P=0.05).

^xResponse = (inoc - noninoc/noninoc) x 100.

Appendix Table 8A. F-test probabilities and mean values for growth characteristics and total phosphorus uptake of mycorrhizal and nonmycorrhizal pepper seedlings raised with three rates of phosphorus.

Effect	Dry Weight (g)		R/S Ratio	Root Length/ Width (m/g)	Total P Uptake (mg)
	Root	Shoot			
Statistics					
Inoc- ulation	0.18	0.38	0.14	0.02	0.16
P Rate	<0.01	<0.01	0.19	0.89	<0.01
I x P	0.35	0.02	0.29	0.74	0.06
Means					
<u>Inoc- ulation</u>					
+	0.15a ^z	0.54a	0.30a	67.9b	1.80a
-	0.12a	0.52a	0.25a	87.9a	1.73a
<u>P Rate^y</u>					
Low (L)	0.08a	0.29c	0.30a	77.7a	0.50c
Medium (M)	0.14b	0.50b	0.31a	74.2a	1.64b
High (H)	0.19a	0.86a	0.22b	79.5a	3.35a
<u>Treatment</u>					
L/+	0.10cd	0.37c	0.30ab	70.9a	0.48e
L/-	0.06d	0.21c	0.29ab	84.5a	0.29f
M/+	0.13c	0.40c	0.39a	59.2a	1.08d
M/-	0.14c	0.60b	0.23ab	89.3a	1.53c
H/+	0.20a	0.83a	0.23ab	71.7a	2.56b
H/-	0.18ab	0.92a	0.19b	92.1a	3.03a

^zValues within comparisons followed by the same letter are not significantly different (Duncan's or t-test, p=0.05).

^yLow = 80 g P/m³ media, medium = 210 g/m³ and high = 600 g/m³.

Appendix Table 8B. F-test probabilities and mean values for transpiration (E) and time to wilting for mycorrhizal and nonmycorrhizal pepper seedlings raised with three rates of phosphorus.

Effect	Whole Plant E (mg/s)	E per unit root length (E/m x 10 ⁻²)	Days to Wilting
Statistics			
Inoc- ulation	0.74	0.64	0.07
P Level	<0.01	0.20	<0.01
I x P	0.03	0.92	0.64
Means			
<u>Inoc- ulation</u>			
+	0.35a ^z	3.5a	7.4a
-	0.34a	3.5a	8.1a
<u>P Rate^y</u>			
Low (L)	0.21b	3.4a	7.9a
Medium (M)	0.40a	4.6a	8.3a
High (H)	0.46a	3.0a	7.0b
<u>Treatment</u>			
L/+	0.25c	3.4a	7.4ab
L/-	0.16c	3.5a	8.2a
M/+	0.37b	4.8a	8.2a
M/-	0.44ab	4.4a	8.4a
H/+	0.43ab	3.0a	6.5b
H/-	0.51a	3.1a	7.6ab

^zValues within blocks followed by the same letter are not significantly different (Duncan's or t-test, p=0.05).

^yLow = 80 g P/m³ of medium, medium = 210 g/m³, high = 600 g/m³.

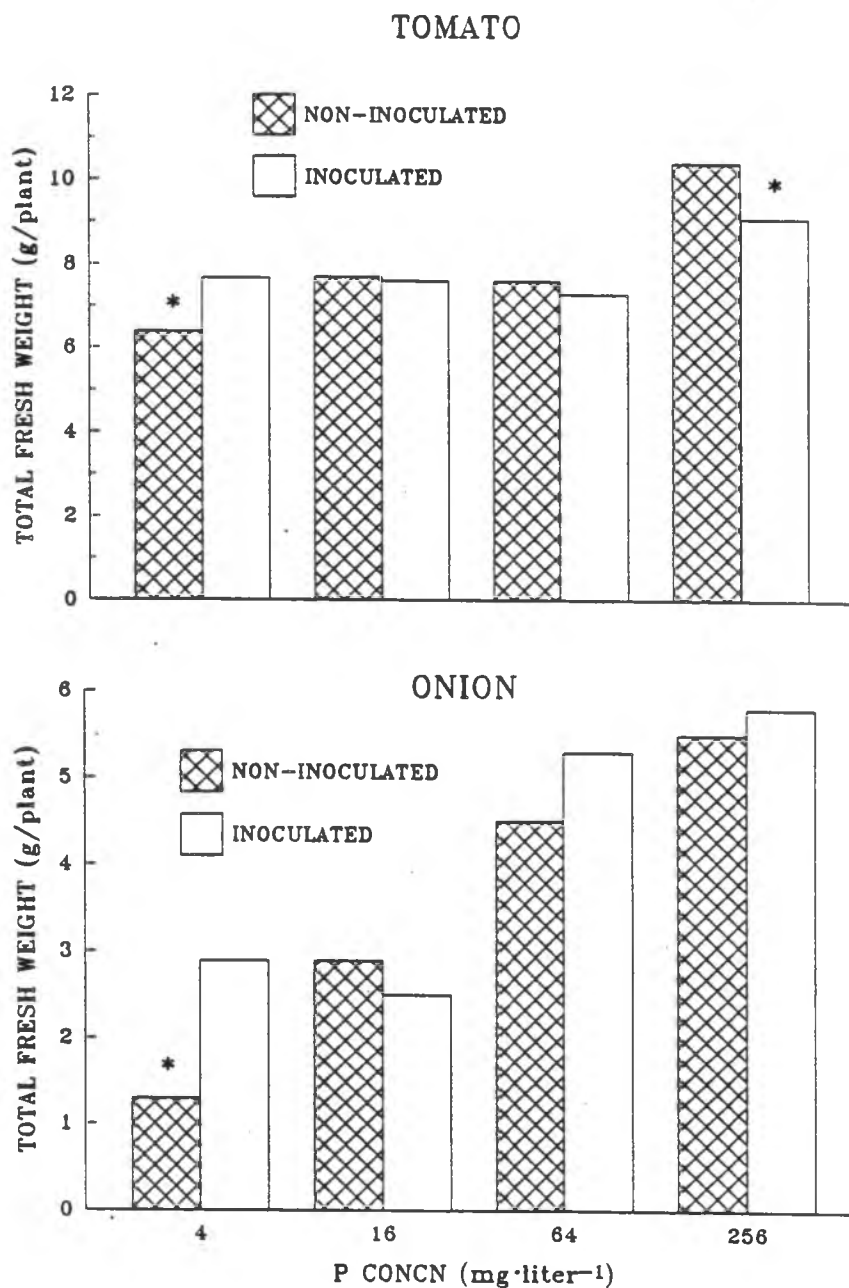
Appendix Table 8C. Classification of pretransplant P and mycorrhizae treatments based on discriminant function analyses of the relationship between soil moisture content and xylem water potentials at wilting of transplanted mycorrhizal and nonmycorrhizal pepper seedlings raised with three rates of phosphorus.

Treatment P/Inoculation	Characteristic of Class ^z
Medium ^y / Low/- High/-	Low Xylem Ψ / High Soil \ominus
High/+ Low/+ Medium/+	High Xylem Ψ / Low Soil \ominus

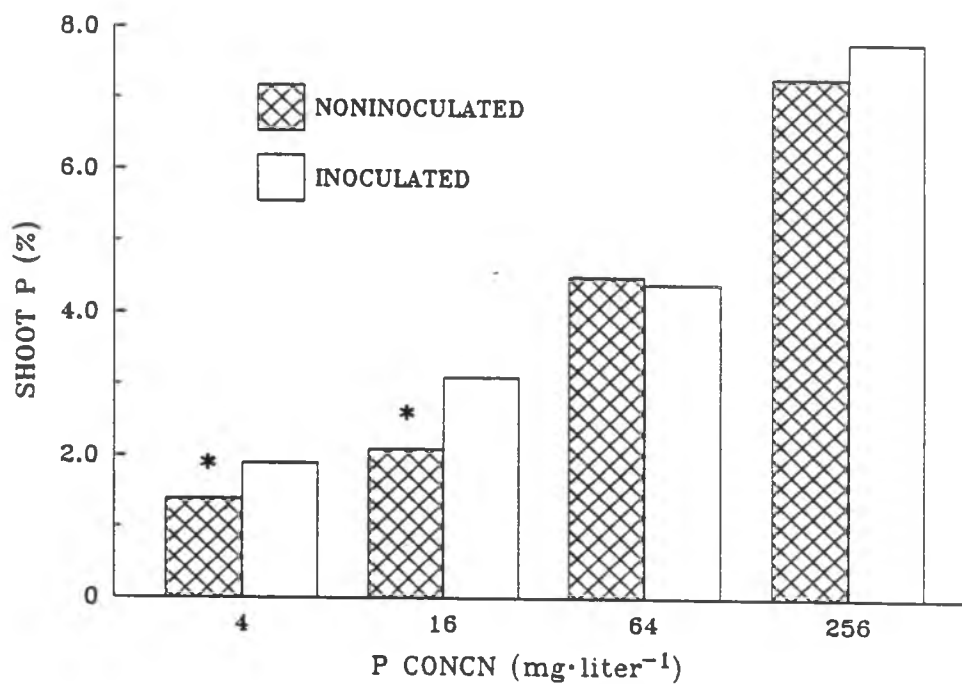
^zDiscriminant functions for members of each class not significantly different ($p=0.05$) by Hotelling's T^2 test.

^yLow = 80 g P/m³ media, medium = 210 g/m³, high = 600 g/m³.

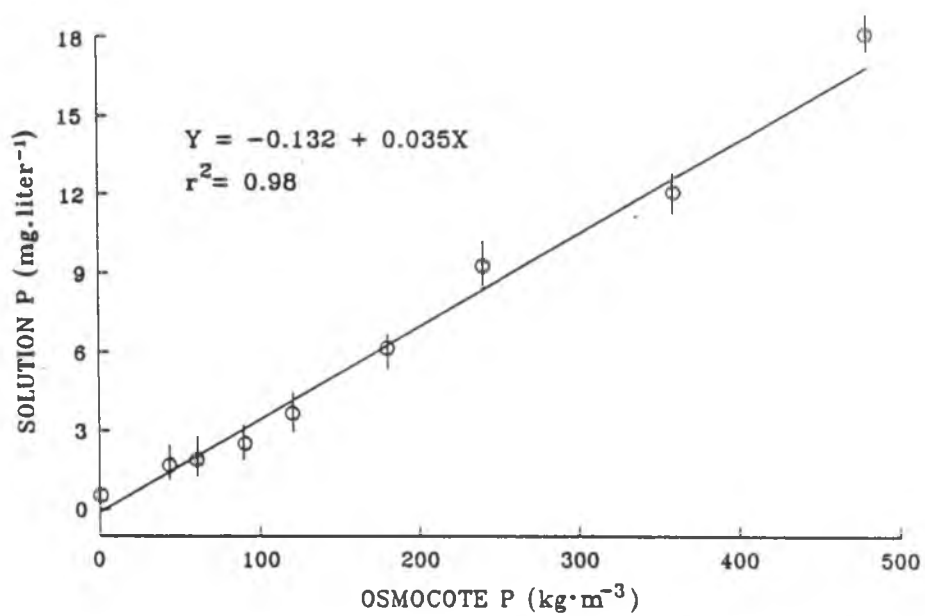
APPENDIX FIGURES



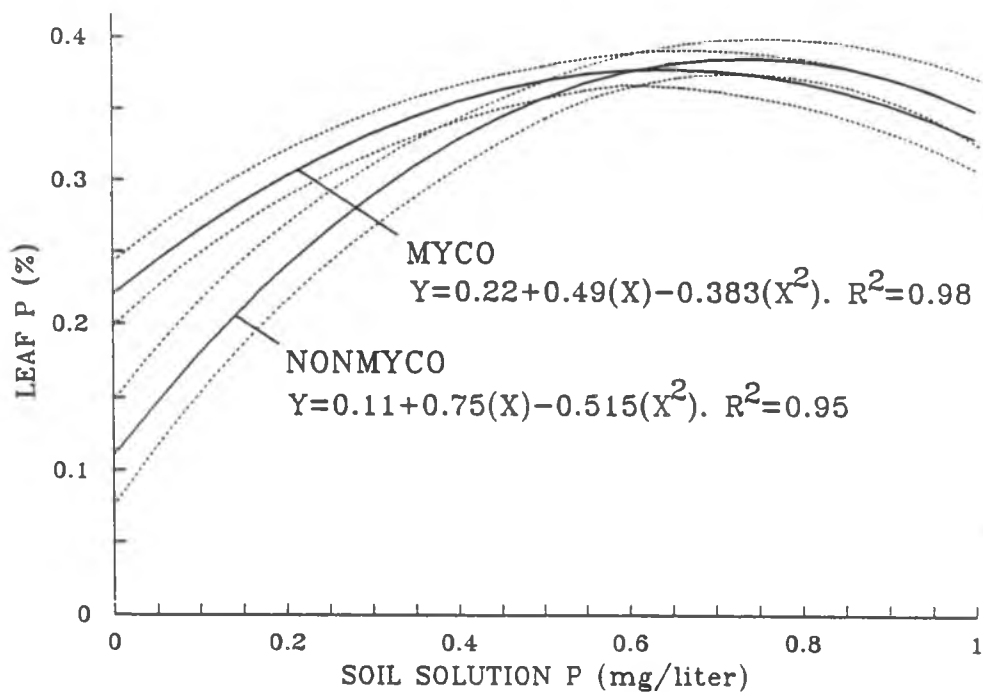
Appendix Fig. 3A. Total fresh weights of inoculated and noninoculated tomato and onion transplants grown with various concentrations of phosphorus fertilizer. *Difference significant at P=5%.



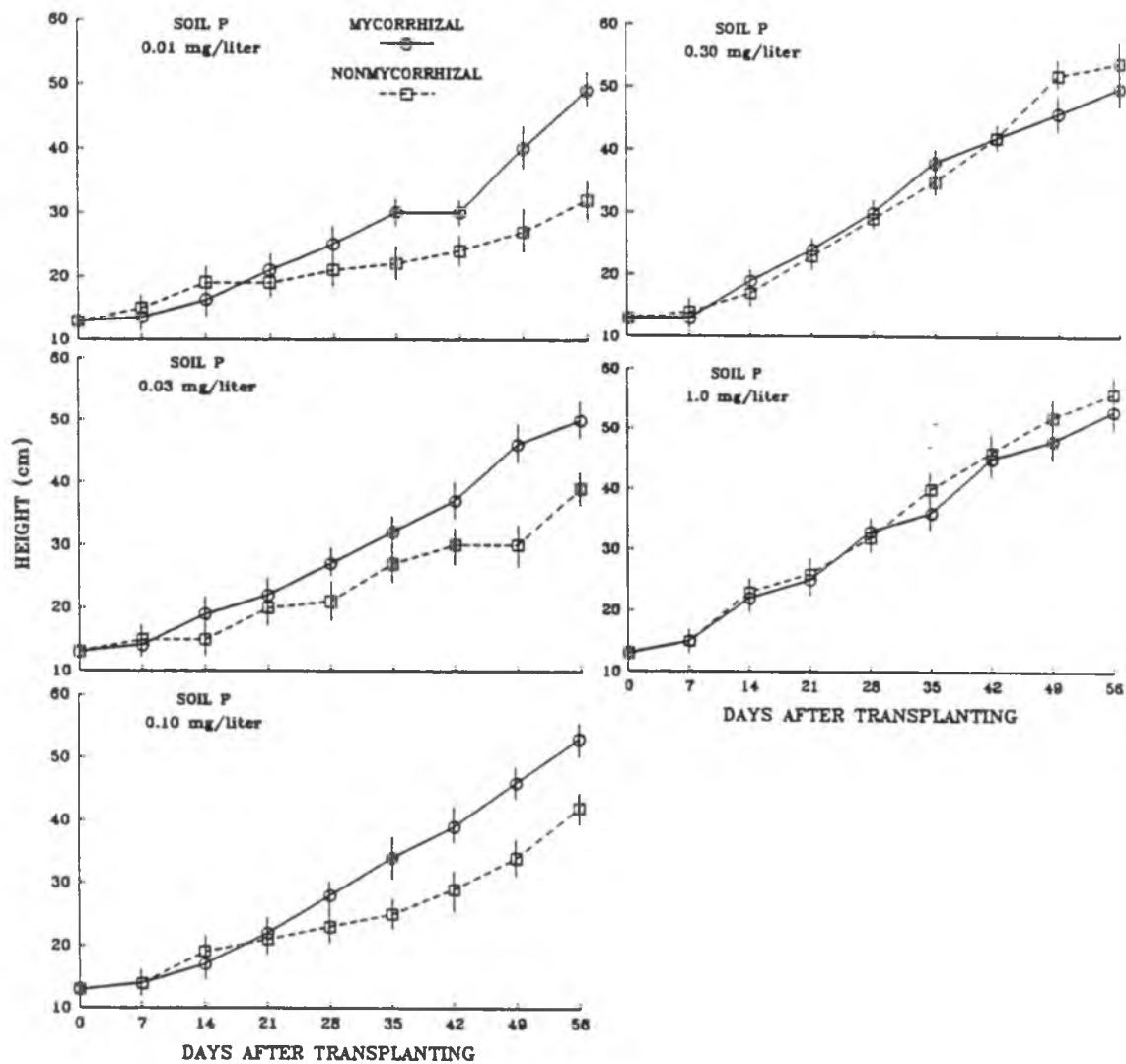
Appendix Fig. 3B. Shoot phosphorus concentrations in inoculated and noninoculated onion transplants grown with varying concentrations of P fertilizer. *Difference significant at P=5%.



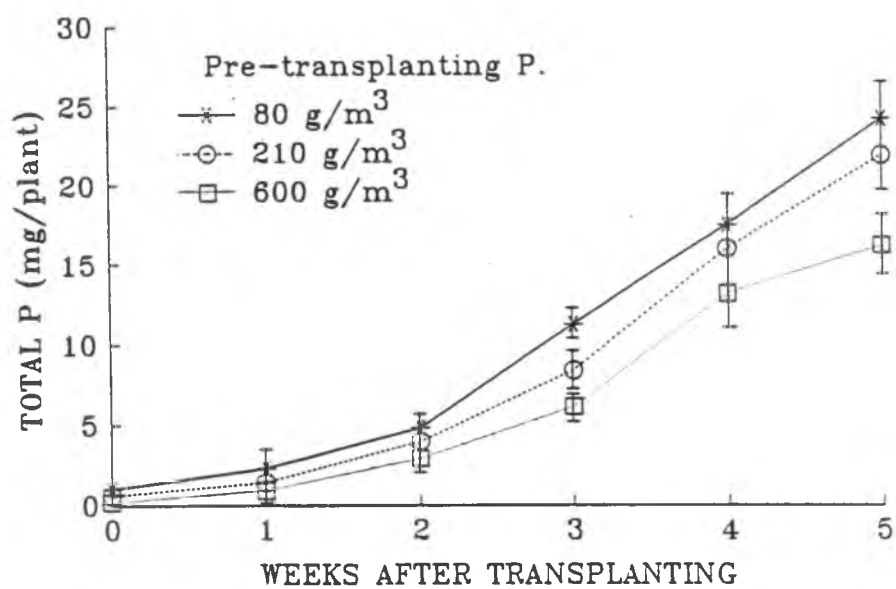
Appendix Fig. 4A. Average solution phosphorus concentrations with different amounts of P from Osmocote 19N-2.6P-10K in a peat/vermiculite medium. Error bars represent SD for 11 samples taken over a 4 week period.



Appendix Fig. 6A. Influence of soil solution phosphorus and mycorrhiza on the average concentrations of P in the newest leaves of peppers over a 56 day growing period. Broken lines represent 95% confidence intervals.



Appendix Fig. 6B. Influence of soil solution phosphorus and mycorrhiza on the weekly heights of peppers (n=15).



Appendix Fig. 7A. Influence of pretransplanting phosphorus on total P uptake of lettuce at varying intervals after transplanting (n=16, means \pm SE).