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THE EFFECTS OF WATER AND NUTRIENT AVAILABILITY ON MYCORRHIZAL AND NON-MYCORRHIZAL ONION PLANTS

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ABSTRACT

THE EFFECTS OF WATER AND NUTRIENT AVAILABILITY ON MYCORRHIZAL AND NON-MYCORRHIZAL ONION PLANTS

By

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Increased growth of higher plants as a result of infection of the roots by vesicular-arbuscular mycorrhizal fungi is well documented. However, the effects of infection on the water relations of the host plant have been described only to a limited extent under well-watered conditions, and have not been investigated under conditions of drought stress. In addition, the influence of different levels of soil phosphorus on the water relations of the host plant under well-watered and drought stressed conditions has yet to be defined.

Experiments were initially carried out under well-watered conditions. The water relations of onions (Allium cepa L.) infected with the mycorrhizal fungus Glomus etunicatus (Becker and Gerdemann) were compared with those of non-mycorrhizal control plants grown under both low and high soil phosphorus conditions. The well-watered mycorrhizal plants had higher leaf water potentials, higher transpiration rates, higher whole plant hydraulic conductivities and lower leaf resistances than did well-watered, non-mycorrhizal plants grown under low soil phosphorus conditions. When non-mycorrhizal plants were grown under high soil phosphorus conditions, all four water relations parameters were essentially the same as those of mycorrhizal plants. The magnitude of the effect of mycorrhizal infection on the water relations of the host appears, in part, to be a function of phosphorus nutrition. The

differences in leaf water potentials, transpiration rates, and leaf resistances are considered to be the result of the differences found in hydraulic conductivities.

When experiments were performed under conditions of cyclic drought-stress, mycorrhizal onion plants were more drought resistant than were non-mycorrhizal onion plants as demonstrated by greater fresh and dry weights and by higher tissue phosphorus concentrations in the mycorrhizal plants. Stressed, non-mycorrhizal plants were phosphorus deficient despite the fact that non-mycorrhizal plants alone were fertilized with high levels of phosphorus, equivalent to 114 kg P/ha. The ability of the mycorrhizal fungus to maintain adequate phosphorus nutrition during stress was the major factor leading to the improved drought resistance. Where conditions of drought stress are common, mycorrhizal infection may be a major factor in improved drought resistance and increased crop yield.

This work is dedicated to the memory of my parents,
Edward H. Nelsen and Marie Eleanor Nelsen,
without whose love, guidance and support in my younger years
I could not have attained this goal.

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TABLE OF CONTENTS

Page

APPENDIX A

THE EFFECT OF SOIL PHOSPHORUS LEVELS ON MYCORRHIZAL INFECTION OF FIELD GROWN ONION PLANTS AND ON MYCORRHIZAL REPRODUCTION

APPENDIX B

ROOT INFECTION AND PLANT GROWTH STIMULATION AS INFLUENCED BY INOCULATION TECHNIQUE

LIST OF TABLES

LIST OF FIGURES

Figure Page

SECTION II

APPENDIX A

APPENDIX B

LITERATURE REVIEW

LITERATURE REVIEW

Introduction

It has been well documented that vesicular-arbuscular (VA) mycorrhizal associations can greatly improve plant growth and nutrition. A number of recent reviews on this subject are available (17, 45, 54). Besides the generally accepted phenomenon of improved growth and nutritional status, VA mycorrhizae also may alter the water relations of the host plant under well-watered conditions (37, 38, 52, 53). Better definition of the effects of mycorrhizal infection on the water relations of the host plant under both well-watered and drought-stressed conditions, and improved understanding of the interaction of soil and plant nutrition on these effects is sorely needed.

Nutrient Uptake and Plant Nutrition

Mycorrhizal root systems are generally more effective at taking up nutrients from the soil than are non-mycorrhizal roots. Since the first report by Mosse (34) there have been several studies showing that mycorrhizal infection can increase the uptake of phosphorus, zinc, sulfur, nitrogen, copper, iron and calcium (for example see 11, 17, 47, 48, 54).

At least 4 hypotheses have been proposed to explain this improved nutrition of mycorrhizal plants. One hypothesis is that the mycorrhizal root surface is a more efficient nutrient absorber, that is, physiological changes due to infection occur in the infected root causing it to

more readily absorb soil nutrients. A second hypothesis is that mycorrhizal root systems are able to use nutrient sources that are unavailable or less available to non-mycorrhizal roots. A third is that the soil network of mycorrhizal hyphae is able to absorb nutrients from a larger soil volume and translocate them to the infected roots. A fourth possibility is that mycorrhizal root segments remain functional as nutrient absorbers longer than do non-mycorrhizal segments. The last hypothesis suggests that mycorrhizal infection alters the length of time over which a root segment can act as a nutrient absorber and enables the entire root system to be larger and more effective at nutrient absorption. These hypotheses are generally described in relation to phosphorus nutrition, because the increased uptake of phosphorus, in the form of phosphate ions, is usually associated with the improved growth of mycorrhizal plants.

Gray and Gerdemann (22) used radioactive phosphorus to demonstrate that infected as well as uninfected portions of mycorrhizal roots can take up more phosphorus than can root segments from non-mycorrhizal plants. This supported the first hypothesis, since non-infected segments of inoculated roots absorbed more phosphorus than non-inoculated control roots. However, the infected segments of mycorrhizal roots absorbed much more phosphorus than did uninfected segments. In addition, hyphal translocation of phosphorus to and within the root and mobilization of P within the root were not ruled out. VA mycorrhizal infection has also been shown to increase the uptake of phosphorus (21) and zinc (2, 3) from aqueous solutions when compared to non-mycorrhizal roots. This suggests a more efficient absorption by the root itself although the differences were small and variable. However, diffusion of phosphorus, as the phosphate ion, in aqueous culture is much more rapid than in soil, and calculations

by Sanders and Tinker (57) indicated that increased absorbing power of mycorrhizal root surfaces cannot account for the observed differences in uptake of phosphorus between mycorrhizal and non-mycorrhizal roots in soil. That is, phosphorus absorption by non-mycorrhizal roots in soil is limited by diffusion of phosphorus in the soil, not by the root absorption rate.

The second hypothesis that mycorrhizal plants utilize different sources of phosphorus than do non-mycorrhizal plants has been suggested by several workers. Murdock et al. (36) demonstrated that mycorrhizal corn plants were larger and had higher phosphorus contents than did non-mycorrhizal corn when phosphorus sources of low availability (rock phosphate) were added to soil. Conversely, mycorrhizal and non-mycorrhizal plants had equal phosphorus contents and grew equally well with a readily available phosphorus supply. Similar results have been obtained by Hall (24), Ross and Gilliam (51), and Powell and Daniel (42). However, studies by Sanders and Tinker (56), Hayman and Mosse (27), Powell (41) and Tinker (61), indicate that mycorrhizal and non-mycorrhizal plants are using the same sources of phosphorus. The latter workers added radioactive phosphorus to the soil and allowed its equilibration with the labile pool of soil phosphorus. Plants were then grown in these soils and the phosphorus specific activities (ratios of ^{32}P to ^{31}P) of the roots and surrounding soils of mycorrhizal and non-mycorrhizal plants were determined. In all cases, the specific activities of the mycorrhizal and non-mycorrhizal roots and soils were similar, which indicates that the same phosphorus sources were being used. Therefore, it appears that the increased "utilization" by mycorrhizal plants of low availability phosphorus sources can largely be

explained by an increased uptake rate by mycorrhizae at the low levels of phosphorus that were available.

Calculations made by Sanders and Tinker (57) based on diffusion theory of the possible rates of phosphorus inflow to cylindrical roots in soil solutions led them to postulate that the greater uptake of phosphorus by mycorrhizal plants was due to uptake of phosphorus by mycorrhizal hyphae from a larger soil volume and transport of this phosphorus along the hyphae to plant roots. Hattingh et al. (26) demonstrated that mycorrhizal hyphae are capable of transporting phosphorus to mycorrhizal roots. This was confirmed by Rhodes and Gerdemann (46), Pearson and Tinker (40) and Rhodes and Gerdemann (47, 49) who used a split plate technique to separate mycorrhizal plant roots from areas of soil into which external hyphae had penetrated. ^{32}P was added to the soil containing the hyphae and ^{32}P uptake by plant roots was measured. Severing the external hyphae from the roots eliminated ^{32}P transport to the roots.

The mechanisms for phosphorus uptake and transport by mycorrhizal hyphae into plant roots are little studied. Preliminary evidence of two kinds favors an active uptake mechanism. First, uptake by germ tubes is temperature sensitive (2) and, second, phosphorus concentration is much higher in the fungal cytoplasm (40) than in the soil solution. Kinetic data by Cress et al. (14), suggest that the absorption sites along external hyphae may have a higher affinity for phosphorus than do non-mycorrhizal roots. However, this may be of limited use to the plant since phosphorus uptake is probably diffusion limited at most soil phosphorus levels. The source of energy for hyphal uptake of nutrients is most probably obtained from the host in the form of organic compounds. Radioactive carbon moves from host to fungal tissue and accumulates in

fungal structures (1, 12).

Phosphorus is probably translocated along the hyphae in the form of polyphosphate as suggested by Cox et al. (12), Tinker (60), and Callow et al. (7). Polyphosphate is apparently restricted to mycorrhizal hyphae and roots, is undetectable in non-mycorrhizal roots, and comprises approximately 90% of total hyphal phosphorus (7). This level of phosphorus agrees with calculations of phosphorus concentration and movement in hyphae (57).

The mechanism of phosphorus movement (probably in the form of polyphosphate granules) has not been determined. Calculations by Sanders and Tinker (57) indicate that diffusion within hyphae is unlikely to account for measured flux rates into the plants, and the authors postulated some form of active transport or mass flow mechanism involving cytoplasmic streaming. They also suggested that water movement in the hyphae may be affected by plant hydraulic fluxes and would partially control phosphorus movement.

The mechanism for transportation of phosphorus from fungal cells to root cells is not understood. Since high root phosphorus levels hinder the establishment of mycorrhizal infection (32, 55), the degree to which high soil or growth medium phosphorus levels lower or delay infection will probably depend on the ability of a plant species to accumulate phosphorus at different soil phosphorus levels. Ratnayake et al. (43) proposed that phosphorus inhibition of mycorrhizal infection was associated with membrane-mediated decreases in root exudation. If true, then mild soil water deficiencies, which are known to increase root exudation, might stimulate infection at soil phosphorus levels that would hinder infection at optimal soil moisture levels. A mycorrhiza-specific

alkaline phosphatase (MSAP) has been isolated which has an activity closely correlated with the mycorrhizal growth response of onion (18, 19). Since MSAP activity is inhibited by increased phosphorus it is possible that this enzyme may be involved with phosphorus uptake by mycorrhizal plants.

Host digestion of arbuscules has been postulated as a mechanism for nutrient transfer between the fungus and the host. However, Cox and Tinker (13) calculated the average life span of an arbuscule to be 4 days followed by host digestion and also that arbuscular digestion could account for less than 1% of the published rates of phosphorus inflow to mycorrhizal roots. They concluded that most phosphorus transfer probably occurs across living membranes of the fungus and host. The data of Cox et al. (12) and Bowen et al. (2) suggest that living arbuscules are unloading sites for polyphosphate. Nutrient exchange probably occurs between the host and all fungal structures, with relative exchange rates between one structure and another depending on factors such as the stage of infection and age of the host. These relative exchange rates remain to be determined.

Nutrients other than phosphorus may also be more effectively absorbed by mycorrhizal than by non-mycorrhizal plants. Sulfur uptake, as the sulfate ion, via hyphal translocation to root and increased uptake at the root surface has been reported (11, 23, 48). Hyphal translocation of sulfur is likely to be less important than for phosphorus because sulfur has greater soil mobility. Increased uptake of sulfur by mycorrhizal plants has been attributed largely to improved phosphorus nutrition of mycorrhizal plants (49). Zinc also moves to mycorrhizal roots through hyphae (11, 50). In addition, zinc deficiency has been associated with

high phosphorus levels in the soil and low mycorrhizal infection levels (20, 29). Rhodes et al. (50) showed that high soil phosphorus levels reduced infection and eliminated both phosphorus and zinc translocation in the hyphae. This may explain the presence of phosphorus induced zinc deficiencies in some field situations.

The hypothesis that mycorrhizal infection enlarges the root system itself, thus increasing the surface area for phosphorus absorption, is unlikely since root/shoot ratios are generally lower for mycorrhizal plants than for non-mycorrhizal plants. There does not appear to be any published information, however, concerning the effectiveness of individual roots (mycorrhizal and nonmycorrhizal) for nutrient absorption over long time periods. Therefore, one cannot ignore the possibility that some root longevity effects may be present.

Interactions of mycorrhizae and other soil organisms have been reported. VA mycorrhizal infection has been associated with improved nitrogen fixation by Rhizobium. Daft and El-Giahmi (15) compared VA mycorrhizal and non-mycorrhizal Phaseolus. The mycorrhizal plants were larger, had increased nodulation, higher rates of nitrogen fixation, and increased levels of phosphorus, leghaemoglobin and total protein in comparison to non-mycorrhizal plants. Application of soluble phosphate to non-mycorrhizal plants duplicated the effects of mycorrhizal infection. Mosse (35) demonstrated that under some conditions the above relationship between VA mycorrhizal infection, phosphorus nutrition and nitrogen fixation may not be straightforward. She found that introduced mycorrhizal species often stimulate nitrogen fixation more than do indigenous species when the phosphorus content of plants associated with the introduced strains was the same or lower than those associated with

the indigenous strains. The possibility of other synergistic interactions between mycorrhizal infection and Rhizobium activity has been suggested by Daft and El-Giahmi (16). It is unlikely, however, that nitrogen is translocated along mycorrhizal hyphae since the nitrogen content of non-legumes is usually not increased by mycorrhizal infections, and since non-nodulating soybean lines were not increased in growth by mycorrhizal infection when soil nitrogen contents were low (8, 58).

The evidence generally supports the hypothesis that when soil nutrients are in limited supply and are relatively immobile in soil (i.e. uptake is diffusion limited), the mycorrhizal network absorbs and translocates sufficient quantities of these nutrients to account for the nutritional differences between mycorrhizal and non-mycorrhizal plants. There may be different translocation mechanisms for different nutrients and for different fungal host combinations. For example, calcium has been shown to be much less mobile than phosphorus in mycorrhizal hyphae (47). The effectiveness of an external hyphal network will probably depend not only on its size but upon its position in relation to soil nutrients. Also, the extent of the nutrient depletion zone surrounding the root will affect the efficiency of hyphal translocation. The increased absorbing power of mycorrhizal roots surfaces will be of major importance to plant nutrition only when uptake is not limited by diffusion of a given nutrient in soil.

Water Relations

Little is known of the effects of infection on plant water relations and the one review written on the topic of mycorrhizae and water (44) was necessarily speculative on the subject of VA mycorrhizae because of the

dearth of information then available. Recently, however, there has been some research progress in this area.

Safir, Boyer and Gerdemann (52) were the first to report changes in the water relations of a plant when infected by a VA mycorrhizal fungus. They inoculated soybeans with the endophyte Glomus mosseae and after about 30 days of growth found that the mycorrhizal soybeans had higher hydraulic conductivities to liquid water flow than did non-mycorrhizal control plants. That is, there was less resistance to water flow through the mycorrhizal plants to the evaporating surfaces in the leaves. Conductivity measurements were determined using two separate methods; one involved determining the rate of recovery of soybean leaves from a single episode of mild water stress (4), and the other involved measuring the rate of transpiration, and soil and soybean leaf water potentials (28). In both cases, the hydraulic conductivity of the mycorrhizal soybeans was about 60% greater than the non-mycorrhizal controls. Additionally, the mycorrhizal plants were larger than non-mycorrhizal controls. These same authors (53) in a later report calculated that the differences in hydraulic conductivities between mycorrhizal and non-mycorrhizal soybeans occurred in the roots and could be eliminated by the addition of a complete nutrient solution to the soil. Fertilized mycorrhizal and non-mycorrhizal plants were larger than their non-fertilized counterparts, although the mycorrhizal plants remained larger than the non-mycorrhizal plants. In the same study, they showed that the addition of the fungitoxicant p-chloronitrobenzene (PCNB) to the soil 48 hours before measurement did not eliminate the differences in hydraulic conductivities. Because the PCNB did not affect hydraulic conductivity but did reduce nutrient uptake by

mycorrhizae (22), and because the addition of nutrients to the soil eliminated the hydraulic conductivity differences, they concluded that the hydraulic conductivity differences were due to enhanced nutrition of the mycorrhizal plants and not due to the fungus providing a low resistance pathway for water to move within the root. Presumably, the 48 hour pre-treatment with PCNB and concurrent lowering of nutrient uptake for only 2 days was over too short a time period to affect the nutritionally controlled hydraulic conductivity when compared to the non-mycorrhizal controls, which were grown the entire length of the experiment under low nutrient conditions. One could hypothesize that a longer pretreatment or constant treatment with PCNB would probably reduce or eliminate mycorrhizal infection and thereby eliminate conductivity differences by maintaining the conductivity of the inoculated plants at the low level of the non-inoculated controls. The addition of nutrients eliminated the differences by causing the conductivity of the non-mycorrhizal plants to increase to the levels of the inoculated, non-fungicide treated mycorrhizal plants.

Christensen and Allen (9, 10) reported changes in the water relations of the prairie grass, Blue Grama (Bouteloua gracilis), when infected with the VA mycorrhizal fungus, Glomus fasciculatus. Under well-watered conditions in the greenhouse, mycorrhizal plants had higher transpiration rates and lower stomatal resistances than did non-mycorrhizal controls at 5 months of age. Because leaf water potentials were similar, these results lead to a higher calculated hydraulic conductivity in the mycorrhizal plants, similar to results found by Safir et al. (52, 53). Christensen and Allen (10) speculated that the increase in hydraulic conductivity was due to an increase in effective surface area of absorption caused by the

mycorrhizal hyphae extending into the soil. They did not, however, determine whether better plant nutrition was responsible for the conductivity differences. This latter point may be important since Sanders and Tinker (57) used differences in transpiration in mycorrhizal and non-mycorrhizal onion plants to calculate potential inflow rates of water (as well as phosphorus) through the hyphae. Their calculations show that an unrealistically high rate of water inflow through the hyphae would be obtained if all the water were conducted into roots through the hyphal strands. Therefore, they concluded that the hyphae are probably not functioning as "low resistance channels" to and into the root (at least under conditions of ample water supply).

Nelsen and Safir (unpublished) used a split plate system to allow hyphae to grow into an area of soil from which the roots of mycorrhizal soybean were excluded. They allowed the soil containing roots and hyphae to dry and leaf water potentials to drop to about -10 bars. The portions of soil which contained only hyphae were partially rehydrated and leaf water status was monitored in a thermocouple psychrometer for up to 10 hours. No detectable changes were evident in leaf water potentials after rehydration of that part of the soil containing only hyphae. Other experiments with onions and tritiated water also showed no differences in water uptake which could be solely attributed to hyphal translocation. These results support the calculations of Sanders and Tinker (57), although they do not eliminate the possibility that very small amounts of water are moving through the hyphae to the roots.

Christensen and Allen (10) reported differences in water relations between mycorrhizal and non-mycorrhizal Boteloua gracilis suffering from various levels of soil water stress. As soil water potential decreased,

the leaf water potentials of the mycorrhizal plants dropped more quickly than did those of the non-mycorrhizal controls. Stomatal resistances of the mycorrhizal plants increased at a slower rate than did those of the non-mycorrhizal plants, and the transpiration rates of the mycorrhizal plants always remained greater than the non-mycorrhizal controls. These findings indicate that mycorrhizal plant hydraulic conductivites may be higher and stomatal resistances lower when compared with non-mycorrhizal plants over a wide range of soil moisture levels.

The hydraulic conductivities calculated by Christensen and Allen were based on the relationship proposed by van den Honert (28) and elaborated by Boyer (5) where:

$$\text{Hydraulic conductivity} = \frac{\text{Transpiration rate}}{\text{Soil water potential-Leaf water potential}} \quad (1)$$

This derivation assumes that soil hydraulic conductivity is negligible; which is true when soil is well hydrated but not true when soil water potential begins to drop (39). Indeed, soil conductivity can easily change by 3 or more orders of magnitude between -0.1 and -1.0 bars of soil water potential (59). The high variability they report in hydraulic conductivities might, therefore, be explained by the effects of changes in soil hydraulic conductivities as the soil dries.

Christensen and Allen's data also showed leaf water potentials higher (closer to zero) than soil water potentials when the soil water potential was at about -6 bars. This would lead to negative values for hydraulic conductivity if equation (1) were used. Leaf water potentials could be higher than soil water potentials if the soil in the pot were drying quickly and the stomates were closed tightly. However, the values they report for transpiration of about 30 to 90 mg m^{-2} sec^{-1} (1.1 - 3.3 g dm^{-2} hr^{-1}) were presumably too high for plants with the stomates closed.

Therefore their values of leaf and/or soil water potentials may be questionable. Hanson et al. (25) have shown that significant gradients of several bars can occur along the leaf blades of long, grass-type leaves when plants are exposed to water stress. In addition, Meiri et al. (31) have shown that pressure bomb measurements of long leaves (wheat) represent the highest water potential in the organ, rather than the average water potential. Finally, Christensen and Allen showed that hydraulic conductivities increased from 98 to 1540 mg MP_a^{-1} M^{-2} S^{-1} as the soil dried. The reasons for an increase are difficult to explain, particularly since previous work has shown that conductivities decrease as plants are water stressed (6, 39). This phenomenon might be explained by the fact that B. gracilis is a dry land plant and thus may actually be more suited to dry conditions. More work on this interesting anomaly is surely needed.

Levy and Krikun (30) reported a number of changes in the water relations of VA mycorrhizal citrus plants (Citrus jambhiri, Lush.). They measured leaf water potential, leaf conductance and photosynthesis, and calculated transpiration rates prior to, during, and upon relief of a single period of mild water stress in 8-month-old seedlings. Leaf water potentials of mycorrhizal and non-mycorrhizal plants of similar size were similar throughout the experiment. During the four days of water stress small, non-significant differences occurred in leaf conductance and transpiration, with the mycorrhizal plants having higher values of both. Leaf water potentials fell from -10 to -35 bars and transpiration approached zero during the 4 day stress period. Mycorrhizal plants recovered faster after rewatering than the non-mycorrhizal controls. Although none of the variables except leaf water potential returned to

prestress levels, leaf conductivity, transpiration and photosynthesis all increased more quickly in the mycorrhizal plants after rewatering. Levy and Krikun concluded that no differences in hydraulic conductivity occurred between mycorrhizal and non-mycorrhizal plants [especially in the root as calculated by Safir et al. (53)], and instead attributed the differences in rate of recovery found in their study to an effect of the mycorrhizal association on the root-shoot hormone balance. However, the hydraulic conductivity analysis from their study was made on mycorrhizal and non-mycorrhizal plants of similar size. This similarity in size was attained by growing the plants under a high nutritional regime (daily watering with a 0.1% solution of 20:20:20, N:P:K, commercial fertilizer), and, therefore, their conclusions on hydraulic conductivity are in agreement with the results of Safir et al. (53).

Mycorrhizae may influence plants in other ways aside from growth and water relations. Levy and Krikun (30) found photosynthetic differences during recovery from water stress between mycorrhizal and non-mycorrhizal plants grown under conditions of high fertility. Christensen and Allen (9, 10) found changes in the levels of several plant hormones, which also may be important since cytokinins are involved in plant development and are produced by some ectomycorrhizal fungi (33).

In addition, when differences in hydraulic conductivities are found, any differences found in leaf water potentials and/or stomatal resistances might be a result of the conductivities in each treatment. If the hydraulic conductivity is low, the leaf water potential would drop for a given evaporative demand. As leaf water potential drops, the plant may respond by increasing stomatal resistance, thus reducing the transpiration rate. This reduction in water loss through the stomates could then

allow partial or total recovery of leaf water potential. It is apparent that these dynamic secondary responses to mycorrhizal infection (or phosphorus fertilization) can have profound effects on the metabolism and growth of the plant.

Not all of the differences between mycorrhizal and non-mycorrhizal plants discussed here have been explained. For example, still to be investigated are the effects of different soil moisture regimes on the fungi involved and on the mycorrhizal relationship itself.

Investigators are close to understanding, qualitatively, the mechanisms by which vesicular-arbuscular mycorrhizae improve plant nutrition and growth. The biochemical mechanisms of fungal nutrient uptake and transport to host plants are still imperfectly understood. If the fungi involved could be grown in pure culture, a more complete understanding of the biochemistry of infection would be feasible and genetic manipulations of fungal strains for practical use might become possible.

The possibility that mycorrhizal plants can better cope with drought may be a fruitful area for future study. Possible involvement of water movement in nutrient translocation by these fungi and hormone changes induced by mycorrhizal infection are other exciting areas for research. Hopefully, the increased interest in mycorrhizal research that has occurred recently will help clarify the role of VA mycorrhizae in plant water and nutrient relationships.

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SECTION I

THE WATER RELATIONS OF MYCORRHIZAL AND NON-MYCORRHIZAL ONION PLANTS UNDER WELL-WATERED CONDITIONS

THE WATER RELATIONS OF MYCORRHIZAL AND NON-MYCORRHIZAL ONION PLANTS UNDER WELL-WATERED CONDITIONS

ABSTRACT

The water relations of mycorrhizal onions (Allium cepa L.) were compared with those of non-mycorrhizal controls grown under low and high soil phosphorus conditions. Mycorrhizal plants had higher leaf water potentials, higher transpiration rates, higher hydraulic conductivities and lower leaf resistances than did non-mycorrhizal plants grown in low soil phosphorus conditions. When controls were grown in high soil phosphorus conditions, all four parameters were not different from those of mycorrhizal plants. The magnitude of the effect of mycorrhizal fungi on the water relations of the host may, in part, be a function of phosphorus nutrition. The differences in leaf water potentials, transpiration rates and leaf resistances are considered to be the result of the differences found in hydraulic conductivities.

Vesicular-arbuscular (VA) mycorrhizal fungi have been shown to improve plant growth by augmenting the phosphorus nutrition of the host plants (9,13,14,18). In addition to changes in growth, changes in the water relations of mycorrhizal plants have been reported (17). In the first report of this type, Safir, Boyer and Gerdemann (15) showed an increase of about 60% in hydraulic conductivity to liquid water flow in soybeans when they were infected with the mycorrhizal fungus Glomus mosseae. Later, these same authors (16) demonstrated that the differences in hydraulic conductivity between mycorrhizal and non-mycorrhizal

soybeans were eliminated after addition of a complete nutrient solution to the soil.

Levy and Krikun (7) reported differences in the water relations of mycorrhizal and non-mycorrhizal citrus plants upon recovery from a single episode of water stress. Upon rewatering, after 4 days of water withholding, the mycorrhizal plants appeared to recover more quickly than the non-mycorrhizal controls to a condition of high stomatal conductance and high photosynthetic rate, although the differences were not statistically significant. They did not find differences in hydraulic conductivity and speculated that differences in leaf conductance reflected altered hormonal status. However, since their mycorrhizal and control plants were grown under conditions of high fertility, their results actually support those of Safir et al. (16) who found that the addition of nutrients eliminated differences in hydraulic conductivity.

In this section, the effects of mycorrhizal infection on the water relations of the well-watered host plants are more fully defined by examining the leaf water potential, transpiration rate, hydraulic conductivity and leaf resistance of mycorrhizal and non-mycorrhizal onions. Furthermore, the effects of soil phosphorus on these same four water relations parameters are described . In doing so the different results reported by Safir et al. (15) and Levy and Krikun (7) on the effects of mycorrhizal infection on hydraulic conductivity were reconciled.

MATERIALS AND METHODS

Single onion plants (Allium cepa, L. cv. Downing Yellow Globe) were grown in plastic cups (8.4 cm high x 7 cm diam) in 200 gm of a 50:50 mix (v/v) of sand and sandy loam soil. The soil mix was sieved through a 2 mm screen and autoclaved for 45 min prior to planting. Soil pH was 7.5±0.1 and soil phosphorus levels were low (10 ppm Bray's P-1 extractable). Plants were grown in a growth chamber with a 14 hr light period (3×10^4 ergs cm^{-2} s^{-1}), air temperatures of 22°C day/16°C night, and relative humidity controlled at either a high (60±5%) or low (40±5%) level.

Pots of onion plants were randomly assigned to 3 treatments and arranged in the growth chamber in randomized blocks, with the blocks parallel to the fluorescent lamps. One-third of the onions were inoculated just below the seeds with 10 gm (600 spores) of soil inoculum from a pot culture containing spores of the mycorrhizal fungus Glomus etunicatus (Becker and Gerdemann). A second third of the plants were fertilized with 30 ml of a 1.58 mg/ml solution of KH_2PO_4 to add 50 ppm P to the soil to stimulate growth of the onion plants in a manner similar to that due to mycorrhizal infection. The final third of the onions were uninoculated and unfertilized. Both sets of non-mycorrhizal onions were treated with a soil wash from the mycorrhizal pot culture from which the spores had been sieved, so that other microbial organisms in the pot culture would be present in all three treatments.

Experiments were begun at plant age between 8 and 11 weeks at which time all pots were watered to a common weight so that soil moisture was near saturation, then carefully enclosed in plastic wrap followed by aluminum foil to eliminate evaporation from the soil surface or through the pot. Transpiration was measured by weighing pots twice daily, then dividing the amount of water transpired by the average of the leaf surface area at the start and at the end of each experiment. Control pots, identical to pots containing plants, except containing cylindrically-shaped wood applicators, were also wrapped and weighed to determine the efficiency of reduction in evaporation due to wrapping. Leaf surface area was determined non-destructively using a regression line of actual leaf surface area versus calculated leaf surface area derived from a separate experiment using a range of leaf sizes from plants grown under similar conditions (Figure 1).

Leaf water potential was measured using a Wescor dew point hygrometer and 12 C-52 sample chambers following a previously described method (10). One cm leaf segments were excised from the midpoint of the leaf being sampled, placed into the sample chambers and the water potential was measured after a 3 hr incubation period.

The hydraulic conductivity of whole onion plants was calculated using a method discussed by van den Honert (20) and Boyer (2). Under steady-state conditions:

$$T = \frac{-(\psi leaf - \psi soil)}{r_p} \qquad (1)$$

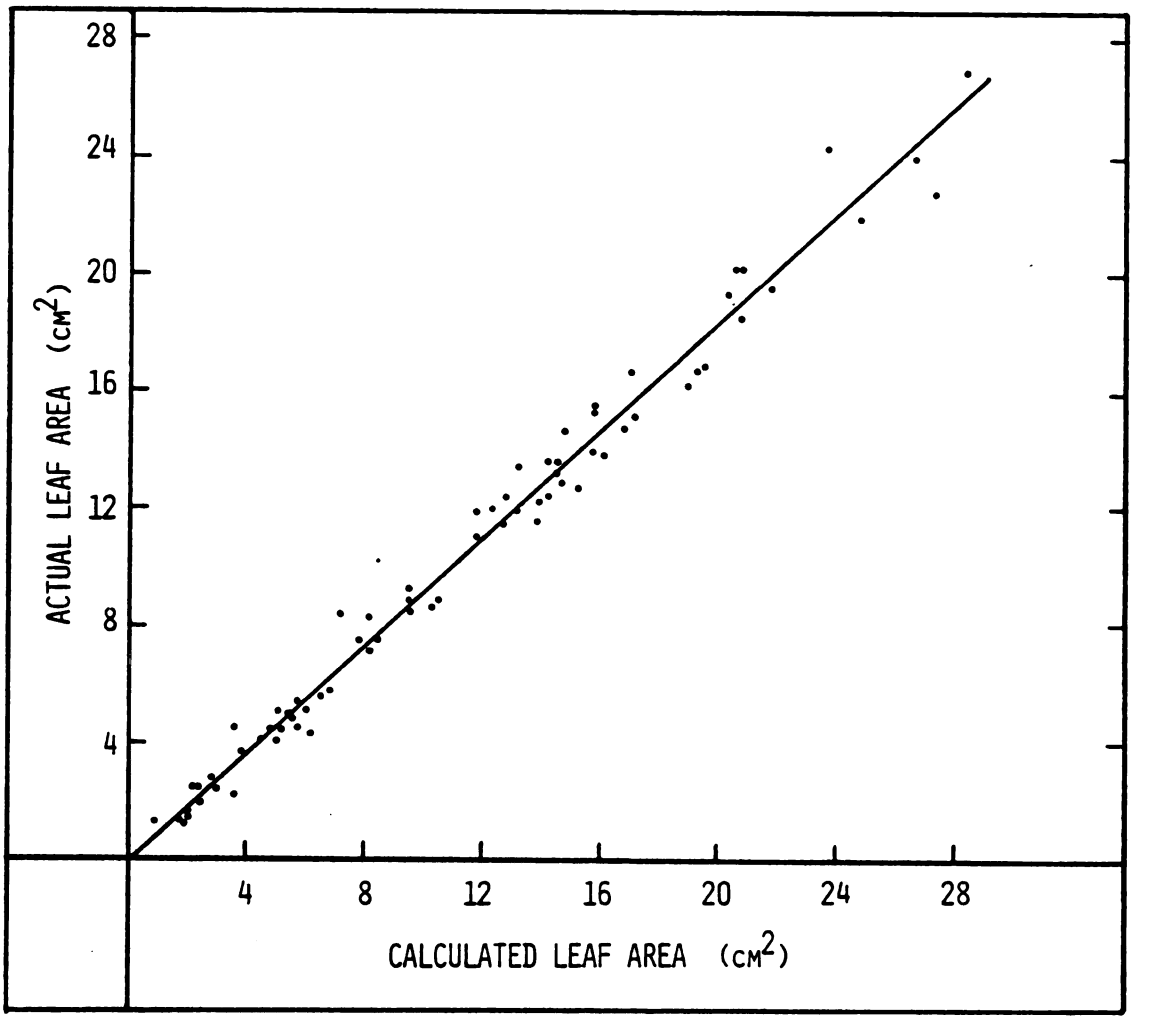
where T = transpiration rate in gm cm^{-2} s^{-1}

ψ = water potential of leaf and soil, respectively in bars

and r_p = plant hydraulic resistance in bars s cm^{-1}

Knowing that hydraulic conductivity (L_p) is equal to the inverse of

Figure 1. Regression line of actual leaf area versus calculated leaf area. Actual leaf area was determined by weighing photocopies of leaves and determining the area by conversion from the weight of each photocopy using the density of the paper. Leaf area was calculated as the surface area of a cylinder equal to π time leaf length times the average of the diameter at 0.5 leaf length and 0.9 leaf length. Regression line = 0.92 x calculated leaf area - 0.24; r^2 = 0.984.


ACTUAL LEAF AREA (cm2)
28
24
20
16
12
8
4
4
8
12
16
20
24
28
CALCULATED LEAF AREA (cm2)

r_p and rearranging equation (1),

$$L_p = - \frac{T}{(\psi leaf - \psi soil)} \qquad (2)$$

Soil water potential was equal to zero bars because the experiments were performed under well-watered conditions.

Leaf resistance was calculated using an equation described by Kramer (6),

$$T = \frac{.622\ \rho}{P} \times \frac{e_{leaf} - e_{air}}{r_{leaf} + r_{air}} \qquad (3)$$

where: T = transpiration rate in gm cm^{-2} s^{-1}

ρ = density of air in gm ml^{-1}

P = atmospheric pressure in mm Hg

e = vapor pressure of leaf and air, respectively in mm Hg

and r = Leaf and boundary layer resistance to vapor flow respectively in s cm^{-1}.

By rearranging equation (3) leaf resistance plus boundary layer resistance can be determined.

$$r_{leaf} + r_{air} = \frac{.622\ \rho}{P} \times \frac{e_{leaf} - e_{air}}{T} \qquad (4)$$

The atmospheric pressure (P) can be measured and the density of air (ρ) can be determined by knowing P and the air temperature, which was measured with a shaded thermocouple held at leaf level. Leaf vapor pressure (e_{leaf}) was determined by measuring leaf temperature with a small thermocouple and assuming the leaf was saturated with water vapor (at 100% Relative Humidity). Air vapor pressure at leaf level was measured using a dew point hygrometer (Yellow Springs Instrument Co.) equipped with a YSI 9102 probe.

The boundary layer resistance was measured by forming filter paper "onion leaves", wetting them, and placing one end of each in a water

source. The weight loss of these "leaves" was then measured with time, and since there was no cuticle, the only resistance to vapor loss was assumed to be the boundary layer resistance. Therefore, equation 2 becomes,

$$r_{air} = \frac{.622\ p}{p} \times \frac{e\ \text{"leaf"} - e_{air}}{T}. \qquad (5)$$

Using 4 replicate filter paper leaves, r_{air} was equal to 0.87 s cm^{-1} for the conditions of these experiments.

Finally, the leaf resistance (r_{leaf}) can be determined by subtracting the value calculated in equation (5) for boundary layer resistance from the value obtained in equation (4) for leaf plus boundary layer resistance.

At the end of each experiment, mycorrhizal infection was checked (12) to ensure that the mycorrhizal plants were infected and that non-mycorrhizal plants had not been contaminated. The experiment was conducted 4 times, 3 times at 60% relative humidity (RH) and 8 weeks of age once at 40% RH and 11 weeks of age.

RESULTS

Figures 2 through 5 show leaf water potentials, transpiration rates, hydraulic conductivities, and leaf resistances of 8 week old onion plants from one of the experiments conducted at 60% RH. Figure 2 indicates that the mycorrhizal (MYC) onions had significantly higher leaf water potentials than did the non-mycorrhizal, non-phosphorus treated (NM minus P) onion plants. Leaf water potentials of non-mycorrhizal plants treated with phosphorus (NM plus P) were not different from that of MYC plants. It was evident that MYC and NM plus P onion plants had a higher (more favorable) water status than did the NM minus P plants.

Transpiration rates exhibited a similar pattern (Figure 3). MYC plants had a transpiration rate which was more than 2 times greater than NM minus P plants on a unit leaf area basis. Again treatment with phosphorus (NM plus P) eliminated the differences between mycorrhizal and non-mycorrhizal plants.

Atmospheric vapor pressure determines the ultimate driving gradient for water from the soil, through the plant, and into the atmosphere (20). Because the MYC plants had a higher leaf water potential than did NM minus P controls when exposed to the same driving force, this indicated that it was easier for liquid water to move through the plant to the evaporating surfaces in the leaf. Results of calculations of hydraulic conductivity (a measure of the ease with which liquid water moves through the plant) are shown in Figure 4. As expected, MYC plants had a

Figure 2. Leaf Water Potentials of 8 week old, well-watered onion plants from experiment number 1. Values are means of 4 replicates and the vertical bars represent the standard error of the mean. MYC = mycorrhizal plants, NON-MYC plus P = non-mycorrhizal plants with phosphorus added, NON-MYC minus P = non-mycorrhizal plants without phosphorus added. Dry weights of plants were: NON-MYC minus P = 14 ± 3 mg, MYC = 173 ± 32 mg, and NON-MYC plus P = 59 ± 3 mg. The leaf water potentials of NM minus P plants were significantly lower than the other 2 treatments at the 5% level by Duncan's Multiple Range Test (DMRT).

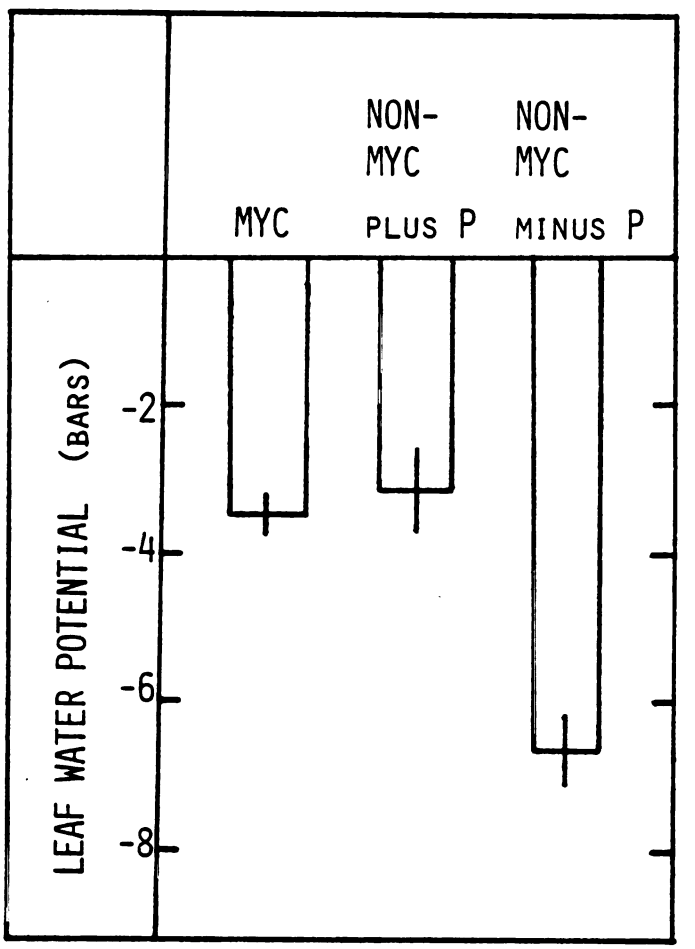

NON-
MYC
NON-
MYC
MYC
PLUS P
MINUS P
LEAF WATER POTENTIAL (BARS)
-2
-4
-6
-8

Figure 3. Transpiration rates of 8 week old, well-watered onion plants from experiment number 1. Values are means of 4 replicates ± the standard error of the mean. The transpiration rates of the NON-MYC minus P plants were significantly lower than the other 2 treatments at the 1% level by DMRT.

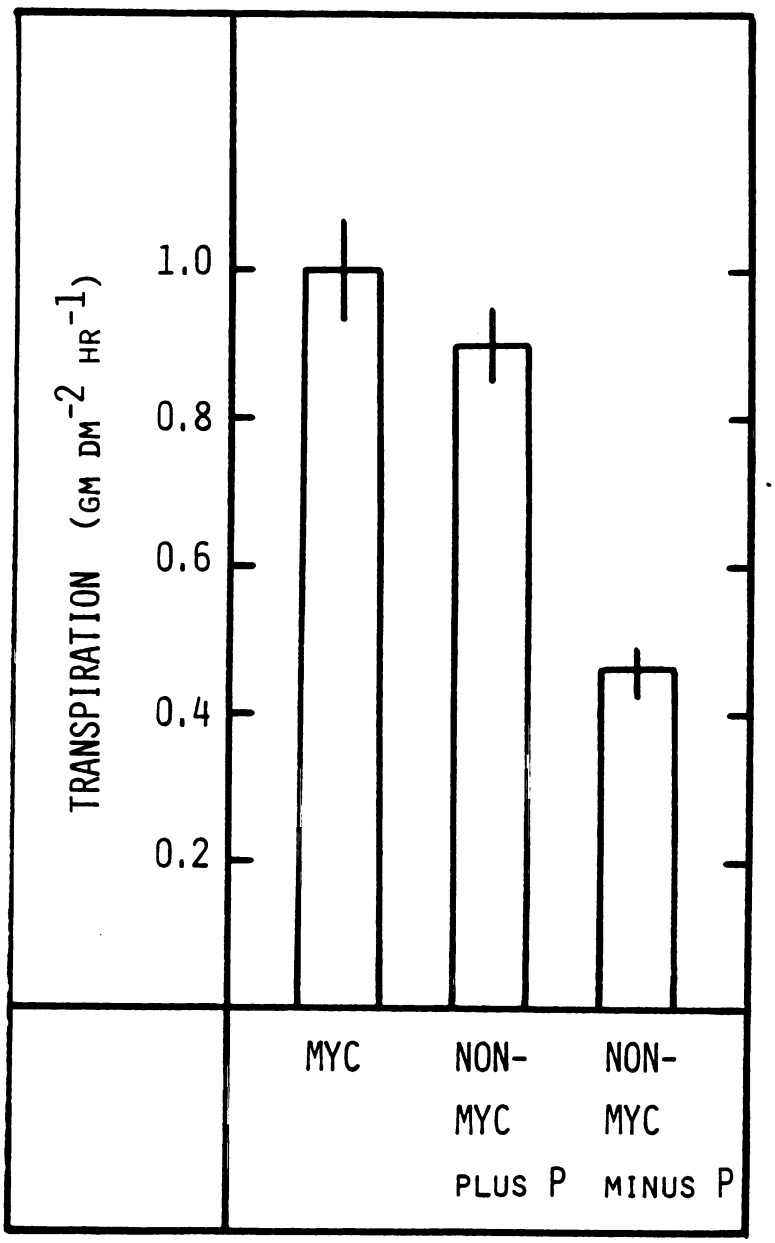
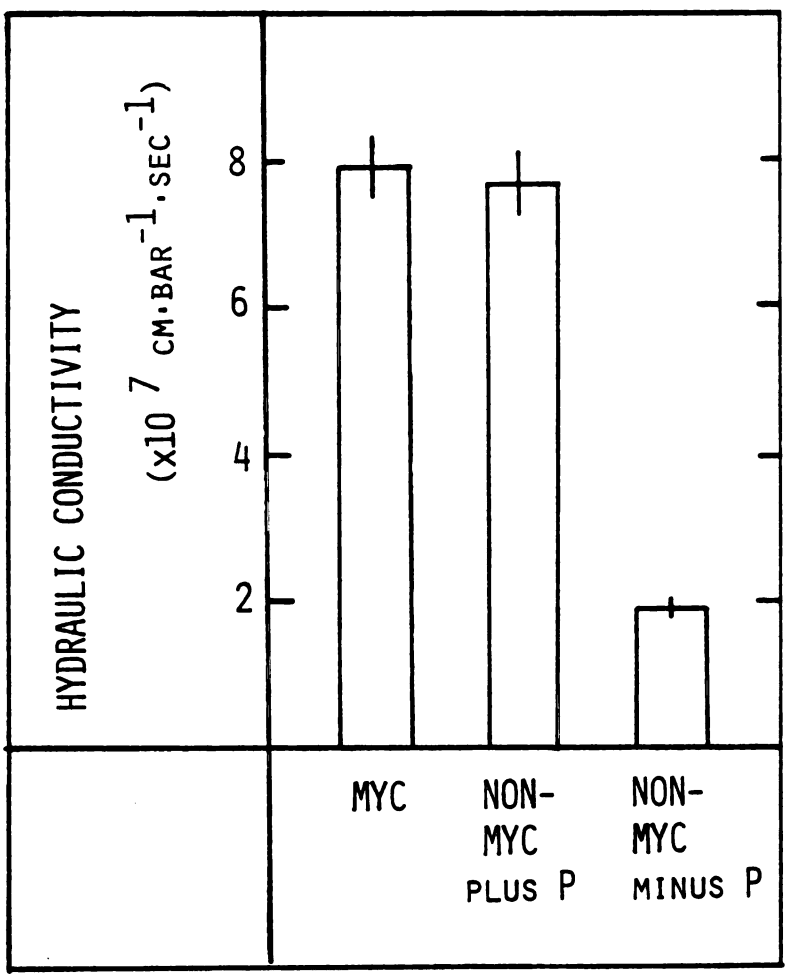

TRANSPIRATION (GM DM^{-2} HR^{-1})
1.0
0.8
0.6
0.4
0.2
MYC
NON-MYC PLUS P
NON-MYC MINUS P

Figure 4. Hydraulic conductivities of 8 week old, well-watered onion plants from experiment 1. Values are the means of 4 replicates ± the standard error of the mean. The hydraulic conductivities of the NON-MYC minus P plants were significantly lower than the other 2 treatments at the 1% level by DMRT.


HYDRAULIC CONDUCTIVITY
(x10 7 CM·BAR-1·SEC-1)
8
6
4
2
MYC
NON-MYC PLUS P
NON-MYC MINUS P

considerably higher hydraulic conductivity than did NM minus P. Since NM plus P plants had leaf water potentials and transpiration rates similar to MYC plants, the hydraulic conductivities of NM plus P plants were also high and not different from MYC plants.

The greater transpiration rates of MYC plants over NM minus P plants when exposed to the same evaporative demand indicated a lower resistance to vapor transfer from inside the leaf to the atmosphere for the MYC plants. Results of calculations of leaf resistance (Figure 5) indicated that MYC plants had leaf resistances much lower than did NM minus P plants (1.0 versus 5.0 sec cm^{-1}). NM plus P plants had a slightly higher resistance than did the MYC plants, but the differences were not significantly different.

The results obtained for the 4 water relation parameters as well as the dry weights of the plants used for the other 3 experiments are presented in Table 1. In every case the results are similar to those reported for experiment 1 (Figures 2-5). Leaf water potentials, transpiration rates, and hydraulic conductivities were always higher for the MYC plants when compared to the NM minus P plants; while leaf resistances were always lower for the MYC plants. Treatment of non-mycorrhizal plants with phosphorus (NM plus P plants) always eliminated the differences between mycorrhizal and non-mycorrhizal plants. In only two cases (leaf water potentials, experiments 2 and 4) the MYC plants were not significantly different from the NM minus P plants at either the 5% or 1% level. In experiment 2 large variability in the NM minus P plants resulted in non-significance despite a large difference in water potential. In experiment 4, conducted at lower RH and at 11 weeks of age rather than 8, the MYC and NM plus P plants were more affected by the

Figure 5. Leaf resistance of 8 week old, well-watered onion plants from experiment number 1. Values are the means of 4 replicates ± the standard error of the mean. The leaf resistances of the NON-MYC minus P plants were significantly higher than the other 2 treatments at the 1% level by DMRT.

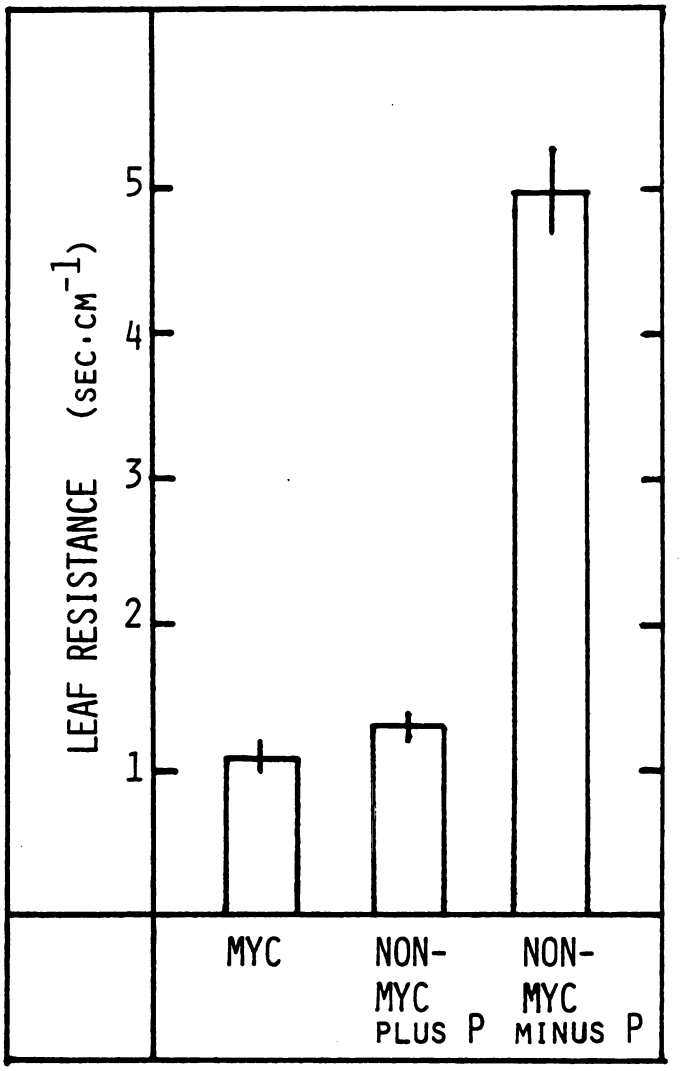

LEAF RESISTANCE (SEC·CM^{-1})
5
4
3
2
1
MYC
NON-MYC PLUS P
NON-MYC MINUS P

Table 1. Leaf water potentials, transpiration rates, hydraulic conductivities, leaf resistances and dry weights from experiments 2, 3, and 4.[W]

Experiment #	2			3			4		
Relative humidity (%)	60			60			40		
	Treatment			Treatment			Treatment		
Parameter[X]	NM minus P[Y]	MYC	NM plus P	NM minus P	MYC	NM plus P	NM minus P	MYC	NM plus P
Leaf water potential (bars)[Z]	11.1 a ±1.1	4.2 ab ±0.2	3.6 b ±0.2	8.1 a ±0.7	4.5 b ±0.4	4.2 b ±0.3	7.4 a ±0.7	5.9 a ±0.9	5.1 a ±0.8
Transpiration rate (gm dm^{-2} hr^{-1})	0.55 a ±0.19	1.00 b ±0.12	0.88 b ±0.11	0.41 a ±0.11	0.78 b ±0.05	0.76 b ±0.04	0.74 a ±0.15	1.13 b ±0.04	1.16 b ±0.05
Hydraulic conductivity (10^{-7} cm bar^{-1} s^{-1})	1.4 a ±0.5	6.6 b ±0.8	6.8 b ±0.3	1.4 a ±0.4	4.8 b ±0.3	5.0 b ±0.2	2.8 a ±0.6	5.3 b ±0.2	6.3 b ±0.2
Leaf resistance (s cm^{-1})	10.9 a ±5.5	1.6 b ±0.3	2.2 b ±0.4	6.0 a ±2.1	2.1 b ±0.2	2.5 ab ±0.2	7.0 a ±1.6	3.3 b ±0.1	3.2 b ±0.2
Top dry weight (mg)	21 a ±2	136 b ±5	102 b ±28	17 a ±1	112 b ±7	77 c ±6	16 a ±2	82 b ±9	209 c ±31

[W]Each value is the mean of 4 replicate measurements or calculations ± std. error.

[X]In certain cases where the variances of the mean within an experiment and parameter were shown to be non-homogenous by Bartlett's test for homogeneity, the data was transformed by a log or square root transformation before statistical analysis was performed.

[Y]NM minus P = Non-mycorrhizal without added phosphorus fertilizer, MYC = mycorrhizal onions, NM plus P = non-mycorrhizal with added phosphorus fertilizer.

[Z]Means followed by the same letter within an experiment and within a parameter are not significantly different by Duncan's Multiple Range Test at the 5% level.

increased evaporative demand; possibly because the NM minus P plants were already suffering from atmospheric water stress in the experiments conducted even at the higher RH (lower leaf water potentials and higher leaf resistances). It appears that at the increased transpiration rates resulting from the increased evaporative demand at the lower RH, the leaf water potentials of the MYC and NM plus P plants dropped enough (to <-5 bars) to eliminate significant differences.

DISCUSSION

It has been shown that mycorrhizal fungi can alter the water relations of the host plants. The effect is pronounced under conditions of low soil phosphorus (Figures 2-5, Table 1) confirming the suggestions of Safir et al. (16) who were able to eliminate the differences between mycorrhizal and non-mycorrhizal plants with a complete nutrient solution. The fact that Levy and Krikun (7) did not find a difference in hydraulic conductivity between their mycorrhizal and non-mycorrhizal citrus plants was probably due to the high levels of available phosphorus supplied by the irrigation solution they used (a 0.1% solution of 20,20,20 N,P,K nutrient solution, applied daily).

Furthermore, the magnitude of the effect of mycorrhizal infection may also depend on the innate capacity of the host to absorb water and/or nutrients. Baylis (1) presented data that show that plants with more primitive root systems (i.e. Magnolioid types) have a greater growth stimulation when infected by a mycorrhizal fungus; and plants with a more advanced, finely divided root system (i.e. grasslike) are less affected. In comparison to soybeans, onions have a shallow and non-extensive root system (5, 19). This may explain the differences between our present results with onions, and those found under low soil phosphorus conditions by Safir et al. (15, 16) with soybean (400% vs. 60% increase in hydraulic conductivity).

The fact that added phosphorus affects the water relations of the

onion in the same way that the mycorrhizal fungus does, suggests that the primary cause of the changes is nutritional. A model root system has been hypothesized (4) where changes in membrane permeability can have large effects on root resistance to water flow. Phosphorus deficiency can affect membrane integrity in any of a number of ways and this may explain the effects of mycorrhizae on whole plant hydraulic conductivity. Conversely, improved phosphorus nutrition may simply increase the amount of vascular tissue throughout the plant (3), thus reducing overall resistance to liquid water flow. It should be noted that the whole plant hydraulic conductivity calculated here does not identify the specific area in the plant where changes are occurring. Two sites which might be involved are in the roots as shown by Safir et al. (15) or the transition zone between root and meristem at the base of the bulb through which all bulb and leaf water must pass.

Finally, the differences found in leaf water potential, transpiration, and leaf resistance (Figures 2, 3, 5, Table 1) may be secondary effects due to the differences found in hydraulic conductivity (Figure 4). For a given evaporative demand, a decrease in hydraulic conductivity (as in the NM minus P plants) would lead to a lower leaf water potential as demonstrated in Figure 2 and Table 1. A plant can counter this decrease in leaf water potential by increasing leaf resistance (Figure 5) by partial or cyclic closing of the stomates, thus allowing partial (or total) recovery of leaf water status. This increase in leaf resistance to vapor transfer would of course reduce the transpiration rate (Figure 3).

It has previously been shown that onions are susceptible to atmospheric water stress (8). This is also evident from the results of experiments 2, 3, and 4 (Table 1). At the higher relative humidities

the NM minus P plants, despite adequate soil moisture, were suffering some symptoms of water stress (low leaf water potential and high leaf resistance). When the humidity was decreased from 60% to 40% for experiment 4, the MYC plants and the NM plus P plants also began to show symptoms of stress. The increased evaporative demand increased transpiration and this resulted in a decrease in leaf water potential. As the leaf water potential decreased below a hypothetical minimum level (perhaps -5 bars as suggested by Millar et al., (8)), the stomates closed and leaf resistance increased. This new dynamic equilibrium resulted in non-significance among the leaf water potential values for the 3 treatments and a decreased difference in leaf resistance among the treatments in experiment 4.

These results suggest that under conditions of high water and phosphorus availability mycorrhizal infection will not have major effects on plant-water relationships. This is supported by the present work as well as field work (11) that demonstrated that high soil phosphorus levels will prevent infection of onion roots by mycorrhizal fungi, without any yield reduction. However, the results reported here and in the field (11) were obtained under conditions of ample soil moisture. Additional results (Section II) indicate that, when exposed to periods of cyclic drought, mycorrhizal onions have greater fresh and dry weights than do non-mycorrhizal onions grown at P levels greater than those used here.

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SECTION II

INDUCTION OF DROUGHT RESISTANCE IN ONION PLANTS BY MYCORRHIZAL INFECTION

INDUCTION OF DROUGHT RESISTANCE IN ONION PLANTS BY MYCORRHIZAL INFECTION

ABSTRACT

Onion plants (Allium cepa L, cv. Downing Yellow Globe) infected by the mycorrhizal fungus Glomus etunicatus (Becker and Gerdemann) were more drought resistant than were non-mycorrhizal onion plants when exposed to several cycles of soil water-stress. Drought resistance was shown by greater fresh and dry weights and higher tissue phosphorus (P) concentrations in the mycorrhizal plants. Stressed, non-mycorrhizal plant tissues were deficient in P, despite the fact that only non-mycorrhizal plants were fertilized with high levels of P (=114 kg/ha). Plant P nutrition was implicated in the ability of the plants to resist drought and it was concluded that the ability of the mycorrhizal fungus to maintain adequate P nutrition in the onions during stress was a major factor in the improved drought resistance.

Plant water stress is considered a major limiting factor in crop yield in dryland areas (3,5,11) as well as in areas considered to have ample rainfall (26). The effects of crop water deficit on plant growth and development and increased drought resistance or tolerance in crops are major areas of research in many parts of the world (3,5,11,12). A number of authors have reported that increased fertilization or improved plant nutrition have increased drought resistance or increased yield when crops were exposed to varying periods of water stress (1,3,13,14,28).

Vesicular-arbuscular (VA) mycorrhizal fungi are known to improve

plant nutrition (generally phosphorus) and can stimulate plant growth and yield under well-watered conditions (16,20,21,24). In addition, VA mycorrhizal fungi have been shown to alter plant water relations when plants are maintained under well-watered conditions or are exposed to a single brief period of water stress (15,18,22,23,24). However, the ability of VA mycorrhizal fungi to improve the drought resistance of their host plants has thus far not been investigated.

In this section evidence is presented, for the first time, for the role of VA mycorrhizal fungi in the increased drought resistance of onion plants exposed to several periods of soil water stress. In addition, the effects of drought and phosphorus fertilization on VA mycorrhizal reproduction are described.

MATERIALS AND METHODS

Onion seeds (Allium cepa L., cv. Downing Yellow Globe) were sown in plastic cups with drain holes which contained 440 g of a 50:50 mix (v/v) of sand and sandy loam soil; pH 7.5 and available P of 6 ppm (Bray's P-1 extractable). The soil was sieved through a 2 mm screen, autoclaved for 45 min, and allowed to cool prior to planting. Before sowing, two-thirds of the pots received 5 g of mycorrhizal soil inoculum containing 2,500 spores of the VA mycorrhizal fungus Glomus etunicatus (Becker and Gerdemann) which was placed 1 cm below the seed position. The final one-third of the pots received a soil wash, from which the mycorrhizal spores had been sieved, to ensure that the non-mycorrhizal pots contained all other microorganisms which would be in the mycorrhizal pots. Mycorrhizal inoculum was obtained from pot cultures maintained on sorghum in the greenhouse.

The plants were grown in a growth chamber with a 14 hour day length, temperatures of 22 C day/16 C night and a light level of 3×10^4 ergs cm^{-2} s^{-1}. The experiment was set up as a completely randomized design with factorial treatments (Table 1). One factor was watering regime, in which pots were either well-watered or drought-stressed, and the second factor was soil treatment. Soil treatments within the well-watered and drought stressed treatments included: non-mycorrhizal plants plus P fertilization (=114 kg/ha), mycorrhizal plants without P fertilization, and mycorrhizal plants plus P fertilization at an intermediate level

Table 1. Experimental design to determine the effect of drought-stress on the mycorrhizal association.[a]

Factor A water level	Well-watered			Drought stressed (-10 bars)[b]		
Factor B soil treatment	Non-mycorrhizal plus 114 kg P/ha	Mycorrhizal no P added	Mycorrhizal plus 57 kg P/ha	Non-mycorrhizal plus 114 kg P/ha	Mycorrhizal no P added	Mycorrhizal plus 57 kg P/ha

[a]Four replications of each treatment combination were harvested at weeks 4, 8, and 12.

[b]Stress was initiated at week 4 and the stressed plants were rewatered when the soil water potential fell below -10 bars.

(=57 kg/ha). Phosphorus fertilization levels were selected after preliminary well- watered experiments conducted in the growth chamber (Figure 1) and the greenhouse. Fertilization of non-mycorrhizal plants with the equivalent of 114 kg/ha P stimulated plant growth so that it was not different from that of the mycorrhizal plants grown at the base soil P level (6 ppm ≈ 12 kg/ha). Fertilization of mycorrhizal plants with the equivalent of 57 kg/ha P appeared to stimulate plant growth somewhat better than P alone or mycorrhizal infection alone (Figure 1), and was included in 2 of 3 experiments to investigate the effects of drought and P fertilization on fungal infection and reproduction. Phosphorus was added to the soils in 20 ml or 10 ml, respectively, of a .04 M solution of KH_2PO_4.

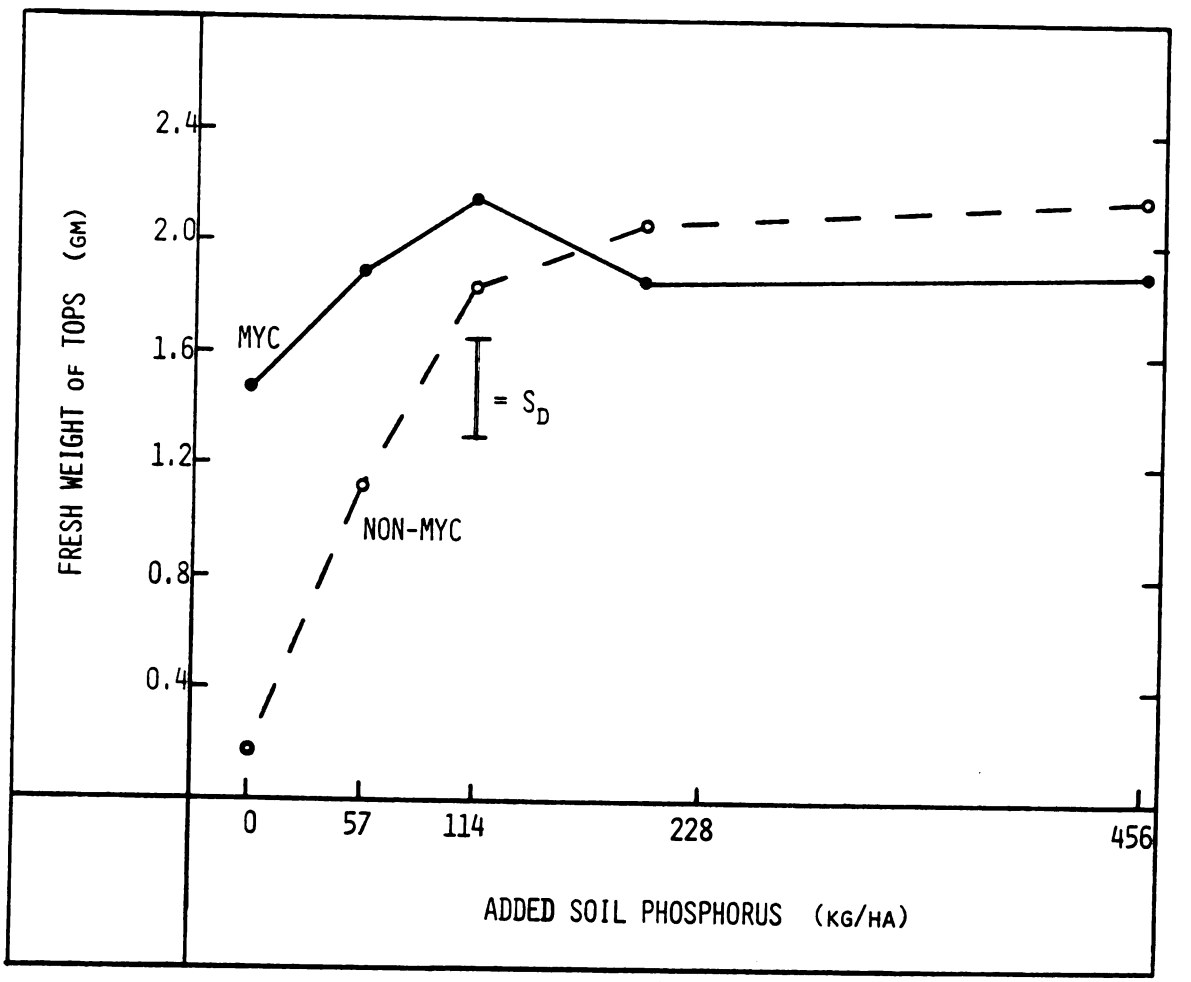
Onion plants were thinned to one plant per pot at 4 weeks after sowing, to ensure emergence and the establishment of mycorrhizal infection, at which time the drought stress treatment was initiated. Drought stress was developed by withholding water and the soil water potential of each stressed pot was monitored daily with individually calibrated ceramic soil moisture blocks (Beckman Instrument Co.). Stressed pots were rewatered to capacity when soil water potential values fell to or below -10 bars. Well-watered pots were irrigated daily with distilled water to approximately saturation.

Four pots of each treatment combination were destructively harvested at weeks 4 (12 pots), 8 (24 pots), and 12 (24 pots) after sowing to evaluate a number of plant and fungal parameters.

At each harvest onion top fresh weight was determined. Top and root dry weights were determined after drying at 75 C to constant weight.

The leaf water potentials of a 1 cm leaf segment from the midpoint

Figure 1. Top fresh weight (leaves plus bulbs) of 8 week old mycorrhizal and non-mycorrhizal onion plants as influenced by added phosphorus fertilizer. Each point is the mean of four replications and S_D = standard error of the difference between 2 means. Plants were grown in a growth chamber under the same conditions as were plants used in the drought-stress experiments except in 200 g soil. Phosphorus was added as an aqueous solution of KH_2PO_4 at seed sowing.


FRESH WEIGHT OF TOPS (GM)
2.4
2.0
1.6
1.2
0.8
0.4
MYC
NON-MYC
= S_D
0
57
114
228
456
ADDED SOIL PHOSPHORUS (KG/HA)

of the youngest fully expanded leaf from each harvested plant was determined by a previously described method (17) using a Wescor dew point hygrometer and C-52 sample chambers.

When determined, the transpiration rate of one plant from each treatment combination was measured as previously described (16). Briefly, pots were wrapped 2 days before harvest with plastic wraps, then aluminum foil. Weight loss per unit time was then determined by weighing pots several times each day until harvest to obtain steady state measurements. Transpiration on a unit leaf area basis was calculated by dividing pot weight loss by the leaf area of the onion in each pot as determined non-destructively using a previously published regression equation (18). Stressed pots used to determine transpiration rates were chosen from those that had been recently watered so that diffusion of water in the soil would not be limiting.

After drying, the tissue phosphorus content in the leaves, bulbs, and roots (concentration and total) for each plant harvested was determined using a modification of the method of Bartlett (2). One to 25 mg of dry tissue of each sample was added to a 10 ml microkjeldahl flask with 0.5 ml of 10N H_2SO_4 and heated to reflux for 30 min on a Labconco microkjeldahl burner. After cooling, 0.5 ml of 30% H_2O_2 was added, shaken gently, and again heated to reflux for 30 min. The H_2O_2 step was repeated if the liquid was not completely clear. After cooling, 5 ml of distilled water was added and the flask was heated for 10 min in a boiling water bath. After cooling, the color was developed as follows: 0.2 ml of a 5% (w/v) solution of ammonium molybdate was added followed by 0.2 ml of Fiske-Subbarow reagent; the flask was vortexed, heated for 7.0 min in a boiling water bath, cooled, and the absorbance read at 620 nm

with a Perkin-Elmer 35 spectrophotometer. All measurements included a blank, treated as the other flasks, except that it contained no tissue. Fiske-Subbarow reagent was made up of 1 g of 1-amino-2-napthol sulfonic acid, 2 g of sodium sulfite, and 58.4 g of sodium meta-bisulfite, mixed and ground as a powder in a dry mortar. Just prior to use, 0.77 g of the mix was dissolved in 5 ml of warm water. The standard curve was determined using KH_2PO_4 as the P source. Recovery of known amounts of organic P (glycerophosphate ≡ 30 μg P) in the presence or absence of plant tissue was essentially 100% (96.6% ± 1.6 and 102.2% ± 1.8, respectively). Equal amounts of tissue P were recovered from flasks refluxed for 1, 3, 5 and 10 hours, indicating P loss during tissue digestion was negligible.

Available soil P levels were determined using the Bray's P-1 extraction technique in air dried soil from each pot harvested.

Mycorrhizal root infection was determined on a number of small random root segments, taken from each plant before the roots were dried, from each plant harvested. Root segments were cleared and stained before inspection with a light microscope (150x) using a method modified from Phillips and Hayman (19). Root pieces were heated in 10-20 ml of 10% KOH (w/v) in an 85 C water bath; then rinsed and placed in 0.1 N HCl for 1 hr at room temperature (22°C). Finally, roots were stained in a solution of 0.1% acid fuchsin (Eastman Chemical) in lactophenol for 24-48 hr at room temperature, followed by removal of unfixed stain by soaking in 1-2 changes of clear lactophenol (without acid fuchsin) for 24-48 hrs. Four to 9 one cm segments were mounted on glass slides and rated using a scale from 0-4 in order to evaluate presence and intensity of infection, where: 0 = no evidence of infection; 1 = entry points only present, no hyphal

development inside the segment, 2 = presence of small areas of internal fungal development, less than about 5% of the segment infected, 3 = presence of moderate fungal development throughout the segment or concentrated development in less than one-half the segment, and 4 = presence of concentrated fungal development throughout the entire 1 cm root segment. This was considered to be essentially an exponential scale rather than a linear scale.

The number of mycorrhizal spores per pot was determined at each harvest. Soil from each pot was thoroughly mixed after the plant roots were removed, then spores were isolated from duplicate 10 g soil samples of the mixed soil and visually counted using a dissecting microscope. Spores were isolated by washing soil samples with running tap water through 2 sieves, the top sieve having a 200 μm mesh and the bottom having a 38 μm mesh. The mycorrhizal spores were trapped on the second sieve (with soil and organic debris) and were washed into a 50 ml centrifuge tube and centrifuged at 1150 g for 3 min in a swinging bucket rotor. The supernatant containing light organic debris was removed. The pellet containing the spores was resuspended in a sucrose solution (45 g/100 ml), and centrifuged again for 1.5 min. The supernatant containing spores was washed through the 38 μm sieve, to remove the sucrose solution, and the spores were washed into a gridded petri dish for counting. The sucrose centrifugation was repeated, since it had previously been determined that 2 isolations in sucrose removed about 90% of the spores in the soil. Because the sandy nature of the soil prevented the formation of a stable pellet, resulting in some difficulty in decanting the first supernatant, the initial water supernatant from each isolation was also checked for the presence of spores.

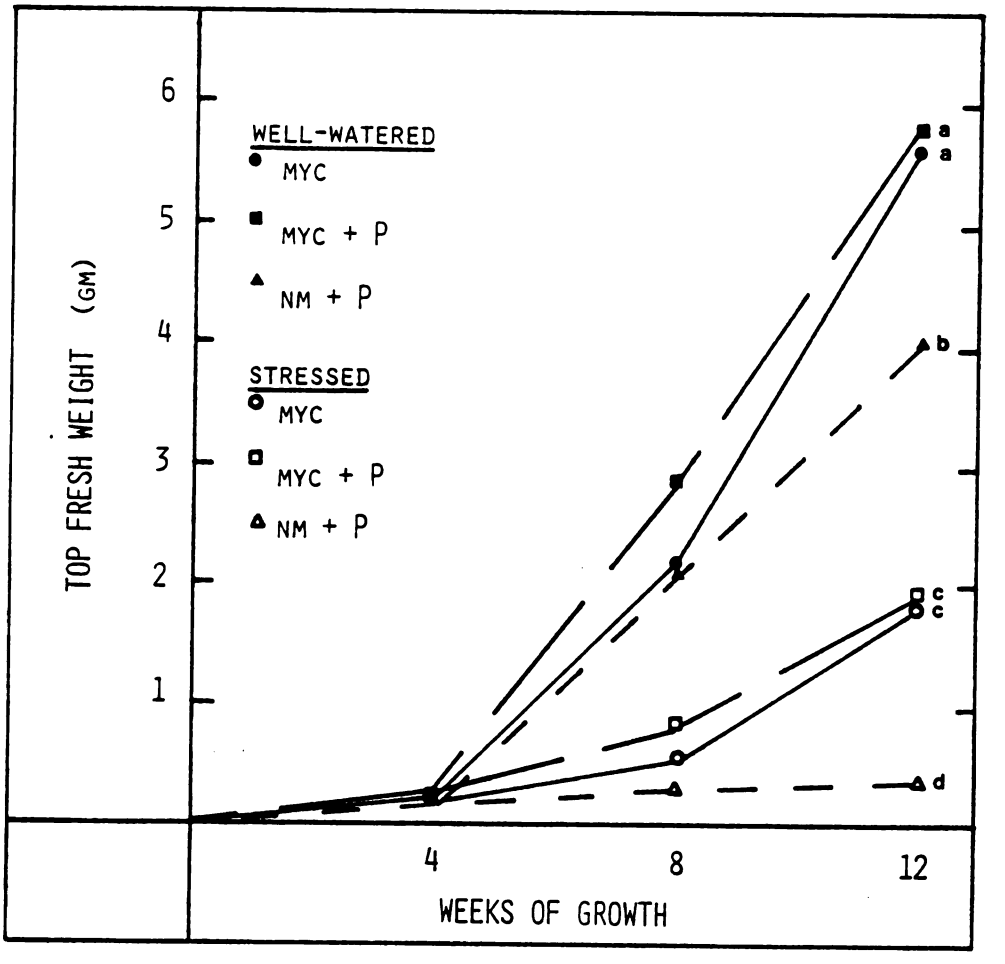
Where appropriate, analyses of variance calculations were performed and when significant F values were found, treatments were compared using Duncan's Multiple Range test. The experiment was conducted 3 times, with similar results obtained each time.

RESULTS

The relationship between soil moisture content and soil water potential is markedly hyperbolic rather than linear. The soil moisture content of the soil used in these experiments can decrease from 32% to 9% with little change in soil water potential (0 to -0.5 bars). A subsequent decrease of 3.5% to 5.5% soil moisture resulted in a large decrease in soil water potential from -0.5 bars to -10 bars. Because of these non-linear changes in soil water potential, plants in pots from which water was withheld were exposed to cycles of wet soil and dry soil where the soil water potential was above -1 bar for 5 days and then fell rapidly to or below -10 bars in about 3 days. Therefore, the average drought cycles lasted about 8 days during which the plants were essentially well-watered for 5 days and increasingly drought-stressed for 3 days at the end of which time they were rehydrated. Because the average drought cycle lasted 8 days and the drought stress section of the experiment lasted 8 weeks the stressed plants experienced 7 cycles of drought stress. There were no significant differences in the drought cycle time among the 3 soil treatments exposed to the stress.

The fresh weights of the onion tops (leaves plus bulbs) for each of the six treatment combinations at the 3 harvest times for one of the 3 experiments are shown in Figure 2. Fresh weights rather than dry weights were reported because onion leaves, like those of other monocots, die back from the tip when stressed (10). Differences in the extent of tip

Figure 2. Top fresh weight (leaves plus bulbs) of well-watered and drought-stressed, mycorrhizal and non-mycorrhizal onion plants versus time. MYC = mycorrhizal plants without added P, MYC + P = mycorrhizal plants with 57 kg P/ha added, NM + P = non-mycorrhizal plants with 114 kg P/ha added. Each point is the mean of 4 replications and points followed by different letters at week 12 are significantly different from the other by Duncan's Multiple Range Test (DMRT) (P=0.05).


TOP FRESH WEIGHT (GM)
6
5
4
3
2
1
WELL-WATERED
MYC
MYC + P
NM + P
STRESSED
MYC
MYC + P
NM + P
a
a
b
c
c
d
4
8
12
WEEKS OF GROWTH

dieback among treatments would be obscured by dry weight data and were better expressed by presenting live (i.e. fresh) weights. Figure 2 demonstrates that drought stress significantly reduced plant growth. There also was a significant effect of soil treatment which was greater within the stressed treatment than within the well-watered treatment. The water-stressed non-mycorrhizal onion plants, despite high levels of P fertilization, were only 23% as large as the stressed, mycorrhizal onions grown at the low P levels.

Root weights, like top weights, of stressed plants were depressed at week 12 when compared to the root weights of well-watered plants. However, root weights of stressed plants were always less affected by drought than shoots resulting in a consistently higher root to shoot ratio for stressed plants than for well-watered plants (Table 2).

Leaf water potentials and transpiration rates were monitored in the last two experiments to determine if there were differences in the water relations of the treatments which might explain the reduced growth of the stressed, fertilized, non-mycorrhizal plants. Leaf water potentials for plants harvested at week 12 from one experiment are shown in Figure 3. There were no significant differences among any of the six treatments.

Transpiration rates, for the two experiments in which transpiration was measured, are shown in Figure 4. Because only one plant per treatment was monitored at each harvest period, the data presented are combined from weeks 8 and 12 for both experiments. Because of this, statistical analysis of the transpiration rate was inappropriate, but inspection of the data in Figure 4 suggests no differences among treatments within a watering regime. However, it was evident that the water-stressed plants, as a group, transpired less water than did the

Table 2. Dry weights and root/shoot ratios of 12 week old, well-watered and drought-stressed onion plants.[a]

Treatment	Dry Weights (mg)				Root/shoot ratio	
	Well-watered		Drought-stressed			
	Tops	Roots	Tops	Roots	Well-watered	Drought-stressed
Non-mycorrhizal plus P	376 ± 75	109 ± 19	43 ± 21	37 ± 10	.304 ± .036	.973 ± .254
Mycorrhizal no P	553 ± 30	195 ± 15	155 ± 23	82 ± 4	.354 ± .030	.568 ± .078
Mycorrhizal plus P	607 ± 90	187 ± 90	165 ± 35	95 ± 14	.345 ± .041	.604 ± .071

[a]Values are means of 4 replicates ± standard error of the mean.

Figure 3. Leaf water potentials of 12 week old, well-watered and drought-stressed, mycorrhizal and non-mycorrhizal onion plants. MYC = mycorrhizal plants without P added, MYC + P = mycorrhizal plants with 57 kg P/ha added, NM + P = non-mycorrhizal plants with 114 kg P/ha added. Each value is the mean of 4 replicates ± standard error of the mean. There were no significant differences between means (P=0.05).

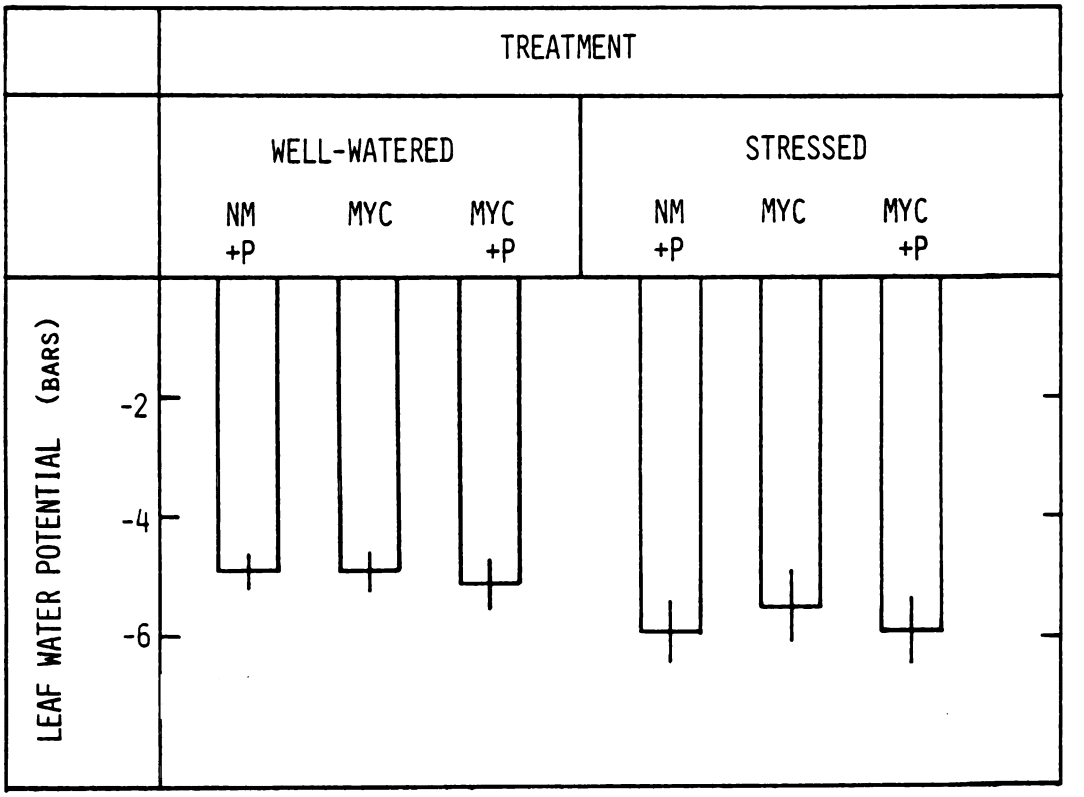
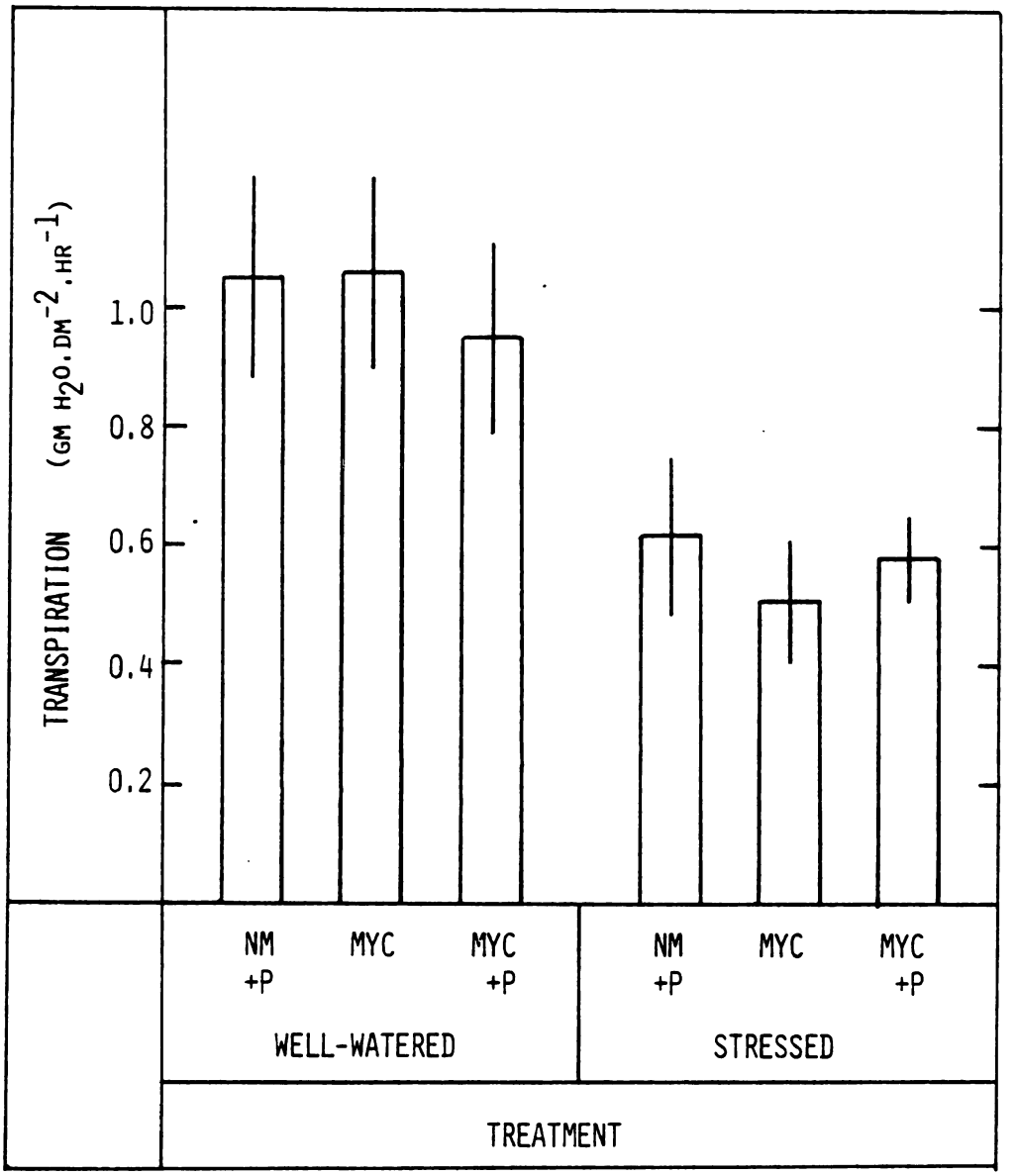

TREATMENT
WELL-WATERED
STRESSED
NM +P
MYC
MYC +P
NM +P
MYC
MYC +P
LEAF WATER POTENTIAL (BARS)
-2
-4
-6

Figure 4. Transpiration rate of 8 and 12 week old, well-watered and drought-stressed, mycorrhizal and non-mycorrhizal onion plants. MYC = mycorrhizal plants without P added, MYC + P = mycorrhizal plants with 57 kg P/ha added, NM + P = non-mycorrhizal plants with 114 kg P/ha added. Because the transpiration rate of only one plant from each treatment was determined at each harvest, each value is the mean of 4 measurements, one each from weeks 8 and 12 of experiments 2 and 3 ± standard error of the mean. Because the data were from different experiments and harvests, no statistical analysis was performed.


TRANSPIRATION (GM H2O.DM-2.HR-1)
1.0
0.8
0.6
0.4
0.2
NM +P
MYC
MYC +P
NM +P
MYC
MYC +P
WELL-WATERED
STRESSED
TREATMENT

plants in the 3 well-watered treatments.

Available soil P was monitored in the last 2 experiments to determine if the added phosphorus became fixed and unavailable to the stressed non-mycorrhizal plant during the cycles of wetting and drying. Throughout both experiments the available soil P did not differ within a soil treatment whether the soil was maintained in a well-watered condition or was dried periodically. Available soil P was always high for the non-mycorrhizal plants which had been fertilized, always low for the mycorrhizal plants without P added and always at an intermediate level for the mycorrhizal plants treated with the intermediate level of P in both water regimes (Table 3).

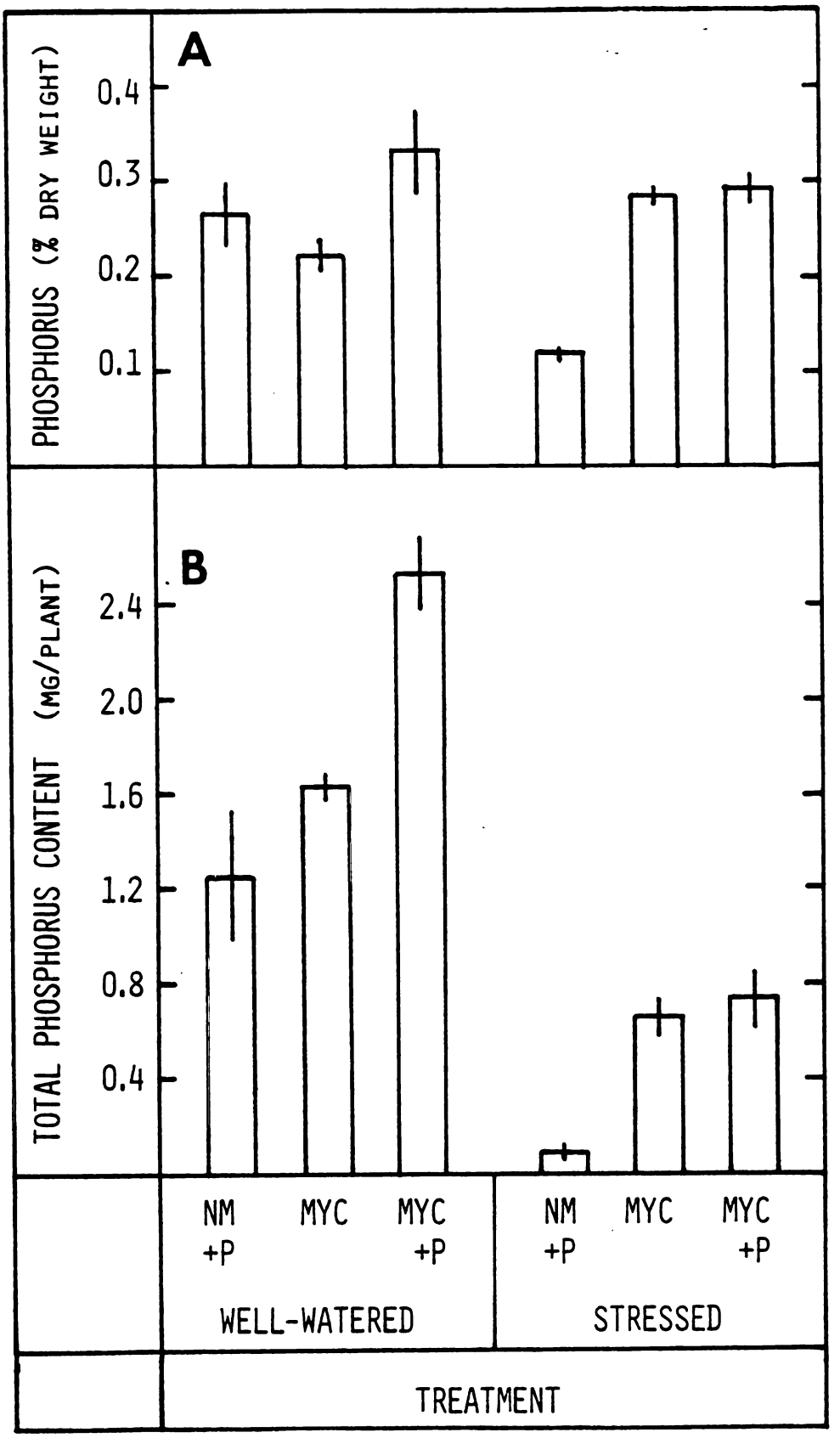
To determine the P nutritional status of the plants, P concentration and total P content for leaves, bulbs, and roots were determined for all plants harvested. Values for week 12 from one experiment are shown in Figure 5. Tissue P concentrations (Figure 5A) were uniformly high (0.22-0.33%) for all treatments except the stressed, non-mycorrhizal plants (0.12%). Total P content per plant (Figure 5B) showed even greater differences between treatments. All plants took up large amounts of P from the soil except the stressed, non-mycorrhizal plants. The 94 µg P in these 12 week old plants is only about 4 times that in the onion seeds used for these experiments (24 µg P per seed). In a separate greenhouse experiment, non-mycorrhizal plants were grown in phosphorus deficient soil at 5 added P rates and kept well-watered throughout the experiment (Figure 1). At eight weeks of age, all plants had phosphorus concentrations above 0.2% except the plants grown at 0 added P. These non-fertilized plants were stunted and had a phosphorus concentration of 0.12%.

Table 3. Available soil phosphorus (kg P/ha) at the 3 harvest dates, 4, 8, and 12 weeks after planting.[a]

Week	Well-Watered			Drought-stressed		
	Non-mycorrhizal plus P	Mycorrhizal no P	Mycorrhizal plus P	Non-mycorrhizal plus P	Mycorrhizal no P	Mycorrhizal plus P
4	107 ± 14	15 ± 1	51 ± 4	-	-	-
8	68 ± 12	11 ± 1	45 ± 3	103 ± 15	13 ± 1	47 ± 1
12	90 ± 14	10 ± 1	37 ± 2	83 ± 2	13 ± 1	45 ± 1

[a]P levels determined using Bray's P-1 extraction technique. Values are means of 4 replications ± standard error.

Figure 5. Phosphorus concentration (A) and total phosphorus content (B) of 12 week old, well-watered and drought-stressed, mycorrhizal and non-mycorrhizal onion plants. MYC = mycorrhizal plants without P added, MYC + P = mycorrhizal plants with 57 kg P/ha added, NM + P = non-mycorrhizal plants with 114 kg P/ha added. Each values is the mean of 4 replications ± standard error of the mean. A. Phosphorus concentration; the stressed fertilized, non-mycorrhizal plants had a significantly lower tissue phosphorus concentration than the other 5 treatments by DMRT (P=0.05). B. Total phosphorus content; the stressed, fertilized, non-mycorrhizal plants had a significantly lower phosphorus content and the well-watered, fertilized, mycorrhizal plants had a significantly higher phosphorus content than the other treatments by DMRT (P=0.05).


A
PHOSPHORUS (% DRY WEIGHT)
0.4
0.3
0.2
0.1
B
TOTAL PHOSPHORUS CONTENT (MG/PLANT)
2.4
2.0
1.6
1.2
0.8
0.4
NM +P
MYC
MYC +P
NM +P
MYC
MYC +P
WELL-WATERED
STRESSED
TREATMENT

Finally, by week 12 there were obvious visual differences between stressed, mycorrhizal and non-mycorrhizal plants. Stressed, mycorrhizal plants, though small, were dark green, with little or no tip dieback on any of the 3 to 5 leaves present on each plant. Conversely, the stressed, fertilized, non-mycorrhizal onions were a paler green, with extensive tip dieback and had only 1 or 2 leaves per plant.

At week 12, there were no statistical differences in root infection by the mycorrhizal fungus in any of the 4 mycorrhizal treatments (Table 4). Neither water-stress nor the intermediate P level had any major effect on root infection, with the possible exception that at week 8, root infection of the stressed, fertilized plants was somewhat lower.

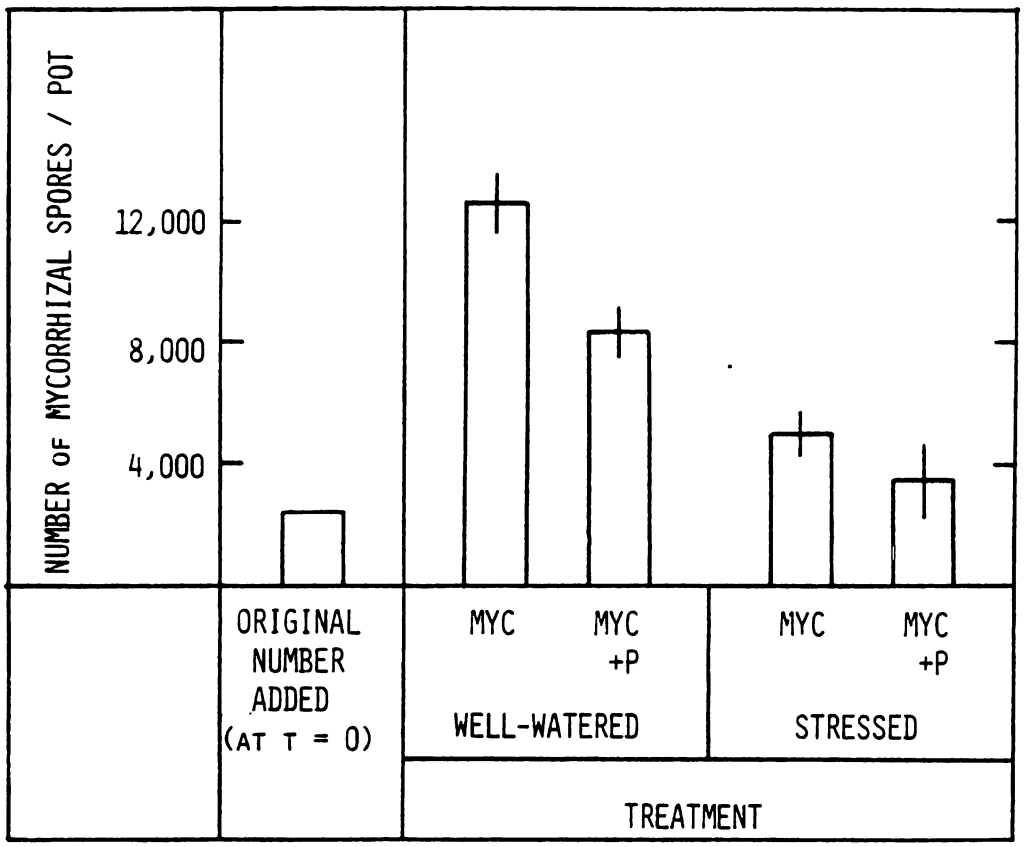
Water-stress and P fertilization affected spore production by the fungus by week 12 (Figure 6). Water-stress decreased the average spore number per pot by 51% at the low soil P level and by 57% at the intermediate soil P level when compared to the numbers of spores found in the well-watered, low soil P pots (top fresh weight was decreased 68% and 67%, respectively). Fertilization with P decreased spore production, by 35% when the pots were well-watered, and by 29% in the water-stressed pots. Spore numbers at week 8 were lower but the trend was similar.

Table 4. Ratings of mycorrhizal infection of onion roots as influenced by water and soil treatments.[a]

Harvest date	Mycorrhizal infection rating			
	Well-watered		Drought-stressed	
	Mycorrhizal no P	Mycorrhizal plus P	Mycorrhizal no P	Mycorrhizal plus P
week 8	2.6 ± 0.1	2.4 ± 0.2	2.5 ± 0.4	1.6 ± 0.7
week 12	3.0 ± 0.3	3.0 ± 0.3	3.3 ± 0.2	2.6 ± 0.3

[a]Values are means of 4 replications ± standard error. Mycorrhizal infection rating: 0 = no infection and 4 = heavy concentration of mycorrhizal hyphae throughout 1 cm root segment.

Figure 6. Numbers of mycorrhizal spores per pot at week 12 as influenced by soil treatment. MYC = mycorrhizal plants without P added, MYC + P = mycorrhizal plants with 57 kg P/ha added. Values are means of 4 replications ± standard error of the mean. Pots of well-watered, non-fertilized plants had a significantly higher number of spores than the other 3 treatments by DMRT (P=0.05). Pots of well-watered, fertilized plants had a significantly higher number of spores than the pots of stressed, fertilized plants (P=0.05).


NUMBER OF MYCORRHIZAL SPORES / POT
12,000
8,000
4,000
ORIGINAL NUMBER ADDED (AT T = 0)
MYC
MYC +P
MYC
MYC +P
WELL-WATERED
STRESSED
TREATMENT

DISCUSSION

Not surprisingly, soil water stress had marked effects on onion growth (Figure 2) development (Table 2) and physiology (Figures 4 and 5). The increased size of the mycorrhizal plants when exposed to drought-stress was less easily predicted (Figure 2).

The larger size of the stressed, mycorrhizal plants relative to the stressed, non-mycorrhizal plants might be due to one or more of the three following possibilities. First, the fungus might have improved the water relations of the host plant. If the fungus helped maintain a higher leaf water potential in the host plant, cell expansion (and growth) might be increased, since cell expansion is generally greater at high leaf water potentials (4,12). In addition, a direct or indirect effect of the fungus on transpiration (and stomatal opening) might have improved the carbon balance of the host plant, resulting in better growth for the mycorrhizal onions. The data presented in Figures 3 and 4 indicate that the effects of the fungus were not due to changes in plant water relations. There were no differences in leaf water potentials (Figure 3) and the reduction in transpiration in the stressed plants (Figure 4) was apparently the same in all three treatments. Certainly, the stressed, non-mycorrhizal plants were not transpiring at substantially lower rates which could have implicated stomatal closure (and carbon starvation) as a possible cause for their slow growth. In these experiments, solute potential levels were not determined so that possible differences in

turgor potential at similar leaf water potentials cannot be eliminated. However, in earlier expeiments, no evidence of differences in solute potentials between mycorrhizal plants and fertilized non-mycorrhizal plants could be found (Nelsen and Safir, unpublished). Because onion plants are considered to be sensitive to drought stress (18) it was interesting to note that leaf water potentials stayed high (Figure 3) despite the low soil water potentials. Leaf water potentials of viable leaves never dropped to -10 bars at week 12. Onion plants can apparently survive short periods of soil water stress by maintaining high leaf water potentials in much of the plant while allowing older leaves to slowly senesce and dry.

Second, the relatively better growth of the stressed, mycorrhizal plants might actually have been due to a lowered growth rate of the stressed, non-mycorrhizal plants. Perhaps during the cycles of wetting and drying, the added phosphorus was fixed and became unavailable to the onion plants. This would result in the stressed, non-mycorrhizal plants having a lower level of soil P available than did the well-watered non-mycorrhizal plants. Because no differences in soil P levels were found within a soil treatment and between well-watered and drought-stressed roots (Table 3), the slower growth of the stressed, non-mycorrhizal plants could not be attributed to a reduced level of available soil P.

The third possibility for the greater growth of the stressed, mycorrhizal plants might be that those plants had improved P nutrition when compared to the stressed, non-mycorrhizal plants despite the presence of lower levels of soil P. Epstein (8) has discussed a "critical concentration" for each nutrient required for plant growth. If the concentration of any nutrient is at or below some critical value in

the plant tissue, then the plant remains stunted. That is, plant growth stops when the concentration of the critical nutrient is diluted to the minimum level. The critical concentration for phosphorus is about 0.1 to 0.13% (of dry weight) for onions (Results and 25,26) as well as for other plants (8). The phosphorus concentrations of the plants from the six treatments are shown in Figure 5A. Only the stressed, non- mycorrhizal plants had a concentration of P in the tissue which was low and in the range of the critical value for P (0.12%). Therefore, there were two factors limiting the growth and development of the stressed, non-mycorrhizal plants-water and P nutrition. Only water appeared to limit the growth of the stressed, mycorrhizal plants. The improved drought resistance of the mycorrhizal onions was related to nutrition and was directly related to the presence of the mycorrhizal fungus.

The stressed, non-mycorrhizal plants took up very little P from the soil (Figure 5), despite the presence of soil P at high levels (Table 3). Two factors were involved. First, onion plants have a reduced capacity for phosphorus uptake when stressed (6,9). Second, the already low diffusion rate of P in soils decreases even further as soil moisture content declines (28). The magnitude of each effect is difficult to determine, but uptake would seem to have been impaired, since the soil was well-watered for 5 days of each 8 day cycle.

Mycorrhizal fungi can overcome both of these problems with which the host plant is confronted. The slow diffusion of P could be overcome by the fungus occupying a larger total volume of the soil, thus extending beyond the depletion zone which surrounds plant roots. This, indeed, is probably the explanation for the typical mycorrhizal growth stimulation that is seen in well-watered soils which are low in available

P (16,20,21). In the experiments reported here, despite the high levels of P available in the soil, the low levels of moisture reduced the diffusion rate of P so that P may have been essentially unavailable to the plants. Second, the mycorrhizal fungus could overcome the reduced uptake of P by the roots by transporting the P directly into the roots.

Although the presence of the fungus increased the drought resistance of the onion plants, the drought-stressed (and P fertilized) plants could not support as high a level of fungal reproduction. When plants were exposed to water-stress, the mycorrhizal spore numbers at week 12 were reduced by about 60% (Figure 6). Since top fresh weight was reduced by a similar percentage, the reduced reproductive ability of the fungus may have been due to reduced carbon availability rather than to a direct effect of reduced water availability. This seems feasible, since many fungi can grow quite well at water potentials of -10 bars and below (7). P fertilization also reduced spore production (Figure 6, Appendix A). Because ample water supply and low soil P levels favored mycorrhizal reproduction, it would appear that maximum spore production can be achieved when host plants are exposed to optimum conditions for growth, with the exception of maintaining low levels of P in the soil.

It has previously been shown that plant growth stimulation due to mycorrhizal infection can be duplicated under well-watered conditions by application of P to the soil (Figure 1 and ref. 18). This observation can lead to a conclusion that mycorrhizal fungi are unnecessary when P fertilizers are available (18). The data presented here show that this may be a naive assumption and that under the common conditions where soil moisture is low or cyclicly available, plant growth stimulation due to mycorrhizal fungi cannot be duplicated by added P. The increased drought

resistance resulting from mycorrhizal infection can benefit plant growth and development and suggests that mycorrhizal infection may be even more significant in dry conditions than when moisture is plentiful.

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APPENDICES

APPENDIX A

THE EFFECTS OF SOIL PHOSPHORUS LEVELS ON MYCORRHIZAL INFECTION OF FIELD GROWN ONION PLANTS AND ON MYCORRHIZAL REPRODUCTION

THE EFFECT OF SOIL PHOSPHORUS LEVELS ON MYCORRHIZAL INFECTION OF FIELD GROWN ONION PLANTS AND ON MYCORRHIZAL REPRODUCTION[1]

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ABSTRACT

Seeds of onion (Allium cepa, L.) were sown on 2 muck soils that were high and low in available phosphorus (P) and which contained an indigenous population of mycorrhizal spores (Glomus sp.). Treatments were 4 levels of P (0, 30, 97, 193 kg/ha) and inoculum of the mycorrhizal fungus Glomus etunicatus. In the soil that was low in available P (3 kg/ha) bulb weight increased with added P. Root infection by the mycorrhizal fungus and mycorrhizal spore numbers in the soil were negatively correlated with added P. Bulb weight and mycorrhizal spore number at harvest increased when mycorrhizal inoculum was added to the soil. In the soil that was high in available P (97 kg/ha), bulb weight, root infection, and spore numbers were not influenced by added P or added mycorrhizal inoculum. Root infection data from both soils suggested a threshold level of soil P below which mycorrhizal infection was high and above which infection was low. The data presented suggest that the levels of P commonly added to muck soils may negate any usefulness of mycorrhizae and that the addition of P might be reduced if mycorrhizal spore numbers were increased through inputs of mycorrhizal inoculum or cultural practices.

[1]Journal of the American Society for Horticultural Science, submitted.

Infection of host plant roots by vesicular-arbuscular (VA) mycorrhizal fungi can improve plant growth especially in soils with low levels of available P (12, 16, 19, 20). However, most studies were carried out in pots under controlled environment conditions with initially sterilized soil. In a limited number of pot studies using nonsterilized soil (1, 5, 13, 14) added inoculum improved plant growth, despite the presence of indigenous VA mycorrhizal fungi. This improvement may have been due to the addition of a more effective strain of the fungus or to more rapid infection rates.

The effects of fertilization on mycorrhizal spore numbers in the soil and on mycorrhizal root infection have been investigated in mineral soils. When wheat (Triticum aestivum L.) was fertilized with calcium nitrate (7), spore numbers and root infection decreased. Spore numbers ranged between 0.1 and 2.0 spores g^{-1}. When potato (Solanum tuberosum L.) and barley (Hordeum vulgare L.) were grown in fields treated with varying levels of P (8), root infection was highest at the lowest level of added P. Spore numbers were highest at intermediate levels of added P (10-20 ppm available P). Mycorrhizal inoculum was not added in either of these studies. Such mycorrhizal studies have not been carried out in muck soil.

Where field soils are fumigated and most of the soil organisms are killed, mycorrhizal inoculation has been useful (6, 10, 18, 23). However, the benefit of added mycorrhizal inoculum has thus far been limited to perennial nurseries where fumigation is practical. Khan (9) demonstrated a mycorrhizal response of corn transplants in non-fumigated field soils low in phosphorus. Pre-infected corn transplanted into natural soil grew as well or better than noninfected or infected transplants which were fertilized with high levels of P. Corn transplants which were not

pre-infected grew poorly despite late infection by indigenous mycorrhizal fungi. In addition, fertilization with P reduced root infection in both pre-infected and non-infected transplants.

In the only study of its type, Black and Tinker (2) added mycorrhizal inoculum to nonfumigated soil in which potatoes were planted. Tuber yield was increased 20% by soil inoculation. Fertilization with high levels of P reduced root infection and increased yield more than did inoculation.

We report here field experiments in nonfumigated muck soil in which onions were seeded after soil treatment with mycorrhizal inoculum and various levels of P fertilizer.

Materials and Methods

Experiments were conducted during the summers of 1979 and 1980 at the Michigan State University Muck Farm near East Lansing, Michigan. The soil used was a Houghton muck that contained 65% organic matter, 97 kg/ha P (Bray's P-1 extractable), and had a pH of 5.8. In 1979 the experimental design was a randomized complete block with 3 replications. Treatments were 2 levels of mycorrhizal inoculum and 2 levels of added P. Plots of each treatment contained 3 rows which were 7.6 m long and 0.4 m apart. Mycorrhizal inoculum (Glomus etunicatus Becker and Gerdemann) produced in the greenhouse on sorghum plants was banded under the rows of one half the plots at an approximate rate of 2500 spores per m of row using a planter with the seed opening set at maximum. Phosphorus as triple superphosphate was applied to the plots at rates of 0 and 97 kg/ha P. Seeds of two onion cultivars, 'Spartan Banner' and 'Downing Yellow Globe', were sown on May 22, 1979. Fresh weight of onion bulbs from 4 m of row and mycorrhizal spore levels in the soil from each row were recorded on October 18, 1979.

In 1980 the experiment included 4 levels of added P and was also conducted on an area of virgin muck soil that contained low P (3 kg/ha P). Since the yields of the 2 cultivars were similar in 1979 only the cultivar 'Spartan Banner' was used. Onion seed was sown on May 23, 1980. Mycorrhizal treatments were the same as in 1979. Phosphorus was banded approximately 5 cm below the soil surface below the seed in plots in both fields at the rate of 0, 30, 97, and 193 kg/ha P. Nitrogen and potassium were added at rates of 170 kg/ha and 234 kg/ha, respectively. Onion bulb fresh weight and mycorrhizal spore numbers in the soil were determined on September 24, 1980. Root infection was determined on July 30 and September 10. Infection was determined on roots from 5-15 cm below the soil surface and 4 cm from the center of the row. One sample per row was taken.

Mycorrhizal spores were isolated from a 5 g soil sample taken from below a randomly chosen plant from the center row of each plot. Spores were isolated by centrifugation in a 45 g/100 ml sucrose solution and counted under a dissecting microscope. Spore numbers are expressed on a soil volume basis ($1/cm^3$) because of differences in bulk densities between the 2 soils used. Roots were cleared and stained (15) and then visually rated using a light microscope. One cm root segments were rated as follows: 0 = no infection; 1 = entry points only present; 2 = small areas of hyphae occupying less than 5% of root; 3 = hyphae present more or less throughout the root, or heavily concentrated in less than 1/2 the root; and 4 = hyphae concentrated throughout the root.

Available soil P in each plot was determined at harvest using Bray's P-1 extraction technique from the same soil sample from which roots were obtained for infection analysis.

Where appropriate, regression analysis was performed on the data.

Results

In 1979 bulb weight was not affected by P level or soil inoculation. Mycorrhizal spore numbers (Glomus sp.) were increased 38% by the addition of inoculum although numbers/cm^3 soil remained low (0.7 vs. 0.5 spore/cm^3). Root infection was not examined. During 1980 in the field containing high levels of available P, bulb weights did not differ with added phosphorus or added mycorrhizal inoculum. However, in the virgin soil, low in available P, there appeared to be a response to both added P and added mycorrhizal inoculum although differences were not statistically significant (Figure A1). Bulb weight was increased 44% with an increase in added P from 0 to 30 kg/ha. Added mycorrhizal inoculum increased bulb weight 34% at the lowest soil P level. Mycorrhizal inoculum plus the lowest P rate (30 kg/ha) increased bulb weight 64%. At the 2 higher P levels, onion yield did not increase further with either increased P or added mycorrhizal inoculum.

In the soil with an initially high level of P, infection was uniformly low ranging between 0 and 1 at all levels of added P. Figure A1 shows the effect of P and inoculum levels on root infection in the soil that contained low P. Similar results were found at midseason and at harvest, with high root infection at the 2 lowest P levels in both the presence and absence of inoculum. At the 2 highest P levels infection was strongly inhibited.

At harvest, mycorrhizal spore numbers were always lower than 1 spore/cm^3 in all treatments on the soil that contained high levels of P (Figure A2). In the soil that contained low P, spore numbers were between 1 and 2.2 spores/cm^3 when infection was high. At the 2 higher levels of

Figure A1. Rating of mycorrhizal infection of onion roots and bulb yield (fresh weight, kg/m of row) as influenced by total soil P (initial level plus added P) and mycorrhizal inoculum in soil that contained low P, 1980. z = mycorrhizal rating, no inoculum added; * = mycorrhizal rating, plus inoculum added; o = bulb weight, no inoculum added; Δ = bulb weight, plus inoculum added, where 0 = no mycorrhizal infection and 4 = concentrated mycorrhizal infection. Solid lines are bulb weight and broken lines are mycorrhizal rating.

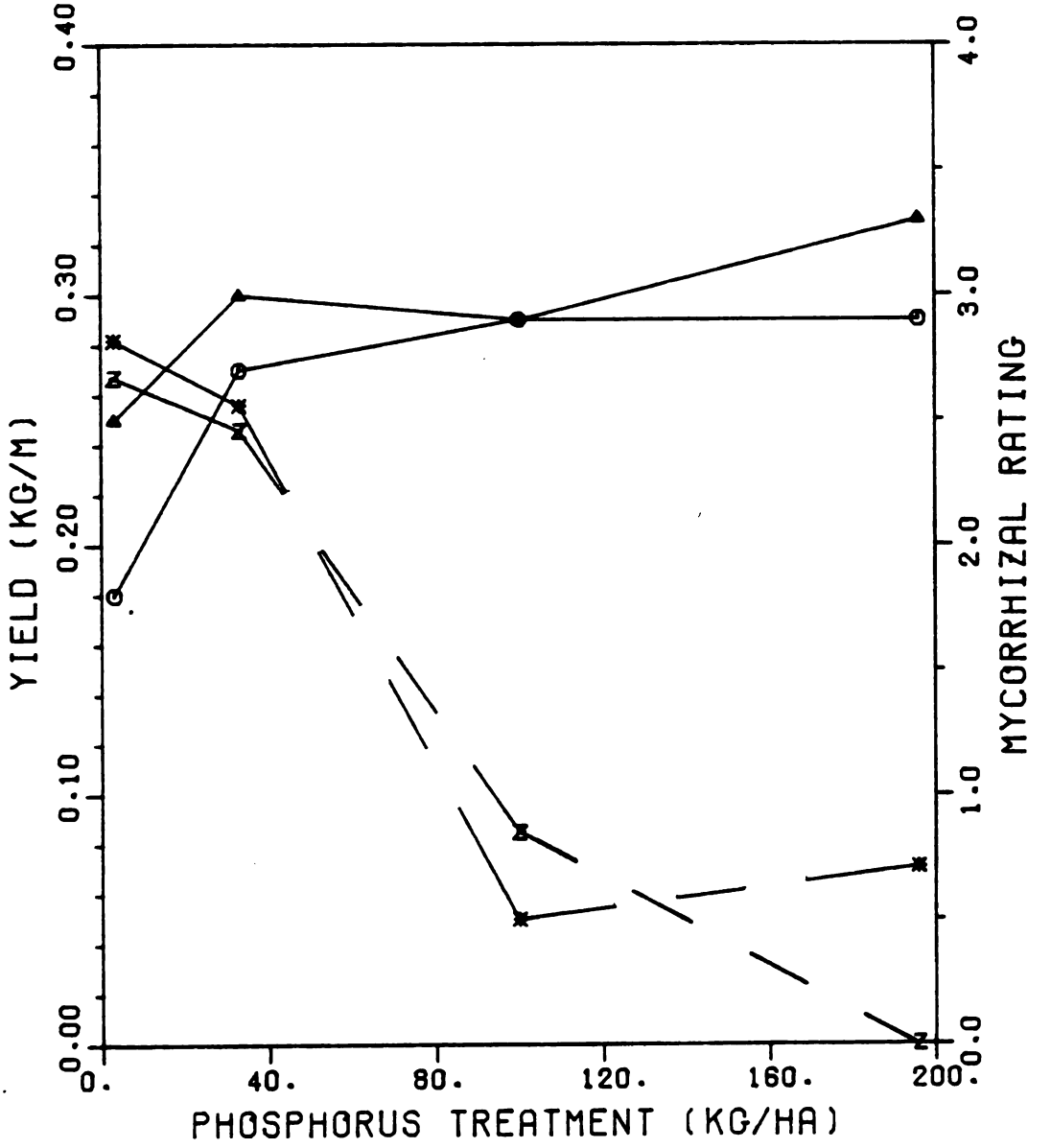
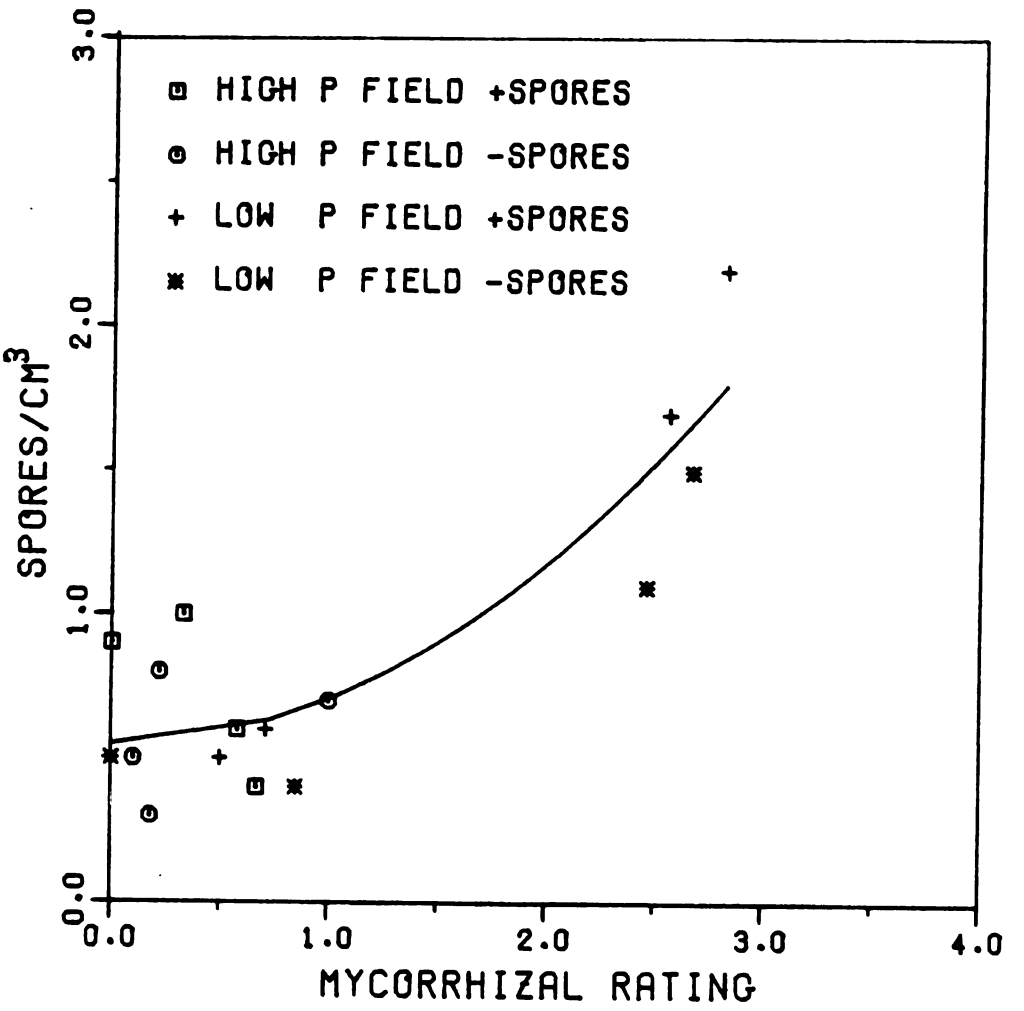

YIELD (KG/M)
0.40
0.30
0.20
0.10
0.00
MYCORRHIZAL RATING
4.0
3.0
2.0
1.0
0.0
0.
40.
80.
120.
160.
200.
PHOSPHORUS TREATMENT (KG/HA)

Figure A2. Mycorrhizal spore number/cm^3 of soil as influenced by mycorrhizal rating of onion roots in the soils which contained high and low levels of P. The regression line is $Y = .157\ X^2 + 0.550$, $r^2 = 0.78$; where Y is the spore number and X is the mycorrhizal rating, where 0 = no infection and 4 = concentrated mycorrhizal infection.


HIGH P FIELD +SPORES
HIGH P FIELD -SPORES
LOW P FIELD +SPORES
LOW P FIELD -SPORES
SPORES/CM3
MYCORRHIZAL RATING
0.0
1.0
2.0
3.0
4.0

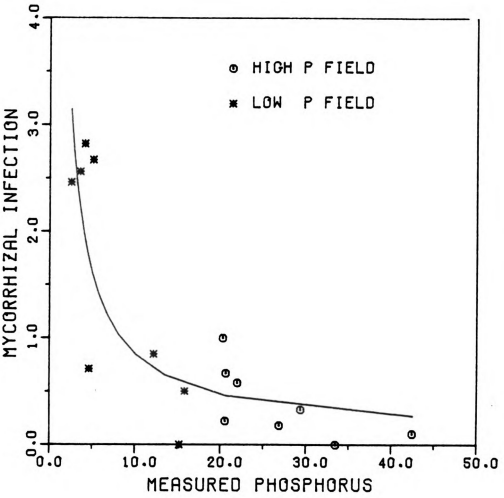
added P, spore numbers were reduced to levels similar to those in the soil that contained high P (below 1 spore/cm^3). Addition of inoculum to the soil that contained low P increased spore numbers at the 2 lower levels of added P (plus inoculum = 2.2 and 1.7 spores/cm^3; without inoculum = 1.5 and 1.1 spores/cm^3). In addition, Figure A2 shows a strong correlation between high infection level (only obtained at low soil P levels) and mycorrhizal spore numbers.

Figure A3 shows that the inhibition of mycorrhizal root infection by added P is not linear. In this soil, above a threshold level of about 10 kg/ha available P, infection is uniformly low (rating = 1 or below). At available soil P levels below this threshold there is generally a much higher level of root infection. The 4 points clustered between a rating of 2.4 and 3.0 are all from the soil that contained low P and had been treated with 0 or 30 kg/ha P. Figure A3 also shows the available P levels at harvest were all considerably lower than that added. This may indicate that suppression of infection by added P during the early growth of the onion plant was not overcome as soil P levels gradually decreased with time.

Discussion

The soil that contained a high initial level of P (97 kg/ha) used in this study was below levels of P often found in Michigan muck fields (150 to 200 kg/ha). However, even at 97 kg/ha, there was a strong inhibition of mycorrhizal infection since ratings at midseason and at harvest ranged between 0 and 1. This inhibition of infection at high levels of soil P, the first reported for muck soil, supported the results found for mineral soils in pots (22) and in the field (9). In addition, there was no growth response in this soil as P levels increased, so that P nutrition

Figure A3. Mycorrhizal infection of onion roots as influenced by available P levels at harvest. The regression equation is $Y = (7.02)x\ 1/X + 0.09$, $r^2 = 0.72$; where Y is the mycorrhizal rating (0 = no infection and 4 = concentrated mycorrhizal infection) and X is the available P level (kg/ha). Inoculum treatments (+/-) are combined within each field.


HIGH P FIELD
LOW P FIELD
MYCORRHIZAL INFECTION
MEASURED PHOSPHORUS
0.0
1.0
2.0
3.0
4.0
0.0
10.0
20.0
30.0
40.0
50.0

was not limiting growth (3, 4). Because adequate P nutrition will inhibit root infection by mycorrhizal fungi (11, 21), there was also no response in plant growth, root infection or mycorrhizal spore numbers to addition of mycorrhizal inoculum in either 1979 or 1980. In 1980, the use of the soil which contained a low level of P (3 kg/ha) resulted in a clear demonstration of the effect of added P on mycorrhizal root infection and the concomitant effect of root infection on plant growth and fungal reproduction.

Growth of onions in mineral soils that contained low levels of P was severely retarded in the absence of any mycorrhizal fungi (14). The indigenous mycorrhizal population in the soil that contained low P in this study apparently stimulated onion growth and bulb development so that a yield was harvested even at zero added P and no added inoculum (Figure A1). Because mycorrhizal growth stimulation did occur, no significant differences in yield were found; however, an increased stimulation in yield of 34% by the addition of mycorrhizal inoculum apparently occurred (Figure A1). In addition, at the lowest level of added P (30 kg/ha) there was a synergistic growth stimulation due to both added P and added inoculum resulting in a 64% increase in yield when compared to zero added P and no added inoculum (Figure A1). This agrees with the results of a number of workers who found added inoculum increased growth more than the indigenous fungal population in pots (1, 14) and in the field (2, 6, 9, 17). At higher levels of added P (97 and 193 kg/ha), yield was not increased further. However, these levels of added P did inhibit mycorrhizal root infection (Figure A1) indicating mycorrhizal stimulation was no longer a factor in onion growth. That is, amounts of P had been added so that the usefulness of the mycorrhizal fungus was eliminated with

no benefit to onion growth and at the expense of the added P fertilizer.

Finally, the data of Figure A3 and Figure A2 show that root infection can be maintained high if soil P levels were held below a threshold level, while fungal reproduction as measured by spore numbers in the soil increased with infection. This increase in spore numbers, without any significant loss in yield, would be beneficial for crops in succeeding years (2).

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APPENDIX B

ROOT INFECTION AND PLANT GROWTH STIMULATION AS INFLUENCED BY INOCULATION TECHNIQUE

ROOT INFECTION AND PLANT GROWTH STIMULATION AS INFLUENCED BY INOCULATION TECHNIQUE

ABSTRACT

Onion plants (Allium cepa L.) were infected with the mycorrhizal fungus, Glomus etunicatus (Becker and Gerdemann), using three different inoculation techniques. Fresh weight of the leaves plus bulbs (tops) and onion root infection by the fungus were determined during 10 wk of growth. Inoculation at a depth of 2 cm with soil from a pot culture containing the spores of the fungus showed the most rapid and most intense reaction to the mycorrhizal fungus, and onion tops were 23 times larger, by fresh weight, than the uninoculated controls. Plants inoculated with isolated spores placed at a soil depth of 2 cm below the seeds, or seedlings inoculated directly on the roots with isolated spores, as they were transplanted into pots, showed an intermediate growth stimulation. This study indicated that some of the variation in mycorrhizal growth stimulation and infection may be due to inoculation technique.

Reports on mycorrhizal growth stimulation vary both in the timing of initial growth stimulation and in the magnitude of response to the infection (Table B1). While some of this variation is obviously due to different species of host plant and fungus being tested, there are still differences when the same host and fungus are used [onion and "laminate spore", Sanders et al. (6) (mycorrhizal = 2.0 times non-mycorrhizal) and Mosse (2) (mycorrhizal = 14.9 times non-mycorrhizal)]. Soil phosphorus, soil type, environmental conditions, and other factors can be expected to influence the extent of the mycorrhizal reaction. However, some

Table B1. Examples of the variation in the timing of initial growth stimulation and in the magnitude of the growth response to mycorrhizal infection as reported in the literature.

Host plant(s)	Mycorrhizal fungus	Initial growth stimulation (days)	Mycorrhizal growth stimulation[a]	Citation
Lavender maize	E_3 spore type	-	1.1-1.2	1
Soybean	Endogone mosseae (Glomus mosseae)	28	1.5	4
onion	Endogone sp.	23	5.0	5
onion	5 species including "laminate" spore type	-	2.0-29.5	2
onion	"laminate" spore type	33	2.0	6

[a]Stimulation = mycorrhizal fresh or dry weight/non-mycorrhizal fresh or dry weight.

experimental methods can also add to the variability. In this report, the variation in root infection rating and growth of the onion plant (Allium cepa L.) when infected with Glomus etunicatus using three different inoculation techniques is described.

Materials and Methods

Onion plants (cv. Downing Yellow Globe) were grown in plastic cups (8 cm high x 7 cm diam.) with drain holes in 200 gm of a 50:50 mix (v/v) of sand and sandy loam soil (pH 7.2 and soil phosphorus equal to 10 ppm [Bray's P-1 extractable]). The soil mix was sieved through a 2 mm screen and autoclaved for 45 min prior to planting. The mycorrhizal fungus Glomus etunicatus was maintained in pot culture in the greenhouse using sorghum as the host plant.

Experiments were conducted in the growth chamber with a 14 hr light period (5.2×10^4 ergs cm^{-2} s^{-1}), temperature of 22 C day/16 C night. The relative humidity was controlled at 70 ± 10 percent.

Three inoculation techniques were used to infect the onions with the fungus and the experiment was conducted 3 times. In the first experiment, the first treatment consisted of 10 gm of soil from the pot cultures which was added to each pot 2 cm under the seeds. This insured maximum exposure of the onion roots to the fungus, from germination, onwards. The second treatment consisted of 150 spores, isolated from pot culture soil, suspended in water by stirring continuously and then added in 2 ml of water using a syringe on a layer of soil at about 2 cm below the seeds in each pot. The onion seeds were planted as in treatment one. In the third treatment, onion seeds were germinated in vermiculite and transplanted to pots at about 2 weeks of age. As the seedlings were placed in the soil, isolated spores (150 per pot), in 2 ml of a water suspension, were added

directly on the roots using a syringe. Control plants, without any fungal spores, were inoculated with soil washings from which mycorrhizal spores were removed by sieving, to ensure the presence of other soil microflora which would have been added in all three of the inoculation techniques used.

Four replicates of treatments one and two were harvested, beginning at week 2 for nine consecutive weeks. Two replicates of treatment three were harvested at weeks 2, 3, and 4 and 3 replicates were harvested at week nine. Four replicates of the control plants were harvested at weeks 2, 3, 4, 5, 6, 8, and 10.

Fresh weights of the leaves plus bulbs (tops) were recorded and root samples were taken for clearing and staining by a method modified from Phillips and Hayman (3) in order to assess root infection. Roots were cleared by heating in 10% KOH at 85 C for 45 min. Roots were then transferred to 0.1 N HCl for one hr and then placed in 0.1% acid fuchsin in lactophenol at 22 C for about 36 hr to stain any mycorrhizal fungus present. Finally, roots were washed in 2 daily changes of clear lactophenol to remove excess acid fuchsin dye. Fifteen random 1 cm root sections from each replicate were mounted on slides and inspected visually with a microscope. A rating system of 0-4 was used to assess the amount of root infection. A rating of 0 means no infection was present in the entire 1 cm section. One equals a limited number of infection points only. Two equals a limited number of small areas of concentrated hyphae, but not spread through root. Three equals hyphae continuous, but not concentrated, throughout the root section; i.e. root fully but not heavily infected. Four equals concentrated hyphae throughout the entire root section.

Because there may have been more than 150 spores in the soil inoculum in experiment 1, the experiment was repeated twice more, with spore levels of 600 spores per pot for all treatments in one experiment and 2500 spores per pot for all treatments in the second repeat. Four replicates of each treatment were harvested at weeks 2, 4, 6, 8, and 10 in experiments 2 and 3.

Results

Figure B1 shows the fresh weights of onion tops versus inoculation technique and time. The controls grew slowly, reaching a weight of about 130 mg at week 5, and stayed near that weight throughout the remainder of the experiment. Treatment one (soil inoculum) showed the quickest and most intense reaction to the mycorrhizal inoculation. There was a slight increase at week 4 and a definite stimulation by week 5. By week 10, the mycorrhizal onions were 23 times larger than the controls.

Treatment two (isolated spores at 2 cm pot depth) showed a much slower and smaller mycorrhizal stimulation. They were still quite small at week 6. At week 10, the treatment two onions were 3.5 times larger than the controls; a significant amount, but small relative to treatment one.

Onions in treatment three (isolated spores on the transplant roots) showed an intermediate reaction to the fungus. At week 9, the onion tops were 10 times larger than the controls, but only 0.4 times as large as treatment one tops.

Figure B2 shows the infection of the onion roots relative to inoculation technique and time. As expected, the control onions showed a rating of zero throughout the experiment. Treatment one onions had infection in all replicates at week 2 at the first check. The infection rating

Figure B1. Time course of onion top fresh weight as influenced by inoculation technique. Standard error bars, omitted for clarity, were about 10% of the mean and exceeded 20% only at week 5, treatment 1. Treatment 1 = soil inoculum with seeds; treatment 2 = spore inoculum with seeds; treatment 3 = spore inoculum with transplant; controls = non-mycorrhizal plants.

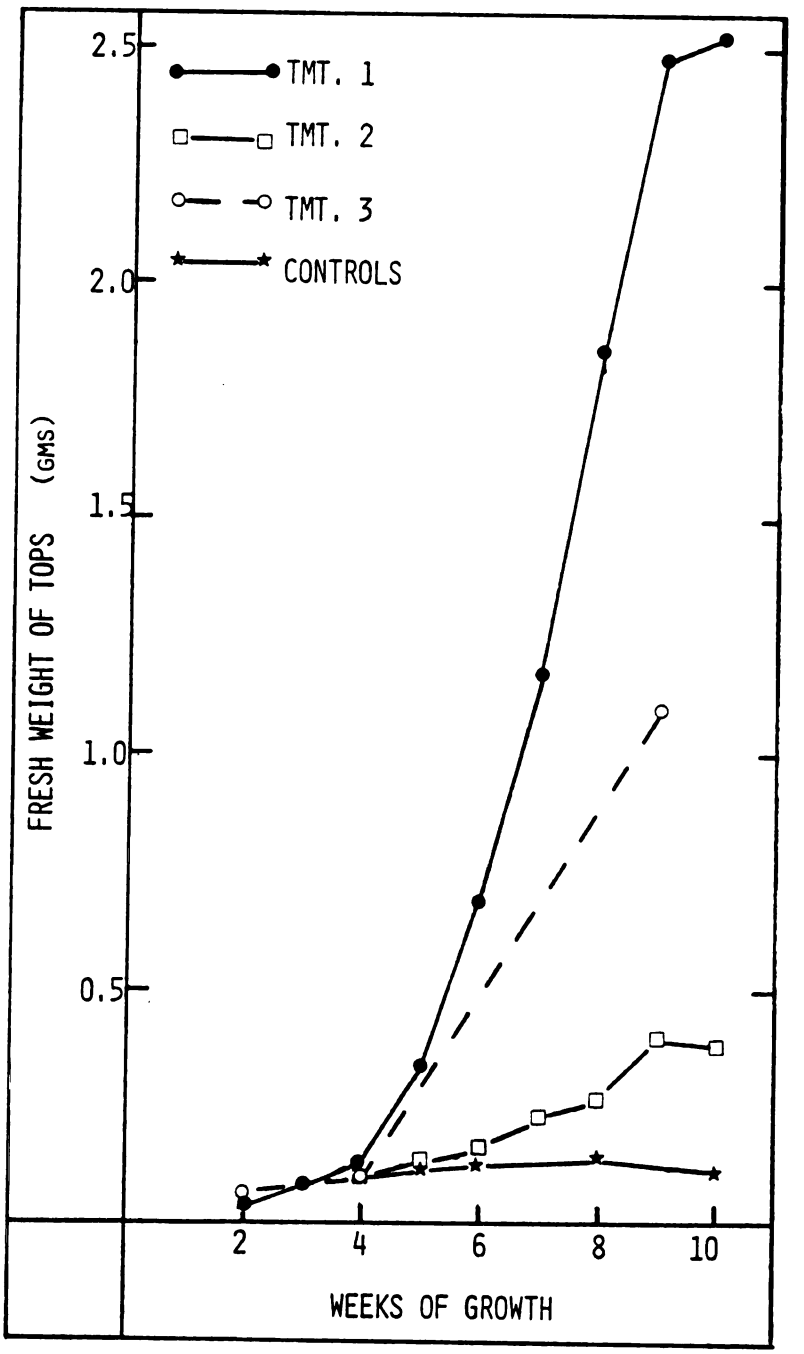
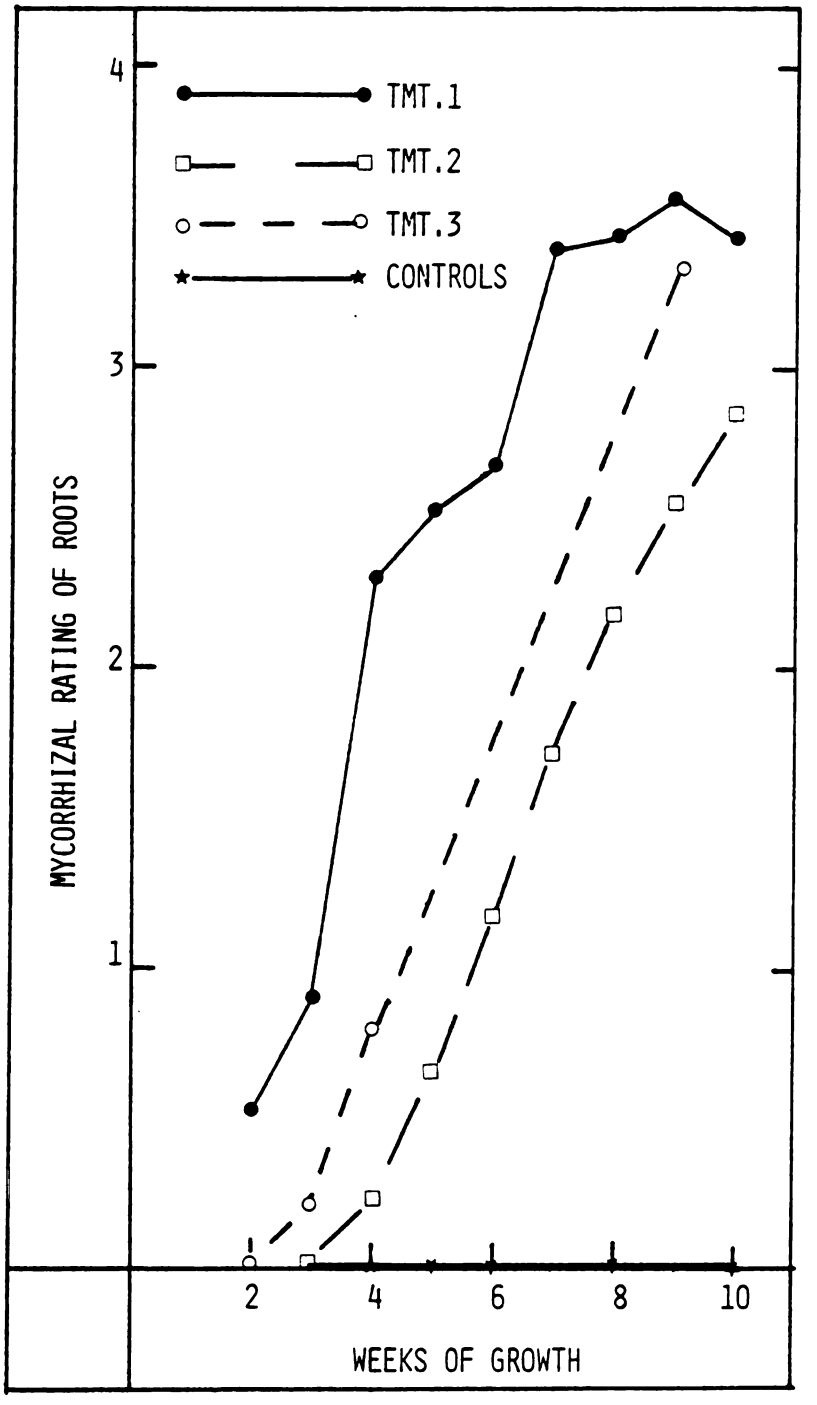

TMT. 1
TMT. 2
TMT. 3
CONTROLS
FRESH WEIGHT OF TOPS (GMS)
2.5
2.0
1.5
1.0
0.5
2
4
6
8
10
WEEKS OF GROWTH

Figure B2. Time course of root infection by the mycorrhizal fungus as influenced by inoculation technique. Treatments listed in figure legend B1.


TMT.1
TMT.2
TMT.3
CONTROLS
MYCORRHIZAL RATING OF ROOTS
4
3
2
1
2
4
6
8
10
WEEKS OF GROWTH

increased quickly to a final level of about 3.5. In treatments two and three, root infection lagged behind that of treatment one both in rate of infection and intensity of infection never equaling treatment one during the course of the experiment. The results of experiment 2 and 3 were essentially identical to the experiment 1 data presented here.

Discussion

The results of this study indicate that at least some of the variability in the literature (Table B1) can be explained by the inoculation technique used by each group of investigators as well as the time at which experiments are measured or terminated. They also suggest that the inoculation technique used might depend on the purposes for which the study was undertaken. If one is interested in comparing results with field data, where spores were distributed through the soil at a rather low concentration, some variation of treatment two may be a realistic inoculation technique. However, if one is interested in maximum stimulation or physiological interactions, where exact replication of field infection rates are not necessary, then treatment one would give the largest and most rapid stimulation and allow maximum differences to be observed.

In addition, treatment three onions, which have the most rapid exposure to the mycorrhizal spores (as well as having a rating close to treatment one onions by week 9) are smaller than treatment one onions. This may indicate some additional mechanism controlling both infection and growth stimulation other than the simple exposure of the host roots to the spores of the mycorrhizal fungus. This may implicate the involvement of other microorganisms in infection and/or direct hyphal infection without the necessity of spore germination.

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