INFLUENCE OF VESICULAR-ARBUSCULAR MYCORRHIZA ON *LEUCAENA LEUCOCEPHALA* GROWTH, WATER RELATIONS AND NUTRIENT ACQUISITION

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ABSTRACT

Knowledge of the dynamics of VA mycorrhizal plant systems is fundamental to the understanding of the relationships between VA mycorrhiza, plants and soil. Pot experiments were conducted to: 1) determine early physiological responses to VA mycorrhizal inoculation, 2) measure the acquisition and utilization of water and nutrients, dry matter production and assimilate partitioning in mycorrhizal and nonmycorrhizal plants, and 3) develop a model of VA mycorrhizal influence and its consequences to the physiology and ecology of VA mycorrhizal plants.

Leucaena leucocephala seedlings, with and without the VA mycorrhizal fungus (Glomus aggregatum), were grown in a Wahiawa soil (Tropeptic Eutrustox) with soil P levels ranging from 0.005 to 0.429 mg L^{-1} of P in 0.01 M $CaCl_2$ extract. Without mycorrhizal infection, leucaena plant growth was stunted under low soil P conditions. Even with high P fertilization, the growth of nonmycorrhizal plants was less than the growth of mycorrhizal plants.

Daily pinnule sampling, pot weighing methods and multiple 5-day-interval harvests revealed a series of changes in nutrient uptake, dry matter production and water transpiration between mycorrhizal and nonmycorrhizal plants. The series of changes was as follows: 1) Five days after inoculation, plant roots had about 7% mycorrhizal infection. 2) At 10 days, root P concentrations were higher in mycorrhizal plants than in nonmycorrhizal plants. By 15 days after inoculation, increases in shoot P, K and S concentrations were observed in mycorrhizal plants.

Shoot Mg and Ca concentrations in mycorrhizal plants were greater than in nonmycorrhizal plants at 20 and 25 days after inoculation, respectively. From 10 to 15 days after inoculation, the flux of P into mycorrhizal roots was greater than that into nonmycorrhizal roots. 3) Elevated nutrient contents in shoots of mycorrhizal plants was followed by superior growth rates. Mycorrhizal plants also allocated more assimilate to leaf growth than did nonmycorrhizal plants. Increased leaf growth was followed by increased transpiration. 4) Leaf area expansion rates and net assimilation rates were greater for mycorrhizal plants than for nonmycorrhizal plants. Greater dry weight was observed in mycorrhizal plants, supporting further growth of the mycorrhizal roots (positive feedback), and 5) Greatest soil volume was explored by the mycorrhizal roots. A scheme to explain these changes is proposed and used to describe processes involved in the soil-mycorrhiza-plant system.

In contrast, the flux of P into nonmycorrhizal roots decreased during the period 10 to 15 days after transplanting. The resulting low P content in nonmycorrhizal plants further reduced relative leaf expansion rates, net assimilation rates and later reduced relative root expansion rates (negative feedback). Nevertheless, when nonmycorrhizal plants were subsequently inoculated they eventually attained a similar size and weight as mycorrhizal plants. The stunting of nonmycorrhizal plants thus appears to be reversible and probably is part of a survival strategy which reduces energy use while retaining the potential for mycorrhizal infection.

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INTRODUCTION

With diminishing reserves of fertilizer and increasing energy cost, there is an urgent need to develop sustainable agriculture which requires minimal fertilizer inputs. Such a system might include: 1) plant species and cultivars which are able to produce well under low nutrient conditions, e.g., legumes for low nitrogen soils; 2) fertilizers which can be more efficiently used by plants; and 3) the use of vesicular-arbuscular (VA) mycorrhizal symbiosis to increase the utilization of both current and residual fertilizer (National Research Council, 1983). There are some mycorrhiza-dependent legume species, such as Leucaena leucocephala (Lam.) de Wit (leucaena). With mycorrhizal symbiosis, leucaena plants are efficient in absorbing soil P, particularly in high P-fixing tropical soils (Yost and Fox, 1979).

Leucaena is one of the most productive leguminous tree species and is widely cultivated in tropical and subtropical regions, including Hawaii, Australia and Southeast Asia (National Research Council, 1984). Annual leaf litter has been as much as 1150 g dry weight m^2 which is greater than litter fall in temperate forests (Mikura et al. 1984). The plant has also been used for cattle forage, as a shade tree in crop fields, or as fuelwood (Gray, 1968; Hill, 1971; International Development Research Center, 1983). Although leucaena plants have few root hairs, they are highly mycorrhiza dependent (Yost and Fox, 1979), even in high P soils.

An understanding of the physiological events and processes of VA mycorrhizal establishment is a prerequisite for manipulating the association for the benefit of agriculture. It has been repeatedly shown that mycorrhizal plants have superior growth in experiments with a range of host plants, VA mycorrhizal fungi or growth conditions. Although these studies give an indication of the scale of differences between plants with or without inoculation, they provide little information about the dynamics of mycorrhizal systems where fungi and plants interact with each other. Consequently, the sequence of events in the host response of mycorrhizal systems is not adequately understood.

Differences in nutrient absorption between mycorrhizal and nonmycorrhizal leucaena plants is initiated during the first few weeks after inoculation (Huang and Fox, 1984). Further impacts including dry matter production and water relations of leucaena plants also were observed by Huang et al. (1985). It is important to identify the primary and secondary influences of VA mycorrhizae and understand the interdependence of nutrient acquisition, growth and physiological aspects of these influences. It is possible by means of frequent harvests to observe the early events of mycorrhizal influence at a time when the mycorrhizae are beginning to infect and establish on the plant's roots.

In these experiments, we investigated the early physiological events of mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings with various phosphorus levels through sequential harvests. The daily

samplings and frequent harvests were conducted during the period which corresponded approximately to the time of initiation and development of VA mycorrhizal influences on the growth and physiology of seedlings. The objectives of these studies were 1) to identify and measure early physiological responses to mycorrhizal colonization, 2) to measure the rates of dry matter production, water and nutrient acquisition and their utilization as influenced by mycorrhiza, and 3) to develop a model to explain the mycorrhizal influences on plants. These experimental results were expected to reveal some of the dynamic mycorrhizal influences on plant nutrient acquisition, growth and water relations. The results of these experiments were expected to be useful in assessing agricultural and ecological significance of the soil-mycorrhiza-plant system.

LITERATURE REVIEW

The Soil-Root System

A remarkable feature of plants is their ability to utilize primary resources: radiation, carbon dioxide, water and essential nutrients from the environment and to synthesize these materials into cellular components or to use them as energy sources. Although mineral nutrients make up a comparatively small proportion of plant dry matter (about 3%) (Epstein, 1972), they are essential for metabolic processes. For optimum plant growth, nutrients should be supplied and maintained above a certain 'critical' level (Mengel and Kirkby, 1982).

Major factors affecting plant mineral relations include: 1) supply of available nutrients, 2) plant requirements for nutrients, 3) nutrient movement from bulk soil to the proximity of plant roots, 4) nutrient absorption capacity of root systems and 5) mycorrhizal associations. Understanding the influence of these factors on phosphorus (P) nutrition is particularly important because P deficiency is a major soil fertility problem of the tropics (Fox and Searle, 1978). Moreover, mycorrhizae play an important role in the availability of this nutrient as indicated by numerous publications on this subject (Harley and Smith, 1983).

Soil Factors Affecting Phosphorus Supply

Phosphorus uptake is influenced by soil-solution P concentration (Wild, 1966), which is often referred to as the intensity factor of nutrient supply. Because P concentration in soil solutions is low, it must be continuously renewed (Barber et al. 1962). Otherwise, the P in solution would decrease rapidly as soil solution P is absorbed by plants. Labile P replenishes soil solution P and is referred to as the quantity factor in P nutrition (Wild, 1966).

Both intensity and quantity factors of nutrient supply to roots are affected by soil water content (Olsen and Watanabe, 1963; Shapiro et al. 1960). Water movement toward roots helps renew the depleted supply of P near the root surface and affects the soil volume which supplies P to plants. Soil water content also influences the cross-sectional area for P diffusion to roots.

Attempts have been made to determine the relative importance of quantity and intensity factors. For example, adsorption isotherms (plots of P sorption vs. P concentration in equilibrated solutions) have been used to obtain information about both the quantity and intensity aspects of P availability (Beckwith, 1965; Fox and Kamprath, 1970). Many P sorption curves and critical concentrations of external P requirement have been published (Fox et al. 1974; Fox and Searle, 1978, Nishimoto et al. 1977; Van der Zaag et al. 1979; Yost and Fox, 1979). The external P requirement of a crop does not appear to be a single-value constant that holds true in all conditions, however, it is a

useful indicator of the P nutrition of that crop.

Plant Factors Affecting Phosphorus Demand

Plants and soils dynamically interact, affecting and being affected by the other. In order to examine such dynamic relationships, information is needed on the nutrient requirements of plants at different stages of growth, and on the size and distribution of the root system in the soil.

Williams (1967) discusses the effects of plant characteristics on the relative importance of quantity and intensity factors for immobile nutrients such as phosphate. A high P intensity in the soil allows rapid P uptake by an expanding root system, and is therefore likely to be particularly favorable for early growth of plants, rapidly-growing crops and for highly-responsive crops.

Early growth of a plant seems to have a particularly high P requirement. Fox et al., (1974) showed that corn required 0.2 mg P L^{-1} in soil solution for maximum growth in the early stage, but 0.06 mg P L^{-1} was sufficient to give 95% maximum grain yield. Similarly, the forage legume Desmodium aparines required 0.2 mg P L^{-1} for establishment but only 0.01 mg P L^{-1} for regrowth after the first harvest (Fox et al. 1974). Webber and Mattingly (1970) cite examples where solution P concentration correlated well with initial growth, but not with total uptake, the latter depending more on quantity. This was also demonstrated by Holford and Mattingly (1976).

Rapidly-growing crops or those with a short growing season typically have high P requirements. Vegetable crops normally require higher P intensity than annual field crops (Nishimoto et al. 1977) and annual crops frequently require higher P intensity than perennial crops such as trees or permanent pasture (Fox, 1979). A low P intensity is more likely to be adequate when extensive root systems supply a slowly-growing crop. Such a system will deplete more soil volume and allow time for P forms of intermediate solubility to be transferred to labile forms.

Nutrient Movement from Soil to Root Surface

Nutrient ions vary in mobility in soil medium both because of differences in ion mobility and of differences in the soil medium. Bray (1954) defined mobility as the 'overall process whereby nutrient ions reach sorbing root surfaces, thereby making possible their sorption into the plant'. The amount of nutrients in contact with the root is small, therefore, 'the significant source of nutrients to the root surface comes from the movement or diffusion into the film of water between the root surface and the soil surface'. On one extreme of the mobility scale is the nitrate ion, which interacts little or not at all with most soil particles and is relatively mobile. At the other end of the scale are relatively immobile (highly soil-reactive) nutrient ions, i.e. P and several cations. The significance of the mobility concept to nutrient availability is that relatively mobile nutrients are drawn from

a large volume of soil (termed 'root system sorption zone' by Bray). Relatively immobile nutrients are exploited from a smaller volume of soil adjacent to each root ('root surface sorption zone'). Bray (1954) points out that the stage of growth when the plant requires the highest nutrient concentration would determine the needed soil level of a relatively immobile nutrient.

Diffusion and mass flow

Since roots normally absorb water as well as solutes, solutes move into the neighborhood of absorbing root surfaces by both diffusion and by mass flow (Bouldin, 1961). It is important to consider how these two processes interact. Quantitative experiments designed to investigate the combined processes have been few and difficult to devise.

The importance of mass flow and diffusion in the movement of ions in soils was considered by Porter et al., (1960). Barber (1962), however, was the first to measure this movement in a soil-plant system and to determine the relative contributions of mass flow and diffusion to nutrient availability. He suggested that the rate of nutrient supply to the root is determined by the sum of the amount of nutrient moving by diffusion and the amount supplied by mass flow of the soil solution, resulting from transpiration loss, to the plant root. He discussed situations in which one or the other mechanism would dominate. In two subsequent papers (Barber et al. 1962 and 1963), it was shown that mass flow may supply much of the plant's requirement for Ca, Mg and N. Mass flow was thought to supply only small amounts of P and K ions because relatively low concentrations are present in solution relative to the

requirement of the plant. If P in solution is assumed to be 10 μmol L^{-1} and plants transpire 500 g H_2O g^{-1} dry matter produced, mass flow will supply 5 μmol to roots for every g of dry matter produced by plants. The P content of most plants is at least 100 μmol g^{-1} plant dry matter, hence, mass flow would supply only 5% of the needed P and diffusion must supply 95% if root interception is neglected. Tinker (1969) pointed out that the actual mass flow passing across the root surface is the water flux to plant roots multiplied by the solution concentration which may be different from that deduced in the concepts of Barber (1962), viz. as the product of transpiration and bulk solution concentration. Furthermore, Tinker (1969) pointed out that Barber's calculations were based on the mean for the growth period of the plant and uncertainty arises from the attempt to average all processes over a whole growing season. As a plant grows, there is a typical exponential increase in transpiration (Brewster and Tinker, 1970) causing mass flow to be greater in the later growth stage than in young seedling stages. Which one is most important--diffusion or mass flow--may change during plant development.

Autoradiographic evidence for diffusion

Verification of concentration gradients around roots has come from autoradiographs showing depletion or accumulation of radio-labelled nutrient elements around individual roots (Barber, 1962). Walker and Barber (1961) developed a procedure for using autoradiographs to verify ion accumulation or depletion around the root. Corn was grown in soil labeled with ^{86}Rb. Radioactive ^{86}Rb is a satisfactory replacement

for K because its properties are similar to those of K. X-ray film exposed to soil labeled with ^{86}Rb showed a concentration gradient extending perpendicular to the root. These workers, therefore, concluded that K depletion near the corn root surface should also occur. Autoradiographic evidence for the supply of P by diffusion is also available (Lewis and Quirk, 1967; Bhat and Nye, 1973; Owusu-Bennoah and Wild, 1979). Autoradiographic and theoretical studies made by Olsen et al. (1962) and Nye (1966) indicate that the zone of depletion may have a diameter of several mm. Phosphate ions in soil have a lower diffusion coefficient (10^{-8} to 10^{-11} cm^2 sec^{-1}) than other ions. Since diffusion over long distances is relatively slow, it may limit nutrient uptake under most field conditions (Drew and Nye, 1970). In young seedlings with small seed nutrient reserves, the balance between nutrient demand and supply is crucial. Loughman (1981) reported that P in solution could be taken up by a barley seedling root system in seconds. Due to the slow rate of diffusion of the P ion to root surfaces (Lewis and Quirk, 1967), immediate root zone becomes readily depleted of plant-available phosphate. When there is no transpiration, nutrients are supplied by soil to root by diffusion. When there is transpiration, nutrients are supplied by both mass flow and diffusion (Fried and Broeshart, 1967). Most autoradiographic results were generally obtained at early plant growth stages. If these seedlings transpired water during the period of exposure to radioactive isotopes, the autoradiographs might indicate the total area of depletion both by mass flow and by diffusion, as suggested by Fried and Broeshart (1967).

Autoradiographic results indicate that diffusion occurs over

relatively short distances when the soil solution concentration of an ion is very low, such as with P. As mentioned above, the diffusion volume must be continuously recharged with the nutrient ions. Recharge must come from mechanisms that involve water movement (Bray, 1954). Soil water movement not only renews the supply of phosphate ion at the root surface, but also controls the soil volume contribution to phosphate replenishment (Shapiro et al. 1960). The magnitude of this volume depends upon the amount of water movement. Richards and Wadleigh (1952) reviewed several experiments where a higher yield was obtained with frequent irrigations than with fewer. This increased growth was attributed to a larger soil volume that was sampled for the renewal of phosphate in the soil solution.

Root Configuration for Nutrient Uptake

The most obvious functions of root systems are the absorption of water and nutrients. The effectiveness of a root configuration is influenced by the mobility of soil nutrients (Barley, 1970) and the porosity of soil structure (Hamblin, 1985). The configuration of the root system (e.g., root diameter, length and distribution) is more important for less mobile nutrients such as P than for more mobile nutrients such as nitrate (Barley, 1970). According to Nye (1966) the most efficient root anatomy appeared to be thin roots covered with root hairs, which was important in exploring a soil volume (Lewis and Quirk, 1967). Silberbush and Barber (1983a and 1983b) conducted a sensitivity

analysis for their simulation model of K uptake by soybean in a silt loam soil (K is an ion with an intermediate diffusion coefficient, but required by plantsin large amounts). Their results indicated that the most sensitive parameters are those related to root morphology: root growth and root radius. Growth in root length was the most sensitive parameter, because both root length and the soil volume explored increase with growth. Under most soil conditions, however, because continued root growth was costly and soil mechanical resistance to root growth was quite high, the soil volume that could be explored was usually limited (Taylor, 1974).

Although plants may differ in root diameter and root configuration, the overall root functions for nutrient uptake could be improved by mycorrhizal associations in which the hyphae penetrate the soil for distances up to 8 cm (Rhodes and Gerdemann, 1975) rather than the mm or fractions of mm penetrated by root hairs. For example, despite a very high P requirement, cassava has a coarse, sparsely branched root system (Jintakanon et al. 1979). Nevertheless, it grew well in soils of low fertility in which P uptake was markedly increased when roots were infected by VA mycorrhizal fungi (Yost and Fox, 1979; Van der Zaag, et al. 1979). Thus even plants with coarse roots and few root hairs have developed an effective absorption mechanism via mycorrhizae for relatively immobile nutrients such as P (Fox, 1981).

Nearly all detailed work on the flow of water to roots has assumed that the root was in full contact with homogeneous soil material similar in composition to the bulk soil (Barber, 1984). However, soil media are heterogeneous complexes of mineral and organic particles, water and

air. Roots often entered cracks or other voids in the soil where contact with the soil would be incomplete (Russell, 1973). Tropical soils with low-activity clays were generally found to be better aggregated than high-activity clays (Uehara and Gillman, 1981). Pore size between aggregates usually increases as aggregate size increases, in many highly weathered, clayey soils of the Tropics, such as the Wahiawa soil. The moisture released from the Wahiawa soil was similar to that of sandy soils (Sharma and Uehara, 1968). Tsuji et al. (1975) suggested that both micropore (intra-aggregate) and macropore (inter-aggregate) regions occurred in the Wahiawa soil.

Large continuous inter-aggregate pores and channels (larger than 50 um) strongly influenced soil water movement (Beven and Germann, 1982) and root growth (Barley and Greacen, 1967). Roots do not normally grow into rigid pores narrower than their own diameters (Wiersum, 1957), hence nutrients within aggregates may not be directly accessible by plant roots. In contrast, the intra-aggregate pores (less than 50 um) because of properties associated with high specific surface area may buffer adsorption and desorption of nutrients. The desorption process was important for plant-P availability (Camargo et al. 1979). However, Tisdall and Oades (1979) found that fine external VA mycorrhizal hyphae could proliferate between aggregates.

Modeling Nutrient Uptake in Plant Roots

Mathematical models of nutrient uptake by plants are useful for

investigating the effect of various soil and plant factors on nutrient flux to plant roots. Caassen and Barber (1976) have attempted to develop a model based on theoretical considerations of the processes of nutrient uptake by a plant root growing in soil. Their model predicts P uptake and plant growth response from independently measured soil and plant characteristics. They stated that nutrient absorption by plant roots growing in soil depends on three factors:

1) Morphology and rate of growth of the root system. Morphological characteristics may be described by root radius, root length, root surface/shoot weight ratio, and root hair density.

2) Nutrient absorption characteristics of the root system. Physiological characteristics strongly affect P uptake. The kinetics of P uptake can be characterized by the maximum rate of net influx (I_{max}), the Michaelis-Menten constant (K_m), and the minimum concentration in solution below which no further new influx occurs (C_{min}).

3) The nutrient supply characteristics of the soil. Barber's consideration of these soil characteristics was patterned after the work of Nye and Marriott (1969).

Barber and his colleagues tested their model under greenhouse and field conditions and found that observed uptake was much greater than calculated uptake at low P levels (Appendix 1). Differences decreased at high P levels, however. In sterilized soils with high available P contents, plant P uptake values as predicted by the model agreed with observed P uptake, whereas at low P levels predicted P uptake was only half of measured uptake.

The results indicated that the parameters and equations of the

Caassen-Barber model satisfactorily predicted P uptake in experiments with sterilized soil if P fertility was high. But if P levels were low the model underpredicted P uptake. This discrepancy probably resulted from not considering the contribution of P by mycorrhizal colonization as suggested below.

Barber's model (1984), furthermore, assumes that chemical effects of root exudates did not influence nutrient flux, and secondly that mycorrhizal effects were minimal. Moghimi et al. (1978), however, isolated 2-ketogluconate from the rhizosphere of wheat roots in amounts that could solubilize significant amounts of phosphate from hydroxy-apatite. It also has been suggested that several plant species are obligatorily mycorrhizal in soils with low P (Tinker, 1980). Inoculation of mycorrhizal fungi in corn (Murdoch et al. 1967) and millet (Bagyaraj and Manjunath, 1980) increased plant growth and P uptake. Both of these crops were included in Barber's experiments. Therefore, in my opinion, it is probable that Barber's plants were mycorrhizal. In some of Barber's experiments, soil solution P was approximately 10 to 50 µmol, i.e., about ten- to fifty-fold greater than might be expected in fertile natural soils (Clarkson, 1985). In such circumstances, plant growth was probably not constrained by P supply. Clarkson (1985) also commented that the predictive power of Barber's models for P uptake deteriorated where nutrient concentrations were low. It was under low nutrient conditions that ill-defined factors which determined the 'efficiency' of absorption became important. The mycorrhizal factor was ignored by Barber and his colleagues.

The Vesicular-Arbuscular Mycorrhizal System

Vesicular-arbuscular mycorrhizal (VAM) fungi are present in most soils throughout the world. VA mycorrhizal fungi form a symbiotic relationship with the host plant. The fungi help the host plant absorb nutrients, particularly P, while the host plant supplies carbohydrate for the fungal growth. As a nutrient-uptake mechanism, the VA mycorrhizal system consists of three components: soil, plant, and the VA mycorrhizal fungus (Mosse and Hayman, 1980). The extent to which a mycorrhizal system increases plant P uptake is determined by 1) the plant species, its P requirement and inherent ability to extract soil P, 2) the P content of the soil, 3) the extent of mycorrhizal infection, which depends on plant nutrient level and fungal adaptation to soil and climate, and 4) the efficiency of the endophyte species.

Structure of VA mycorrhizae

VA mycorrhizae that colonize plant roots belong to the family Endogonaceae (Gerdemann and Trappe, 1974). The main diagnostic feature of VA mycorrhizal fungi is the presence of vesicles and arbuscules in the root cortex. The endodermis, stele and root meristem are not invaded. Inter- and intra-cellular hyphae are also present in the cortex. The hyphae inside the roots are directly linked to external mycelium which spread into the soil. This enlarged zone is referred to

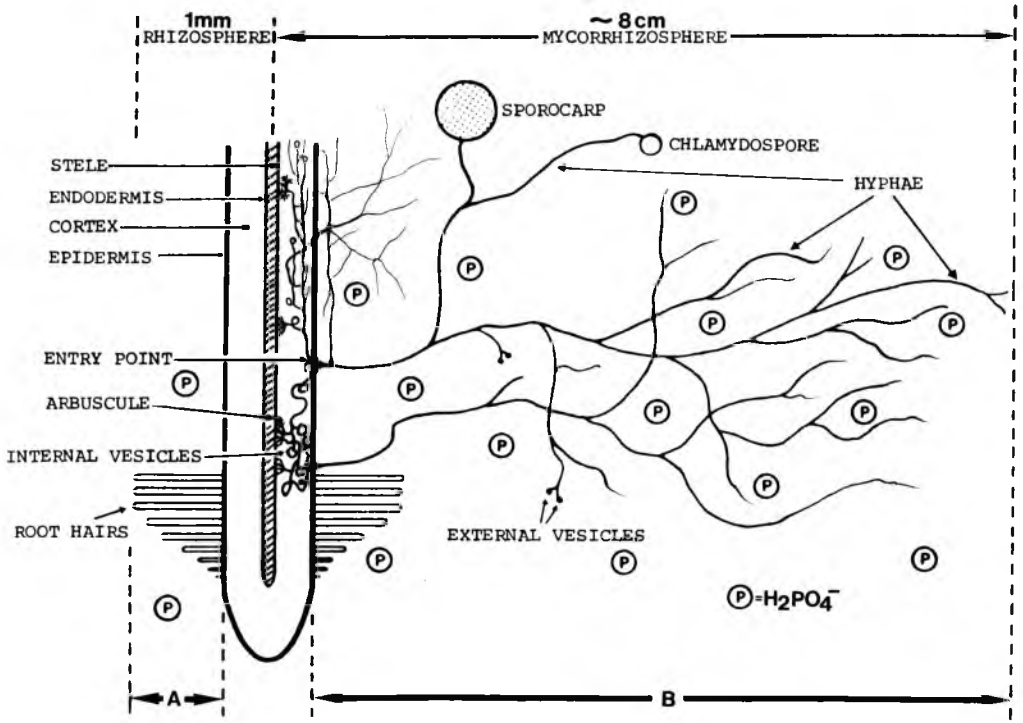
as the 'mycorrhizosphere', as compared to the rhizosphere surrounding plant roots (Plenchette, 1982) (Fig. 1).

A VA mycorrhizal infection usually begins with an appressorium on the root surface from which hyphae penetrate the epidermal cells. The hyphae spread inter- and intra-cellularly through the outer cortex where they often form coils. In the middle and inner cortex, and to a lesser extent in the outer cortex, intercellular hyphae grow parallel with the root axis and lateral branches of hyphae penetrate cortical cells forming arbuscules. Vesicles develop inter- or intra-cellularly as swellings along or at the tips of hyphae. The external mycelia form a loose network in the soil around the root. The main network is formed from coarse hyphae 20 to 30 μm in diameter that are thick-walled. Finer thick-walled hyphae branch from these and the ultimate branches are thin-walled structures with a diameter of 2-7 μm (Nicolson, 1967).

Function of VA mycorrhizae

Carbon metabolism

Because the fungus requires carbohydrate for its growth from the host, the question of carbon supply is important to an overall understanding of the VA mycorrhizal symbiosis. In fact, mycorrhizal growth responses are strongly influenced by two opposing processes: a growth stimulating effect due to enhanced P uptake (Sanders and Tinker, 1973; Mosse, 1973), and a growth detrimental effect caused by fungal drain of host photosynthate (Buwalda and Goh, 1982; Koide, 1985).



A — ZONE OF RHIZOSPHERE P UPTAKE
B — ZONE OF MYCORRHIZOSPHERE P UPTAKE

Fig. 1. Diagram of the association between vesicular-arbuscular mycorrhizal fungus and a plant root in soil (Plenchette, 1982).

^{14}C-labeled experiments have shown that mycorrhizal inoculation increased transport of fixed carbon to the roots of citrus, leek and faba bean by about 6, 7 and 10%, respectively, (Koch and Johnson, 1984; Snellgrove et al. 1982; Pang and Paul, 1980). However, it is not possible to state whether or not this carbon utilization by the fungal symbiont is an extra energy cost to the plant. For some plants, mycorrhizal symbiosis is an indispensible mechanism to absorb sufficient P from the soil (Mosse, 1973) to support growth and physiological processes such as photosynthesis (Allen et al. 1981).

Under nonlimiting growing conditions, mycorrhizal plants may compensate for this carbohydrate drain. For example, although roots of mycorrhizal faba beans had higher respiration rates than nonmycorrhizal roots, there was no relative change in plant dry weight (Pang and Paul, 1980). Photosynthetic rates and CO_2 fixation rates may increase in mycorrhizal plants (Levy and Krikun, 1980; Allen et al. 1981) by as much as 8 to 17% (Kucey and Paul, 1982). Higher chlorophyll levels in mycorrhizal plants may indicate a greater number of photosynthetic units (Allen et al. 1981). Indirect effects of mycorrhizal infection on leaf thickness (Krishna et al. 1982) and leaf number (Daft and Okusanya, 1973) may also improve the carbon balance of the host plant.

Nutrient acquisition

In the majority of cases, improved P uptake is the primary cause of growth and yield enhancement in VA mycorrhizal plants. Possible models of mycorrhizal enhancement of P uptake are summarized as follows:

1) Physical exploration: VAM hyphae extend beyond the zone of depletion around plant roots and decrease the distance that ions must move in soil solution to roots (Rhodes and Gerdemann, 1975).

2) Higher nutrient affinity and lower threshold concentration for nutrient uptake: Mycorrhizal plants took up P from solution faster than did nonmycorrhizal plants (Cress et al. 1979; Howeler et al. 1982). It had also being suggested that mycorrhizal plants have a lower threshold concentration for absorption from solution than nonmycorrhizal plants (Mosse, 1973; Howeler et al. 1982).

3) Chemical solubilization: Mycorrhizal plants may produce exudates which increase the amount of P available to plants (Tinker, 1975).

4) Rhizosphere modification: Differences between mycorrhizal and nonmycorrhizal plants in the absorption of anions and cations (Buwalda et al. 1983) may lead to differences in rhizosphere pH.

Other nutrients are also absorbed by mycorrhizae. For example, sulfate uptake via hyphal translocation to root and increased uptake at the root surface has been reported (Gray and Gerdemann, 1969; Cooper and Tinker, 1978; Rhodes and Gerdemann, 1978a). Hyphal translocation of sulfate is likely to be less important than that of P because sulfate has greater mobility in soil. Increased uptake of sulfate by mycorrhizal plants has been attributed largely to improved P nutrition of mycorrhizal plants (Rhodes and Gerdemann, 1978b). Zinc also moved to mycorrhizal roots through hyphae (Cooper and Tinker, 1978). In addition, Zn deficiency was associated with high P levels in the soil and low mycorrhizal infection levels (Gilmore, 1971; LaRue et al. 1975).

Rhodes and Gerdemann (1975) showed that high soil P levels reduced infection and eliminated both P and Zn translocation in the hyphae. This may partially explain the problem of P-induced Zn deficiencies in some field situations.

Water relations

Mosse and Hayman (1971) observed that mycorrhizal onions did not wilt when transplanted, but that nonmycorrhizal plants did. Subsequently several similar observations (Menge et al. 1978; Janos, 1980) have been made, consistent with the suggestion that mycorrhizal fungi may influence the water relations of host plants. Several conclusions can be drawn from numerous studies in this area:

1) Stomatal physiology is affected by mycorrhizal infection. Decreased stomatal resistances to water and CO_2 movement and increased transpirational fluxes and rates of photosynthesis have been shown (Allen et al. 1981; Johnson et al. 1982). Under well-watered conditions, mycorrhizal plants generally had both higher leaf area and transpiration rates than nonmycorrhizal plants. However, Huang et al. (1985) found that, during the day, mycorrhizal leucaena plants had more rapid stomatal responses to humidity deficits of the air than did nonmycorrhizal plants. Such stomatal responses might protect the leaf from excessively rapid transpiration and tissue desiccation.

2) Greater hydraulic conductivity per unit length of mycorrhizal root relative to nonmycorrhizal roots may contribute to better use of soil water. Safir et al. (1972) estimated soybean root resistance to

water transport. Water uptake resistance was reduced by about 40% with mycorrhizal infection. Greater root hydraulic conductances and higher leaf transpiration at lower soil water potentials were observed in mycorrhizal clover by Hardie and Leyton (1981). The increased water transport in the root corresponded to improved P nutrition to which growth was directly correlated. Atkinson and Davidson (1973) similarily found that drought tolerance in plants was related to their P status. Nelsen and Safir (1982a and 1982b) demonstrated that additions of P fertilizer to nonmycorrhizal plants essentially eliminated the differences in resistance to water transport. Allen (1982) suggested that the effect of infection on plant water relations was due to increased water uptake by hyphae, but Sanders and Tinker (1973) and Cooper and Tinker (1981) showed evidence that hyphal water movement could not account for increased water uptake by mycorrhizal plants. An indirect mechanism enabling water uptake by roots of mycorrhizal plants was to prevent the development of significant gaps between the root and soil, thereby maintaining liquid continuity across the soil-root interface (Reid, 1984). VA mycorrhizal hyphae were shown to bind soil particles into water-stable aggregates (Tisdall and Oades, 1979).

Proposed mechanisms of increased carbon, nutrient and water uptake by mycorrhizal plants have been discussed above. These interpretations reflect the complex and sometimes paradoxical nature of mycorrhizal systems. Some concepts and approaches were described that are needed to study mycorrhizal systems.

Methods for Studies of Mycorrhizal Systems

The mycorrhizal influence on plant growth and nutrient acquisition is dynamic and sequential. The time dimension is, however, largely ignored in many mycorrhizal studies. Some researchers realize that instantaneous measurement of growth or one destructive harvest alone is unlikely to improve our knowledge of physiological mechanisms which affect growth responses and determine yield differences between mycorrhizal and nonmycorrhizal plants (Abbott and Robson, 1984). Sequential harvesting of plants is recommended to allow studying the development of mycorrhizae in relation to plant nutrient acquisition, growth and physiological responses (Sanders et al. 1983). A series of sequential harvests with samplings both before and after the occurrence of growth differences is almost the only way to determine cause and effect. One attempt to provide such a continuous and integrated picture of the dynamics of mycorrhizal plant growth was reported by Huang and Fox (1984) who developed a sampling technique to assess the effectiveness of mycorrhizal association by tracing daily changes in pinnule P content between mycorrhizal and nonmycorrhizal leucaena plants.

Other considerations for evaluating the effect of VAM on plant growth include selection of control treatments so that plant growth response to added P or some other growth factor in each experiment is examined. This would permit resolution of different degrees of mycorrhizal dependency. Moreover, description and analysis of the growth of root systems, soil mycelium, host growth, tissue nutrient contents, soil measurement and mathematical analysis of data are

essential (Sanders et al. 1983).

Models are valuable tools in a number of disciplines. However, modeling of mycorrhizal symbioses is still in its infancy with some of the first efforts reported about ten years ago (Tinker, 1975). Recent efforts have focused on the complex interactions in host/fungus physiology in mycorrhizal systems, and have indicated that mycorrhizal associations may be described using modeling, or system analysis techniques (Sanders et al. 1983). These approaches focused on the interaction between the symbionts through nutrient uptake, growth and feed-back control.

Mineral Nutrition and Plant Growth

Relations between Plant Growth and Nutrient Acquisition

Mineral nutrient acquisition by plants strongly influences plant growth and yield (Mengel and Kirkby, 1982). However, there is no unified interpretation of such influences. A common approach to the interpretation of fertilizer experiments is to fit a mathematical model to the observed yields, and then attempt to relate the parameter estimates to environmental measurements such as soil test values in order to provide a basis for prediction on other soils. This has been only moderately successful because it over-simplifies a complex pattern

of nutrient supply, demand and physiological responses of the plants (Scaife and Smith, 1973).

Barrow (1977) suggested that a P-deficient plant can be considered a 'double-ended feedback' mechanism in which the phosphate supply ultimately determines the rate of photosynthesis and vice versa. Barrow's (1977) model involved four sets of relationships. In one, the rate of uptake is determined both by external nutrient supply and the efficiency with which the plant's roots used photosynthate to obtain P; in a second P is partitioned between roots, stems and leaves; in a third P in the leaves influences the rate of photosynthesis; and in the fourth, photosynthate is partitioned between the leaves, stems and roots. Scaife (1976) suggested that a dynamic model of plant growth (i.e., one incorporating feedback) might be used to predict the responses of plant species at each growth stage. This would require inserting appropriate parameter values of soil solution concentration, specific nutrient uptake rates, and optimal and minimal concentrations of the nutrient in the plants. Hence, if we wish to understand and predict nutrient influence on plant growth we need a model in which the relationships between nutrient acquisition and physiological processes of plant growth are represented (Soil nutrient status-plant nutrient status-growth rate-yield), instead of simply (Soil nutrient status-yield) as in the case of typical growth-nutrient equations.

Plant Response to Nutrient Deficiency

Nutrient absorption is required to maintain plant growth. If rate of nutrient uptake is insufficient to maintain maximum relative growth rate the plants will be under nutrient deficiency stress (Greenwood, 1976). Plant nutrient deficiency may be accentuated not only by a small quantity of available nutrients in a soil but also by ineffective processes of nutrient acquisition and utilization from applied or native sources (Clarkson and Hanson, 1980).

Plants may modify their growth structures and efficiency of nutrient acquisition and utilization in order to cope with these nutrient limitations by altering the following: 1) ion uptake efficiency, 2) root/shoot ratio, 3) effective rooted zone explored by root hairs, 4) root diameter and branching, 5) mycorrhizal symbioses, 6) rhizosphere chemistry, 7) metabolism, 8) element re-distribution and re-utilization in the plant and 9) plant growth rate. These parameters are discussed below.

1) Increasing ion uptake efficiency. The rate of ion transport by the root (V_{max}) and the affinity of transport mechanisms for the ions they carry (K_m) may increase as nutrient deficiency develops in the plant (Epstein, 1976). Using flowing nutrient solutions Asher and Edwards (1983) showed that optimum plant growth is obtained at very low external phosphate concentrations (3-13 μM), despite the high concentrations in the plant cell (1-10 mM) (Bieleski, 1973). Loughman (1981), however, indicated that the plant root already had an efficient

biochemical mechanism operating in order to absorb such large quantities of phosphate from low concentrations in soil solution. He suggested that the entry of P ion into the plant root was largely limited by physical rather than metabolic factors, hence there would be little to gain from increased biochemical efficiency.

2) High root/shoot ratio. Some plants allocate a much greater proportion of their assimilate to root growth under low nutrient conditions. For example, P deficiency resulted in increased root/shoot ratio in sugar beets (Ulrich and Berry, 1961) and in corn genotypes (Schenk and Barber, 1979a and 1979b). A larger root system should explore a greater volume of soil and perhaps tap new sources of nutrients. A larger root system, however, will consume a larger proportion of nutrient resources within the plant and this may, to some extent, offset the advantage in acquisition (Clarkson, 1981).

3) Increase effective rooted zone by root hairs. Because of an obvious effect of increasing root surface area, it has been suggested that additional root hairs would contribute substantially to nutrient uptake, particularly of nutrients for which access at root surfaces is diffusion-limited (Nye, 1977). Evidence for a significant role of root hairs in P absorption was given by Lewis and Quirk (1967). Bole (1973) found genotypic differences in root hair numbers, but soil P uptake was not closely related to those differences. In such conflicting instances, Clarkson (1981) suggested that the duration of the experiment may be important. With experiments of long duration, four weeks in the work of Bole (1973), the geometrical benefit of the hair may be obviated by extensive depletion of the soil.

4) Change of root diameter and branching. In P-deficient conditions, root systems of several plant species were thought to become more finely branched and suitable for physical exploration (Hackett, 1968; Taylor and Goubran, 1976). Considering the low mobility of phosphate in soil, this type of response to phosphate stress could be of greater practical benefit than alterations in the activity of the P transport system in the root. Most root characteristics such as relative size, diameter and branching pattern, however, are genetically determined. Because roots are unable to readily decrease their diameter, root growth was restricted to certain soil pore sizes and nutrients within fine aggregates were physically unavailable for plant roots (Russell and Goss, 1974).

5) Mycorrhizal symbioses. VA mycorrhizal infection can increase the uptake of P to host plants (Mosse, 1973) (See above discussion).

6) Inducing changes in the rhizosphere. Certain plant roots, under nutrient deficiency or with nitrogen regimes that included ammonium absorption, were able to modify conditions within the rhizosphere by changing the pH (Riley and Barber, 1971), releasing chelating or reducing substances (Moghimi et al. 1978), releasing acid phosphatase (Bieleski, 1971) or by exuding substances beneficial to rhizosphere microorganisms (Barber and Martin, 1976). As a consequence, mobilization of some ions may be enhanced in the rhizosphere.

7) Capacity for normal metabolism at relatively low tissue concentration. It has been suggested that even a low tissue concentration of P (Whiteaker et al. 1976), N (O'Sullivan et al. 1974) or K (Makmur, et al. 1978) was adequate to support normal metabolic

process. However, an alternative explanation for metabolism at low nutrient concentration was that greater re-distribution to terminal growing points would maintain required elements at relatively high concentrations in the most active regions of growth. Proof that growth and metabolism do continue at reduced tissue concentrations must await comparative studies of specific biochemical reactions in which the elements participate (Gerloff and Gabelman, 1983).

8) Element re-distribution and re-utilization in plants. Due to the high mobility of N, P and K in plants, these nutrients are usually re-distributed from senescing plant parts to young tissue which leads to an increase of the ratio in biomass production to nutrient content (White, 1972).

9) Low plant relative growth rate. Chapin and Bieleski (1982) reported that some plants were tolerant of nutrient stress and their growth rates appeared to be genetically or physiologically restricted to a low level. This may be a survival strategy in nutrient-poor surroundings.

Plant response to nutrient stress, as discussed above, was manifested through differences in ion uptake and distribution, morphological modification of individual organs, as well as in nutrient re-utilization and role in the metabolic processes. It is important to identify the mechanism of nutrient acquisition and to understand the potential and limitation of that mechanism.

Mathematical Models in Agricultural Research

Biological systems are highly inter-reactive and developing models is one way of sorting out the complexity and integrating knowledge of component behavior. Important attributes of modeling include: 1) helps to define and categorize the state of knowledge of the subject; 2) helps to locate gaps in knowledge and to make hypotheses explicit, and thus helps to set priorities for research; 3) provides a tool to make the integrated information operational; 4) establishes an effective link between scientists from adjacent disciplines, between researchers studying different levels of biological organization, and between fundamental and applied scientists (Penning de Vries, 1983).

Static and Dynamic Plant Growth Models

Thornley (1978) suggested that there are two types of plant growth models--static and dynamic. A static model, for example, uses a typical response function of yield to applied fertilizer, and takes no account of the time course of events (such as growth and development) through the season, or variable factors such as weather. A dynamic model attempts to describe the growth and development of the plant throughout the season.

Regression approaches are usually used in static models, because of

the simple and straightforward relation between yield and one or more environmental variables. However, they are less accurate, and cannot be easily generalized a priori to other areas, other crops or other years. For example, Van Keulen et al. (1976) simulated biomass production in fertilized pastures with natural rainfall in a dry zone of Israel and concluded that 5 out of 13 consecutive years, water was the primary limiting factor of plant growth. In the other, wetter years, production was determined by soil fertility, which was not related to precipitation in the same way. Indeed, a regression technique could not cope with such variation and tended to underestimate the complexity of biological and agricultural systems and their environments.

In dynamic models, a system is described by a set of state variables (such as the weights or areas of various organs) that are updated at each iteration of the model by rate variables (such as the flux of carbon in photosynthesis). The calculation of rate variables depends upon information from external driving variables (such as radiation and air temperature) and internal auxiliary variables (such as the root/shoot ratio) drawn from the current state of the system (France and Thornley, 1984). Penning de Vries (1983) compared various approaches for modeling of plant growth and production and concluded that dynamic models predict yields from defined biological processes while a regression model provides only the yield estimate. However, the more accurate the dynamic model, the more data are required. If these data are unavailable, the regression model is probably the best option.

Whole Plant Growth Analysis

Quantitative studies of dynamic plant growth are commonly based on the analysis of sequential harvest data, using polynomial or exponential equations to describe changes in plant dry weight or leaf area as a direct function of time. The techniques and applications of this type of analysis have been reviewed by Hunt (1982). The use of growth functions is largely empirical: a polynomial can nearly always be found to adequately describe a particular set of data (Hughes and Freeman, 1967); the derived functions may be valid statistically, but meaningless biologically (Milthorpe and Moorby, 1979; Causton and Venus, 1981). France and Thornley (1984) suggested that it is preferable to try to select or construct a function that has some biological plausibility, and whose parameters may be meaningful--that is, they may characterize some underlying physiological or biochemical mechanism. For example, exponential plant growth in the seedling stages is an analogue of autocatalysis in metabolic process (France and Thornley, 1984).

Whole plant growth analysis has been used for estimating various aspects of plant growth and development. For example, relative growth rate is defined as the amount of biomass produced per amount of biomass per unit of time. Other indexes of physiological processes include net assimilation rate (the amount of biomass produced per unit of leaf area per unit of time) (Evans, 1972), nutrient flux (the amount of nutrient increased per unit of root area per unit of time) (Williams, 1946;

Brewster and Tinker, 1972) and nitrogen productivity (the amount of biomass produced per amount of nitrogen in the biomass and per unit of time (Ingestad, 1979).

Functional Approach to Dynamic Plant Systems

Understanding plant life forms on a functional basis requires at least a general understanding of the plant carbon, water and nutrient relations. Lockhart (1976) suggested that it is feasible to investigate the energy and material balances of plants in order to describe and interrelate the various plant structure and physiological processes. Huck and Hillel (1983) pointed out that one may divide the plant into functional morphological classes (leaves, stems and roots) and estimate the carbon, water and nutrient fluxes into and within each class. The advantages of using a 'functional approach' in modeling whole plant systems (Bowen and Cartwright, 1977; Loomis et al. 1979; Penning de Vries, 1983; France and Thornley, 1984) are summarized as follows:

1) It captures the interdependence of basic plant biophysical processes (e.g., transpiration, photosynthesis and water and nutrient acquisition). A change in any one factor alters transitions between growth states, resulting in changes in the assimilate allocation and resource acquisition and utilization of further growth patterns.

2) It considers the partitioning of assimilate to plant growth sinks (e.g., roots and leaves). The partitioning of assimilate to plant absorption surface (leaves and roots) changes with alterations in

available water and nutrients and with symbiotic associations.

3) It includes the plant functional surfaces which are useful in the estimation of important fluxes occurring in the interfacial region between soil-plant and plant-atmosphere.

4) It helps examine and describe how specific physiological processes and controls relate to and interact with other plant activities. Penning de Vries (1983) have reviewed dynamic models involving carbon, water and nutrient balances and morphogenesis at the plant-environment level. However, no dynamic model of mycorrhizal system which reflects the carbon, water and nutrient relations of the symbiosis is known to exist.

MATERIALS AND METHODS

Plant Inoculation and Growing Conditions

Leucaena leucocephala (Lam.) de Wit var. K-8 (leucaena) was grown from seed in a greenhouse near Honolulu, Hawaii (21°19' N, 157°58' W). Wahiawa soil (Tropeptic Eutrustox) was collected from the Poamoho Experiment Station, College of Tropical Agriculture, Island of Oahu, Hawaii. After being crushed and passed through a 2-mm sieve, 1.7 kg of soil (oven dry weight) was placed into 15-cm diameter plastic pots. The pots and soil were fumigated with 652 g methyl bromide and 14 g chloropicrin m^{-3} for 2 days, then aerated for 21 days before planting. The soil pH was adjusted with $CaCO_3$ to 6.5. Three levels of phosphorus were added (as KH_2PO_4): 14, 135 and 678 mg P per kg of soil. These rates of phosphate corresponded approximately to 0.005, 0.080 and 0.140 mg P L^{-1} of phosphorus in 0.01 M $CaCl_2$ extracts (1 g of oven-dry weight soil mixed with 10 ml of extracting solution), respectively. In all plantings, basal nutrients were mixed with the soil before it was transferred to pots. The nutrients were applied to each pot as KNO_3, 513 mg kg^{-1}; $MgSO_4 \cdot 7H_2O$, 577 mg kg^{-1}; $ZnSO_4 \cdot 7H_2O$, 32 mg kg^{-1}; $CuSO_4 \cdot 5H_2O$, 19 mg kg^{-1}; H_3BO_3, 13 mg kg^{-1}; $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 22 mg kg^{-1}.

Seeds of leucaena were scarified in concentrated H_2SO_4 for 10 min, then rinsed four times with tap water and germinated in flats of fumigated sand. Five days after germination, six seedlings of leucaena were transplanted to each pot. About 500 g of coarse sand was placed on top of these pots and the pot were placed on greenhouse benches. The

moisture content of the soil was maintained near field capacity through regular watering by weighing. The average gravimetric soil moisture content was 40%. A strain of Glomus aggregatum Schenck & Smith emend. Koske (Appendix 2) (Schenck and Smith, 1982; Koske, 1985) was isolated from the Poamoho Experiment Station and maintained in a greenhouse pot culture. Appendix 3 shows the method followed for the isolation and examination of VA mycorrhizal fungi from mineral soil (Huang, Yost and Pinchin, 1985). The spores used in these experiments have been examined and identified by Dr. N. C. Schenck, University of Florida, Gainesville and Dr. R. E. Koske, University of Rhode Island, Kingston. Twenty-five g of inoculum was added as a layer at a depth of 2 cm to each pot before transplanting the leucaena seedlings. Control pots received the same amounts of root and sand from a pot culture of Glomus aggregatum which had been fumigated. Filtered washings from both inocula were added to each pot. These experiments were prepared and conducted during April and May 1985 (Appendix 4).

Daily solar radiation was measured by a quantum sensor (LI-COR Model LI-190, Lincoln, NE, USA) and a pyranometer (LI-COR Model LI-200). The quantum flux density varied from 500 to 1200 $\mu E\ s^{-1}\ m^{-2}$ depending on cloud cover. Air temperature was recorded daily at noon and ranged from 25 to 32^{o}C. Relative humidity varied from 75 to 95 %.

Main Experiment

Experiment I: Daily Pinnule Sampling

The pinnule (Fig. 2) sampling method (Huang and Fox, 1984) (Appendix 5 and 6) was used to monitor daily changes in P content of leucaena in order to assess mycorrhizal effectiveness. The experiment was a randomized complete block design with two treatments with or without mycorrhizal inoculation and three levels of soil P (described above). There were four replicates per treatment. A most recently fully-expanded pinnule was sampled daily from each pot beginning 5 days after inoculation. Pinnules were weighed after drying at 76^{o}C for 12 h and then ashed at 500^{o}C for 3 h in a muffle furnace. Phosphate was measured by the method of Murphy and Riley (1962).

Experiment II: Frequent Transpiration Measurement and Destructive Plant Harvesting

The experiment consisted of two treatments with or without mycorrhizal inoculation, three soil P levels (0.005, 0.080, and 0.140 mg P L^{-1}) and six harvests (5, 10, 15, 20, 25 and 30 days after inoculation). There were three replicates per treatment arranged in a randomized complete block design. Four pots were prepared to measure the water evaporated from pots without plants. During the experiment,

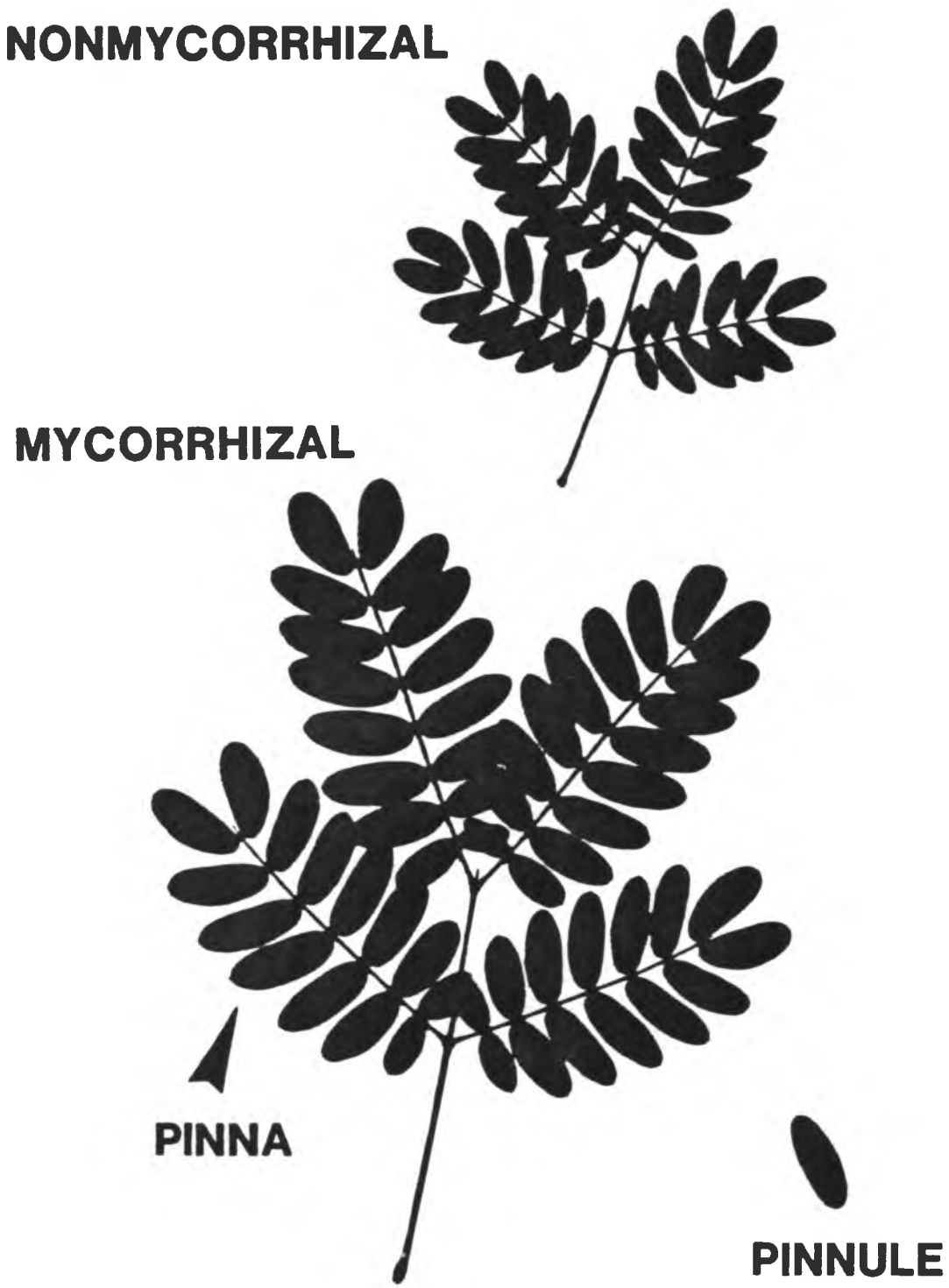


Fig. 2. Pinnules and leaves of mycorrhizal and nonmycorrhizal Leucaena leucocephala (actual size).

water loss was measured at intervals ranging from 1 to 4 days, depending on the rate of water use. The plants were weighed to 0.1 g on an electronic balance (Sartorius Model 6100, New York, USA) generally at the same time on each sampling date. The mean daily water transpired per pot, E (g day^{-1}), was adjusted for variation in free-water evaporation as follows: $E = (dW - S)/(dT/D)$ where dW is the change in weight (g) since the previous weighing; S is the mean water loss directly from the soil (g) estimated from four sample pots without plants; dT is the time interval in daylight hours; and D is daylength (h day^{-1}). Plants from each treatment were harvested destructively at each of the 6 intervals after planting.

Supplementary Experiment

An attempt was made to increase soil P sufficiently high in order to determine whether nonmycorrhizal leucaena plants could achieve growth and nutritional status equivalent to that of VA mycorrhizal plants. In this supplementary experiment, phosphorus was added as KH_2PO_4 at three levels: 0, 600 and 1800 mg per kg of soil (dry weight basis). These rates of phosphate corresponded approximately to 0.013, 0.117 and 0.429 mg P L^{-1} of phosphorus in 0.01 M $CaCl_2$ extracts, respectively. After mixing with basal nutrients and P additions, 1.0, 1.3, 1.8 and 3.5 kg of soils were placed into either 2.0 or 4.0 liter plastic pots. The 4-liter pots were used for plants grown to 40 days to reduce the effects of restricted soil volume. The experiment consisted of two treatments

(with or without mycorrhizal inoculation), three levels of soil P (0.013, 0.117 and 0.429 mg P L^{-1}) and four harvests (12, 24, 32 and 40 days after inoculation). There were three replicates per treatment. The general measuring and harvesting procedures were the same as in the main experiments.

Growth Measurement and Chemical Analysis

At each harvest, leaf area of all plants in each pot were measured by a LI-COR leaf area meter Model LI-3100 (Lincoln, NE, USA). Leaves which had senesced during the experiment were not included in the measurement and were retrieved from the pot surface as close as possible to the time of abscission. Following harvesting, oven-dry weights and phosphorus content of the leaves were determined. Fresh and dry weights of leaves, stems and root dry weights were recorded. Length of feeder root was estimated by the modified line intersect method (Tennant, 1975). Preliminary tests using fine wires indicated that this method could predict wire lengths within 8% of the actual length. Root diameter was measured by averaging diameters of 200 root segments in each treatment using a compound microscope with micrometer disc at 100X-200X magnification. Root area (RA) was calculated from RA = 2 X 3.1416 X rL, where r = average root radius and L = root length. Roots from each sample were cleared and stained by the method of Philips and Hayman (1970) for measurement of mycorrhizal colonization.

Nutrient concentration was determined in ball-milled subsamples of

plant tissue with an X-ray quantometer (Applied Research Laboratory Model 72000).

An Analytical Model for the Dynamic VA Mycorrhizal System

System Structure

A compartment diagram of the soil-mycorrhiza-plant system is shown in Fig. 3. This diagram presents a series of external growth factors (e.g., water, nutrients, solar radiation), plant biomass (total dry weight), and assimilatory structures (leaf and root). The interfaces between the plant and atmosphere or the plant and soil were used to represent each of the main functional plant components, e.g., leaf area was associated with transpiration and photosynthesis and root area was associated with water and nutrient acquisition. Other functions represented in the diagram include growth (dry matter production), structural partition (assimilate allocation to leaf or root), and water and nutrient utilization. This model was used to suggest calculations and relationships describing the system.

In the main experiment, the dynamic soil-mycorrhiza-plant system was divided into six growth stages, each of five day duration beginning 5 days after inoculation. Fig. 4 illustrates the state and auxiliary variables of growth, water relations and nutrient acquisition of leucaena seedlings from a single harvest resulting from the analytical

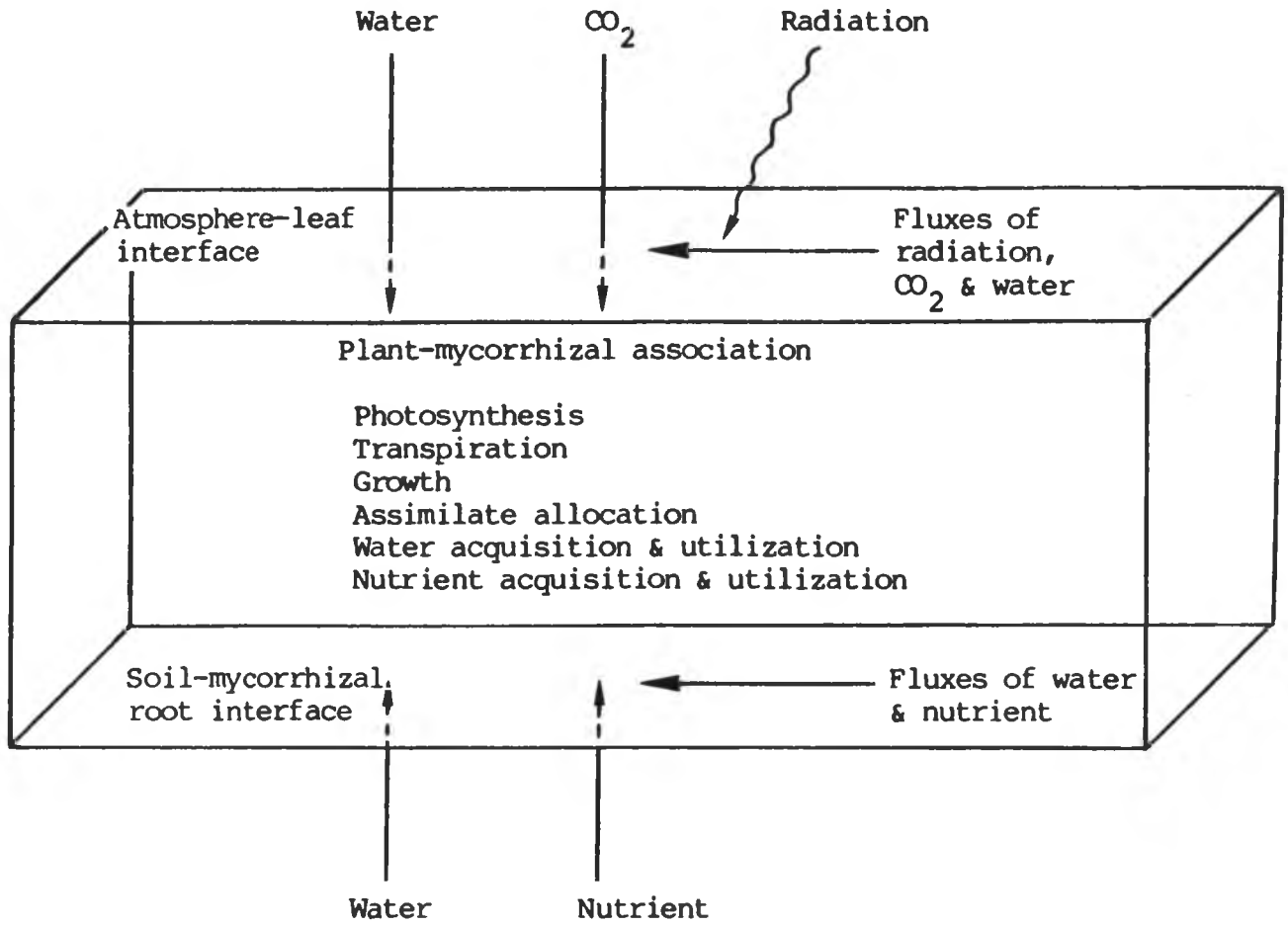
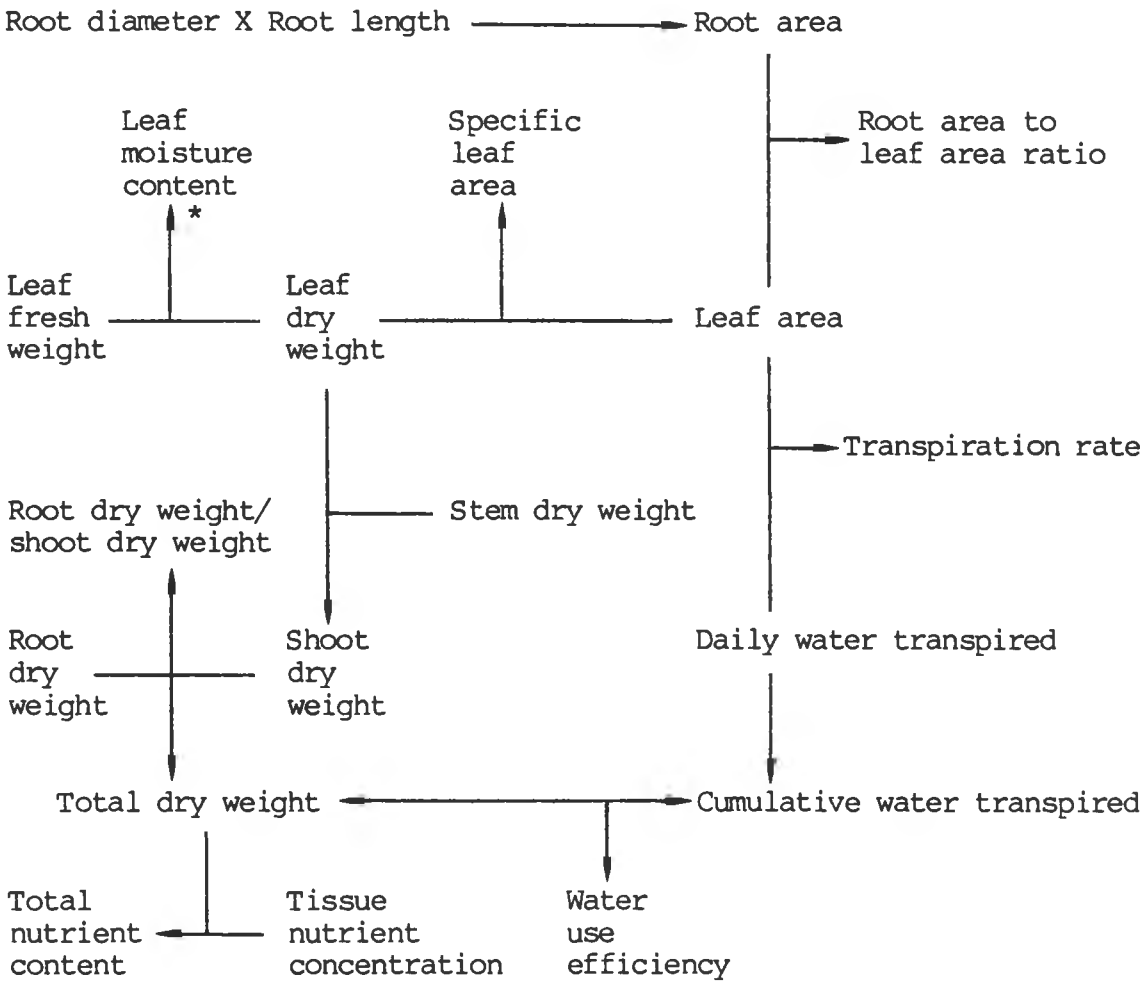


Fig. 3. Diagram of components and fluxes of radiation, carbon dioxide, water and nutrients and major physiological reactions in the soil-mycorrhiza-plant system.



State variable: Variable describes plant quantitative characteristic at certain time (e.g. root dry weight and shoot dry weight).

Auxiliary variable: Variable derives from state variables (e.g. total dry weight and root/ shoot dry weight ratio).

* Arrows point to auxiliary variables.

Figure 4. Schematic diagram of state and auxiliary variables used to quantify dry matter production, transpiration and nutrient absorption of Leucaena leucocephala seedlings from a single harvest.

model of the system. Dry matter production, biomass allocation patterns and nutrient uptake and utilization efficiency of mycorrhizal and nonmycorrhizal plants were compared by mathematical growth analysis techniques (Evans, 1972). Abbreviations, definitions and formulas for the calculations of rate variables are given in Table 1.

There were 15 rate variables (Table 1) calculated from five variables of total dry weight (TDW), leaf area (LA), root area (RA), cumulative water transpired (CWT) and nutrient content (M). These variables were grouped into three functional parameters of the system: 1) an assimilate parameter (total dry weight) which was determined by the interaction of carbon, water and nutrient metabolism, 2) growth form parameters (leaf area and root area) which were determined by morphology and configuration, and 3) resource parameters (water and nutrients) which were determined by the supply, movement and accessibility to plant root and leaf surfaces.

Assumptions

The following assumptions were used to obtain values of the rate variables for these experiments.

1) Mathematical analyses of growth were based on a simple exponential growth equation. This approach assumes that the growth rate of a plant was related to its mass, which is generally true for young seedlings (Evans, 1972).

2) Nutrient absorption and water transpiration were assumed to be

Table 1. Definitions, units, and mathematical relationships of rate variables used in these studies.

Calculations require data from at least two harvests over a time interval, dT = length of harvest interval (days), TDW1 and TDW2 = total plant dry weight between the intervals, respectively. LA, RA, CWT and M are the leaf area, root area, cumulative water transpired and tissue nutrient content, respectively., then:

1. Relative transpiration rate = (ln CWT2 - ln CWT1)/(CWT2 - CWT1) X dT, (mg/g/day).
2. Relative growth rate = (ln TDW2 - ln TDW1)/(TDW2 - TDW1) X dT, (mg/g/day).
3. Relative leaf area expansion rate = (ln LA2 - ln LA1)/(LA2 - LA1) X dT, (cm^2/cm^2/day).
4. Relative root area expansion rate = (ln RA2 - ln RA1)/(RA2 - RA1) X dT, (cm^2/cm^2/day).
5. Relative nutrient accumulation rate = (ln M2 - ln M1)/(M2 - M1) X dT, (µmol/mol/day).
6. Water flux to root = (CWT2 - CWT1) X (ln RA2 - ln RA1)/(RA2 - RA1) X dT, (µg/cm^2/day).
7. Water flux to leaf = (CWT2 - CWT1) X (ln LA2 - ln LA1)/ (LA2 - LA1) X dT, (µg/cm^2/day).
8. Nutrient flux to root = (M2 - M1) X (ln RA2 - ln RA1)/(RA2 - RA1) X dT, (nmol/cm^2/day).
9. Net assimilation rate= (TDW2 - TDW1) X (ln LA2 - ln LA1)/(LA2 - LA1) X dT, (mg/cm^2/day).
10. Unit root rate = (TDW2 - TDW1) X (ln RA2 - ln RA1)/(RA2 - RA1) X dT, (mg/cm^2/day).
11. Component leaf production rate = (LA2 - LA1) X (ln TDW2 - ln TDW1)/ (TDW2 - TDW1) X dT, (cm^2/mg/day).
12. Component root production rate = (RA2 - RA1) X (ln TDW2 - ln TDW1)/ (TDW2 - TDW1) X dT, (cm^2/mg/day).
13. Water utilization rate = (TDW2 - TDW1) X (In CWT2 - In CWT1)/ (CWT2 - CWT1) X dT, (µg/g/day).
14. Nutrient utilization rate = (TDW2 - TDW1) X (In M2 - In M1)/ (M2 - M1) X dT, (g/mol/day).
15. Nutrient mass flow rate = (M2 - M1) X (In CWT2 - In CWT1)/ (CWT2 -CWT1) X dT, (nmol/g/day).

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uniformly distributed over the entire root or leaf surface.

3) Changes in the water-storage capacity of plant tissue were assumed to be small compared with the rate of water transpiration by plants. Total water uptake by all roots should therefore equal cumulative water transpired. In these experiments, frequent weighing (usually at one to two day intervals) should have minimized the bias in calculating the amounts of water transpired.

Statistical Analysis

Analysis of variance procedures were used to estimate statistical significance of inoculation treatments at each harvest date. All data were analyzed by Statistical Analysis System procedures (SAS Institute, Inc., 1985) and significance was tested with F and Duncan's tests.

RESULTS

Effects of Mycorrhizal Inoculation and P Fertilization on Plant Dry Weight

Statistically significant data for plant dry weight from the main (Table 2) and supplementary experiments (Table 3) are provided. Mycorrhiza X P fertilization interactions and P fertilization treatments were generally not significant. For this reason, mycorrhizal treatment means were used by combining different P fertilization treatments within each mycorrhizal inoculation treatment of the main and supplementary experiments.

Daily Change in Pinnule P Status

Differences in pinnule P content between mycorrhizal and nonmycorrhizal plants were divided into three major phases (Fig. 5). During phase one, 1 to 10 days after inoculation, differences in pinnule P contents were minor. An initial decline in the P contents of pinnules of both mycorrhizal and nonmycorrhizal plants probably resulted from a decrease in P supply to the pinnules as seed P reserve were used.

During phase two, 11 to 24 days after inoculation, pinnule P contents of mycorrhizal plants were much greater than than that of

Table 2. Statistical significance of effects of P fertilization and mycorrhizal inoculation on total dry weight of Leucaena leucocephala seedlings (main experiment).

Solution P#	Treatment	Days after inoculation					
		5	10	15	20	25	30
mg L^{-1}		g					
		Total dry weight					
0.005	Mycorrhizal	0.26	0.47	0.83	1.29	1.74	4.25
	Nonmycorrzal	0.26	0.52	0.73	1.10	1.28	1.51
0.080	Mycorrhizal	0.25	0.41	0.66	1.05	1.71	3.61
	Nonmycorrzal	0.25	0.40	0.66	1.17	1.45	1.82
0.140	Mycorrhizal	0.26	0.46	0.76	1.13	2.31	3.86
	Nonmycorrzal	0.23	0.53	0.75	1.30	1.60	2.55
Mycorrhizal		NS	NS	NS	*	**	**
Soil P		NS	*	**	NS	*	*
Mycorrhizal X Soil P		NS	NS	NS	NS	NS	NS

Extracted by 0.01 M $CaCl_2$ solution.

Table 3. Statistical significance of effects of P fertilization and mycorrhizal inoculation on total dry weight of Leucaena leucocephala seedlings (supplementary experiment).

Solution P#	Treatment	Days after inoculation 12	24	32	40
mg L^{-1}		g			
		Total dry weight			
0.013	Mycorrhizal	0.46	1.11	2.43	6.84
	Nonmycorrzal	0.40	1.01	1.44	1.39
0.117	Mycorrhizal	0.51	1.57	4.54	10.36
	Nonmycorrzal	0.43	1.46	2.88	5.47
0.429	Mycorrhizal	0.53	2.26	5.44	12.24
	Nonmycorrzal	0.50	2.00	4.10	9.74
Mycorrhizal		**	*	**	**
Soil P		**	**	**	**
Mycorrhizal X Soil P		NS	NS	NS	*

Extracted by 0.01 M $CaCl_2$ solution.

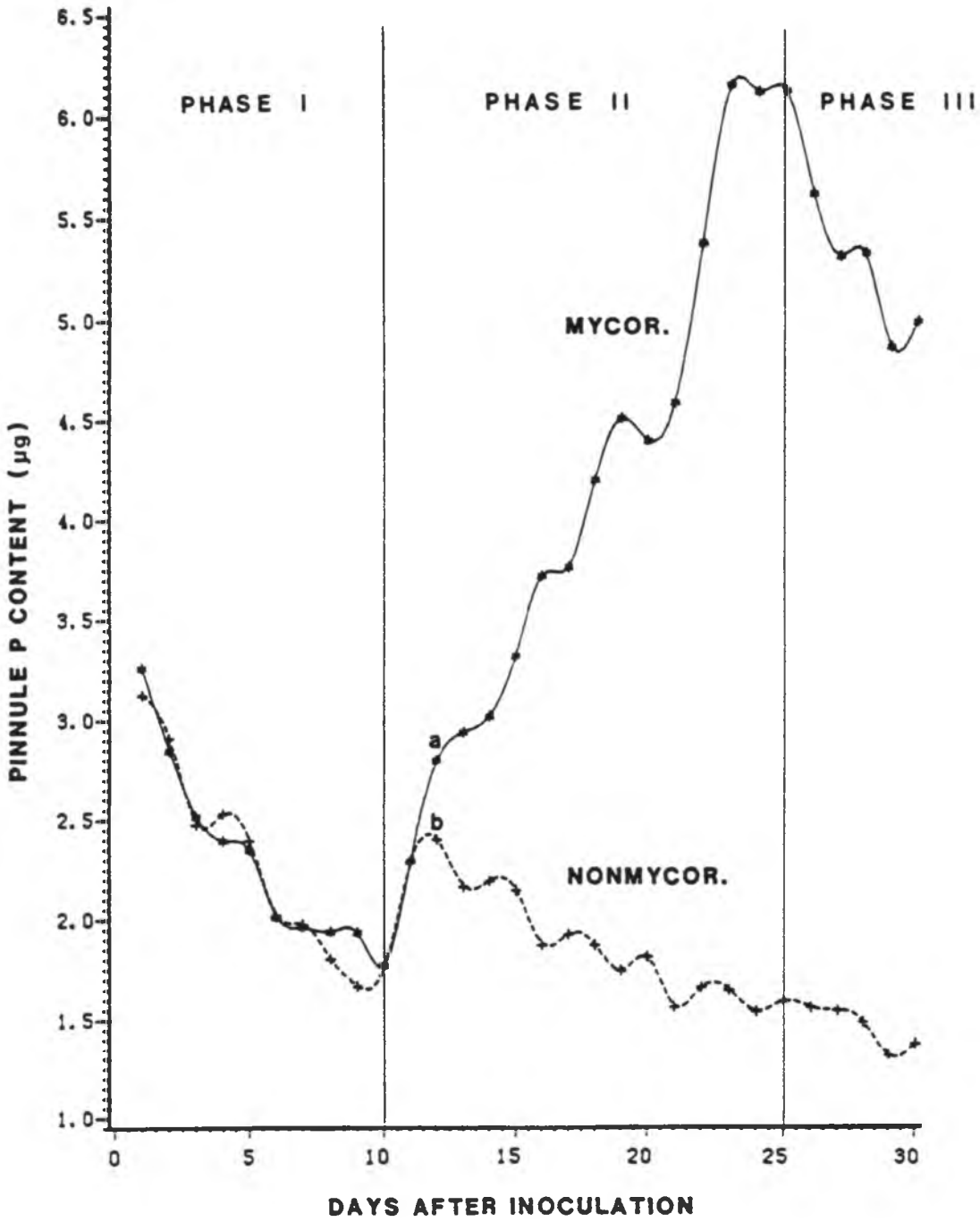

Fig. 5. Time course of change in pinnule P content of mycorrhizal and nonmyccorhizal Leucaena leucocephala seedlings.

nonmycorrhizal plants. In nonmycorrhizal plants, there were small increases in pinnule P content during the first two days of this phase, then P concentration gradually dropped (Fig. 6). The decrease in pinnule P concentration in nonmycorrhizal plants was comparable to that observed in phase one. Pinnule dry weight (Fig. 7) of nonmycorrhizal plants however, increased slightly during phase two.

By 12 days after inoculation differences were distinctly evident between pinnule P contents of mycorrhizal and nonmycorrhizal plants (Fig. 5). Pinnule-P content of mycorrhizal plants increased from 1.7 to 6.2 µg then gradually declined until the beginning of phase 3 (25 to 30 days after inoculation). Five pulses in pinnule-P concentration (Fig. 6) were observed at: 12, 15, 18, 21 and 24 days after inoculation. These pulses occurred approximately 1 to 2 days before peaks in pinnule dry weight (Fig. 7). The increases in dry weight were observed 15, 17, 19, 22 and 25 days after inoculation. This sequential pattern was probably due to absorption, growth and dilution effects.

Following maximum P accumulation in the pinnule at the end of phase two, growth of the pinnule tended to decrease P concentration even though P content of pinnules has continued to increase. There were also more pinnules, but of smaller sizes, which may have been responsible for decreased P contents per pinnule. Nevertheless, pinnule P concentrations (Fig. 6) remained between 0.14 and 0.16%, which was about double the P concentrations in pinnules of nonmycorrhizal plants (0.06 to 0.08%). By the end of phase three, the average pinnule dry weight of nonmycorrhizal plants (1.7 mg) had declined to almost half that of mycorrhizal plants (3.2 mg) (Fig. 7).

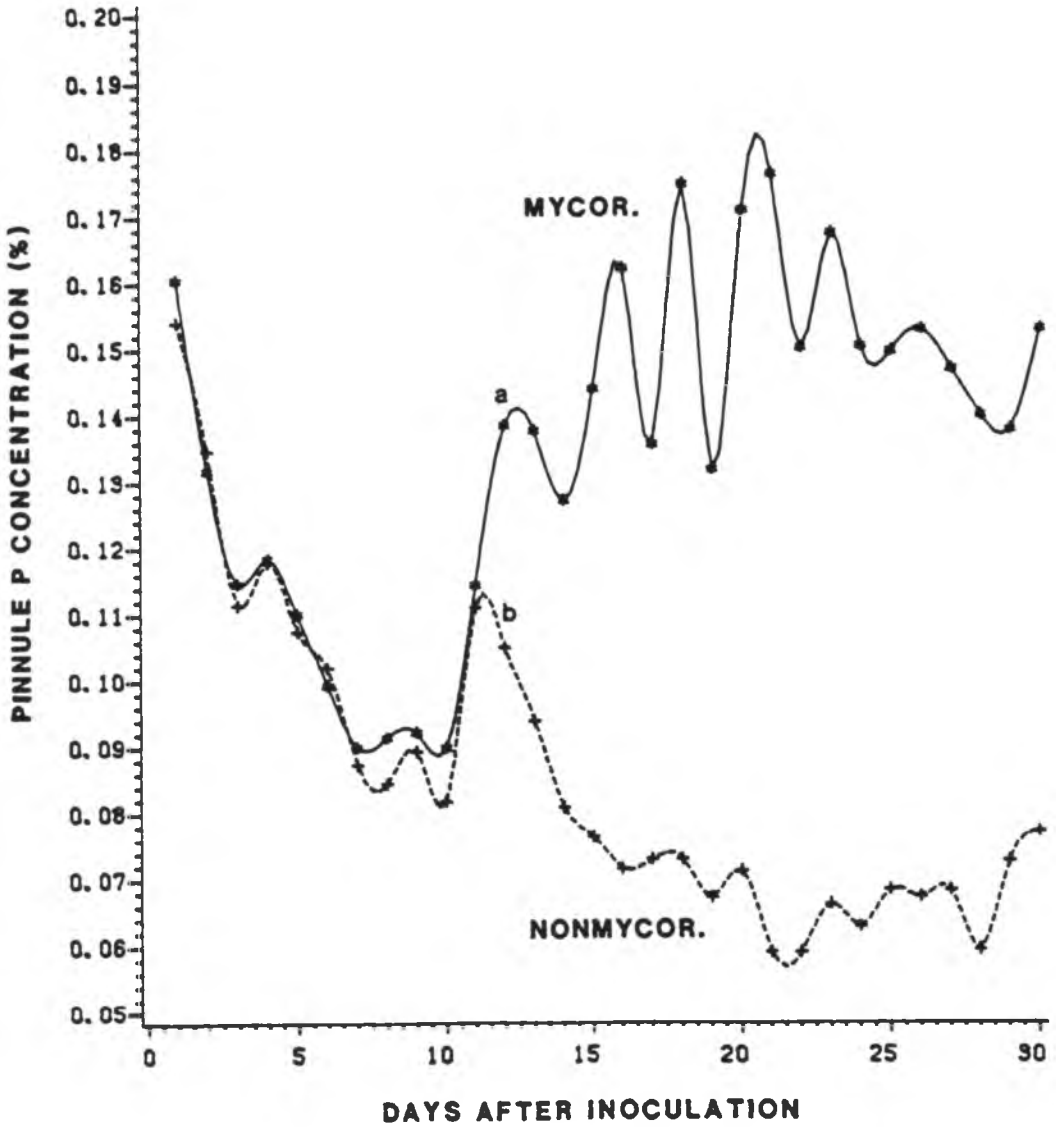

Fig. 6. Time course of change in pinnule P concentration of mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings.

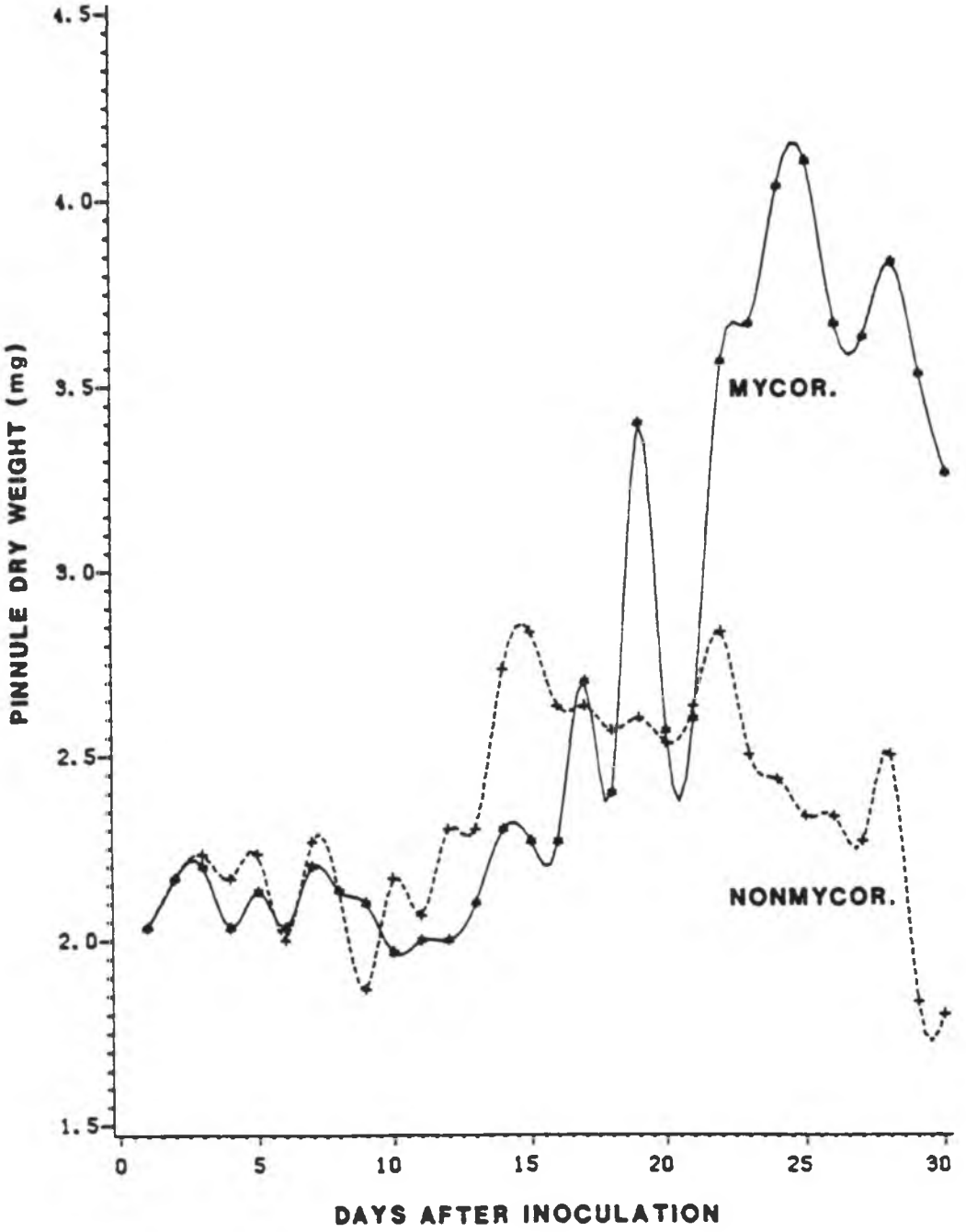

Fig. 7. Time course of change in pinnule dry weight of mycorhizal and nonmycorrhizal Leucaena leucocephala seedlings.

Results from the supplementary experiment were similar to the main experiment except for higher P fertilization treatments and larger pot size. These modifications were made in an attempt to obtain similar growth in nonmycorrhizal treatments as in mycorrhizal ones. As shown in Figures 8, 9 and 10, differences in pinnule P contents between mycorrhizal and nonmycorrhizal plants were greatest in plants grown at the low soil P level whereas these differences decreased with increasing P applications. However, the nonmycorrhizal leucaena seedlings did not have as high a pinnule P content as the mycorrhizal plants at the highest soil P level (0.429 mg P L^{-1} in solution, equilibrated with 0.01 M $CaCl_2$ solution). The minimum pinnule P content of nonmycorrhizal plants was about 0.7 µg per pinnule (Fig. 8) which was identical to the result of a previous experiment (Appendix 6).

Daily Changes in Transpiration

Water transpired by mycorrhizal and nonmycorrhizal plants as a function of time is shown in Fig. 11. As plant growth progressed, an oscillating pattern of increasing transpiration was observed in both mycorrhizal and nonmycorrhizal plants. Daily transpiration appeared to have responded to environmental variables such as solar radiation (Appendix 7). In general, transpiration increased with increasing leaf area. By the sixth measurement (11-14 days after inoculation), transpiration by mycorrhizal plants was significantly greater than that of nonmycorrhizal plants (LSD 5% = 2.0 g of water transpired).

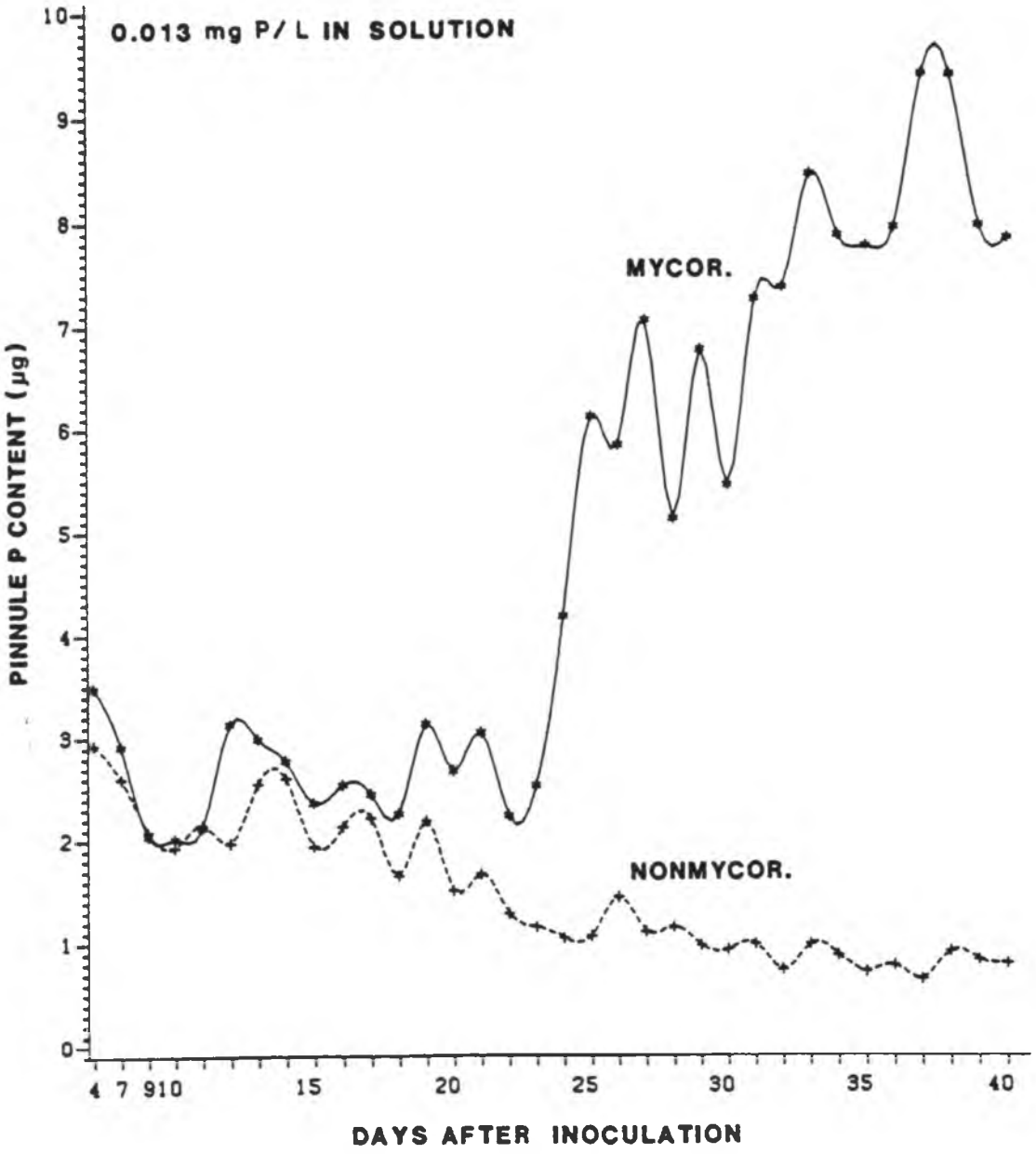

Fig. 8. Time course of change in pinnule P content of mycorrhizal and nonmycorhizal Leucaena leucocephala seedlings grown in soil with 0.013 mg P L^{-1} (supplementary experiment).

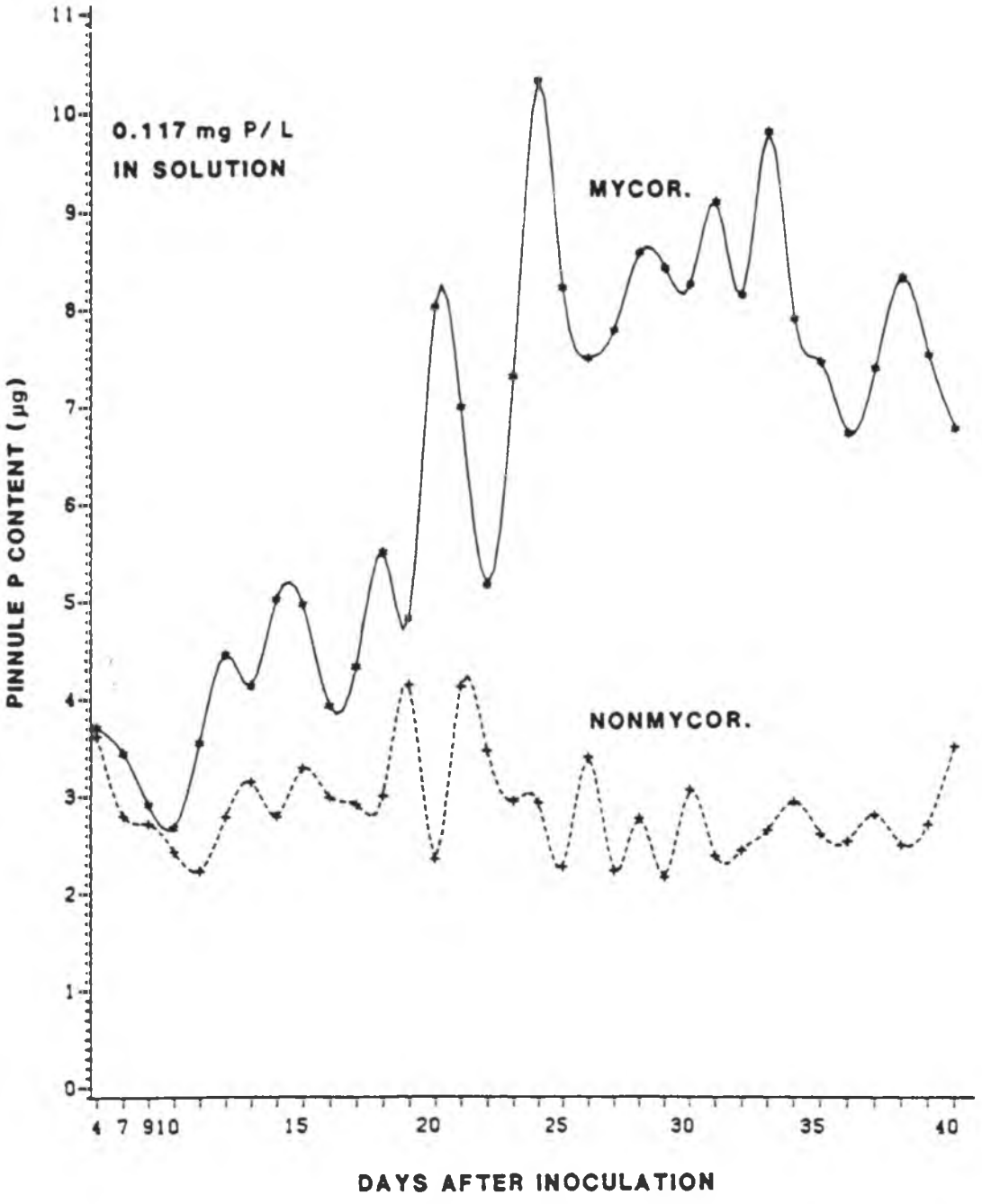

Fig. 9. Time course of change in pinnule P content of mycorrhizal and nonmycorhizal Leucaena leucocephala seedlings grown in soil with 0.117 mg P L^{-1} (supplementary experiment).

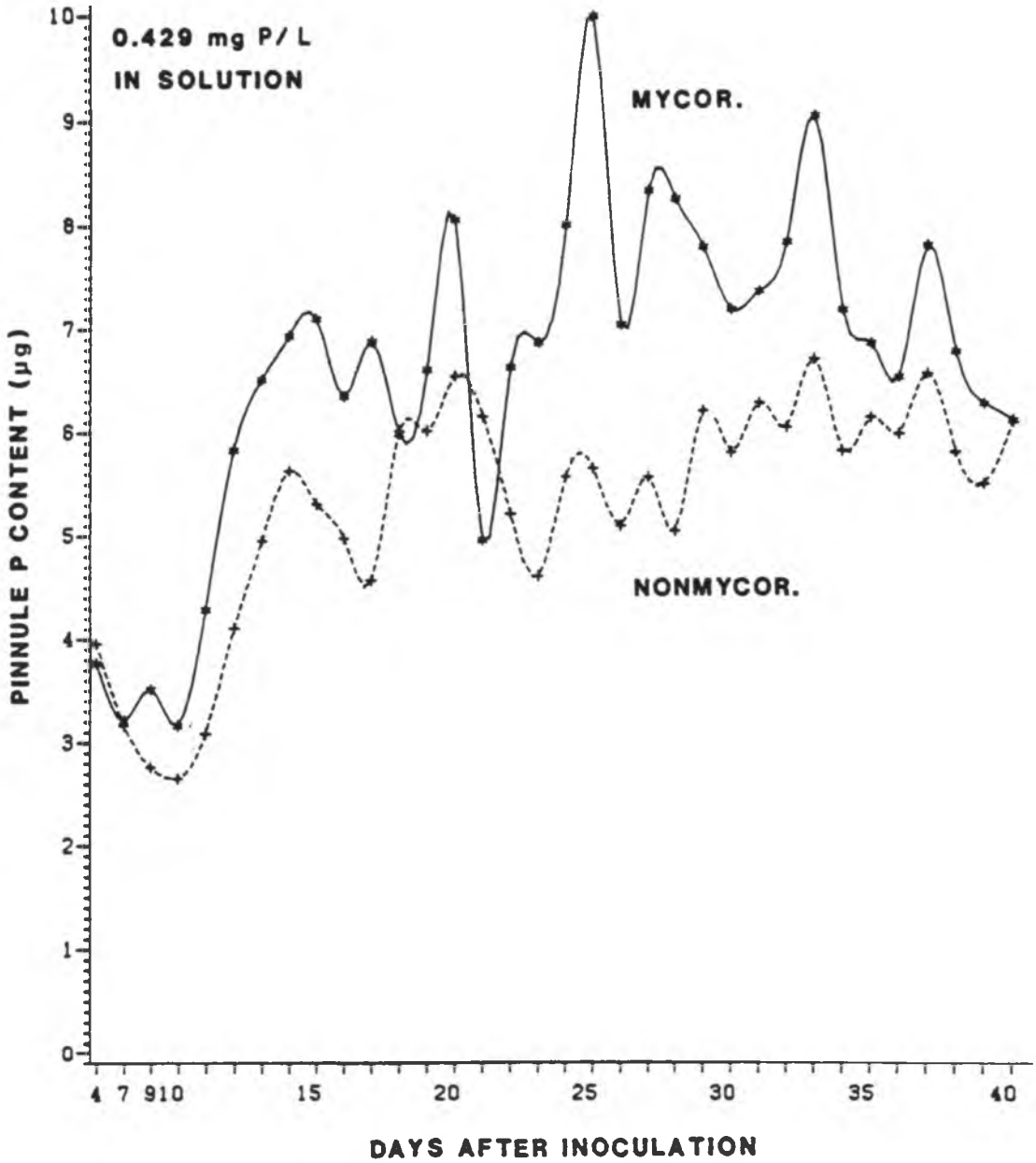

Fig. 10. Time course of change in pinnule P content of mycorrhizal and nonmycorhizal Leucaena leucocephala seedlings grown in soil with 0.429 mg P L^{-1} (supplementary experiment).

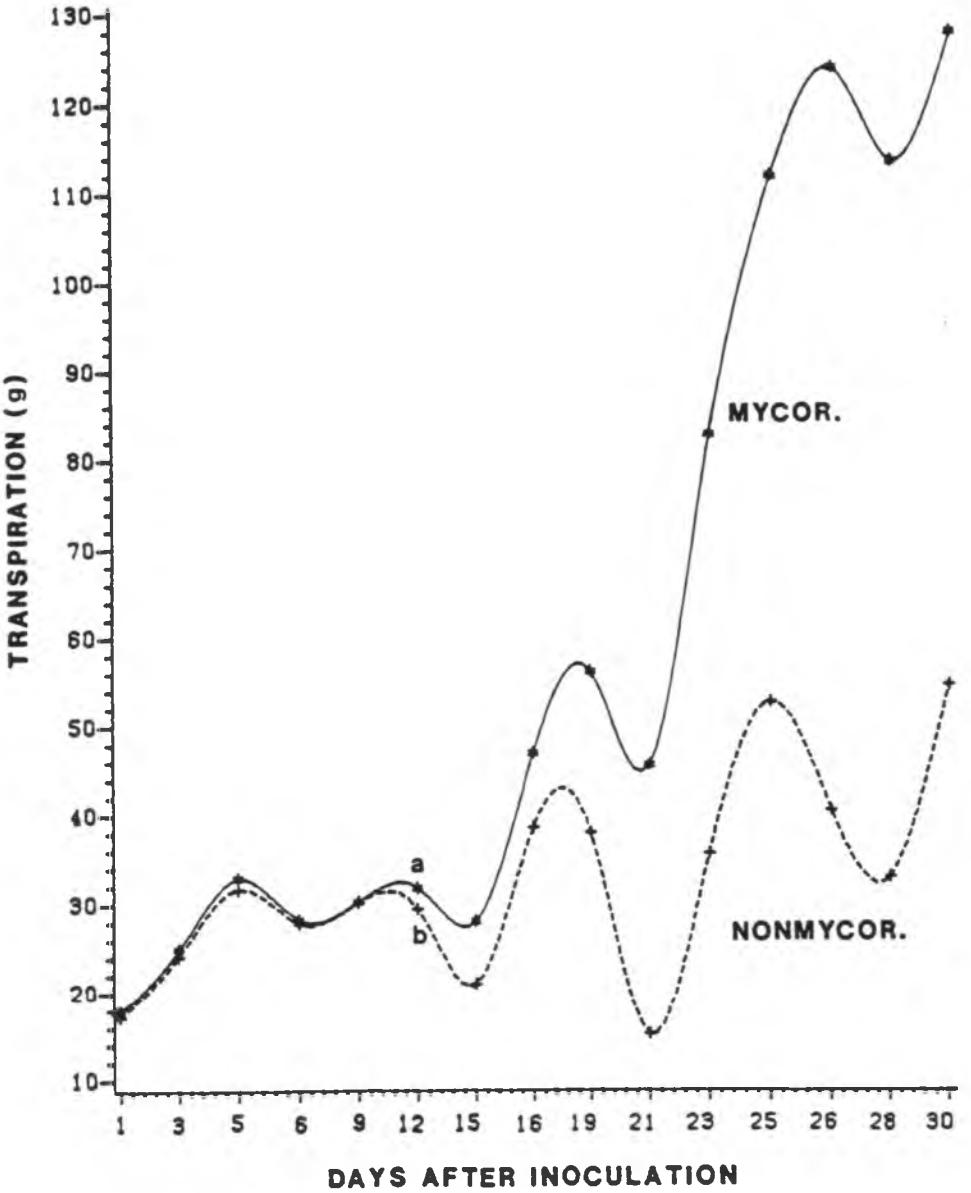

Fig. 11. Time course of transpiration of mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings.

Transpiration varied more during cloudy days than during sunny days (Fig. 11). The coefficients of variation at the 7th, 10th and 14th measurements (15, 21 to 22 and 28 to 29 days after inoculation, respectively) were higher than those taken before or after the low transpiration days (Appendix 8). Evidently stomatal conductance, leaf orientation or other plant factors within treatments responded differently to environmental variation.

In the supplementary experiment, mycorrhiza and high levels of P also led to increased transpiration of leucaena seedlings (Figures 12, 13 and 14). Daily transpiration was significantly higher in mycorrhizal plants. Low soil P levels (0.013 mg P L^{-1} in solution) led to the greatest difference in transpiration between mycorrhizal and nonmycorrhizal plants (Fig. 12). Under high soil P levels (0.429 mg P L^{-1} in soil solution), mycorrhizal plants still transpired more water than nonmycorrhizal plants over the growth period (Fig. 14), and they responded to environmental fluctuation at approximately the same time but in greater magnitude.

Nutrient Acquisition

Changes in nutrient concentrations, nutrient contents and nutrient content ratios over time of mycorrhizal and nonmycorrhizal plants are given in Tables 4, 5 and 6, respectively.

Concentrations in leaves of most nutrients decreased during 5 to 10 days after inoculation (Table 4). At the 10th day after inoculation,

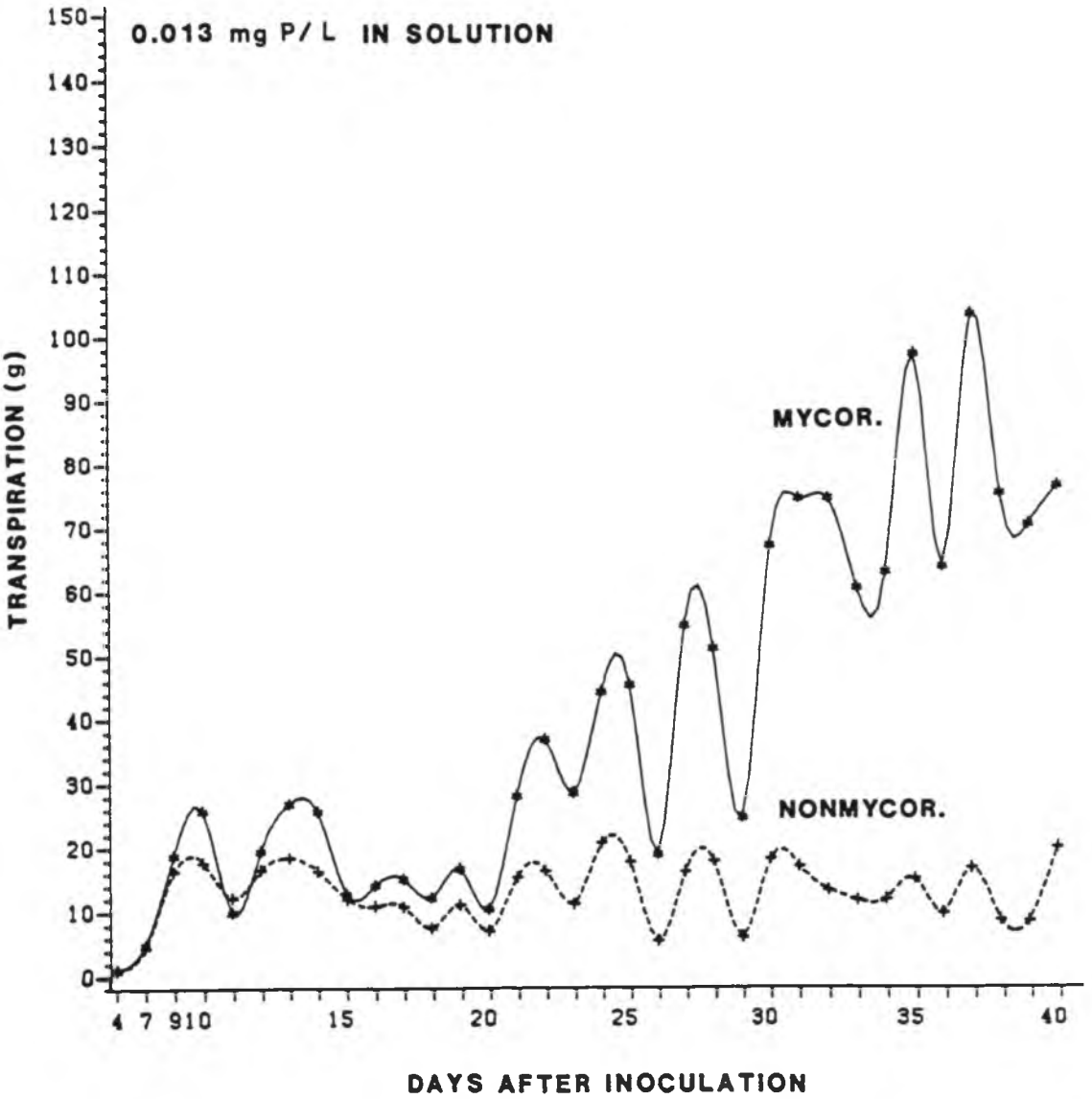

Fig. 12. Time course of change in transpiration of mycorrhizal and nonmycorhizal Leucaena leucocephala seedlings grown in soil with 0.013 mg P L^{-1} (supplementary experiment).

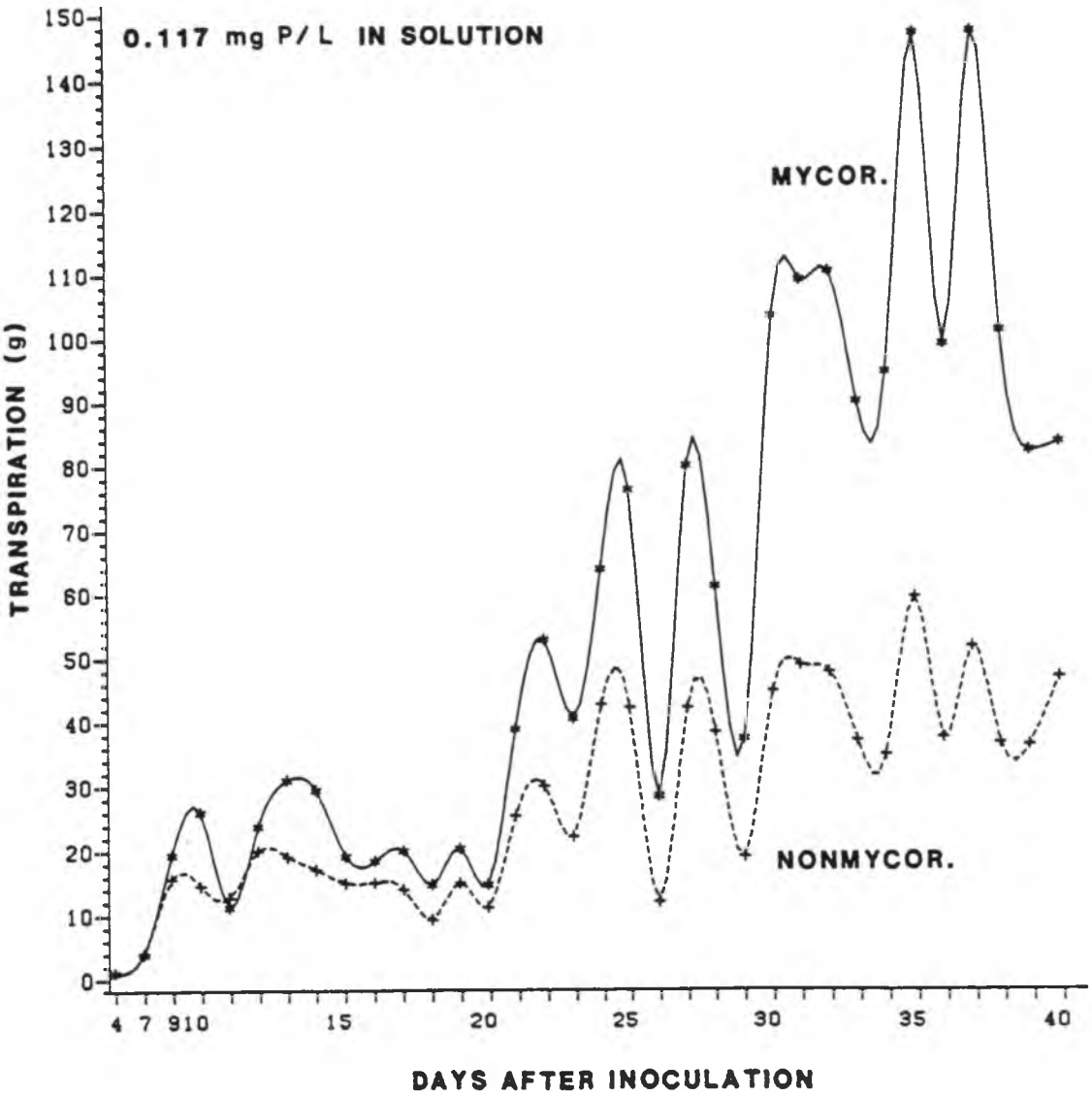

Fig. 13. Time course of change in transpiration of mycorrhizal and nonmycorhizal Leucaena leucocephala seedlings grown in soil with 0.117 mg P L^{-1} (supplementary experiment).

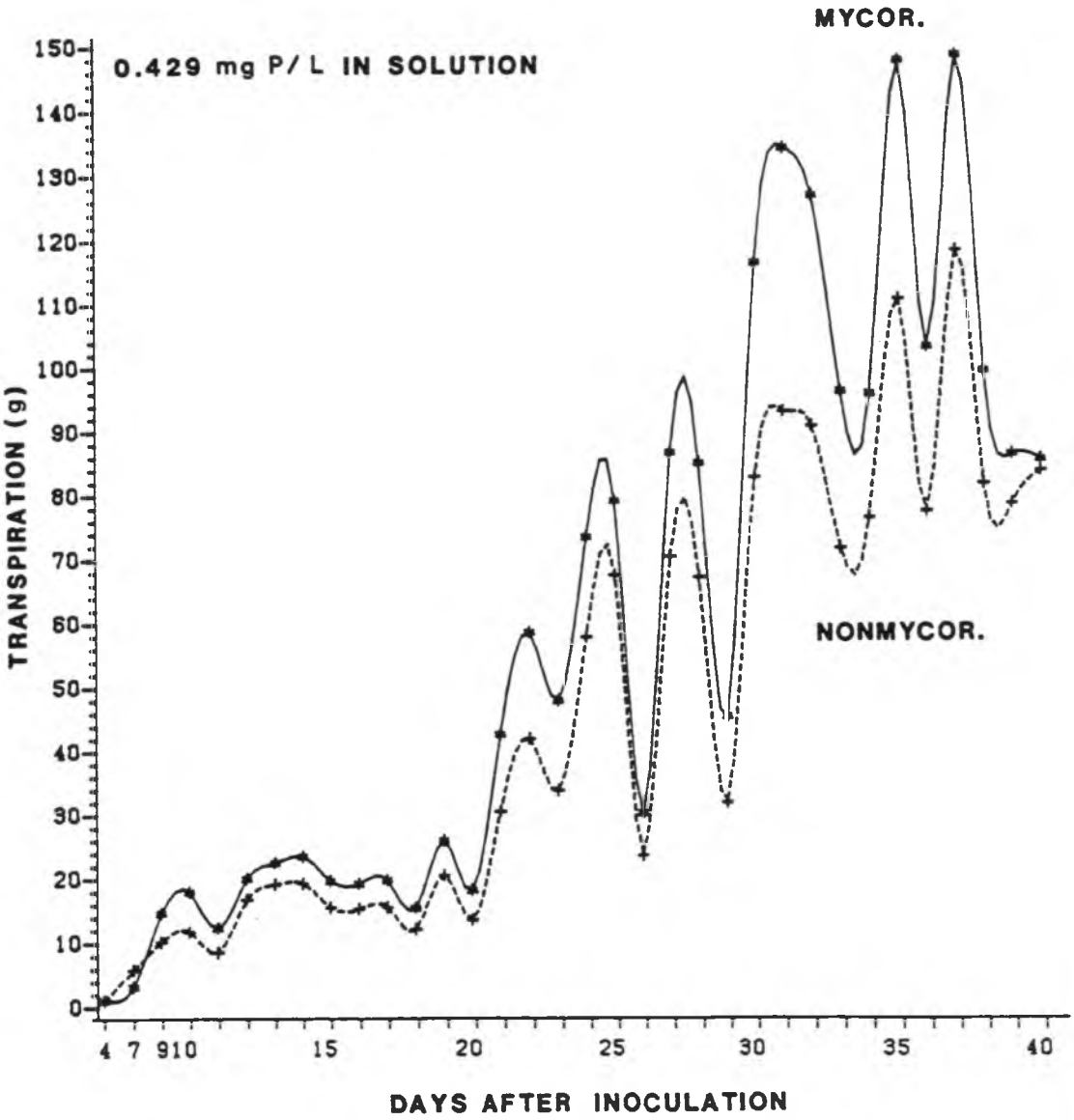

Fig. 14. Time course of change in transpiration of mycorrhizal and nonmycorhizal Leucaena leucocephala seedlings grown in soil with 0.429 mg P L^{-1} (supplementary experiment).

Table 4. Effect of mycorrhizal inoculation on tissue nutrient concentration in Leucaena leucocephala seedlings.

Treatment	Days after inoculation					
	5	10	15	20	25	30
	%					
	Root P concentration					
Mycorrhizal	0.36 a[#]	0.23 a	0.19 a	0.23 a	0.25 a	0.26 a
Nonmycorrhizal	0.39 a	0.17 b	0.14 b	0.15 b	0.09 b	0.08 b
	Shoot P concentration					
Mycorrhizal	0.33 a	0.25 a	0.23 a	0.25 a	0.32 a	0.30 a
Nonmycorrhizal	0.35 a	0.25 a	0.18 b	0.18 b	0.12 b	0.11 b
	Shoot K concentration					
Mycorrhizal	2.21 a	2.81 a	3.45 a	3.46 a	3.79 a	3.37 a
Nonmycorrhizal	2.21 a	3.19 a	3.10 b	3.04 b	2.59 b	2.35 b
	Shoot S concentration					
Mycorrhizal	0.63 a	0.49 a	0.40 a	0.35 a	0.35 a	0.25 a
Nonmycorrhizal	0.61 a	0.45 a	0.36 b	0.31 b	0.27 b	0.24 b
	Shoot Ca concentration					
Mycorrhizal	1.22 a	1.54 a	1.54 a	1.47 a	1.49 a	1.27 a
Nonmycorrhizal	1.24 a	1.51 a	1.51 a	1.43 a	1.38 b	1.29 a
	Shoot Mg concentration					
Mycorrhizal	0.47 a	0.51 a	0.43 a	0.41 a	0.38 a	0.36 a
Nonmycorrhizal	0.47 a	0.47 a	0.42 a	0.38 b	0.35 b	0.32 b

Means in the same columns within harvests followed by the same letter are not significantly different at the 5% level.

Table 5. Effect of mycorrhizal inoculation on tissue nutrient content and P translocation in Leucaena leucocephala seedlings.

Treatment	Days after inoculation 5	10	15	20	25	30
	µmol					
Root P content						
Mycorrhizal	5.5 a[#]	10.9 a	15.8 a	25.1 a	47.8 a	113.4 a
Nonmycorrhizal	5.8 a	8.1 b	10.4 b	19.4 b	16.6 b	21.1 b
Shoot P content						
Mycorrhizal	22.3 a	24.1 a	36.6 a	64.8 a	141.6 a	248.9 a
Nonmycorrhizal	22.7 a	26.4 a	27.6 b	44.6 b	35.9 b	45.7 b
Shoot K content						
Mycorrhizal	117 a	215 b	437 a	725 a	1317 a	2214 a
Nonmycorrhizal	113 a	272 a	386 b	606 b	596 b	731 b
Shoot S content						
Mycorrhizal	41 a	45 a	61 a	89 a	148 a	200 a
Nonmycorrhizal	38 a	46 a	54 b	76 b	76 b	90 b
Shoot Ca content						
Mycorrhizal	63 a	113 a	189 a	298 a	499 a	814 a
Nonmycorrhizal	62 a	126 a	182 a	279 a	308 b	388 b
Shoot Mg content						
Mycorrhizal	40 a	62 a	87 a	135 a	211 a	381 a
Nonmycorrhizal	39 a	63 a	80 b	123 b	129 b	158 b
P translocation = (shoot P content/total P content) X 100%						
Mycorrhizal	80 a	69 b	70 b	71 a	75 a	69 a
Nonmycorrhizal	80 a	77 a	73 a	70 a	68 b	68 a

Means in the same columns within harvests followed by the same letter are not significantly different at the 5% level.

Table 6. Effect of mycorrhizal inoculation on shoot nutrient content ratio in Leucaena leucocephala seedlings.

Treatment	Nutrient	Days after inoculation 5	10	15	20	25	30
		Shoot nutrient content ratio# (K = 100)					
Mycorrhizal	K	100	100	100	100	100	100
	Ca	54	53	43	41	38	37
	Mg	34	29	20	19	16	17
	S	35	21	14	12	11	9
	P	19	11	8	9	11	11
		Shoot nutrient content ratio# (K = 100)					
Non-mycorrhizal	K	100	100	100	100	100	100
	Ca	55	46	47	46	52	53
	Mg	34	23	21	20	22	22
	S	34	17	14	13	13	14
	P	20	10	7	7	6	6

Numbers indicate the shoot nutrient content ratios relative to K within each growth period and mycorrhizal treatment.

root P concentrations in mycorrhizal plants (0.23%) were significantly higher than in nonmycorrhizal plants (0.17%). This increase corresponded to approximately 17% mycorrhizal infection. Differences in shoot P, K, and S concentrations were significant five days later. These differences continued throughout the experiment. Shoot Mg and Ca concentrations differed significantly 20 to 25 days after inoculation. Root and shoot P concentrations in nonmycorrhizal plants declined continuously. For example, 30 days after inoculation root and shoot P of mycorrhizal plants were about 0.26 and 0.30%, respectively. In nonmycorrhizal plants these were 0.08 and 0.11%, respectively. Changes in total P contents (mg/pot) of mycorrhizal and nonmycorrhizal plants (plotted in a log scale) as a function of time are presented in Fig. 15. P content of mycorrhizal plants increased in a linear fashion ($\log_e$ total P content = $-3.34 + 0.11$ day, $r = 0.98^{**}$). In contrast, nonmycorrhizal plants slowly accumulated P ($\log_e$ total P content = $-2.99 + 0.05$ day, $r = 0.84^{**}$) from the soil. As foliar abscission occurred from 21 to 23 days after inoculation, there was a slight decline in P content in nonmycorrhizal plants by the 25th day after inoculation (Fig. 15).

The ratio of shoot P to total P content (Table 5) in mycorrhizal plants was significantly lower than that in nonmycorrhizal plants for harvests at 10 and 15 days after inoculation but was higher than nonmycorrhizal plants at 25 days inoculation.

Shoot nutrient contents relative to K in leucaena seedlings were calculated for each harvests (Table 6). At 5 days after inoculation, there was no difference in these nutrient ratios between mycorrhizal and

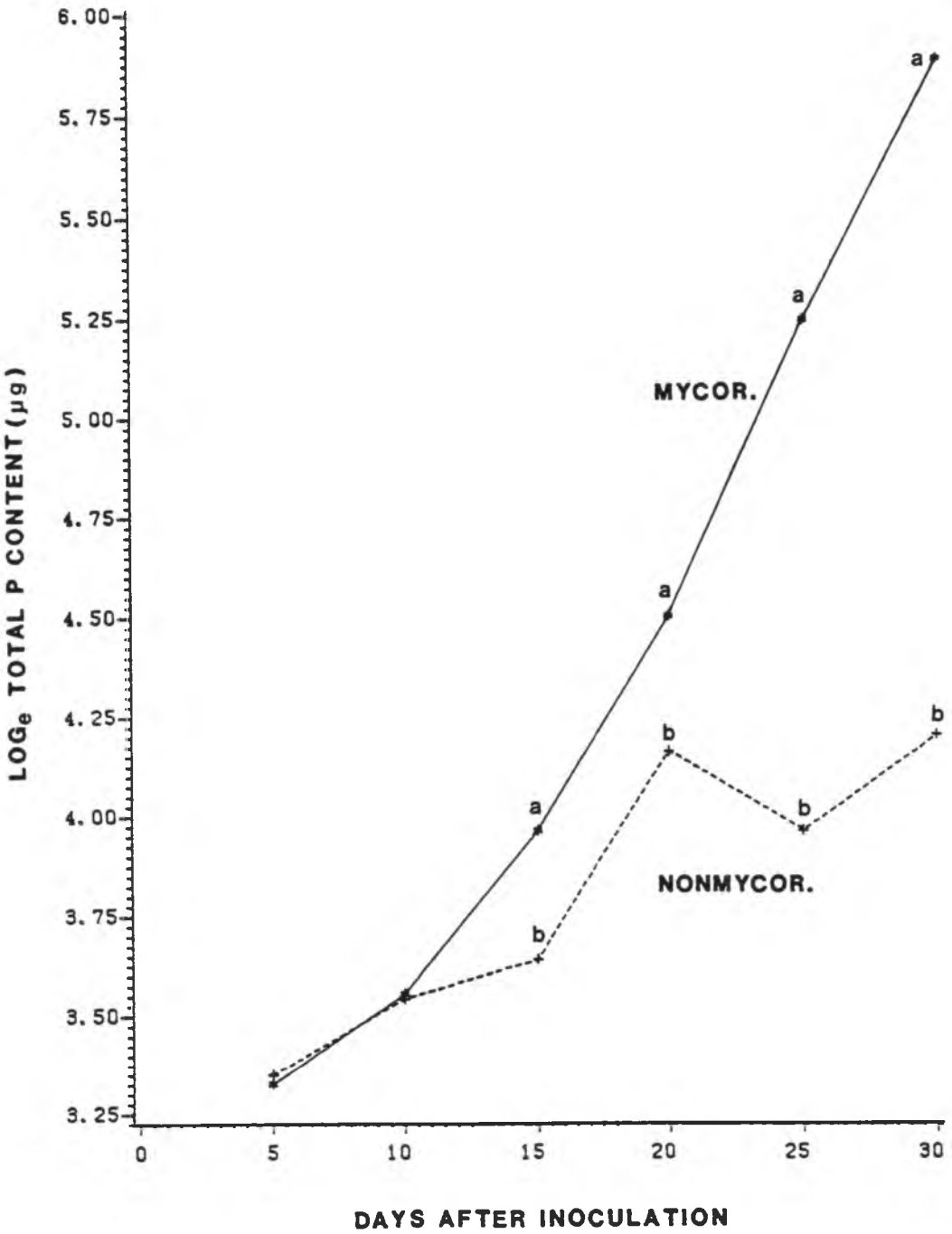

Fig. 15. Time course of total P content of mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings.

nonmycorrhizal plants. After that, these nutrient/K ratios changed every 10 days. Ratios of shoot Ca, Mg and S to K in mycorrhizal plants were lower towards the end of experiment. However, the ratio of shoot P content to K content in mycorrhizal plants was nearly constant (between 9 to 11%). In contrast, 10 days after inoculation the ratio of shoot P content to K content in nonmycorrhizal plants at began to decrease to approximately 6 to 7%. The P/K ratio of nonmycorrhizal plants was especially low and high in other nutrient/K ratios as compared to values in mycorrhizal plants, probably indicating P deficiency in nonmycorrhizal seedlings. Although we have shown that approximately 10 days after inoculation P accumulation first appeared in mycorrhizal roots. It is not definitely clear which element limits growth. We have assumed that it was P that was limited nonmycorrhizal plant growth. Data in Table 7, which is calculated using the nutrient concept of Greenwood (1976), demonstrates that nonmycorrhizal plants had higher nutrient stress factor for P than for other nutrients. P deficiency was probably the most limiting factor in slowing nonmycorrhizal plant growth.

Content of P, K, S, Ca and Mg in relation to cumulative water transpired during 30 days are plotted in Fig. 16 to 20. As indicated in Fig. 16, there were marked differences in total P content as well as cumulative water transpired between mycorrhizal and nonmycorrhizal plants. Both mycorrhizal and nonmycorrhizal plants displayed S-shaped response curves. As shown in Fig. 16, an estimate of initial differences between mycorrhizal and nonmycorrhizal of plants was 400 g of water transpired per pot and which occurred about 10 days after

Table 7. Linear regression coefficients for relative tissue nutrient contents[#] and relative total dry weight[##] for mycorrhizal and nonmycorrhizal, and nutrient stress factor of nonmycorrhizal Leucaena leucocephala seedlings.

Nutrient	Treatment	Intercept (a)[†]	Slope (b)	r	Nutrient stress factor[††] of nonmycorrhizal seedlings
Total	Mycorrhizal	-2.33	1.03	0.99**	
P	Nonmycorrhizal	6.73	0.25	0.88**	0.76[§]
Shoot	Mycorrhizal	0.51	1.03	0.99**	
K	Nonmycorrhizal	4.43	0.62	0.96**	0.40
Shoot	Mycorrhizal	16.02	0.90	0.95**	
S	Nonmycorrhizal	16.19	0.60	0.98**	0.33
Shoot	Mycorrhizal	4.54	0.99	0.99**	
Ca	Nonmycorrhizal	4.28	0.91	0.99**	0.09
Shoot	Mycorrhizal	5.38	0.96	0.98**	
Mg	Nonmycorrhizal	7.64	0.71	0.98**	0.26

\# Relative tissue nutrient content (Y) = 100% X (the tissue nutrient content of each seedling between harvests/the highest tissue nutrient content of a seedling between harvests).

Relative total dry weight (X) = 100% X (the total dry weight of each seedling between harvests/the highest dry weight of a seedling between harvests).

† $Y = b + a X$.

†† Nutrient stress factor of nonmycorrhizal plant = $((a_{Mycor.} - a_{Nonmycor.})/a_{Mycor.})$.

§ For example, P stress factor of nonmycorrhizal plant = ((1.03 - 0.25)/1.03) = 0.76.

** Significantly different at 0.01 level.

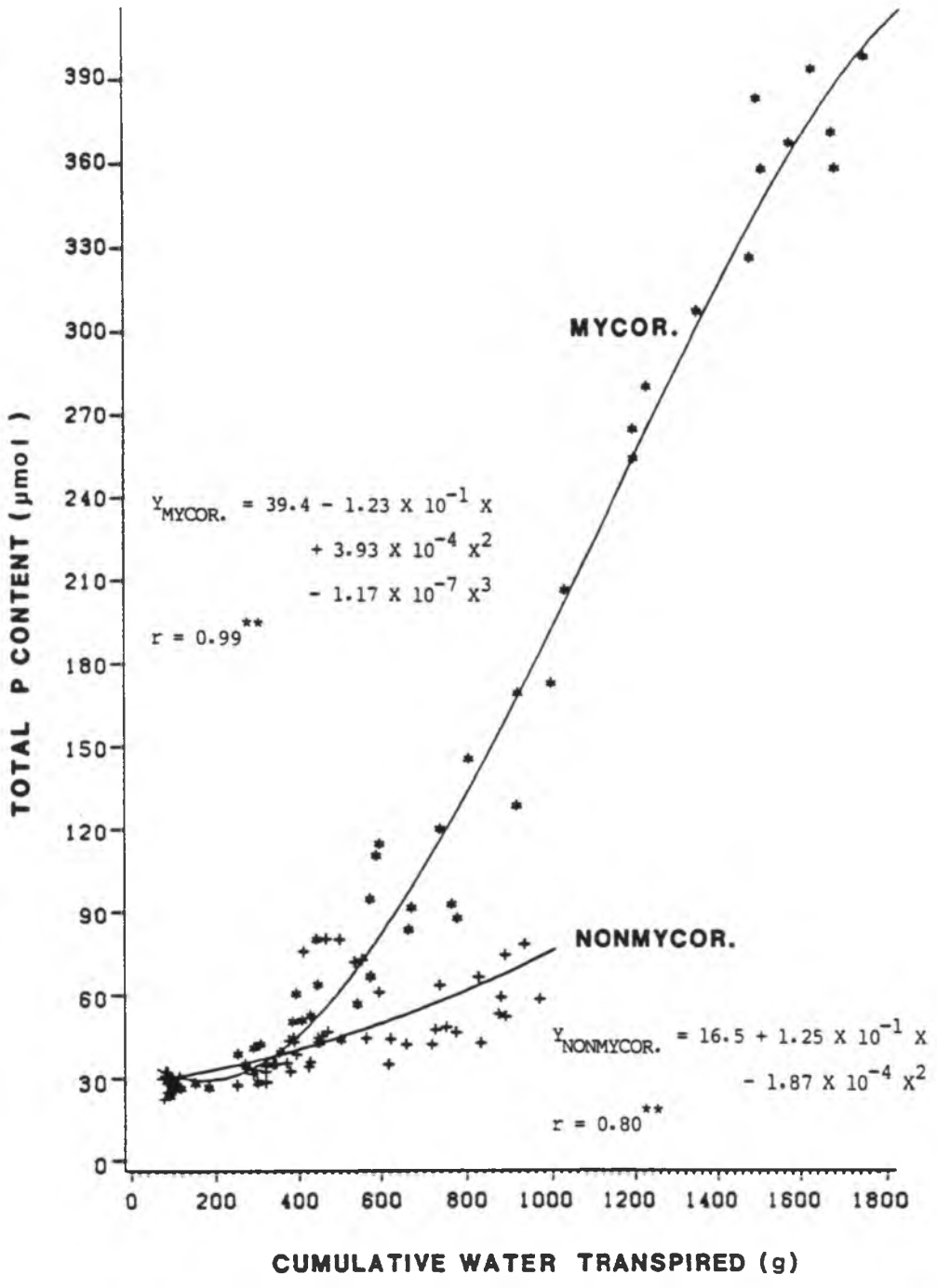

Fig. 16. Total P content as a function of cumulative water transpired by mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings.

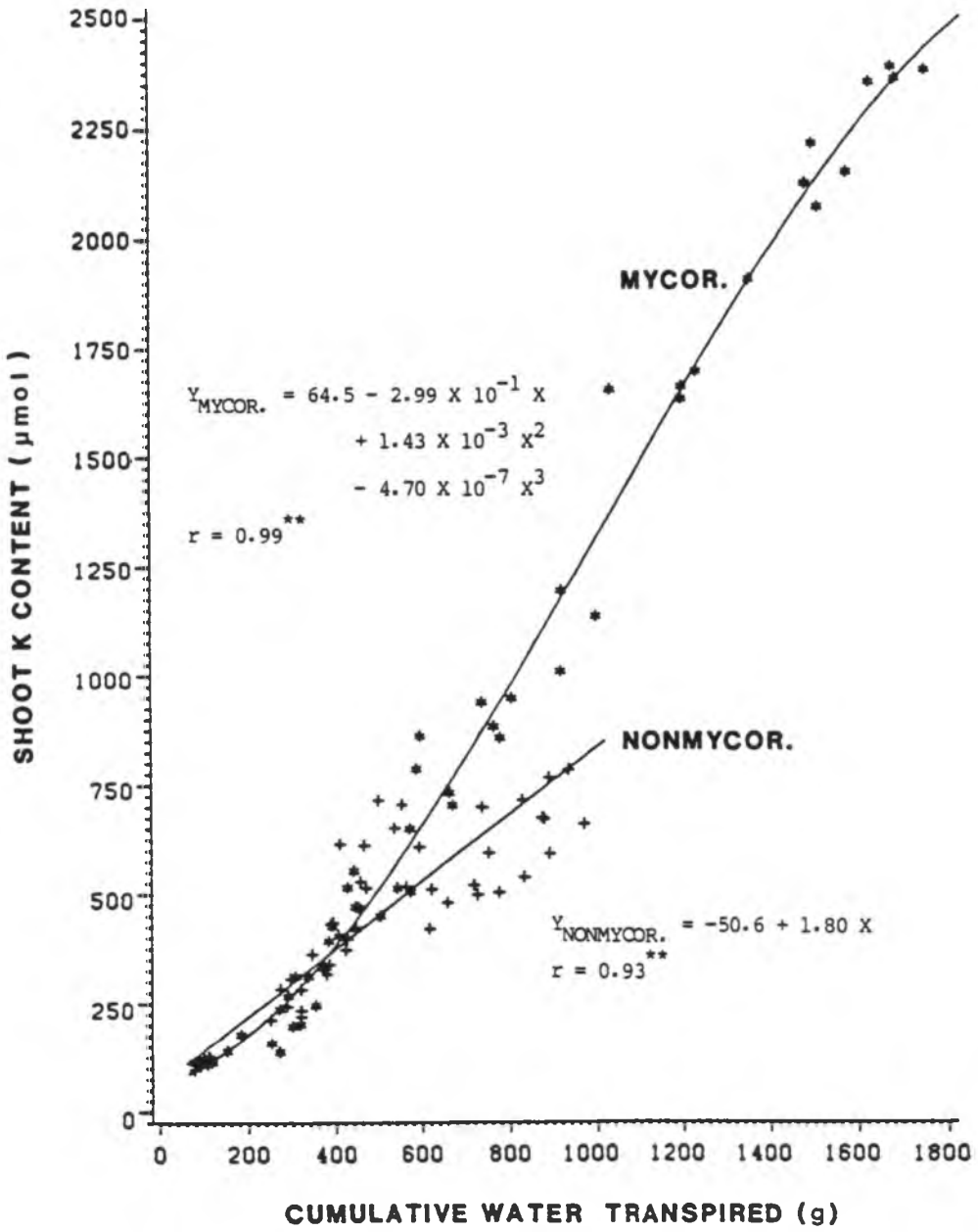

Fig. 17. Shoot K content as a function of cumulative water transpired by mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings.

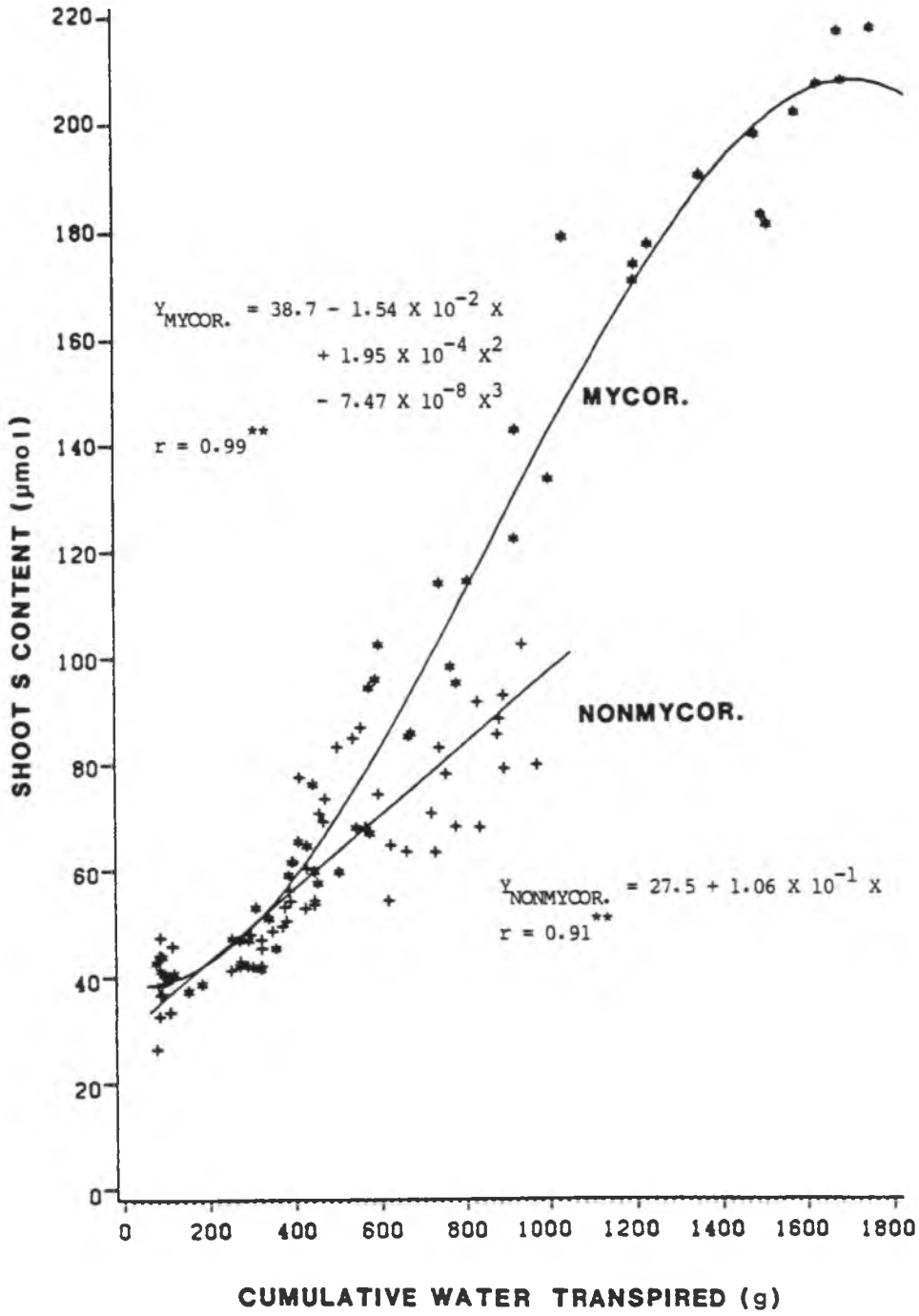

Fig. 18. Shoot S content as a function of cumulative water transpired by mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings.

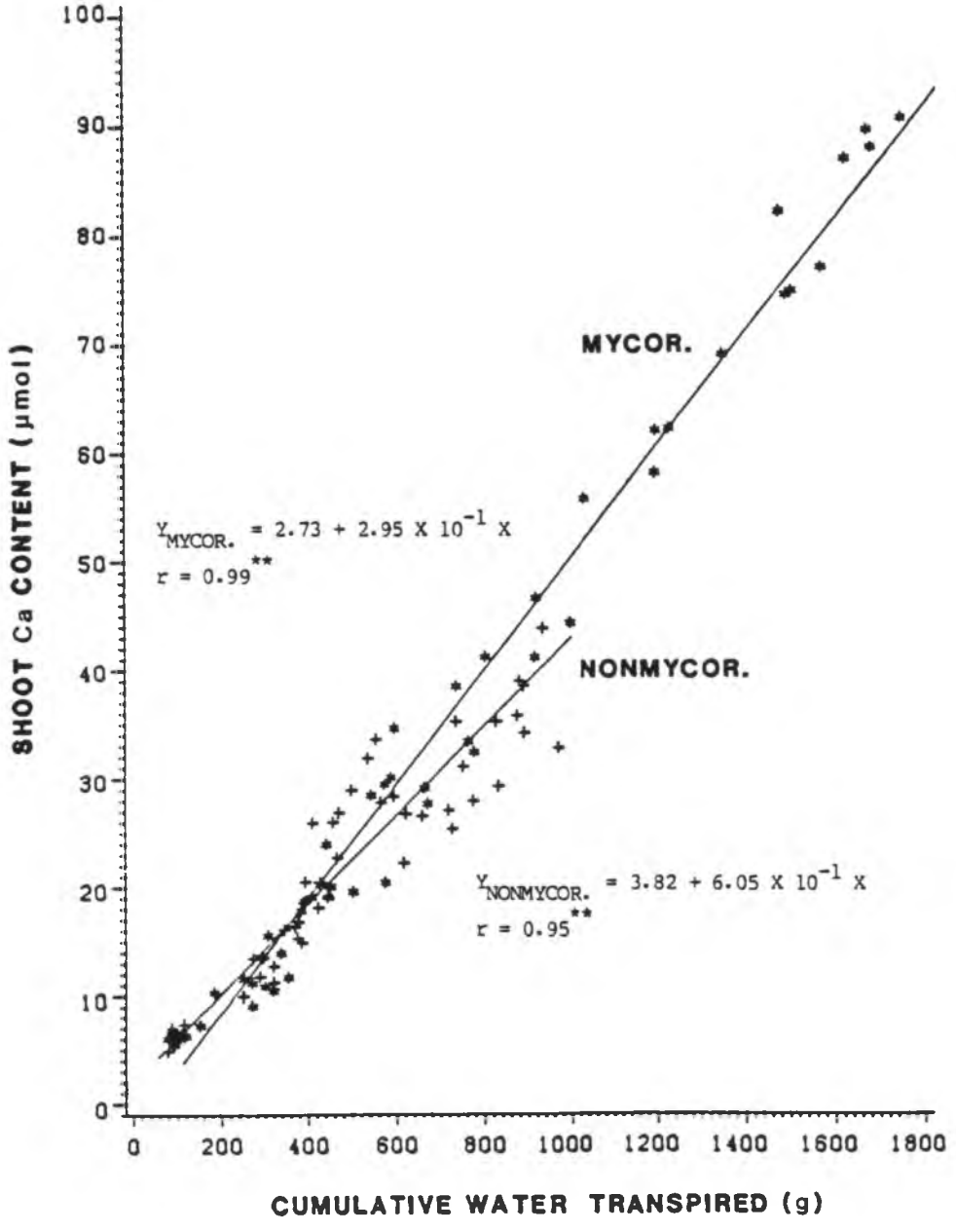

Fig. 19. Shoot Ca content as a function of cumulative water transpired by mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings.

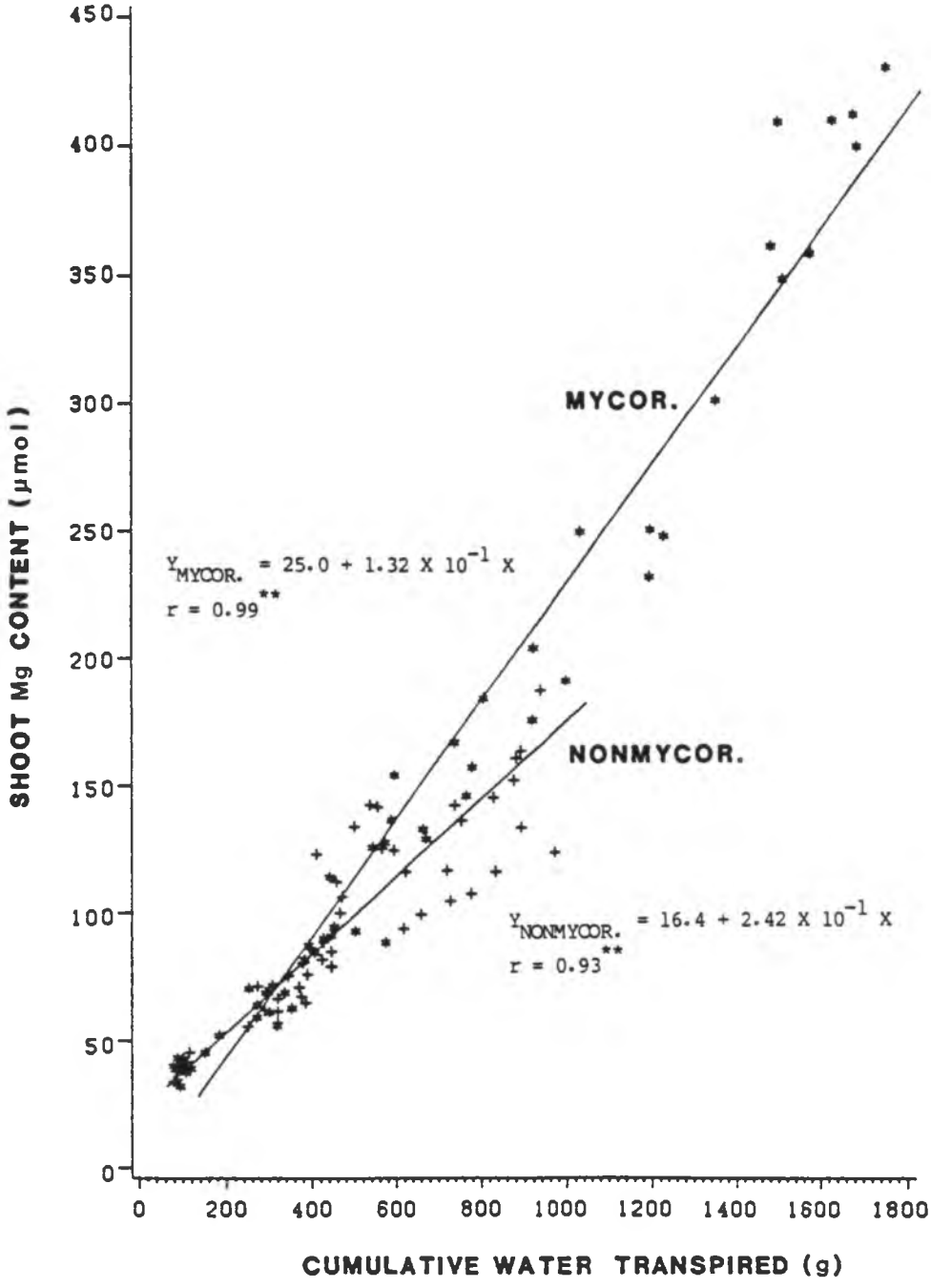

Fig. 20. Shoot Mg content as a function of cumulative water transpired by mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings.

inoculation. Plots of K (Fig. 17) and S content (Fig. 18) also showed an S-shaped pattern. The initial lag phase of K and S contents was not as apparent as that of P, however. In contrast to total P content, plots of shoot Ca and Mg contents against cumulative water transpired were linear (Figs 19 and 20) and the slopes of those curves were similar regardless mycorrhizal status.

The relationship between total P content and root area of mycorrhizal and nonmycorrhizal plants is plotted in Fig. 21. In nonmycorrhizal plants, total P content and root area were not closely related. In contrast, total P contents in mycorrhizal plants were closely correlated with root area. P concentration in nonmycorrhizal plant roots was low (Table 4) which might have limited root absorptive ability. Slopes of total P content plotted vs. the product of root area and root P concentration were similar (Fig. 22).

Growth and Assimilate Partitioning

Differences in growth and assimilate partitioning between mycorrhizal and nonmycorrhizal plants are summarized in Table 8. By 15 days after inoculation, leaf area of mycorrhizal plants was significantly greater than that of nonmycorrhizal plants while shoot dry weight significantly differed at 25 days after inoculation (Table 8). Root dry weights of nonmycorrhizal plants, however, were greater than those of mycorrhizal plants at 20 days after inoculation but with no differences apparent at 25 days. The general trends observed in

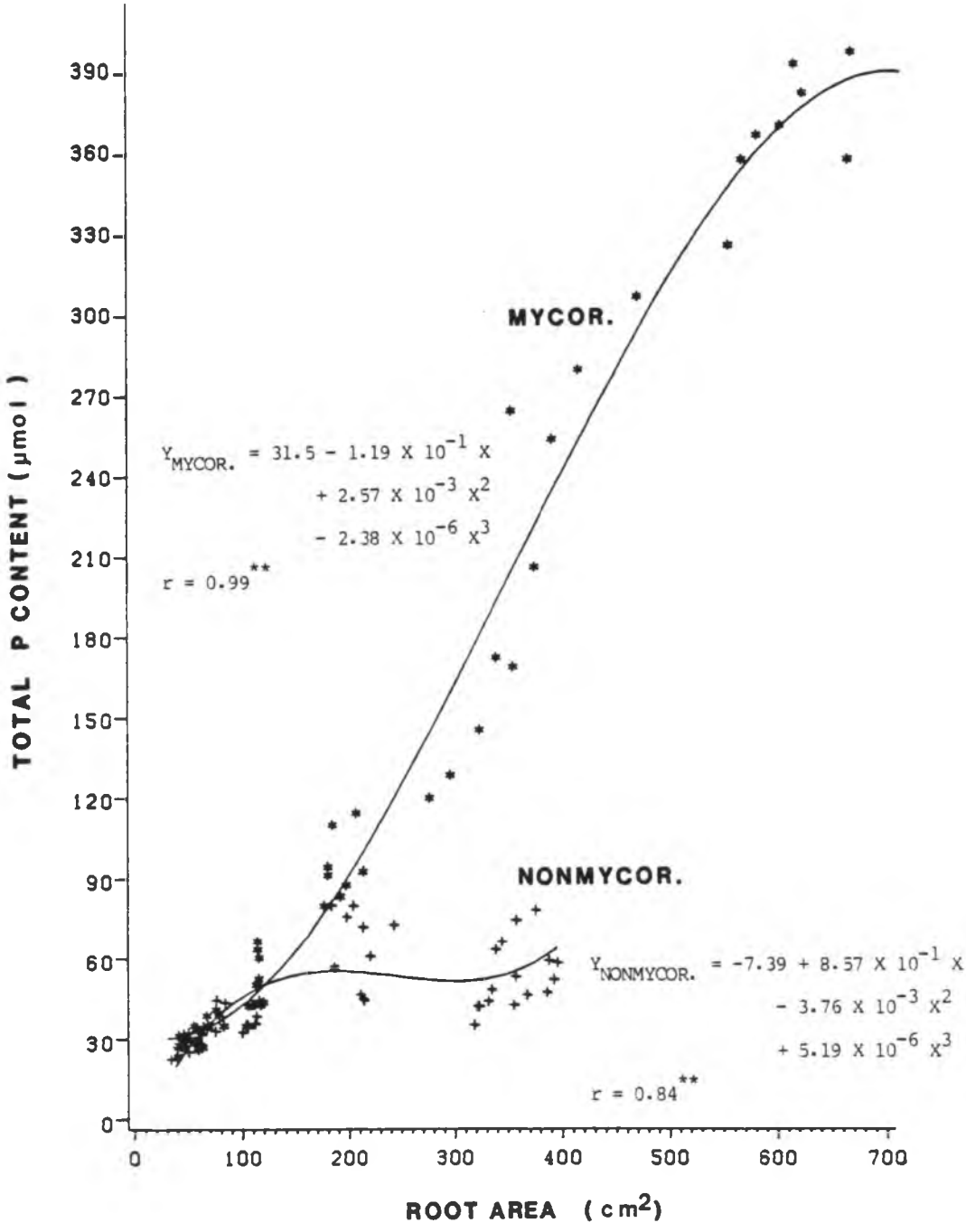

Fig. 21. Total P content as a function of root area of mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings.

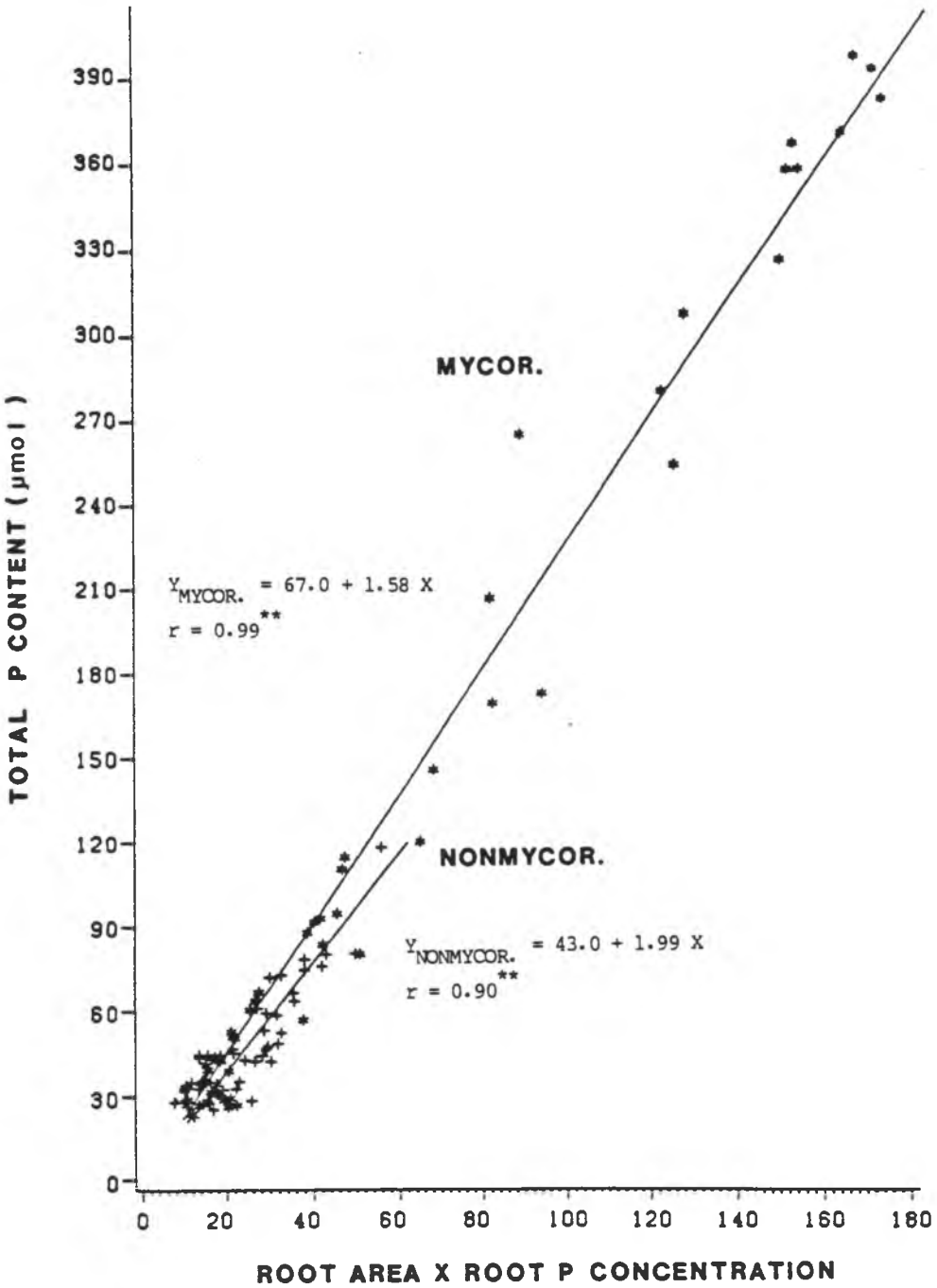

Fig. 22. Total P content as a function of the product of root area and root P concentration of mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings.

Table 8. Effect of mycorrhizal inoculation on root infection, dry weight of shoots and roots, area of leaf and roots in Leucaena leucocephala seedlings.

Treatment	Days after inoculation					
	5	10	15	20	25	30
	Mycorrhizal infection (%)					
Mycorrhizal	6.9 a[#]	17.3 a	22.6 a	28.0 a	41.0 a	47.1 a
Nonmycorrhizal	0 b	0 b	0 b	0 b	0 b	0 b
	Shoot dry weight (g)					
Mycorrhizal	0.21 a	0.30 a	0.50 a	0.82 a	1.35 a	2.57 a
Nonmycorrhizal	0.20 a	0.33 a	0.49 a	0.78 a	0.89 b	1.21 b
	Root dry weight (g)					
Mycorrhizal	0.05 a	0.15 a	0.25 a	0.34 b	0.58 a	1.33 a
Nonmycorrhizal	0.05 a	0.15 a	0.23 a	0.41 a	0.55 a	0.76 b
	Leaf area (cm^2)					
Mycorrhizal	31 a	60 a	109 a	193 a	274 a	480 a
Nonmycorrhizal	30 a	68 a	98 b	168 b	146 b	177 b
	Root area (cm^2)					
Mycorrhizal	49 a	67 a	114 a	190 a	346 a	590 a
Nonmycorrhizal	48 a	66 a	108 a	211 a	336 a	399 b

Means of each variable in the same columns within harvests followed by the same letter are not significantly different at the 5% level.

increasing total dry weight of mycorrhizal and nonmycorrhizal plants were shown by the exponential pattern (Fig. 23). There were no differences between mycorrhizal and nonmycorrhizal plants in total dry weight during 5 to 20 days after inoculation. Data from subsequent harvests (25 and 30 days), however, reflect enhanced accumulation of P in mycorrhizal plants.

Root weight to shoot weight ratio (Fig. 24) and root area to leaf area ratio (Fig. 25) varied with harvests according to the different patterns of assimilate partitioning between mycorrhizal and nonmycorrhizal plants. At 10 days after inoculation, mycorrhizal plants had a higher ratio of root area to leaf area than the nonmycorrhizal plants, however by 20 days after inoculation the relation reversed.

Nonmycorrhizal plants began abscising leaves after 21 to 24 days resulting in temporarily reduced leaf area at the harvest made 25 days after inoculation. P content of abscised leaves was very low (ca. 0.3 µg P per pinnule) compared with the content of attached pinnule (ca. 1.5 µg), suggesting that approximate 80% of the leaf P was translocated before leaf abscission.

Estimated total root area per pot was of the same order of magnitude as the leaf area per pot (Table 8). Mycorrhizal plants had a significantly lower ratio of root area to leaf area than nonmycorrhizal plants. From day 15 to day 20, nonmycorrhizal plants apparently increased root area in response to P deficiency (Table 8) while P concentration in nonmycorrhizal roots dropped from 0.15 to 0.09% (Table 4).

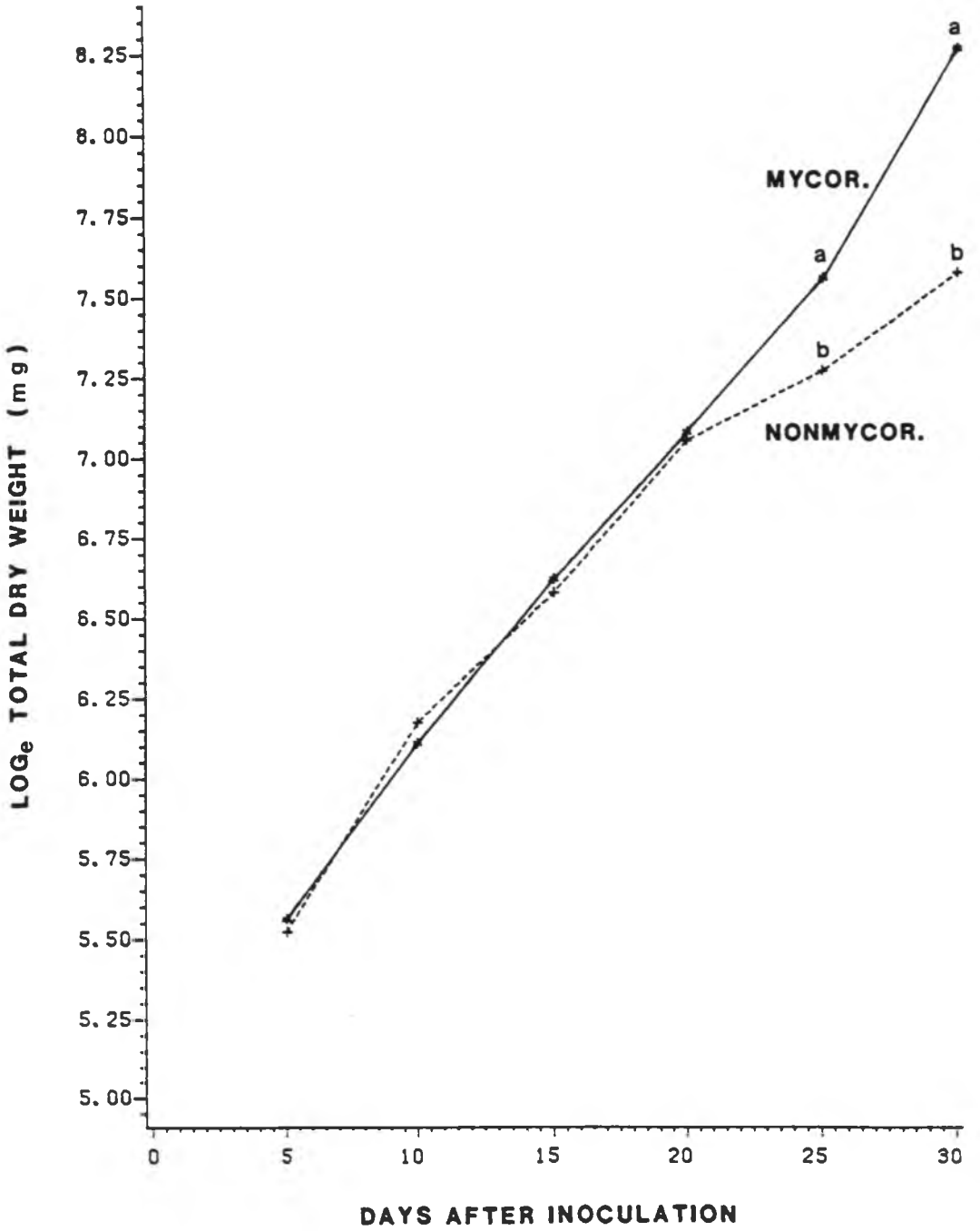

Fig. 23. Time course of total dry weight of mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings. seedlings.

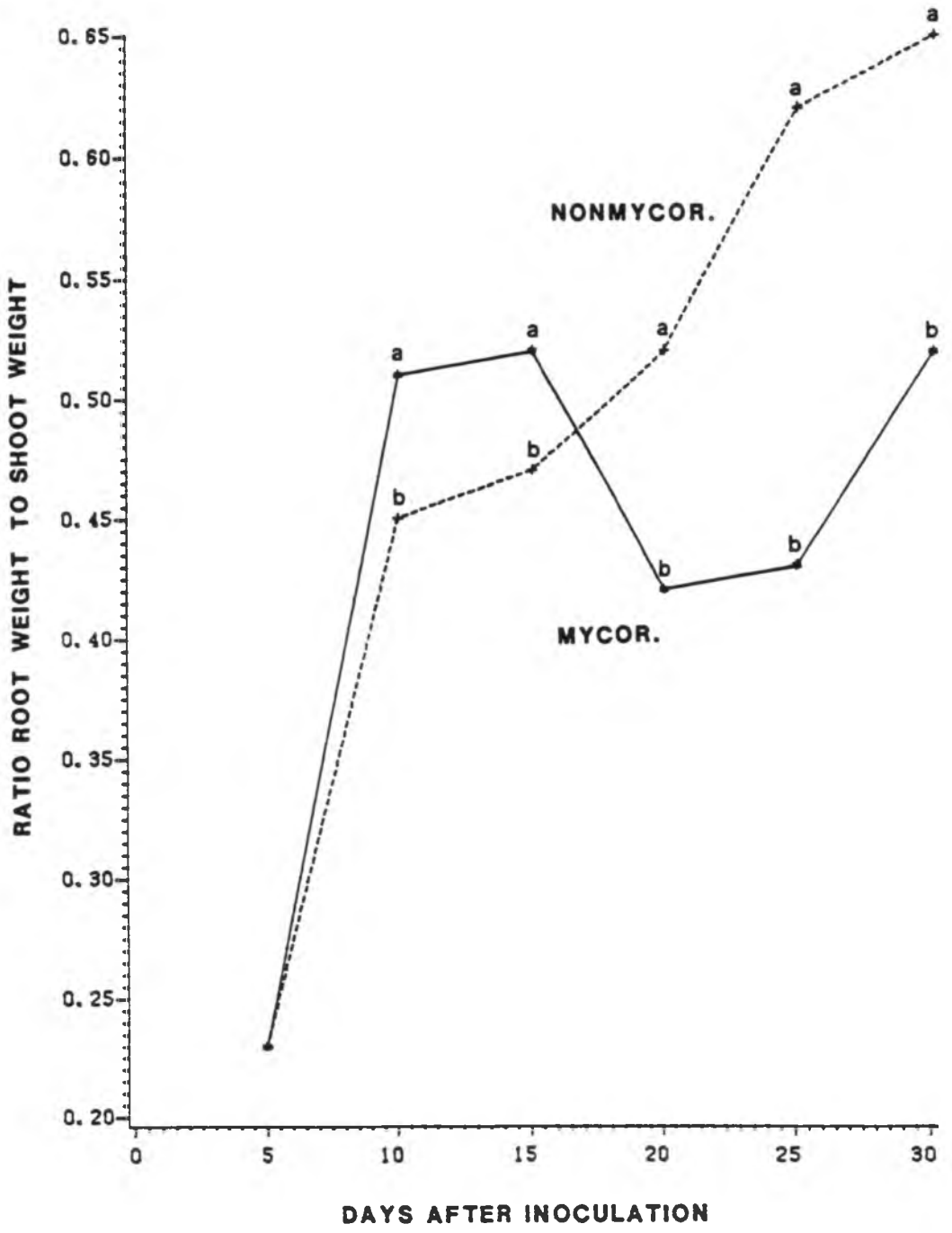

Fig. 24. Time course of root dry weight/shoot dry weight of mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings.

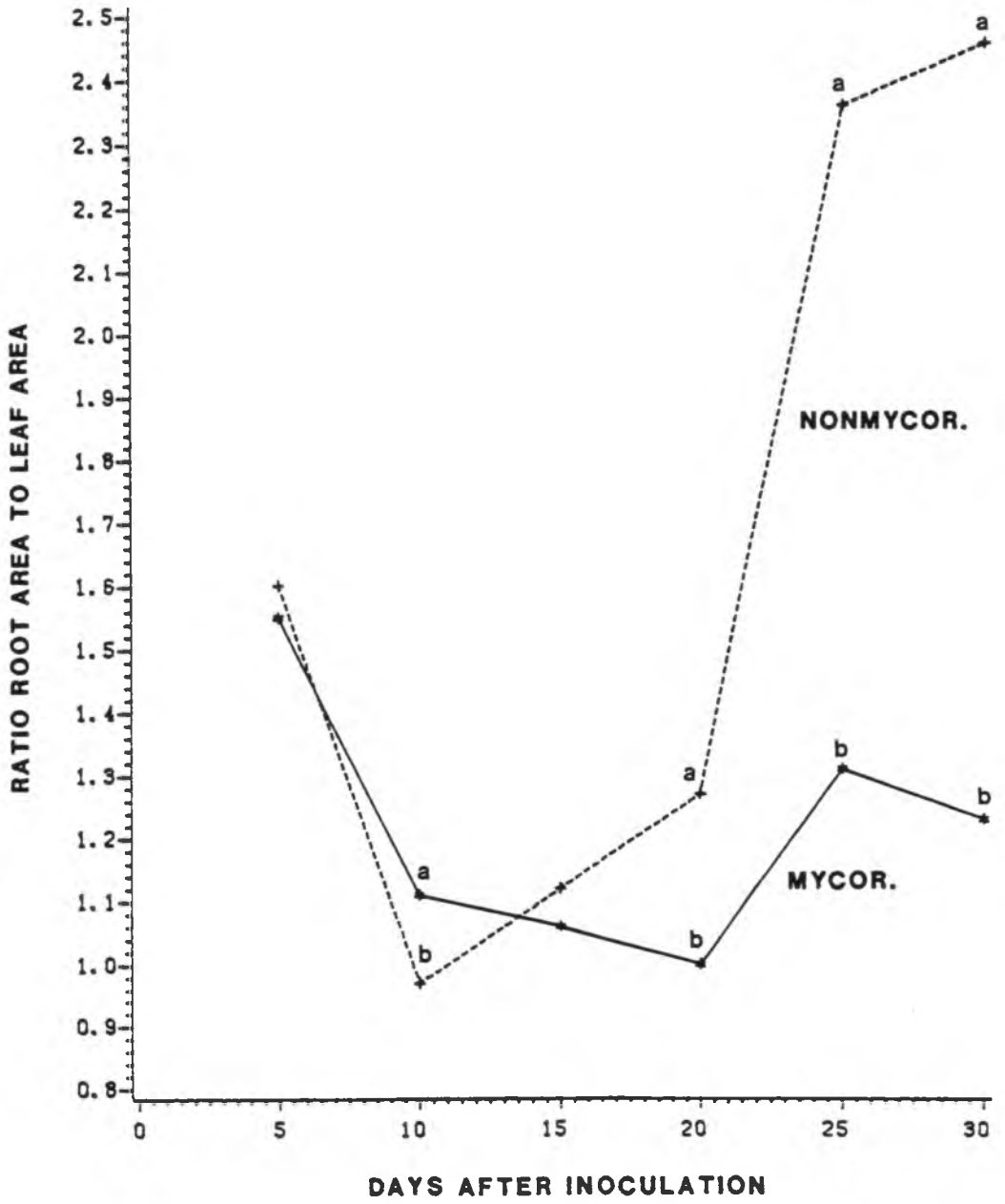

Fig. 25. Time course of root area/leaf area of mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings.

Water Relations and Leaf Morphology

Water transpiration and leaf measurements are listed in Table 9. Beginning 15 days after inoculation, the amount and rate of transpiration in mycorrhizal plants was higher than in nonmycorrhizal plants whereas the cumulative water transpiration was significantly different only at 20 days after inoculation. Significant differences in leaf moisture content (leaf fresh weight/leaf dry weight) during the 15 to 25 days interval and specific leaf area (leaf area/leaf dry weight) during the 15 to 30 days interval were observed between mycorrhizal and nonmycorrhizal plants. After a drastic increase in cumulative transpiration, mycorrhizal plants transpired about 120 g of water per pot per day (as much as 50% of the available water) at 30 days after inoculation. The additional water transpired by mycorrhizal plants probably resulted in increased water stress. This was apparent from the reduced leaf moisture content at the 30 days harvest.

Correlations between total dry weight, leaf area, root area and cumulative water transpiration are shown in Table 10. These four variables were closely related. Coefficients of correlation were smaller, probably because of the rapid response of leaf area to P stress.

Table 11 summarizes the significant differences between mycorrhizal and nonmycorrhizal plants; growth, water transpiration and nutrient acquisition of each harvest. These parameters were used to evaluate the mycorrhizal effects on early physiological events in leucaena seedlings.

Table 9. Effect of mycorrhizal inoculation on daily water transpired, cumulative water transpired, transpiration rate, water use efficiency, leaf moisture content and specific leaf area of Leucaena leucocephala seedlings.

Treatment	Days after inoculation					
	5	10	15	20	25	30
	Daily water transpired (g)					
Mycorrhizal	23.6 a#	29.0 a	31.0 a	50.5 a	73.6 a	120.5 a
Nonmycorrhizal	22.8 a	28.7 a	27.6 b	38.3 b	30.8 b	40.2 b
	Cumulative water transpired (g)					
Mycorrhizal	103 a	286 a	432 a	624 a	972 a	1561 a
Nonmycorrhizal	91 a	314 a	401 a	506 b	744 b	935 b
	Transpiration rate ($g\ m^{-2} sec^{-1}$)					
Mycorrhizal	0.28 a	0.12 a	0.06 a	0.06 a	0.06 a	0.06 a
Nonmycorrhizal	0.18 a	0.11 a	0.04 b	0.05 a	0.05 b	0.07 a
	Water use efficiency ($g\ kg^{-1}$)					
Mycorrhizal	2.56 a	1.59 a	1.76 a	1.88 b	1.97 a	2.50 a
Nonmycorrhizal	2.73 a	1.55 a	1.79 a	2.36 a	1.94 a	2.08 b
	Leaf moisture content					
Mycorrhizal	4.13 a	4.49 a	4.67 a	4.92 a	5.11 a	4.51 a
Nonmycorrhizal	4.03 a	4.63 a	4.34 b	4.42 b	4.68 b	4.51 a
	Specific leaf area ($cm^2\ mg^{-1}$)					
Mycorrhizal	2.28 a	3.22 a	3.26 a	3.42 a	2.92 a	2.80 a
Nonmycorrhizal	2.75 a	3.25 a	2.97 b	3.02 b	2.42 b	2.23 b

Means of each variable in the same column within each harvest date followed by the same letter are not significantly different at the 5% level. Comparisons are valid only within each harvest.

Table 10. Correlation coefficients between total dry weight, cumulative water transpired, leaf area, root area of mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings.

Parameters	Mycorrhizal	Nonmycorrhizal
	r	
Total dry weight vs. Cumulative water transpired	0.99**	0.97**
Total dry weight vs. Leaf area	0.99**	0.89**
Total dry weight vs. Root area	0.99**	0.98**
Cumulative water transpired vs. Leaf area	0.98**	0.82**
Cumulative water transpired vs. Root area	0.99**	0.98**
Leaf area vs. Root area	0.98**	0.79**

** Significantly different at 0.01 level.

Table 11. The sequence of nutrient concentration and growth events following mycorrhizal inoculation of Leucaena leucocephala seedlings.

Plant parameters	Days after inoculation	Comparisons between mycorrhizal (M) and nonmycorrhizal (N) plants
Pinnule P concentration	12	M > N#
Pinnule P content	12##	M > N
Pinnule dry weight	14##	M < N
	19##	M > N
Root P concentration	10	M > N
Shoot P concentration	15	M > N
Shoot K concentration	15	M > N
Shoot S concentration	15	M > N
Shoot Mg concentration	20	M > N
Shoot Ca concentration	25	M > N
Root/shoot ratio	10##	M > N
(dry weight basis)	20##	M < N
Root/leaf ratio	10##	M > N
(area basis)	20##	M < N
Leaf area	15##	M > N
Root area	30##	M > N
Root dry weight	20##	M < N
	30##	M > N
Shoot dry weight	25	M > N
Total dry weight	25	M > N
Daily water transpired	11-14	M > N
Transpiration rate	15	M > N
Leaf moisture content	15	M > N
Specific leaf area	15	M > N
Cumulative water transpired	20##	M > N
Water use efficiency	20##	M < N
	30##	M > N

Result either M > N or M < N was significantly different at the 5% level.

Indicate two or three alternate differences between mycorrhizal and nonmycorrhizal treatments.

Differences in state variables (cf. Fig. 4), except for pennule dry weight, root area and root dry weight, were statistically significant between mycorrhizal and nonmycorrhizal plants. Once the difference appeared it remained consistent during subsequent harvests.

Rates of Physiological Processes

Relative growth rates, relative leaf area and root area expansion rates, and relative water transpiration rates are presented in Table 12. Mycorrhizal plants had a nearly constant relative growth rate during the growth period, indicative of exponential growth (Fig. 21).

During the first harvest interval (5 to 10 days after inoculation), nonmycorrhizal plants showed significantly greater relative growth rate, relative leaf area expansion rate (Table 12), net assimilation rate and unit root area rate and component leaf production rate (Table 13) than that of mycorrhizal plants.

During the second harvest interval (10 to 15 days after inoculation), however, mycorrhizal plants showed a significantly higher relative leaf area expansion rate (Table 12) and component leaf production rate (Table 13) than nonmycorrhizal plants. These results were paralleled by significantly different P accumulation rate (Table 14), P flux to roots (Table 15) and P mass flow rate (defined as the changes of P content per unit of time per unit of cumulative water transpired) (Table 16). These data are consistent with enhanced P

Table 12. Effects of mycorrhizal inoculation on relative growth rate, relative transpiration rate, relative leaf area expansion rate and relative root area expansion rate of Leucaena leucocephala seedlings.

Treatment	Harvest interval, days after inoculation				
	5-10	10-15	15-20	20-25	25-30
	Relative growth rate (mg $g^{-1} day^{-1}$)				
Mycorrhizal	11 b[#]	10 a	9 a	10 a	14 a
Nonmycorrhizal	13 a	8 a	10 a	4 b	6 b
	Relative transpiration rate (mg $g^{-1} day^{-1}$)				
Mycorrhizal	21 b	8 a	7 a	9 a	9 a
Nonmycorrhizal	25 a	5 a	4 b	7 a	4 b
	Relative leaf area expansion rate (cm^2 cm^{-2} day^{-1})				
Mycorrhizal	0.013 b	0.012 a	0.011 a	0.006 a	0.012 a
Nonmycorrhizal	0.016 a	0.007 b	0.011 a	-0.003 b	0.003 b
	Relative root area expansion rate (cm^2 cm^{-2} day^{-1})				
Mycorrhizal	0.006 a	0.011 a	0.010 b	0.012 a	0.011 a
Nonmycorrhizal	0.006 a	0.010 a	0.013 a	0.009 b	0.003 b

Means of each variable in the same columns within harvests followed by the same letter are not significantly different at the 5% level.

Table 13. Effects of mycorrhizal inoculation on net assimilation rate, unit root rate, component leaf production rate, and component root production rate of Leucaena leucocephala seedlings.

Treatment	Harvest interval, days after inoculation				
	5-10	10-15	15-20	20-25	25-30
	Net assimilation rate (mg cm^{-2} day^{-1})				
Mycorrhizal	0.86 b#	0.75 a	0.56 a	0.64 a	1.12 a
Nonmycorrhizal	1.01 a	0.58 a	0.73 a	0.32 b	0.60 b
	Unit root rate (mg cm^{-2} day^{-1})				
Mycorrhizal	0.67 b	0.69 a	0.55 a	0.58 a	0.87 a
Nonmycorrhizal	0.84 a	0.55 a	0.61 a	0.19 b	0.27 b
	Component leaf production rate (cm^2 mg^{-1} day^{-1})				
Mycorrhizal	1.93 b	1.94 a	2.44 a	0.85 a	1.27 a
Nonmycorrhizal	2.65 a	1.03 b	1.69 a	0.96 a	0.31 b
	Component root production rate (cm^2 mg^{-1} day^{-1})				
Mycorrhizal	0.73 a	1.79 a	1.93 a	2.63 b	1.32 a
Nonmycorrhizal	0.80 a	2.01 a	2.94 a	5.39 a	0.45 b

Means of each variable in the same columns within harvests followed by the same letter are not significantly different at the 5% level.

Table 14. Effects of mycorrhizal inoculation on relative nutrient accumulation rates of Leucaena leucocephala seedlings.

Treatment	Harvest interval, days after inoculation				
	5-10	10-15	15-20	20-25	25-30
	μmol mol^{-1} day^{-1}				
	Total P accumulation rate				
Mycorrhizal	4.5 a[#]	8.0 a	10.7 a	14.7 a	13.4 a
Nonmycorrhizal	3.7 a	2.1 b	10.0 a	-4.2 b	4.3 b
	Shoot K accumulation rate				
Mycorrhizal	11.5 b	14.6 a	10.0 a	11.7 a	10.9 a
Nonmycorrhizal	17.3 a	7.1 a	9.0 a	-0.6 b	3.4 b
	Shoot S accumulation rate				
Mycorrhizal	4.1 a	6.2 a	7.5 a	9.9 a	6.4 a
Nonmycorrhizal	1.8 a	3.0 b	6.9 a	-0.3 b	3.0 b
	Shoot Ca accumulation rate				
Mycorrhizal	11.7 b	10.2 a	9.1 a	10.1 a	10.0 a
Nonmycorrhizal	13.9 a	7.6 b	8.5 a	1.8 b	4.0 b
	Shoot Mg accumulation rate				
Mycorrhizal	8.9 a	6.6 a	8.9 a	8.7 a	12.0 a
Nonmycorrhizal	9.7 a	4.6 a	8.5 a	0.8 b	3.4 b

Means of each variable in the same column followed by the same letter are not significantly different at the 5% level. Comparisons are valid only within each harvest interval.

Table 15. Effects of mycorrhizal inoculation on nutrient fluxes to roots of Leucaena leucocephala seedlings.

Treatment	Harvest interval, days after inoculation				
	5–10	10–15	15–20	20–25	25–30
	nmol cm^{-2} day^{-1}				
	Total P flux				
Mycorrhizal	25 a#	40 a	50 a	75 a	77 a
Nonmycorrhizal	22 a	9 b	34 a	-9 b	8 b
	Shoot K flux				
Mycorrhizal	335 b	504 a	386 a	448 a	399 a
Nonmycorrhizal	563 a	269 b	287 a	-11 b	70 b
	Shoot S flux				
Mycorrhizal	13 a	37 a	38 a	45 a	24 a
Nonmycorrhizal	30 a	18 b	29 a	-1 b	7 b
	Shoot Ca flux				
Mycorrhizal	176 b	171 a	147 a	153 a	139 a
Nonmycorrhizal	226 a	135 a	125 a	21 b	42 b
	Shoot Mg flux				
Mycorrhizal	79 a	55 a	65 a	57 a	75 a
Nonmycorrhizal	88 a	39 a	55 a	4 b	15 b

Means of each variable in the same column followed by the same letter are not significantly different at the 5% level. Comparisons are valid only within each harvest interval.

Table 16. Effects of mycorrhizal inoculation on nutrient mass flow rates of Leucaena leucocephala seedlings.

Treatment	Harvest interval, days after inoculation				
	5–10	10–15	15–20	20–25	25–30
	nmol g^{-1} day^{-1}				
	Total P mass flow rate				
Mycorrhizal	8 a[#]	10 a	14 a	25 a	28 a
Nonmycorrhizal	7 a	2 b	12 a	-5 b	3 b
	Shoot K mass flow rate				
Mycorrhizal	107 b	128 a	110 a	148 a	147 a
Nonmycorrhizal	175 a	66 b	98 a	-7 b	29 b
	Shoot S mass flow rate				
Mycorrhizal	9 a	9 a	11 a	15 a	9 a
Nonmycorrhizal	4 a	4 b	10 a	-1 b	3 b
	Shoot Ca mass flow rate				
Mycorrhizal	57 a	44 a	42 a	50 a	51 a
Nonmycorrhizal	70 a	32 a	43 a	9 b	18 b
	Shoot Mg mass flow rate				
Mycorrhizal	25 a	14 a	19 a	19 a	28 a
Nonmycorrhizal	27 a	9 a	19 a	2 b	6 b

Means of each variable in the same column followed by the same letter are not significantly different at the 5% level. Comparisons are valid only within each harvest interval.

concentrations of roots (10 days after inoculation) and shoots (15 days after inoculation) (Table 4). Increased P accumulation rate and P flux to roots were evident in mycorrhizal plants. Similarly, mycorrhizal plants demonstrated increased nutrient accumulation rates for S and Ca (Table 14) and increased nutrient flux and nutrient mass flow rates for K and S (Tables 15 and 16, respectively).

During the third harvest interval (15 to 20 days after inoculation), a sudden increase in relative root area expansion rate in nonmycorrhizal plants was observed (Table 12). During this period there were no differences between mycorrhizal and nonmycorrhizal plants in nutrient accumulation rates (Table 14) and fluxes to root (Table 15). Before and after this period, however, P accumulation rates and fluxes in mycorrhizal plants were greater than nonmycorrhizal plants. Although nutrient accumulation rates were not different during this period, nonmycorrhizal plants showed significantly higher specific utilization rates for all nutrients (Table 17) than mycorrhizal plants for all nutrients.

During the fourth and fifth harvest intervals (20 to 30 days after inoculation), nonmycorrhizal plants shed leaves prematurely and growth slowed. The component root production rates (Table 13), nutrient accumulation rates (Table 14), nutrient fluxes (Table 15) and nutrient mass flow rates (Table 16) for all nutrients of nonmycorrhizal plants were significantly lower than those of mycorrhizal plants. There was no difference in specific utilization rate of P between mycorrhizal and nonmycorrhizal plants (Table 17), but other nutrients differed at certain harvest intervals.

Table 17. Effects of mycorrhizal inoculation on nutrient utilization rates of Leucaena leucocephala seedlings.

Treatment	Harvest interval, days after inoculation				
	5-10	10-15	15-20	20-25	25-30
	g mol^{-1} day^{-1}				
	Total P utilization rate				
Mycorrhizal	1221 b[#]	1448 a	1189 b	1106 a	1556 a
Nonmycorrhizal	1497 a	1312 a	1945 a	907 a	1604 a
	Shoot K utilization rate				
Mycorrhizal	236 a	199 a	144 b	150 a	237 a
Nonmycorrhizal	260 a	146 a	195 a	85 b	145 b
	Shoot S utilization rate				
Mycorrhizal	90 a	117 a	110 b	128 a	235 a
Nonmycorrhizal	113 a	94 a	147 a	66 b	117 b
	Shoot Ca utilization rate				
Mycorrhizal	443 a	411 a	341 b	379 a	629 a
Nonmycorrhizal	521 a	311 a	415 a	172 b	277 b
	Shoot Mg utilization rate				
Mycorrhizal	75 b	82 a	74 b	87 a	140 a
Nonmycorrhizal	94 a	66 a	95 a	40 b	67 b

Means of each variable in the same column followed by the same letter are not significantly different at the 5% level. Comparisons are valid only within each harvest interval.

Table 18 shows that, during the first harvest interval (5 to 10 days after inoculation), water flux to leaves of nonmycorrhizal plants was significantly greater than that of mycorrhizal plants. However, during the third (15 to 20 days after inoculation) and fifth (25 to 30 days after inoculation) harvest intervals water fluxes in nonmycorrhizal plants were lower than in mycorrhizal plants.

Water flux to leaves of mycorrhizal plants was almost constant during the third, fourth and fifth harvest intervals and approximately 100, 40 and 160%, higher than in nonmycorrhizal plants, respectively (Table 18). Water flux to the roots of mycorrhizal plants was higher than flux to nonmycorrhizal plants only during the third harvest interval. While the growth of nonmycorrhizal plants was stunted during the last two harvests, the specific utilization rate of water in nonmycorrhizal plants was significantly lower than that of mycorrhizal plants (Table 18). The calculated transpiration flux based on leaf area was nearly the same as water flux based on root area.

Table 19 summarizes the comparisons of rate variables between mycorrhizal and nonmycorrhizal leucaena seedlings in different development phases, including the initial phase, represented by harvest at 5 and 10 days, the transitional phase (after intervals of 10 to 20 days) and the quasi-steady state phase (after intervals of 20 to 30 days).

Table 18. Effects of mycorrhizal inoculation on water flux to root, water flux to leaf, and water utilization rate of Leucaena leucocephala seedlings.

Treatment	Harvest interval, days after inoculation				
	5-10	10-15	15-20	20-25	25-30
	Water flux to root ($\mu g\ cm^{-2}\ day^{-1}$)				
Mycorrhizal	0.84 a#	0.35 a	0.26 a	0.31 a	0.23 a
Nonmycorrhizal	0.97 a	0.22 a	0.16 b	0.30 a	0.23 a
	Water flux to leaf ($\mu g\ cm^{-2}\ day^{-1}$)				
Mycorrhizal	0.66 b	0.33 a	0.26 a	0.26 a	0.26 a
Nonmycorrhizal	0.80 a	0.21 a	0.13 b	0.18 b	0.10 b
	Water utilization rate ($\mu g\ g^{-1}\ day^{-1}$)				
Mycorrhizal	91 a	78 a	72 a	75 a	131 a
Nonmycorrhizal	114 a	65 a	79 a	44 b	54 b

Means of each variable in the same columns within harvests followed by the same letter are not significantly different at the 5% level.

Table 19. Comparison of rate variables between mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings in different development phases.

Rate variable		Development phases: Initial phase (5-10 DAI#)	Transitional phase (10-20 DAI)	Quasi-steady phase (20-30 DAI)
Relative water transpiration rate		M < N##	M > N	M > N
Relative growth rate		M < N	M > N	M > N
Relative leaf area expansion rate		M < N	M > N	M > N
Relative root area expansion rate		M = N	M < N	M > N
Relative nutrient accumulation rate	P	M = N	M > N	M > N
	K	M < N	M = N	M > N
	S	M = N	M > N	M > N
	Ca	M < N	M > N	M > N
	Mg	M = N	M = N	M > N
Water flux to root		M = N	M > N	M > N
Water flux to leaf		M = N	M > N	M = N
Nutrient flux to root	P	M = N	M > N	M > N
	K	M < N	M > N	M > N
	S	M = N	M > N	M > N
	Ca	M < N	M = N	M > N
	Mg	M = N	M = N	M > N
Nutrient mass flow rate	P	M = N	M > N	M > N
	K	M < N	M > N	M > N
	S	M = N	M > N	M > N
	Ca	M = N	M = N	M > N
	Mg	M = N	M = N	M > N
Net assimilation rate		M < N	M = N	M > N
Unit root rate		M < N	M = N	M > N
Component leaf area production rate		M < N	M > N	M > N
Component root area production rate		M = N	M < N	M > N
Utilization rate for water		M = N	M = N	M > N
Utilization rate for nutrients	P	M < N	M < N	M = N
	K	M = N	M < N	M > N
	S	M = N	M < N	M > N
	Ca	M = N	M < N	M > N
	Mg	M < N	M = N	M > N

DAI represents days after inoculation.

Result either M > N or M < N was significantly different at the 5% level.

DISCUSSION

Early Events in the VA Mycorrhiza-Leucaena Symbiosis

To help us understand early events and succeeding responses in Leucaena leucocephala-Glomus aggregatum symbiosis, a series of sequential measurements were made. A pinnule sampling method was used to monitor daily changes in P content and pot weighing methods were used to measure transpiration. In addition to these daily measurements, sequential destructive harvests were made in order to measure morphological and physiological changes in host plants.

Ten days after inoculation, an infection rating of approximately 17% was observed in mycorrhizal plants (Table 8), and root P concentrations were also greater at this time (Table 4). Two days later (12 days after inoculation) mycorrhizal plants contained higher pinnule P contents (Fig. 5) than nonmycorrhizal plants. These results indicated that during this time Glomus aggregatum germinated, infected the roots, developed external mycelium in the soil and transported P to plant roots which was then translocated to the shoots. Mycorrhiza formation in these circumstances was probably similar to that described by Brundrett et al., (1985). They estimated that at least one day was required for hyphae to contact the root and penetrate it, two to three days to develop arbuscules and three to four days to form vesicles. In our study, large amounts of inoculum (about 25 g per pot) were placed directly beneath the leucaena transplants in order to provide near

optimal conditions for colonization. Abbott and Robson (1982) suggested that rapid colonization in the early stages of plant growth is required for good host plant response to mycorrhizae.

Mycorrhizal Influences on Root/Shoot Ratio

Although mycorrhizal symbioses can be parasitic (Bethlenfalvay et al. 1983; Buwalda and Goh, 1982; Koide, 1985), no significant depression in host dry weight was observed during the mycorrhizal establishment (Table 8). Nevertheless, mycorrhizal plants showed a lower rate of leaf expansion during the 10 to 15 days harvest interval (Table 12) and displayed a higher root to shoot ratio at 15 days after inoculation (Fig. 25). Other studies of mycorrhizal symbioses suggested that in early growth stages mycorrhizal plants allocate significantly more assimilate to roots than to shoots compared to nonmycorrhizal plants do (Snellgrove et al. 1982). This was apparent in this study by 15 days after inoculation. However, this temporary parasitic phase was soon shifted to a symbiotic phase, apparently after the additional P taken up by mycorrhiza resulted in greater assimilation, thus compensating for the carbon cost of mycorrhiza. The following discussion will further consider how mycorrhizal leucaena plants grew larger and absorbed higher nutrient contents than nonmycorrhizal plants during early seedling growth.

Mycorrhizal Influences on Nutrient Absorption

Dynamic changes in pinnule P contents (Fig. 5), pinnule P concentration (Fig. 6) and pinnule dry weight (Fig. 7) of leucaena plants with or without mycorrhizal inoculation were apparent. Pinnule P content increased only 2 days after root P concentration increased, which occurred 10 days after inoculation. The repeated observations provided by daily pinnule sampling clearly demonstrated mycorrhizal effects on P absorption. Responses of individual plants to treatments can be measured through time, thus reducing the size and variability of experiments.

Plant nutrient status was also measured by sequential destructive harvests. During the first stage of mycorrhizal infection (10 days after inoculation), only root P concentration was enhanced (Table 4). Wieser et al., (1986) observed that mycorrhizal roots contained higher concentrations of adenine nucleotides than nonmycorrhizal roots under conditions of P-starvation and even after P-fertilization. With an increase in the contents of central metabolites such as ATP, ADP and AMP, an increase in metabolic activity should also be expected. In Fig. 22, plots of total P content vs. (root area x root P concentration) for mycorrhizal and nonmycorrhizal plants were coincident. This may indicate that both mycorrhizal and nonmycorrhizal plants have the same absorption capacity per unit root area at a given root P concentration. Several studies have indicated that the relationship between the rate of uptake of P and its internal concentration is controlled by the concentration of P in the roots (Anghinoni and Barber, 1980; Lefebvre

and Glass, 1982; Cumbus and Nye, 1985). Root P concentration, although rarely determined in mycorrhizal studies, may be a major factor controlling root absorbing power and also may serve as the an early indicator of growth and nutritional improvements resulting from a VA mycorrhizal symbiosis.

Mycorrhizal plants contained greater shoot P, K, Mg and S contents than nonmycorrhizal plants 15 days after inoculation. By 20 days after inoculation, mycorrhizal plants showed higher levels of all measured variables than did nonmycorrhizal plants (Table 5). Secondary mycorrhizal effects have been demonstrated in several studies showing that mycorrhizal plants not only displayed higher P uptake, but also increased absorption of other nutrients (Mosse, 1973; Powell, 1975). Rhodes and Gerdemann (1978a) suggested that mycorrhizal hyphae could absorb and translocate S in much the same manner as P. A similar enhancement of K uptake by mycorrhiza has been observed by Powell (1975). There is as yet no experimental basis for postulating direct involvement of the fungus in absorption and translocation of K. It is unlikely that absorption of Ca and Mg would depend on along mycorrhizal hyphae because Ca and Mg in the soil solution are more mobile than P (Barber, 1984). Moreover, Ca is apparently much less mobile than P in mycorrhizal hyphae (Rhodes and Gerdemann, 1978c). Most of the methods involve a diffusion-limited uptake process. Barber (1962) suggested that when nutrient concentrations in soil solutions were high, as was the case with Ca and Mg in these experiments, mass flow would be dominant and could explain the linear correlation of Ca (Fig. 19) and Mg (Fig. 20) uptake with cumulative water transpired.

Greater P, K and S fluxes into mycorrhizal roots were observed during the 10 to 15 days harvest interval. Subsequently, fluxes of all nutrients into mycorrhizal roots, including Ca and Mg, were greater than into nonmycorrhizal roots during the 20 to 30 days harvest interval. It has been suggested (Smith, 1982) that plants with greater transpiration and greater water flux to roots, as seen in mycorrhizal plants in these studies, may increase water movement from the unrooted zones to rooted zones. This effectively increases the soil volume accessible to plant roots and expands the labile pool which replenishes nutrients such as Ca and Mg (Shapiro et al. 1960).

Movement of water through the soil may increase nutrient desorption from the solid phase. The general indication is that for water flow velocity less than 0.2 cm/day the contribution of hydrodynamic dispersion to nutrient movement will be small (Nielsen and Biggar, 1961 and 1962). The flow velocity to mycorrhizal leucaena roots was about 0.63 cm/day 30 days after inoculation (Table 20), which was higher than that of nonmycorrhizal plants (0.31 cm/day) and also higher than the suggested critical flow rate given above. If only a fraction of the roots were 'active' in water uptake or in 'contact' with the soil water film (20 to 50%), then mass flow would have moved more nutrients to the plant than would diffusion. A speculative theory may be given as follows: a pulse of water transpiration and carbon assimilation is triggered by an input of phosphate by mycorrhizal roots while water flow through the soil-plant-atmosphere continuum induces water movement in adjacent soil, carrying additional nutrient to the roots/hyphae. Nutrient movement in the soil thus influenced plant growth and it, in

Table 20. Effect of mycorrhizal inoculation on water flow velocity at the root surface of mycorrhizal and nonmycorrhizal Leucaena leucocephala seedings.

	Days after inoculation					
Treatment	5	10	15	20	25	30
	Water flow velocity (cm day^{-1})					
Mycorrhizal	1.53 a[#]	1.35 a	0.84 a	0.82 a	0.65 a	0.63 a
Nonmycorrhizal	1.51 a	1.37 a	0.80 a	0.56 b	0.28 b	0.31 b

Means of each variable in the same columns within harvests followed by the same letter are not significantly different at the 5% level.

turn, is influenced by plant growth. This suggests a shift in dominance from diffusion to mass flow of ions by water movement as a result of increased plant transpiration. In this manner, mycorrhizae may be seen as a catalyst, whereby infection helps plants cross a threshold and initiates a positive feedback mechanism of nutrient absorption, growth and water relations of young seedlings.

Comparing the above sequential differences between mycorrhizal and nonmycorrhizal plants, it would not be expected that mycorrhiza can directly cause all these events, however. Fitter (1985 and 1986) proposed that mycorrhizal infection is likely to be benefical only at times when phosphate supply is particularly limiting, especially under drought conditions and possibly also at seedling establishment. He did not explain why P absorption is most critical in the seedling stage. Further experiments are necessary to clarify the speculation that the mycorrhizal contribution to nutrient absorption is more important in young seedlings than at later growth stages.

Mycorrhizal Influences on Growth and Transpiration

By 15 days after inoculation, mycorrhizal plants displayed greater leaf area (Table 8), higher specific leaf area (ratio of leaf area to leaf dry weight) and higher transpiration rate than nonmycorrhizal plants (Table 9). Mycorrhizal infection increased specific leaf area in leek (Snellgrove et al. 1982) and soybean (Harris et al. 1985). A higher specific leaf area enabled plants to achieve a higher rate of

carbon fixation from a given amount of leaf biomass (Allen et al. 1981). In contrast, nonmycorrhizal plants exhibited lower leaf P concentration than mycorrhizal plants which (Table 4) could be responsible for lowering net assimilation rate in the leaf (Table 13).

An increase in water transpiration of mycorrhizal plants (Table 9 and Fig. 11) was observed 15 days after inoculation, following increased P concentration in plant roots. Increased water transport from root to leaf and from leaf to the atmosphere probably were secondary effects of improved nutritional (P) status (Safir et al. 1972; Levy and Krikun, 1980). As shown in Table 8, the leaf area of mycorrhizal plants was of the same order of magnitude as the root area and the calculated water flux per unit leaf area approximated water flux per unit root area (Table 18).

After the onset of differences in root P concentration, differences were observed in leaf area, shoot dry weight, transpiration rate, specific leaf area, root dry weight and root area between mycorrhizal and nonmycorrhizal plants (Table 11). This sequence suggests that improved P nutrition amplifies other physiological functions of the plant such as improved growth and water relations. These results are similar to those obtained by Atkinson and Davison (1971, 1972 and 1973) who showed that P deficiency reduced not only plant growth, but also decreased leaf water content and stomatal conductance.

After inoculation, water use efficiency of mycorrhizal plants increased more (from 1.58 to 2.50 g of total dry weight/kg of water transpired) than that of nonmycorrhizal plants (from 1.55 to 2.08). The increased water use efficiency reflected the improved growth of

mycorrhizal plants. In contrast, nonmycorrhizal plants continued to transpire even though growth was stunted.

Increased leaf area and transpiration generally brought an increase in net assimilation rate (Table 13) and thus increased dry matter production (Table 8). As the water requirement of mycorrhizal plants increases, there may be more abrupt daily changes in water potential of mycorrhizal plants than of nonmycorrhizal plants with less leaf area (Huang et al. 1985). In this study, greater root surface of mycorrhizal plants probably led to more water extraction (Table 9). As growth progressed and plants became larger soil moisture was depleted more rapidly, leading to decreased plant leaf water content and lower transpiration rates per unit leaf area (Table 9). Although pots were watered daily to near field capacity, 30 days after inoculation mycorrhizal plants transpired approximately 120 g of water per day per pot (one-half of the available water). Water supply can be particularly limiting in pot conditions when plants have been growing for a long time. In such experiments it is necessary to frequently monitor plant water content to ensure that only treatment factors are the dominant growth effects (de Vries, 1980).

Thus far, the role of mycorrhizal associations in improving the nutrition of mycorrhizal plants in the early stages of the symbiosis has been discussed. Responses displayed in the greenhouse were also observed in the field (unpublished data) in which mycorrhizal plants were 4 times as tall (210 vs. 53 cm) and showed nearly 80 times as much stem dry weight (361.2 vs. 4.6 g) as nonmycorrhizal plants after 190 days of growth (Appendix 9).

A Proposed Scheme For Sequential Mycorrhizal Influence on Nutrient Uptake, Plant Growth and Water Relations

The dramatically improved growth of mycorrhizal plants may be regarded as a consequence of sequential events influenced by mycorrhizal colonization. Each event was seen as a function of preceding events. By comparing the time-course of these experimental results (Table 11 and 19), it is possible through the phasic changes (Table 19) to point out the primary and secondary mycorrhizal influences. A flow diagram for a mycorrhizal plant model of nutrient uptake, plant growth and water relations is proposed (Fig. 26). Possible explanations of sequential mycorrhizal influences are as follows:

1) After colonization by the fungus, mycorrhizal plants showed greater root P concentration (day 10), pinnule P content (day 12) and shoot P concentration (day 15) than nonmycorrhizal plants. All of these differences in P status could be attributed to increased P flux into mycorrhizal roots 10 to 15 days after inoculation. Increased P uptake is probably the major influence of the mycorrhiza on host plant physiology (Rhodes and Gerdemann, 1980).

2) With enhanced P status, mycorrhizal plants showed greater leaf area (day 15), transpiration rate per unit leaf area (day 15) and cumulative water transpired (day 20) than nonmycorrhizal plants. Enhanced P nutrition appears to increase transpiration (Atkinson and Davidson, 1973) and root conductivity to water (Radin and Eidenbock, 1984). The close relationship between P status and water relations in

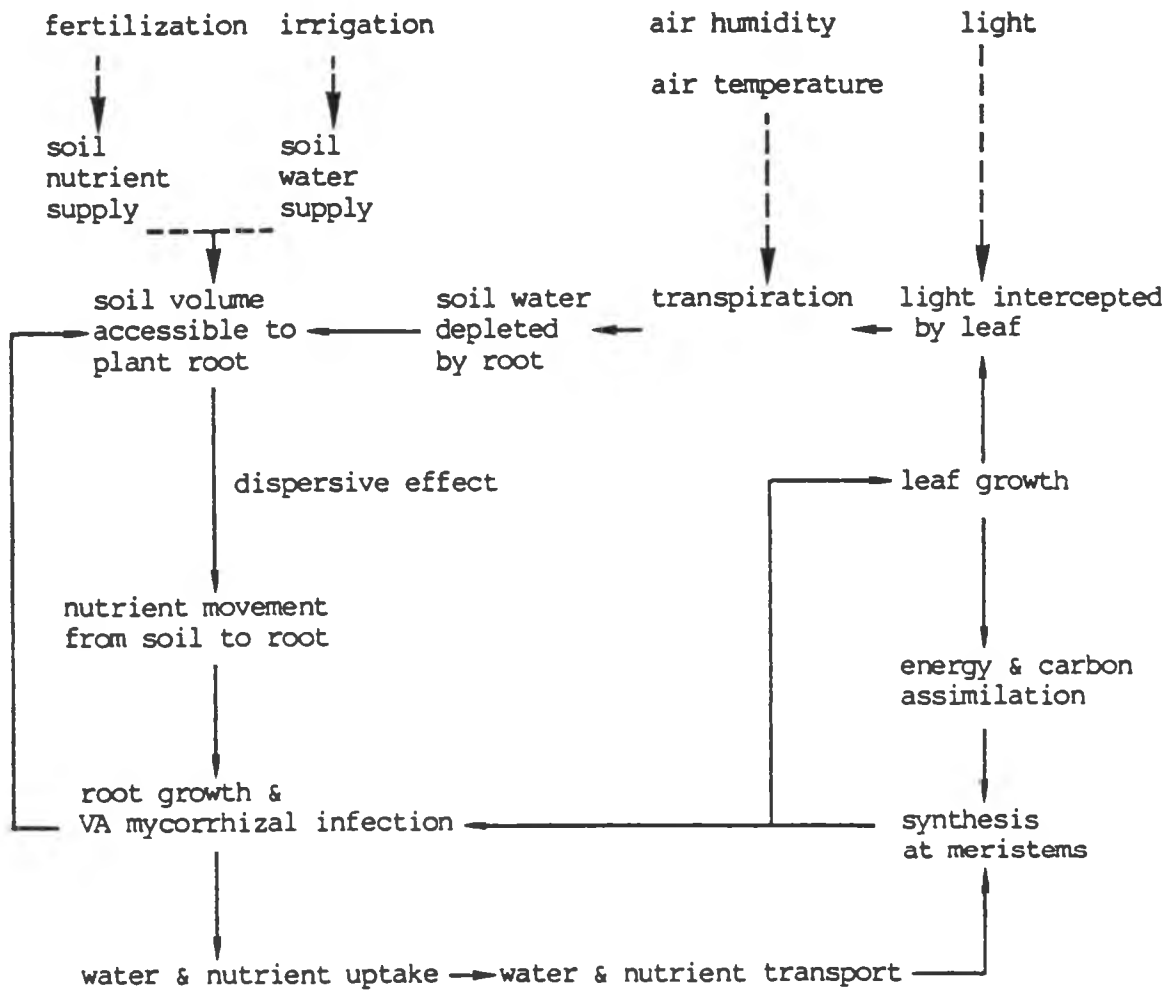

Fig. 26. Flow diagram for a proposed model of mycorrhizal plant growth, nutrient acquisition and water relations.

plants probably explains many of the reported benefits of mycorrhizae on host plant water uptake, translocation and transpiration (Levy and Krikun, 1980; Nelsen and Safir, 1982a and 1982b).

3) Under well-watered conditions, mycorrhizal plants displayed greater leaf area for photosynthesis and also developed higher net assimilation rates than those of nonmycorrhizal plants. An increased assimilation rate of VA mycorrhizal host plants can lead to compensation for the increased energy and carbon demands of both the fungi and the roots (Kucey and Paul, 1982; Snellgrove et al. 1982). Having greater net assimilation rates, mycorrhizal plants can allocate more assimilates for greater root growth. This would provide more absorbing area for further nutrient and water absorption. Thus responses of mycorrhizal plants to increased nutrient absorption, transpiration and dry matter production can be visualized as a positive feedback process (Fig. 26).

Possible Mechanisms of Nutrient Uptake by Mycorrhiza

For all soil P levels (0.005 to 0.429 mg P L^{-1} in solution), mycorrhizal plants usually showed higher plant dry weights (Table 2 and Table 3) and total P contents than nonmycorrhizal plants (Table 5). In this section discussion will focus on why mycorrhizal plants were able to grow so much better than nonmycorrhizal plants even in high-soil P.

Physical Dimensions of Mycorrhizal Hyphae vs. Plant Roots

It is generally believed that mycorrhizal plants derive P from the same chemical pools as uninoculated plants (Tinker, 1980). The uptake of less mobile nutrients through extensive exploration of soil volumes by extramatrical mycorrhizal hyphae is an important role of mycorrhiza (Harley and Smith, 1983). Fine, long mycorrhizal hyphae, such as observed in these experiments (Fig. 27), can accumulate more P and S than the host roots (Rhodes and Gerdemann, 1975, 1978a and 1978b). One reason is that the small diameter of hyphae is associated with greater surface area per unit weight than can be attained by coarse roots. Assuming equal specific gravity, 1 mg of hyphae of 5 μm diameter has the same length as 6000 mg of leucaena root of 390 μm diameter.

In addition, mycorrhizal hyphae would be expected to permit higher radial conductance than can be attained by coarse roots (Fowkers and Landsberg, 1981). A sensitivity analysis for P uptake conducted by Silberbush and Barber (1983a and 1983b) indicated that, with fixed root biomass and soil volume, altering root radius produced a greater effect on P absorption than known variations in physiological characteristics of the roots. Root radius effects on nutrient uptake have also been analyzed with a diffusion model (Bouldin, 1961). He suggested that nutrient flux per unit of root surface area may increase tenfold as the radius is decreased from 500 μm to 7.5 μm. The difference in root radius of leucaena (195 μm) and mycorrhizal hyphae (1 to 5 μm) may also lead to large increases in nutrient flux. The data showed that the P flux into mycorrhizal roots was 4 to 10 times greater than the flux into

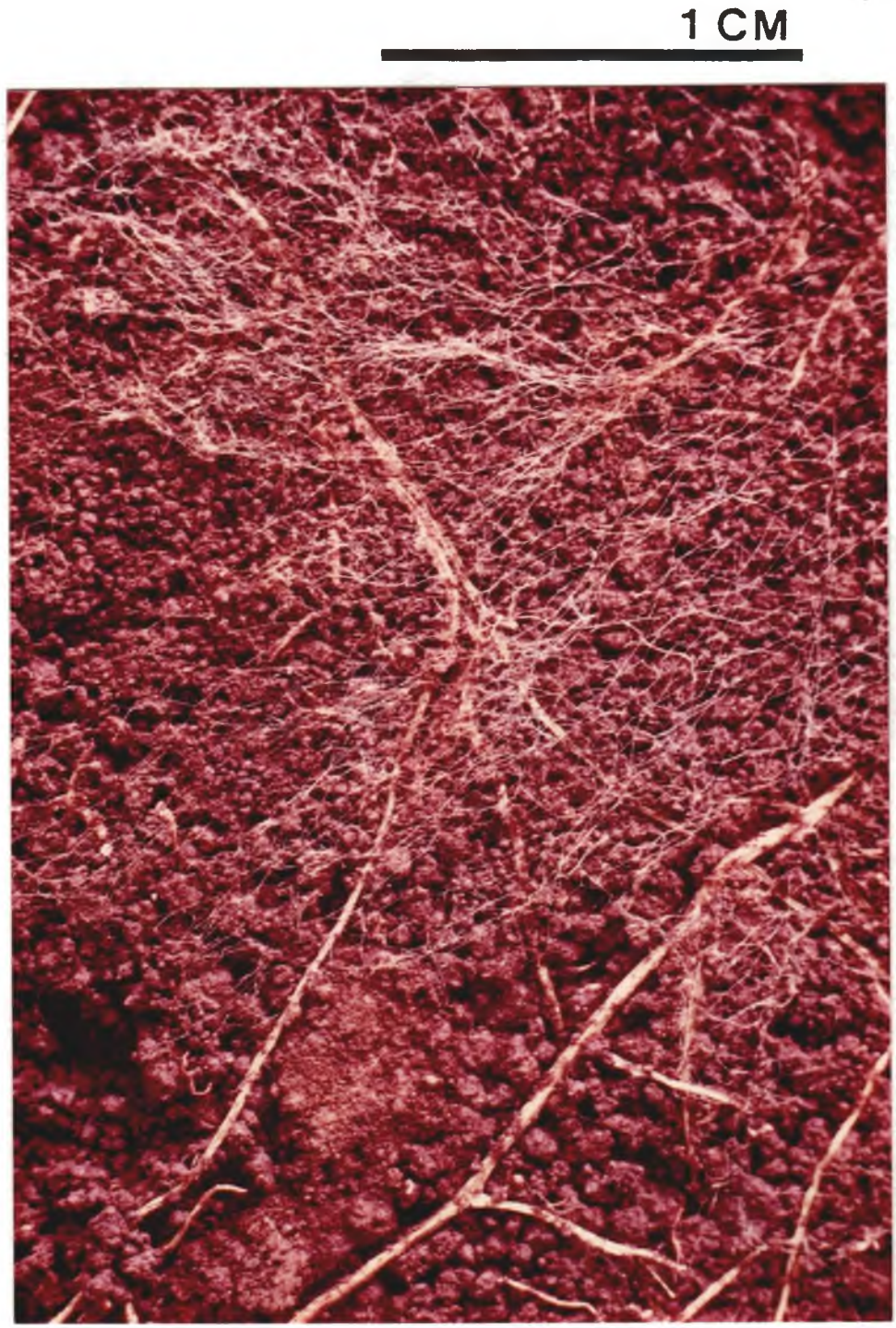

Fig. 27. Magnified view of mycorrhizal Leucaena leucocephala roots in potted Wahiawa soil.

nonmycorrhizal roots (Table 15). Based on these results we believe that leucaena seedlings responded strongly to mycorrhizal infection because of low rooting intensity and low production of root hairs, even though their root diameters are smaller than that of 'magnolioid' roots (Baylis, 1975).

Configuration of VA mycorrhizal Root System in the Soil

It is interesting to examine geometrical properties of soil pores, leucaena roots and mycorrhizal hyphae. Evidence from water release curves of the Wahiawa soil used in these experiments indicated the presence of both intra-aggregate micropores and inter-aggregate macropores (Sharma and Uehara, 1968). The diameter of intra-aggregate micropores (2-10 μm) of the Wahiawa soil (Tsuji et al. 1975) approximates the diameter of mycorrhizal hyphae (2 to 20 μm) but was much smaller than the diameter of leucaena roots (100 to 600 μm, with an average of 390 μm). Wiersum (1957, 1961 and 1962) reported effects of soil physical properties on plant growth and nutrient uptake. He observed that plants absorbed less P when their roots grew in soils with large clods. Olsen and Watanabe (1963) pointed out that if two soils have the same 'available P' soil test values, the amount of P taken up by growing plants is less on the coarser-textured soil. These considerations suggested that hyphae have access to micropores not accessible to leucaena roots.

The small distance P diffuses and the inaccessible intra-aggregate

micropores render soil P relatively unavailable for leucaena roots in contrast with mycorrhizal hyphae which in this soil were several cm long, thin and highly branched with loops (Fig. 27). Fowkers and Landsberg (1981) discussed contradictory requirements for absorption (small diameters and long lengths) and for conducting (coarse diameter and short lengths) within the root system. Mycorrhizal hyphae, with a smaller diameter and an improved branching configuration, apparently overcame the impeded diffusion of some nutrients, particularly P, to the roots and fulfilled the nutrient absorption function. Greater branching of mycorrhizal hyphae provides a good configuration for accessing less-mobile nutrients. With their small diameter, mycorrhizal hyphae should be able to contact water films in intra-aggregate micropores which supply P from the solid phase. While speculative, this could explain the higher flux of P to mycorrhizal roots than to nonmycorrhizal roots (0.040 vs. 0.009 μmol P cm^{-2} day^{-1}, respectively) (Table 15).

Soil aggregate size and its potential influence on nutrient supply and movement and root accessibility in a soil-plant system is summarized in Table 21. In contrast to rapid water movement in the inter-aggregate macropores, the high water retention and high specific surface area in the intra-aggregate micropores may act as an important site for P supply.

The earliest fossil VA mycorrhizae are associated with primitive plants from the Rhynie chert, approximately 370 million years old (Pirozynski and Malloch, 1975). These were morphologically similar to modern VA mycorrhizae (Nicolson, 1975). The evidence for mycorrhizae

Table 21. Soil aggregate size and potential influence on nutrient supply, nutrient movement and root accessibility in a soil-plant system.

	Intra-aggregate micropores	Inter-aggregate macropores
Average pore size:	2 to 10 μm	10 to 500 μm
Possible role in nutrient supply:	higher specific surface area	lower specific surface area
	longer water retention time	shorter water retention time
	higher P concentration in soil solution	lower P concentration in soil solution
Possible role in nutrient movement:	higher matric potential	lower matric potential
	smaller pore diameter	larger pore diameter
	lower pore water velocity	higher pore water velocity
Pores more easily accessed by:	VA mycorrhizal hyphae (2 to 20 μm diamter, average 8 μm)	leucaena root (50 to 600 μm diameter, average 390 μm)

was reviewed by Pirozynski and Malloch (1975) who concluded that available soil P and other nutrients might have been deficient in the earliest terrestrial environment, so that the intervention of the fungi was important to the success of terrestrial plants. Selective pressure towards an efficient root system probably favored the symbiosis of plant root and mycorrhizal hyphae. The combined structures allow greater exploitation of soil resources and also serve to improve both absorption and conduction of nutrient and water.

This discussion, however, does not exclude the possibility that mycorrhizae have a greater ability to absorb P at very low external concentrations than roots of many higher plants as suggested by Mosse (1973), Cress et al., (1979) and Howeler et al., (1982). Further investigations are needed to determine whether greater ability to absorb P is due to improved geometry and configuration or due to improved physiological efficiency.

Response of Nonmycorrhizal Plants to Phosphorus Stress

As reviewed earlier, plants usually make several compensatory adjustments to P stress such as increased ion uptake efficiency at the root surface, increased P solubility in the rhizosphere due to increased root exudates, reduced root diameter, increased root growth relative to top growth, reduced nutrient requirements as a result of lower relative growth rates, and more efficient P utilization by enhanced P re-

distribution within the plant. It is useful to explore how these responses to P stress help plants tolerate adverse conditions.

It has been amply demonstrated in solution cultures that low plant nutrient status can increase short-term P flux into plant roots (Epstein, 1976). However, if the major limiting step for P acquisition is not root function per se, increased P absorption capacity at root surfaces is of limited value in overcoming slow P diffusion through the soil (Nye, 1977). Several reports have shown that plant roots can excrete organic acids in the rhizosphere leading to increased nutrient availability (Jungk and Caassen, 1986; Marschner et al. 1986). It has been speculated that the quantity of organic acids exuded by plant roots is high enough to render soil P more soluble (Nye, 1968). However, the low P status and stunted growth of nonmycorrhizal leucaena plants observed in these experiments demonstrated that, if present, this mechanism did not supply sufficient P from the soil. Further study is necessary to quantify the influence of root exudates on nutrient availability in the rhizosphere.

Root diameter, as discussed above, is important for the exploration and absorption of less-mobile nutrients. This root characteristic, however, is to a large degree genetically controlled (Russell, 1977). In spite of large responses to mycorrhizal infection and to P fertilization, no differences in average leucaena root diameters were detected either among mycorrhizal inoculation or P fertilization treatments.

It is well documented that a reduction in nutrient supply during plant growth can limit leaf growth and increase root growth (Brouwer,

1962a and 1962b). Indeed increased root growth rate in nonmycorrhizal plants during the 15 to 20 days harvest interval (Table 12). Twenty days after inoculation, root dry weights of nonmycorrhizal plants were greater than those of mycorrhizal plants (Table 8). Greater root growth appeared to sharply increase P absorption (Fig. 15). This was accompanied by an increase in P accumulation rate during the 15 to 20 days harvest interval (Table 14). This rapid increase was short-lived, however, with net P absorption approaching zero and P content remaining nearly constant after 20 days (Fig. 15). In these studies, even enhanced root growth of nonmycorrhizal leucaena was still insufficient to counteract low P uptake. It is probable that during the early growth period (10 to 15 days) P ions around growing roots had decreased sufficiently to limit P flux into nonmycorrhizal roots (Table 15). The increased growth of coarse roots was apparently inadequate to maintain the necessary P flux for continued growth (Robinson and Rorison, 1983).

In these experiments, plant growth was restricted by pots. In field conditions, however, unimpeded root growth of nonmycorrhizal plants may increase chances for mycorrhizal infection by exploring larger soil volumes.

After day 25, the P concentrations in nonmycorrhizal roots and shoots declined to 0.09 and 0.12%, respectively. Typical symptoms of P deficiency, leaf shedding (Table 8) and slow root area expansion rate (Table 12), became apparent in nonmycorrhizal plants. Negative feedback, i.e., decreased nutrient absorption because of decreased growth, is frequently observed when plants are under severe P stress (Chapin, 1980). Reduced plant growth rate, however, is presumably

advantageous for plants because it minimizes nutrient requirement and prevents exhaustion of internal nutrient reserves (Chapin and Bieleski, 1982).

Even though nonmycorrhizal plant growth was stunted during 20 to 30 days, both mycorrhizal and nonmycorrhizal plants utilized P with nearly equal efficiency (Table 17), whereas the specific utilization rate of other nutrients was lower in nonmycorrhizal than mycorrhizal plants. Phosphorus concentrations were lower in old, senesced leaves than in young nonmycorrhizal leaves. Enhanced translocation of P from mature to young leaves may serve as one of the mechanisms for increasing efficiency for P utilization, as suggested by Greenway and Gunn (1966) and Clarkson et al., (1982).

When seed reserves were depleted, growth of nonmycorrhizal leucaena plants was retarded. In spite of various changes in root physiological and morphological characteristics, nutrient acquisition was quite limited. Nevertheless, Huang and Fox (1984) noticed that when nonmycorrhizal plants were later inoculated they eventually attained similar plant size and weight as mycorrhizal plants (Appendix 6). The stunting of nonmycorrhizal plants appeared to be reversible and probably is a survival strategy which reduces energy use while retaining the potential for mycorrhizal infection.

Water Use Strategy in Leucaena and Its Ecological Significance

From previous discussions, it is apparent that mycorrhizal association permits increased P uptake, enabling leucaena plants to grow well. It is interesting to know how improved P nutrition affects other ecophysiological characteristics in leucaena which, in turn, affect growth and adaptation of leucaena in semi-arid regions.

In these studies, nonmycorrhizal and mycorrhizal plants were dramatically different in water relations. Root surface area of nonmycorrhizal plants was limited (Table 8), water transpiration, specific leaf area, leaf moisture content were low (Table 9) and shedding of leaves and leaflet folding increased (Huang et al. 1985). Stomatal closure, as shown by reduced water flux through the leaf (Table 18), is important to conserve water and avoid severe water stress. However, it may also reduce photosynthesis.

In contrast, mycorrhizal plants had greater root area (Table 8), higher specific leaf area (Table 9), and displayed more rapid stomatal adjustment to ambient humidity than nonmycorrhizal plants (Huang et al. 1985). Those plants would transpire more water (Table 9) and grow larger than nonmycorrhizal plants (Table 8). Although mycorrhizal plants depleted soil water more quickly than nonmycorrhizal plants in these studies and in other pot experiments (Levy et al. 1983), the water supply in natural habitats may not be as limiting as for potted soil.

It has been suggested that plants with higher potential

transpiration rates can deplete soil water quickly and thus gain a competitive advantage in field conditions, especially in the semi-arid regions (Hill, 1971; Mikura et al. 1984). The high transpiration rate of leucaena plants may result in higher carbon gain which would further enhance shoot and root growth for more nutrient acquisition and carbon assimilation (Huang et al. 1985). Besides leucaena plants, other "water-spending" plant species have been observed in natural ecosystems (Bunce et al. 1977; Fischer and Turner, 1978). Many crop plants appear to behave in this way (Ritchie, 1973 and 1974). Moreover, high water use probably stimulates soil water movement and nutrient in the soil solution from unrooted zones to rooted zones, which may effectively enlarge the soil volume accessible to plant roots (Smith, 1982). The ecophysiological consequences of increased soil volume have been suggested by Sarmiento et al., (1985). They pointed out that the adaptive strategy of woody species in savannas was to maintain a high water flux as long as possible, as a way of simultaneously improving nutrient acquisition and maintaining a favorable carbon gain.

In considering successional shrubs, as leucaena, in the semi-arid environment, Passioura (1976) suggested that the better strategy for plant competition would be to use water faster, perhaps less efficiently, grow quickly and form seeds while soil water is available. As the soil dries, the plant then must have some mechanisms for drought avoidance to prevent damage from water deficits. Leucaena adapts well to wet-dry climate regions and exhibits such drought avoidance characteristics as deep taproot, leaf shedding, leaf folding (Gates, 1916; National Research Council, 1984) and stomatal closure in response

to decreases in leaf water potential or increases in the leaf-to-air water deficits (Huang et al. 1985).

To understand the physiological and ecological significance of mycorrhizal contribution to water relations of plants, it is necessary to relate models of nutrients, water and carbon. Such knowledge is essential not only to understanding the mycorrhizal systems but also to managing our natural ecosystems.

CONCLUSIONS

An understanding of the dynamics of mycorrhizal systems is a prerequisite for the manipulation of these beneficial associations for agriculture. This thesis describes an effort to identify and integrate some of the important feedback processes involved in nutrient acquisition, growth and water relations of mycorrhizal systems. The results reported here demonstrate how the concept of feedback helps in understanding dynamic processes in mycorrhizal systems involving Leucaena leucocephala. In these experiments, daily pinnule sampling and pot weighing were used in order to measure changes in P acquisition and transpiration. In addition, destructive harvests at 5-day intervals were applied in differentiating primary and secondary differences between mycorrhizal and nonmycorrhizal plants.

A sequence of measurements of mycorrhizal and nonmycorrhizal seedlings permitted a time-course description of critical events in the soil-mycorrhiza-plant system. First, the onset of mycorrhizal activity affects root functioning and nutrient uptake. Differences in root P concentration were observed by day 10, and were then followed by differences in pinnule P content (day 12), shoot P concentration, leaf area (day 15), cumulative water transpired (day 20), shoot dry weight (day 25) and then root dry weight (day 30). Based on this sequence, a model of a soil-mycorrhiza-plant system and associated effects of mycorrhiza on nutrient uptake, plant growth and water relations is proposed (Fig. 27).

We also discussed how nonmycorrhizal leucaena seedlings responded

to P stress. Despite greater initial root growth, leucaena roots alone could not acquire sufficient P to maintain growth. To cope with low nutrient absorption, nonmycorrhizal plants appear to slow their relative growth rates and recycle nutrients to new leaves from old leaves before they are shed. The outcome of P deficiency and stunted growth in nonmycorrhizal seedlings seems to be a typical sequence of negative feedback response: 1) lowered nutrient uptake, 2) reduced carbon assimilation rate, 3) decreased rate of root proliferation, and 4) less nutrient uptake and stunted growth.

Mycorrhizal plants absorbed more P from the soil than did nonmycorrhizal plants. The following mechanisms are suggested: 1) specific surface area (per unit of assimilate) of mycorrhizal roots is increased, 2) fine and long hyphae increase the potential for effective physical exploitation of soil pores. Phosphorus may be acquired from such micro-pores even though they are inaccessible to plant roots, and 3) mycorrhizal roots extract P more efficiently because the threshold concentration for uptake is lower than for nonmycorrhizal roots.

To better understand the complexity of nutrient acquisition, a systematic approach is needed which deals with the physiology of plant roots and the soil physical, chemical and biotic factors. A unified concept of 'nutrient bioavailability' and methods to study and characterize dynamic soil-mycorrhiza-plant systems should be developed. A framework to do this is proposed in Fig. 28.

Results from numerous pot and field experiments demonstrate that without mycorrhizal associations, leucaena plants are stunted. It is now necessary to ask some fundamental questions. What are the major

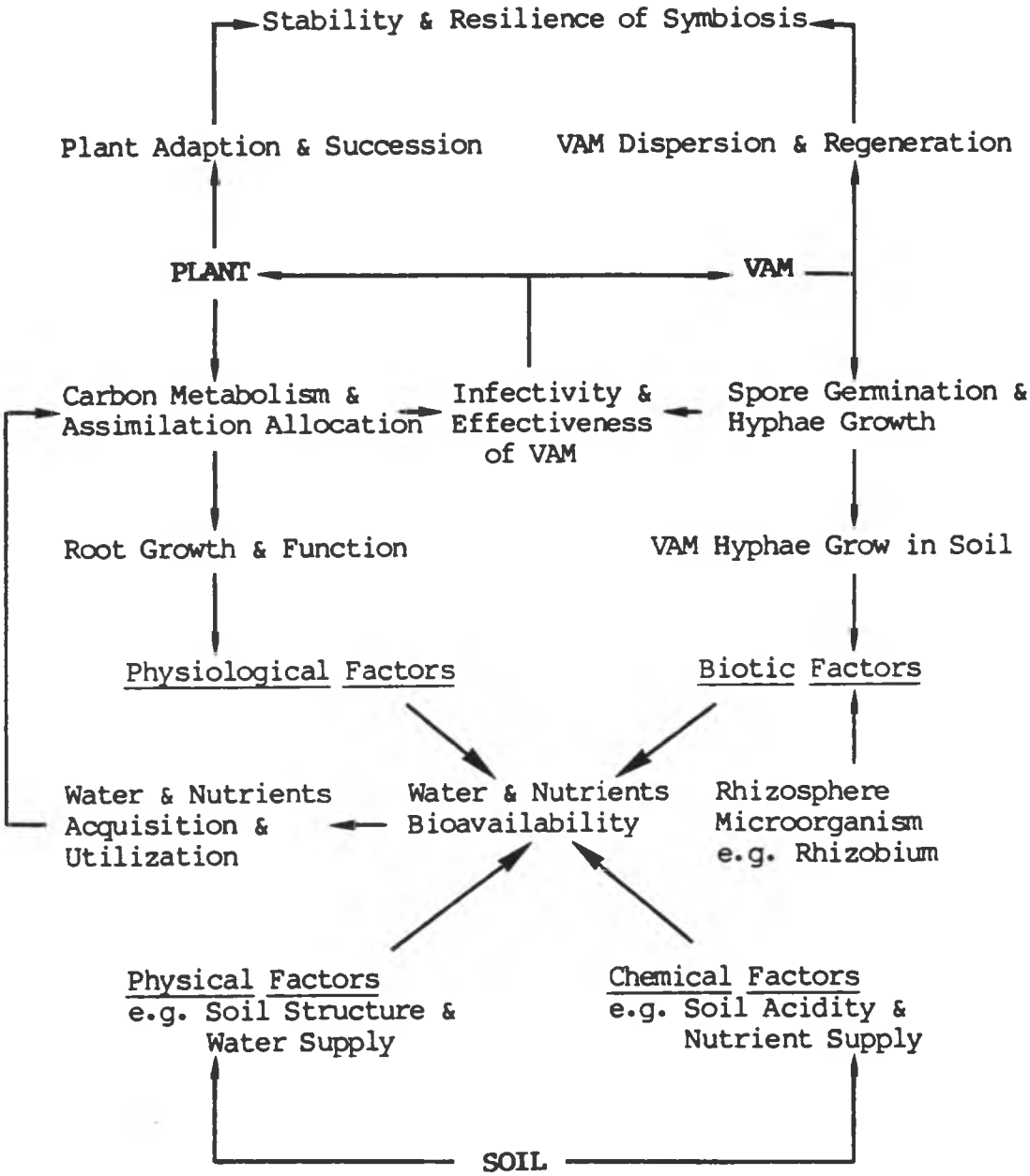

Fig. 28. Water and nutrient bioavailability as influenced by structural and functional relationships in the soil-mycorrhiza-plant system.

critical factors in natural soil environments that limit nutrient acquisition and growth? Is the leucaena plant in some sort of co-evolution, by which a special obligate symbiotic relationship is developed between plants and mycorrhizal fungi? Does a stunted nonmycorrhizal plant exhibit 'optimal' survival strategies for preventing energy exhaustion while retaining the potential for mycorrhizal infection? Obviously, there are numerous problems among the enigmatic facets of these associations.

APPENDICES

Appendix 1. Summary of Barber's experiments to observe and predict phosphorus uptake by the plant root growing in soil.

Experimental no.	Crops	Experimental condition	P concentration in soil solution (μm)	Predicted value/ observed value	Reference
1	Millet	Field	0.65	1/14-25	Adepetu & Barber (1978)
2	Millet	Field	48.50	1/0.66-1.2	Adepetu & Barber (1978)
3	Corn	Sterilized soil	47.6	1/0.97	Schenk & Barber (1979b)
4	Corn	Sterilized soil	47.1	1/0.92	Schenk & Barber (1979a)
5	Corn	Sterilized soil	7.4	1/0.49	Schenk & Barber (1979a)
6	Corn	Field	10.6	1/1.49	Schenk & Barber (1979b)

Appendix 2. Vesicular-arbuscular mycorrhiza formed between Macroptilium atropurpureum and the fungus Glomus aggregatum. Note the sporocarps formed in the sand medium.

Appendix 3. A method for the isolation and examination of vesicular-arbuscular (VA) mycorrhizal fungi from mineral soil.

Huang, Ruey-Shyang, R. S. Yost and J. E. Pinchin.

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A great proportion of nitrogen fixing trees are leguminous and have symbiotic associations with both vesicular-arbuscular (VA) mycorrhizae and nitrogen-fixing microorganisms. This triple symbiosis has great importance in nutrient absorption and in nutrient cycling of forest ecosystems.

Symbiotic fungi of VA mycorrhizae, Family of Endogonaceae, have never been cultured without the presence of a living host plant. In most cases spores collected from soil are the only basis for experimental or taxonomic study. The following method (see below flow chart) describes techniques we use to isolate VA mycorrhizal spores from soils and is derived from the 'wet-sieving and decanting' and 'sucrose centrifugation' methods.

Phase I: Sampling

1. Scrape the litter from the soil surface and collect 500 ml of soil with plant roots (samples should be from a relatively homogeneous area. If the area is highly hetergeneous then subsample from the areas of variation. Do not mix across areas of great variation).
2. Crush soil and pass through a 6 mm sieve to remove large gravel and debris.

3. Air dry the soil samples (low soil moisture content will prevent spore germination and reduce the activity of hyperparasitic microorganisms).
4. If soils of different bulk-densities are to be compared, the dry-weight of an equivalent sample should be determined. Express spore abundance on g dry weight basis.

Phase II: Isolation

1. Soak 250 ml of soil in one liter of tap water in a 2-liter container for 5-10 min.
2. Suspend the sample by stirring briskly and then allow it to settle for 30 sec.
3. Decant the suspension through a 500 µm sieve, collecting the liquid that passes through.
4. Wash the sieve in a stream of water to ensure that all small particles have passed through.
5. Save the sievings as some sporocarps may be present.
6. Resuspend sievings in the liquid that passed through the 500 µm sieve by stirring and allow the heavier particles to settle for 15 sec.
7. Pass the suspension through a sieve fine enough (generally 54 µm) to retain the desired spores.
8. Resuspend and thoroughly mix the sievings with a strong stream of water and allow suspension to settle for 15 sec.
9. Repeat steps 7-8 four to eight times depending on soil type.
10. Wash the material retained on the sieve to ensure that all colloidal

material passes through.

11. Transfer the remaining sievings to a beaker.
12. Prepare a large sucrose gradient: a) place 200 ml of 30% (W/V) sucrose solution in a one liter beaker; b) layer 600 ml of water over the sucrose solution by using a wick to transfer the water from another one liter beaker (keep the supply beaker full during the entire process).
13. Gently layer the sievings onto the gradient with a dropper pipette and allow them to settle by gravity for 10 min.
14. Retrieve the spores and debris which gather at the sucrose/water interface by vacuum aspiration.
15. Pour the retrieved spore suspension through a series of stacked sieves (250, 106 and 53 µm) and rinse thoroughly with water.
16. Wash the sievings into separate 500 ml beakers.

Phase III: Separation

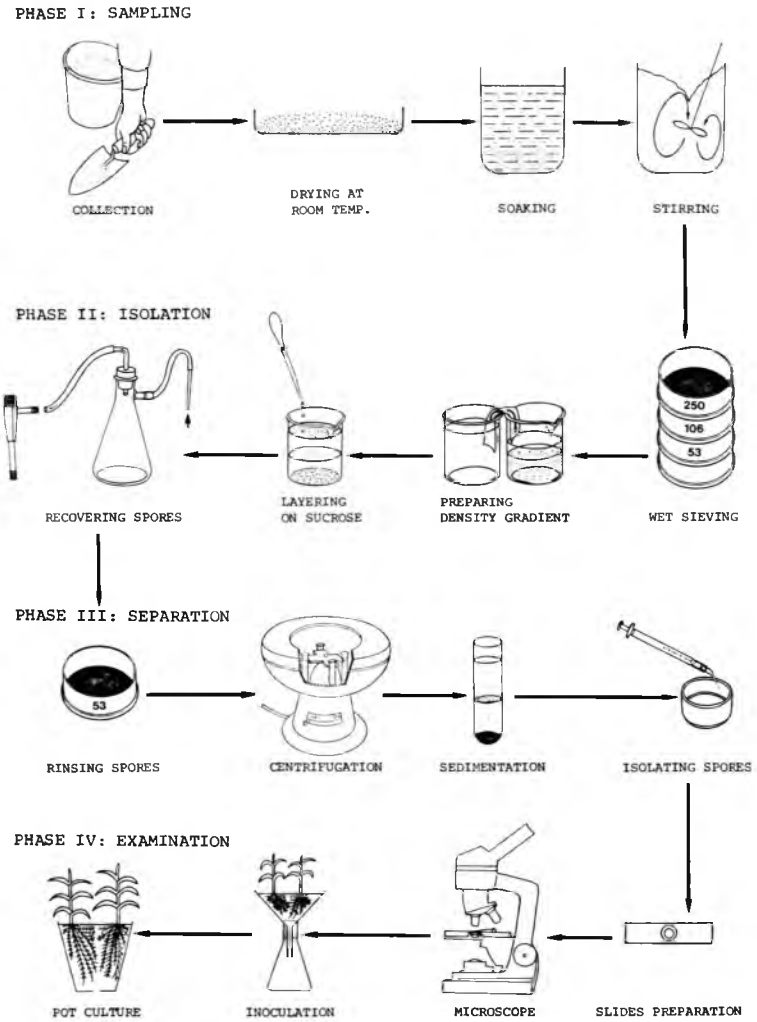
1. Place 25 ml of 30% (W/V) sucrose solution in a 50 ml centrifuge tube.
2. Gently layer a maximum of 15 ml of the suspension of sievings over the sucrose solution using a dropper pipette.
3. Centrifuge for 5 min at 3100 rpm (approx. 1100 XG). Note: Centrifuge should only be run with an even number of tubes positioned opposite each other balanced.
4. Draw the spores off the sucrose/water interface by vacuum aspiration.
5. Pour this supernatant (containing spores) onto a fine sieve (53 µm)

and rinse well with water to remove the sucrose.

6. Transfer the spore suspension to a petri dish.

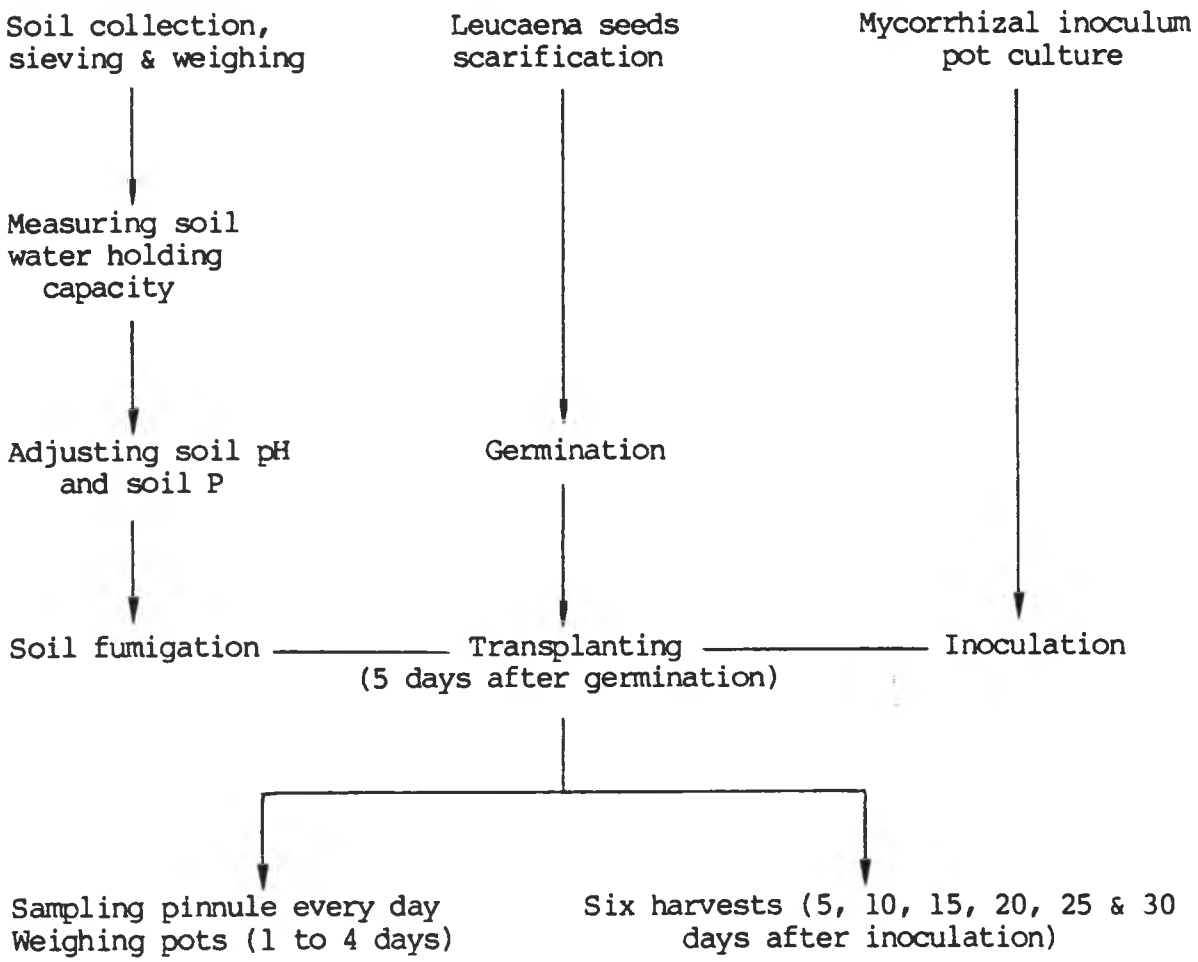
Phase IV: Examination

1. Soak the spore suspension in 5% (W/V) Na-hexametaphosphate for 10 min to disperse fine clay particles which may be adhering to the spore surface.
2. Carefully remove Na-hexametaphosphate and as much remaining debris as possible with a syringe. Add a small amount of water to resuspend the spores. Repeat until most of the debris has been removed.
3. Pick out a few spores with a modified syringe (be sure the opening is large enough to accomodate the size of spore being isolated). Place these spores in a drop of lactophenol-trypan blue on a plain microscope slide and cover with a cover slip. Save the unstained spores.
4. Examine the stained spores at 100X-400X magnification and record specific morphological features of the stained spores (e.g., spore size, color, spore wall structure, etc.).
5. Group un stained spores with similar morphological characteristics by using needles and inoculate for establishing separate pot cultures.



FLOW CHART OF PROCEDURES USED TO OBTAIN SPORES OF VA MYCORRHIZAL FUNGI FOR EXAMINATION BY MICROSCOPY.

Appendix 4. Stages in preparation and conducting a mycorrhizal *Leucaena leucocephala* experiment



Appendix 5. Use of single-leaflets to track the effectiveness of mycorrhizae associated with Leucaena leucocephala.

Huang, Ruey-Shyang and R. L. Fox.

(Published in **Leucaena Research Reports 5:79-81. 1984**).

Yost and Fox (1979) determined that the phosphorus percentage of nonmycorrhizal Leucaena leucocephala (leucaena) plants grown in a fumigated, phosphorus-deficient Oxisol was much less than the phosphorus percentage of mycorrhizal plants grown in soils that had not been fumigated. The influence of mycorrhizae was not replaced completely by phosphate fertilizer--even by very high phosphate additions that raised soil solution concentrations to 1.6 mg P L^{-1}. Such results suggest a high dependency of leucaena on mycorrhizae. It is important to understand the role of mycorrhizae in the phosphorus economy of leucaena during early stages of development because young plants are subjected to many hazards; their survival may depend upon good mineral nutrition.

The purpose of this study was to measure, as a function of time, the influence of mycorrhiza on phosphorus uptake by leucaena seedlings beginning with the earliest fully-expanded leaf. It is hoped that techniques we have developed may be used to compare the relative efficiency of mycorrhizae (in this case indicated by phosphorus uptake) in various soils or rooting media.

Materials and Methods. Four days after germination, three seedlings of leucaena (var. K8) were transplanted into 24 cm pots

containing 5 kg of fumigated soil (Tropeptic Eutrustox, Wahiawa series). The soil pH was 5.9 and sodium bicarbonate extractable P was very low, 2.0 mg L^{-1} on a dry soil basis. There were three rock phosphate treatments, zero, 5 gram and 10 gram per pot of North Carolina phosphate rock, but the effects of the phosphate rock could not be detected by the leucaena. Therefore data from the three phosphate treatments were composited. All treatments were in two replicates. At planting, seedlings were either inoculated with VA mycorrhiza fungus (Glomus aggregatum), or, in the case of control plants, were treated with fumigated inoculum.

Pinnules (leaflets) were removed every 12 to 24 hours from fully-expanded leaves (the most recent leaf to attain maximum green color) from each plant beginning 8 days after germination. This sampling continued for 12 days after which sampling was continued from one plant only.

Thirty-eight days after germination the previously non-inoculated, non-mycorrhizal plants were inoculated with Glomus aggregatum by placing inoculum into small holes in the soil near the plants. Pinnule sampling continued until 88 days had elapsed for the early inoculation treatment and 98 days for the late inoculation treatment.

Pinnules were dried at 55°C, weighed on an analytical balance, placed in either 30 ml pyrex beakers or 25 ml pyrex tubes and ashed at 500°C in a muffle furance for 3 hours. The ash was taken up in dilute HCl after which P was determined colormetrically.

Results. Phosphorus uptake patterns of inoculated (mycorrhizal) and non-inoculated (nonmycorrhizal) plants were essentially identical

during the first 12 days following germination (see Appendix 5). In both cases P contents of pinnules decreased rapidly with time. After 12 days pinnules of the nonmycorrhizal plants continued to lose phosphorus. This was associated with extreme symptoms of pinnules from the lower leaves as the deficiency became more intense. The mean minimum P content attained at days 41-42 was 0.84 µg P per pinnule in pinnules weighing 1.15 mg each, giving a phosphorus percentage of 0.073 which, we assume, represents approximately ultimate P depletion. The contrast between mycorrhizal plants and nonmycorrhizal ones is so evident in Appendix 5 that comments are scarcely required. The curves are based on mean values. They do not adequately indicate the rapid increase in P contents of individual plants after the mycorrhiza became effective. In some individual cases, P contents of pinnules doubled in 12 hour sampling interval. Phosphorus uptake were noted at intervals of approximately 10 days. Surges in root growth and activity. Perhaps these observations are related. Plants which were inoculated soon after germination, and those inoculated at day 38, attained the same level of leaf P, approximately 13 g P per pinnule, 40 days after inoculation. Such quantities of P in pinnules of approximately 5 mg weight suggests a maximum P percentage of 0.26 above which this particular mycorrhiza was not able to increase P percentage further.

In the case of both early and late inoculated plants the initial major peaks in P contents were immediately followed by a drastic decrease in P uptake and percentage. This decrease is so precipitious that we do not believe it is caused by exhaustion of the 'available' P pool. Instead we believe the evidence supports the concept of control

mechanism that operates on the effectiveness of the mycorrhizae. The results suggest that a new, stable level of 6.5 µg P per pinnule is attained. Pinnules of these plants weighed about 3.6 mg, giving a leaf P concentration of approximately 0.18%.

Discussion. The 12 leucaena plants used for this study were sampled repeatedly (more than 100 times each) without substantial damage. This 'non-destructive' sampling method permits the same plant to be used for other determinations relating mycorrhizal function to plant development—for example, studies on water relations (Huang et al. 1983). We intend to use this technique to measure the relative effectiveness of naturally-occuring mycorrhizal inocula.

The data reported here demonstrate that mycorrhizae will permit leucaena to extract phosphorus from soils which are otherwise very phosphorus deficient. The data also suggest that mycorrhizae alone will not support, for an extended period of time, sufficient phosphorus uptake from pots for optimum growth of leucaena. Additional work should be done to determine how these results can be applied to field conditions.

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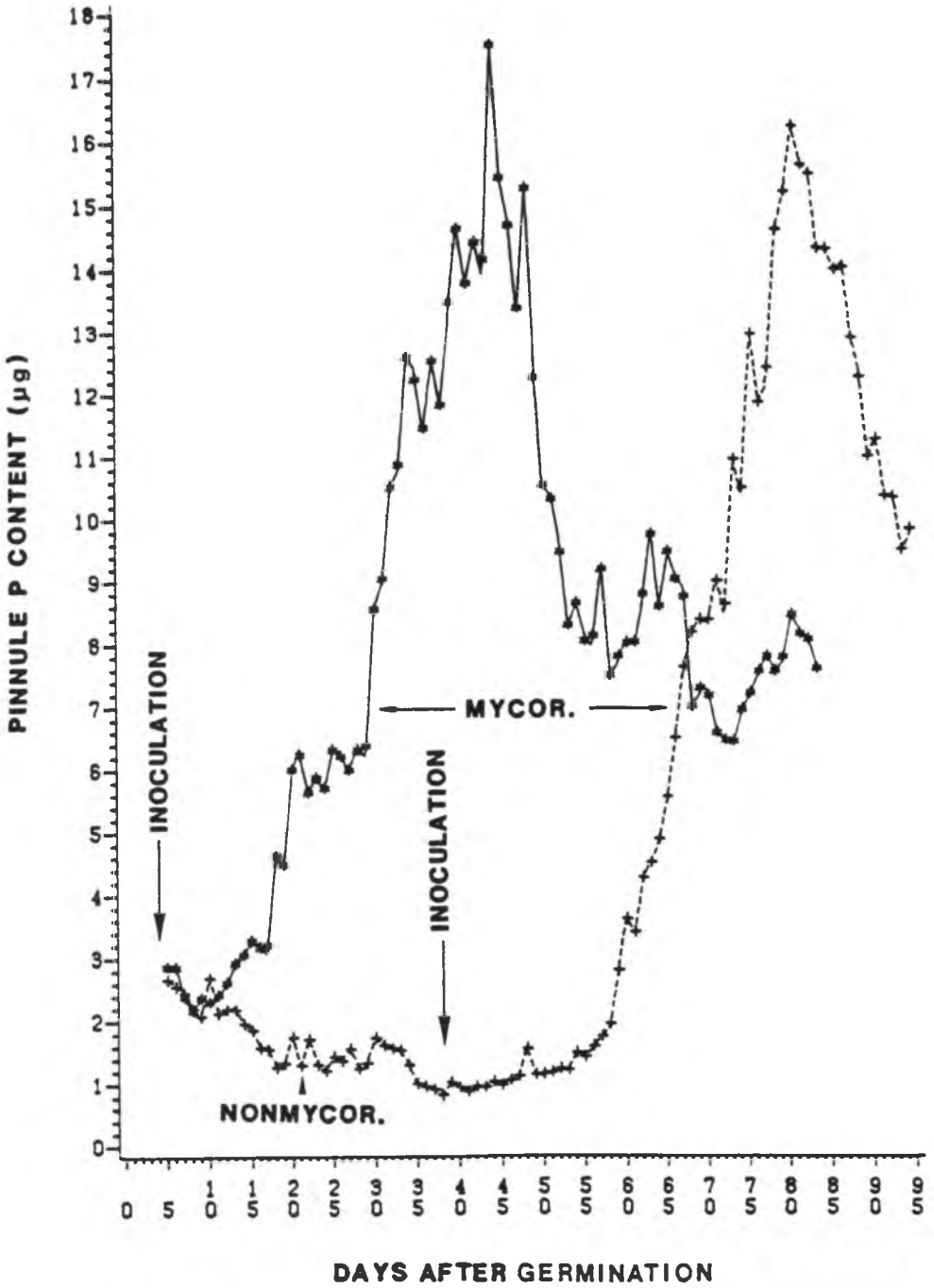
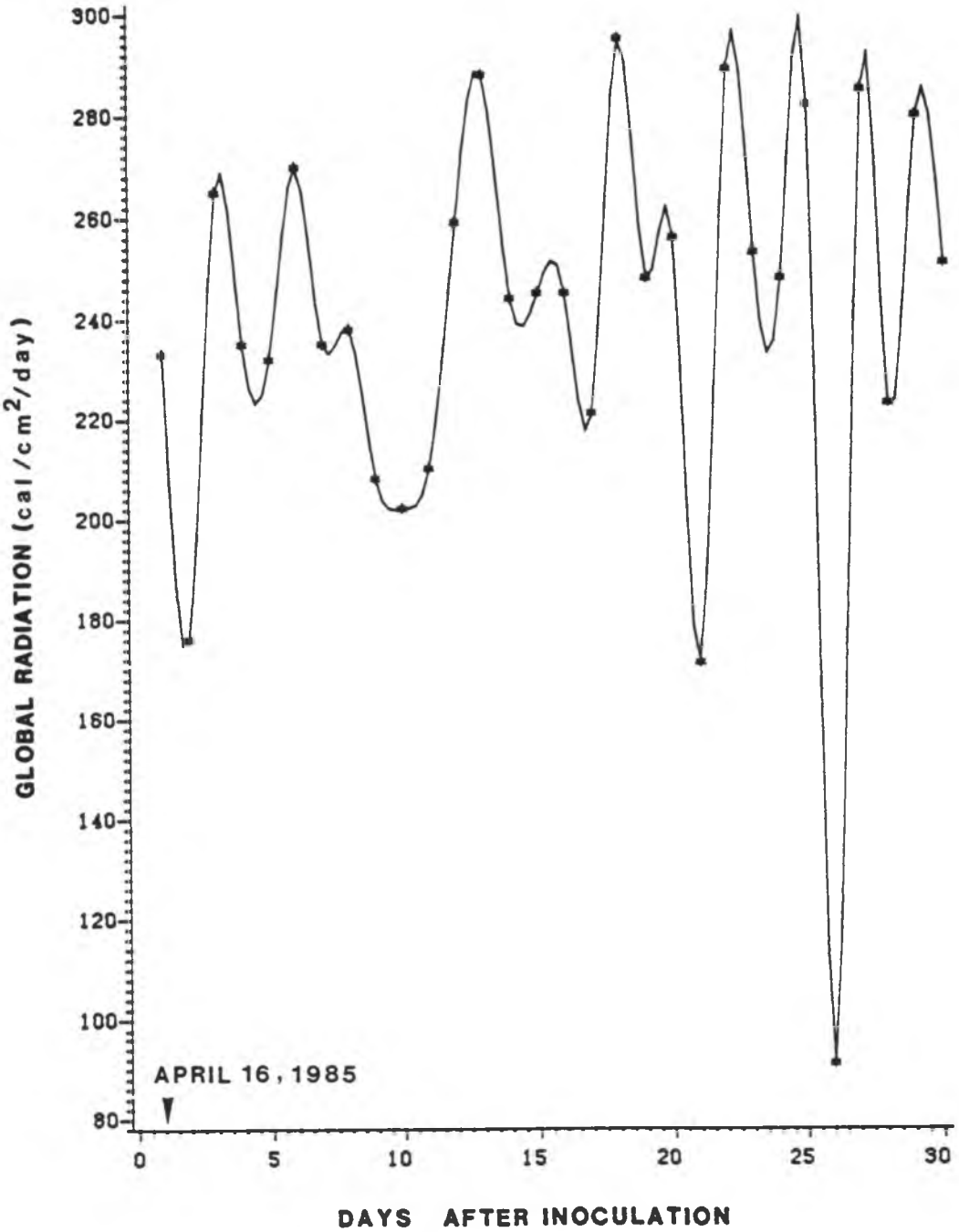


Appendix 6. Time course of pinnule P content of mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings from the experiment of Huang and Fox (1984).

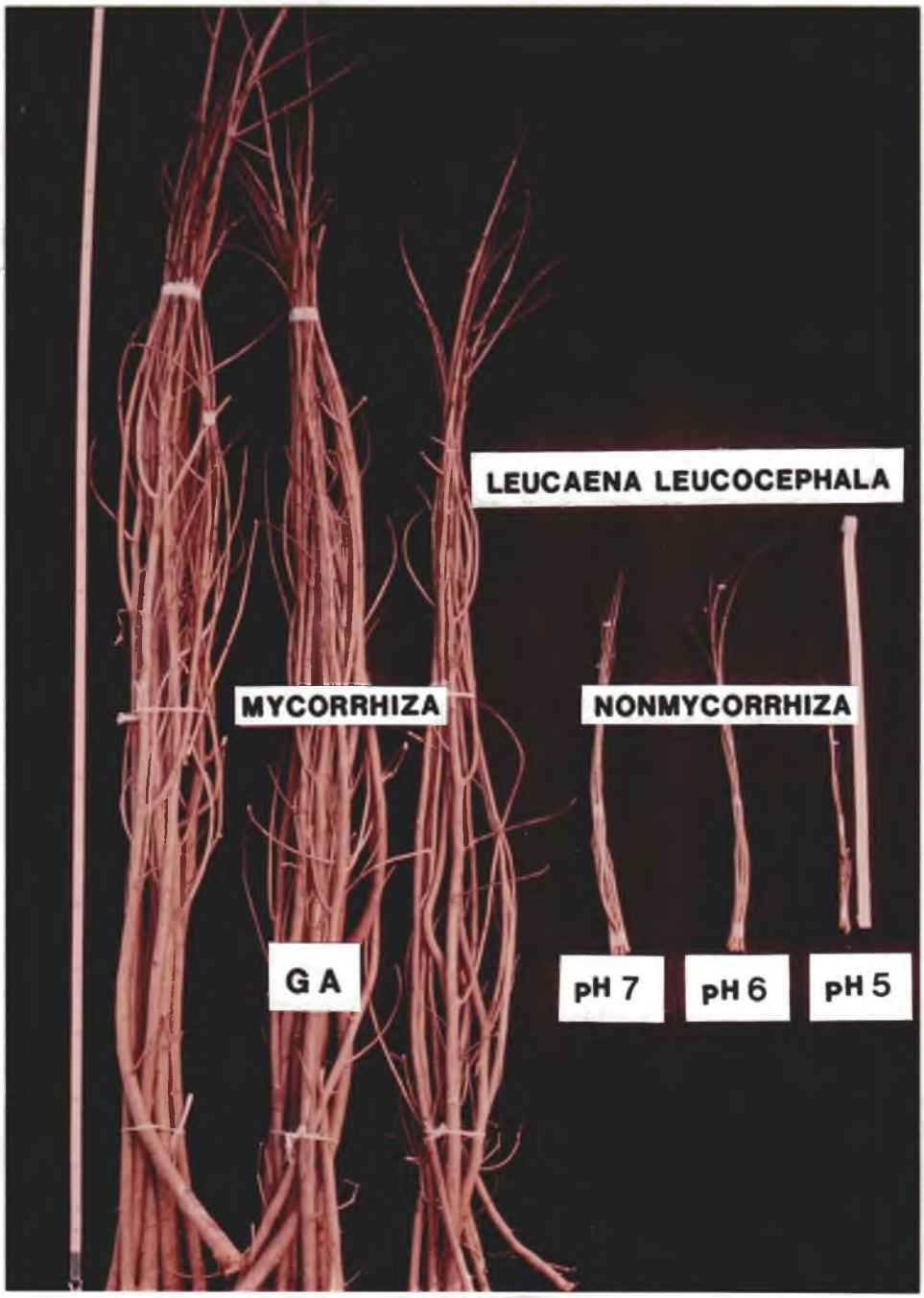


Appendix 7. Global radiation recorded at Manoa campus, University of Hawaii from April 16 to May 17, 1985.

Appendix 8. Daily transpiration and associated variance of mycorrhizal and nonmycorrhizal Leucaena leucocephala seedlings (Main experiment).

	Daily transpiration														
DAI##	1	3	5	6	9	11	15	16	19	21	23	25	26	28	30
Sample no.	108	108	108	90	90	72	72	54	54	36	36	36	18	18	18
CV† of mycorrhizal plants (%)	20	19	20	20	15	14	24	16	20	24	15	14	7	16	6
CV of nonmycorrhizal plants (%)	27	24	24	21	19	15	27	12	16	27	19	15	17	22	15

\# Corresponded to Fig. 11.
DAI represents days after inoculation.
† CV respresents coefficient of variance.



Appendix 9. At 190 days Leucaena leucocephala plants inoculated with Glomus aggregatum (GA) (left) in fumigated Wahiawa soil were an average of 70 times the stem dry weight of uninoculated plants (right).

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