SOIL P BUFFER CAPACITY EFFECTS

ON *LEUCAENA LEUCOCEPHALA* RESPONSE

TO VA MYCORRHIZAE

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Of course, all mistakes in this document are mine.

ABSTRACT

In two glasshouse experiments, Leucaena leucocephala was grown in different soils adjusted to several levels of available P, with half of the treatments inoculated with the vesicular-arbuscular mycorrhizal (VAM) fungus Glomus aggregatum.

The first experiment used two soils with different mineralogies but similar P buffer capacities. Each soil was adjusted to three P levels, and half of the treatments were fumigated with methyl bromide. Fumigation decreased plant growth, while VAM inoculation and added P increased it.

In the second experiment, four soils were used. The soils were selected to provide a wide range of P buffer capacities. All soils were fumigated and each was adjusted to nine P levels. An equation incorporating soil solution P concentration, soil P buffer capacity, and VAM inoculation accounted for 91% of the variation in plant P uptake.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND SYMBOLS

α, β, Γ . . . . . coefficients of P sorption equation (8)
b . . . . . buffer capacity
B_0, B_1 . . . . . parameters of P uptake equation (14)
C . . . . . soil P buffer capacity: dQ/dI
Cl . . . . . concentration of P in soil solution
Cl_0 . . . . . solution concentration of nutrient at the root surface
Cmin . . solution concentration at which there is no nutrient influx
Cthr . . . . . threshold P concentration
DAP . . . . . days after planting
D_e . . . . . effective diffusion coefficient
DZ . . . . . depletion zone
E . . . . . rate of loss of nutrient from root
F . . . . . fumigation of soil with methyl bromide
H, Ho . . . . . Honokaa soil
I . . . . . inoculation of soil with VAM
I . . . . . soil solution P intensity (concentration)
Imax . . . . . uptake rate at "infinite concentration"
In . . . . . rate of nutrient uptake per unit area
Km . . . . . Michaelis-Menten constant
Kr . . . . . solution P concentration where Up = 0.5*Umax
L . . . . . length
L, Lu . . . . . Lualualei soil
L . . . . . liter
M . . . . . substrate concentration
M . . . . . mass
P . . . . . phosphorus
P . . . . . probability
P, Pa . . . . . Paaloa soil
PBC . . . square root of estimated P buffer capacity at 0.02 mg P l^{-1}
Q . . . . . quantity of soil P
r . . . . . radial distance from root
R . . . . . replication
R . . . . . radius
S . . . . . soil series
t . . . . . time
T . . . . . time
Umax . . . . . maximum P uptake potential
Up . . . . . total plant P uptake
V . . . . . rate of an enzyme-catalyzed reaction
V . . . . . volume
v_0 . . . . . rate of water flux into root
VAM . . . . . vesicular-arbuscular mycorrhizae
Vmax . . . . . enzyme concentration
W, Wa . . . . . Wahiawa soil

REVIEW OF THE LITERATURE

Introduction

Vesicular-arbuscular mycorrhiza (VAM) infections of plant roots can increase plant growth (Baylis 1967, 1970; Mosse 1973; Yost and Fox 1979; Plenchette et al. 1983; Schubert & Hayman 1986). This growth increase is often attributed to improved phosphorus (P) uptake by mycorrhizal plants (Baylis 1967; Mosse 1972), and depends on plant and VAM fungus species, soil and environmental characteristics, and interactions among these components (Mosse 1982; Bolan and Robson 1984; Hayman and Tavares 1985; Giovannetti and Hepper 1985; Subba Rao et al. 1985). Inoculating soils with VAM fungi may increase crop yields, especially where soils are low in P. Such soils are common in Hawaii and the tropics (Sanchez and Uehara 1980).

To successfully manage the plant-VAM symbiosis, we must know how soils, plants, and mycorrhizae interact. We need to know the capacity of the soil to supply P and other nutrients to different crops, and how each crop responds to inoculation with mycorrhizae at different levels of soil phosphorus.

We must learn the degree to which specific crops depend on mycorrhizae (Yost and Fox 1979) and how species of VAM differ in their capacity to supply P to these plants (Hall 1984; Mosse 1972). We must investigate how differences in soil P affect crop responses to different mycorrhizal species (Mosse 1972; Bethlenfalvay et al. 1982b; Ojala et al. 1983; Giovannetti and Hepper 1985). We must be able to

measure the existing VAM population in the soil (Mosse 1977) and to determine whether toxic elements are present in detrimental amounts (Gildon and Tinker 1983a).

Vesicular-Arbuscular Mycorrhizal Fungi (VAM)

Vesicular-arbuscular mycorrhizal fungi belong to the family Endogonaceae (Gerdemann and Trappe 1975), and are obligate symbionts of plant roots. These endophytes exist quiescently in the soil until contact with roots or root exudates stimulates them to germinate and produce hyphae (Graham et al. 1981; Schwab et al. 1983). The hyphae then penetrate the plant roots, where they develop distinctive arbuscules (highly branched organs of contact and exchange) and vesicles (globular storage organs). Hyphae also extend from root surfaces into the soil, sometimes to 7 centimeters (Rhodes and Gerdemann 1975).

Because mycorrhizal infection and P fertilizer application have each augmented plant growth in similar circumstances, the fungi have been credited with increasing the P supply of the host plant (Baylis 1967; Mosse 1972). In return, the fungus obtains carbohydrate from its host (Lewis 1975). However, where P is readily available in soil, VAM infections may reduce crop yields (Bethlenfalvay et al. 1982a; Bethlenfalvay et al. 1985; Pacovsky and Fuller 1985; Schubert and Hayman 1986).

In addition to enhancing plant P nutrition, mycorrhizae may increase plant uptake of zinc, copper, iron, sulfur, magnesium, and

potassium (Rhodes and Gerdemann 1978; Swaminathan and Verma 1979; Gildon and Tinker 1983b; Ojala et al. 1983; Kucey and Janzen 1987).

Many species of vesicular-arbuscular mycorrhizal fungi form effective symbioses with a variety of hosts (Mosse 1972, 1975; Schubert and Hayman 1986). The fungal species differ in their capacity to increase plant growth, and some host-endophyte associations may be more effective than others (Giovannetti and Hepper 1985). This effect also depends on soil and environmental conditions, particularly on the availability of P in the soil and whether other nutrients limit plant growth (Miller et al. 1986).

Plant Associations with VAM

Many plant species, both wild and cultivated, form symbiotic associations with mycorrhizal fungi and depend on them for a part of their nutrient supply (Kruckelmann 1975; Read et al. 1976), but to varying degrees (Mosse et al. 1973; Kruckelmann 1975; Yost and Fox 1979). Some plants require high levels of soil P to grow in the absence of VAM; others show little or no response to VAM inoculation at any soil P level (Yost and Fox 1979; Sparling and Tinker 1978). Plants that need high levels of soil P when grown without VAM ("mycorrhiza dependent" plants) often have relatively thick roots with few or short hairs (Baylis 1970 and 1972). Root morphology may not suffice to predict mycorrhizal dependency, because the inability of some nonmycorrhizal roots to obtain P when soil solution concentrations

are low may also be, to some degree, a physiological factor (Mosse et al. 1973).

Leucaena leucocephala

Leucaena leucocephala ("leucaena") readily forms symbioses with vesicular-arbuscular mycorrhizal fungi and responds to inoculation with VAM even at high levels of available phosphorus. Leucaena also forms symbiotic associations with nitrogen-fixing soil bacteria belonging to the genus Rhizobium, which enable it to utilize atmospheric nitrogen (N_2).

The growth habit of leucaena ranges from shrubby to arboreal, depending on the variety and the environment, with the largest trees reaching a height of about 20 meters. Leucaena is a native of semi-arid tropical lowlands, and it competes well in areas that have occasional droughts. It has bipinnate leaves with small pinnules, and avoids drought stress by dropping the pinnules when water is not available. As soon as water becomes available it initiates flowers, and continues to produce buds as long as the water supply lasts. During wet periods one can easily find branches of L. leucocephala bearing, in progression from the meristem of the branch to the trunk, flower buds, mature flowers, and young, older, and ripe seed pods.

Leucaena grows quickly, and the wood can be used for fuel and the trunks for poles in fences and small buildings. It also regrows rapidly after cutting, which enables it to survive grazing and makes it

useful as a perennial fodder and green manure crop. In some places, people eat the unripe seedpods.

Because of its many uses, its fast rate of growth, and its ability to thrive in areas of unpredictable rainfall, lines of Leucaena leucocephala have been selected and bred for use in developing countries. In a small farmer's homestead, leucaena can provide animal feed, firewood, green manure, and poles for construction. In government projects, large-scale plantings of leucaena have been done in marginal areas for reforestation, erosion control, pulpwood production, and biomass production for energy.

A psillid insect parasite of Leucaena leucocephala has recently spread through Asia and the Pacific. This insect feeds voraciously on emerging shoots, deforming leaves and severely reducing plant growth. This has stimulated a search for other woody plants that are less susceptible to insect damage than leucaena, yet share leucaena's ability to form symbiotic associations with N_2-fixing bacteria, grow quickly, supply firewood and poles, produce green manure, provide food for animals, survive drought, and regrow rapidly after grazing or cutting.

Soil Phosphorus: Intensity, Quantity, and Capacity

Phosphorus is essential to plants. Low availability of soil P is the primary constraint to plant growth in many highly weathered tropical soils (Sanchez and Uehara 1980).

Phosphorus occurs in soil in both the liquid phase (soil solution) and the solid phase (mineral and organic matter). The liquid phase, where P exists in the form of the orthophosphate ions $H_2PO_4^-$ and $HPO_4^=$ (Ozanne 1980), is the direct source from which plants and mycorrhizal fungi assimilate P (Sanders and Tinker 1971; Mosse et al. 1973; Tinker 1975). As phosphate is absorbed by plants and fungi, the soil solution is replenished by the dissolution of P from the solid phase (Barber 1984).

Orthophosphate ions migrate between the soil solution and the solid phase. Solid-phase P that cannot exchange with solution P is not accessible to plants; therefore, from the standpoint of plant nutrition, the P of interest is that in solution and the labile P (the solid-phase P that exchanges with the solution). Availability of P to plants is largely determined by the magnitudes of these two forms of P and their relationships (Barrow 1967; Rajan 1973; Holford 1976; Holford and Mattingly 1976b).

In most soils, nearly all the phosphorus is in the solid phase: some is incorporated in the organic matter; some is sorbed on the surfaces of clay particles; some is trapped (occluded) by formation of mineral coatings on top of the layer of sorbed P; and some is secured in the crystal lattices of P minerals (Sample et al. 1980). The labile P is primarily that on the surfaces of clay particles (Beckett and White 1964). In a given soil, labile P depends on several factors, including soil mineralogy, amount of P in the soil, and time allowed for reaction between soil and added P (John 1972; Mattingly 1973; Lindsay 1979; Sample et al. 1980; Jones 1981).

The ratio of solution P to labile P differs among soils. It also changes within the same soil when P is removed or added. The slope of the curve relating the quantity "Q" of labile P to the intensity "I" of solution P defines "C", the capacity of the soil to buffer the solution P concentration (Barrow 1978; Nair and Mengel 1984).

$$dQ/dI = C \qquad (1)$$

In most soils the slope of a plot of this relationship decreases as P is added to the soil (Figure 1). Conversely, as P is removed from the soil, the slope of Q/I increases. Two mechanisms explaining this behavior are described below.

(1) Surfaces of clay particles might have an assortment of sites that sorb P with different energies (Barrow 1983a). Sites with higher bonding energies would retain P more strongly and would be occupied before sites having lower bonding energies. As more P is added, the additional P is held less strongly (lower bonding energy), thus the buffer power of the soil is decreased.

(2) Sorption of phosphate on the surfaces of variable-charge clay increases the negative charge on those surfaces (Sample et al. 1980), and decreases the affinity of those surfaces for additional phosphate (Barrow 1978).

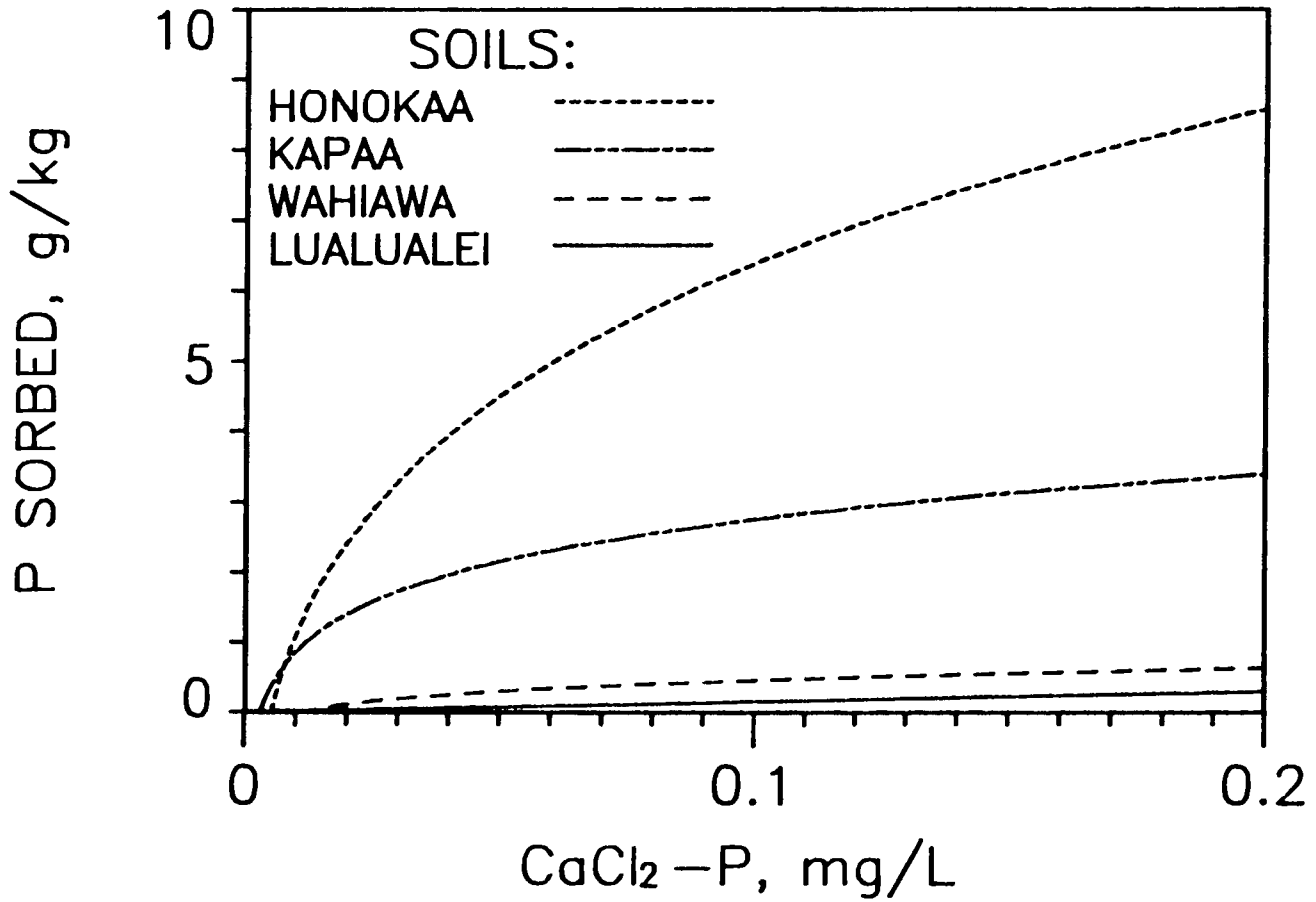

Figure 1. P sorption curves for four soils.

Reactions Between Soils and P Fertilizers

When P is added to the soil solution, perhaps by the dissolution of fertilizer, it can be removed from solution (fixed or retained) by any or all of the following mechanisms: absorption by plants and soil microbes; adsorption on the surfaces of clay particles; occlusion, in which mineral coatings form on top of a layer of sorbed P; or precipitation as P minerals (Sample et al. 1980). On a short term basis, adsorption by soil minerals seems to control P from added phosphate (Thomas and Peaslee 1973; Holford 1979).

P sorption is effected by the formation of chemical bonds of varying strengths between orthophosphate ions and Ca carbonates and Al and Fe oxides on the surfaces of soil particles (Thomas and Peaslee 1973; Sample et al. 1980). Differences in P sorption by soils are attributed to differences in specific surface area and mineralogy, original P status, amount of P added, amount of water present, temperature, and time allowed for reaction (Henry 1961; Barrow and Shaw 1981; Jones 1981; Mendoza and Barrow 1987).

Following the initial, relatively fast sorption reactions, slower chemical processes occur in which orthophosphate ions migrate from sites of relatively high free energy to sites of lower free energy, possibly even penetrating inside the crystal lattices of mineral particles by solid-state diffusion (Barrow 1983a). Diffusion of P into the micropore space, which is extensive in highly aggregated tropical soils, would render this P physically inaccessible to many plants (R.

S. Yost, personal communication). These processes decrease P availability as time elapses after phosphate fertilizer applications.

Generally, oxides of iron have a higher affinity for P than those of aluminum, with the amorphous hydrated oxides of these metals retaining P most strongly, followed by crystalline hydrated oxides, and finally by nonhydrated crystalline oxides (Henry 1961; Fox et. al. 1968). But because it is a chemical reaction between orthophosphate ions and reactive sites on the surfaces of soil particles, P sorption is more closely related to the character and extent of the different P-sorbing surfaces than it is to the soil's percentage mineral composition (Jones 1981).

Soil Properties Affecting the Supply of P to Plants

The concentration of P in the soil solution at the root's surface must be maintained at a nonlimiting level to support good plant growth. As plants extract P from solution at the root surface, two processes sustain solution P intensities: release of P from the solid phase into the solution, and movement of P to the root through the soil.

Three soil properties--the P concentration in the liquid phase, the capacity of the solid phase to deliver P, and the rate of movement of P through the soil--are the main soil factors that control the delivery of phosphorus to plants (Olsen et al. 1962; Olsen and Watanabe 1970; Schenk and Barber 1979; Silberbush and Barber 1983a). The nature of these factors and how they operate in a given situation is determined by such soil properties as clay content of the soil

(Olsen and Watanabe 1970), soil mineralogy and surface area (Henry 1961; Fox et al. 1968; Jones 1981), and moisture content of the soil (Olsen et al. 1962; Holford et al. 1985).

Soil P Intensity

Some researchers have advised that maximum plant growth can be attained by adjusting soil solution P to a concentration ("critical intensity") that is characteristic for each plant species or variety (Ozanne and Shaw 1967; Fox and Kamprath 1970; Peaslee and Fox 1978; Meyer 1980; Moody and Standley 1980; Dandy et al. 1981; Moody et al. 1982; Moody et al. 1883). Others have reported that critical intensities decrease as soil P buffer capacity increases (Barrow 1967; Olsen and Watanabe 1970; Rajan 1973; Holford 1976; Holford and Mattingly 1976b).

The effect of capacity on critical intensity may differ between greenhouse and field trials. The limited volume of soil in pots limits the quantity of P accessible to plants grown in the greenhouse as compared to those grown in the field. Root exploitation of soil in greenhouse pots is intensive and root competition is greater than in the field. Thus, the pool of labile P is usually drained more severely in greenhouse situations, accentuating the importance of quantity and capacity factors in greenhouse experiments relative to field trials (White and Haydock 1968; Salmon 1973; Holford et al. 1985; Holford and Cullis 1985).

Diffusion of P in Soils

There are three mechanisms by which roots can get phosphate: (1) roots can grow into undepleted soil (extension); (2) P can move to roots with the transpirational stream (mass flow); or (3) P can move to roots down a concentration gradient (diffusion) that plants produce in absorbing phosphate (Barber et al. 1963).

A simple calculation shows that most of the P incorporated by plants reaches root surfaces by diffusion, not by mass flow or by root extension (Bouldin 1961; Olsen et al. 1962). Using 0.2 mg P L^{-1} as a figure for soil solution P concentration (a value that is high; Barber et al. 1962), plant P content of 1 mg P g^{-1} (a value that is low; Andrew and Robins 1969), and transpirational efficiency of 500 g H_2O g^{-1} plant material, a plant weighing 10 g will have transpired 5 L of water and have obtained 1 mg P with that water. This plant would require 10 mg P, which is 9 mg (10 times) more than the amount it obtained by mass flow. The proportion of P reaching plant roots by mass flow diminishes as soil solution P concentrations decrease, as water use efficiency decreases, and as plant P concentrations increase.

Ions in solution tend to diffuse evenly throughout the solution by Brownian motion. The overall mass (M) movement across an area (L^2) down a concentration gradient ($M*L^{-3}*L^{-1}$) over time (T) is proportional to the steepness of the gradient (Olsen et al. 1962). Representing the diffusion coefficient by D, we find that

$$M*L^{-2} = D*M*L^{-3}*L^{-1}*T \text{ , or} \quad (2)$$

$$D = M*L^{-2}*M^{-1}*L^{3}*L*T^{-1} = L^{2}*T^{-1} \quad (3)$$

The units of the diffusion coefficient are cm^2s^{-1}. For ion diffusion in soils, more factors must be considered: the volumetric water content (L^3*L^{-3}), which corrects for the relative volume of solution available for ion diffusion; the tortuosity factor ($L*L^{-1}$), which corrects for the additional path length that ions must take; and the inverse of the buffer capacity for that ion, ($dI/dQ = M*L3*M^{-1}*L^{-3}$), which accounts for the ratio of ions in solution to ions in the solid phase (note that all of these compute as dimensionless factors). The rate at which P moves in a soil by diffusion is directly proportional to the soil's moisture content, inversely proportional to the tortuosity of the path the ions must take, and inversely proportional to the soil's P buffer capacity. (For a more complete discussion, see Barber 1984).

Plants absorb phosphorus from the soil solution at the immediate root surface. This reduces the concentration of P in the adjoining solution. The solution is replenished by solvation of solid-phase P from the adjacent soil. As roots continue to extract P from the soil solution, the soil and solution nearest the roots are drained of P. This creates a concentration gradient in the soil around plant roots. This is the gradient that drives the diffusion of P toward roots from zones of higher P concentrations that exist farther away from the roots (Olsen et al. 1962; Barber et al. 1963; Olsen and Watanabe 1966; Lewis and Quirk 1967).

Soil P Buffer Capacity

Soil P buffer capacity affects plant phosphate supply in two opposing ways: (1) Soils with higher capacities to buffer solution P reduce the rate at which solution P concentrations decline as plants take up P from the soil (Nair and Mengel 1984). The capacity of soil to maintain a higher intensity of P in the liquid phase immediately adjacent to plant roots increases the availability of P to plants. (2) Phosphate ions are usually less mobile in soils with high P buffer capacities (Barrow, 1975). This decreased mobility reduces the distance that phosphate ions move per unit time, decreasing the volume of soil that can replenish the soil solution and diminishing the quantity of available P.

In Barber's model of soil nutrient bioavailability (Barber 1984), both the soil P buffer capacity and the effective soil phosphate diffusion coefficient have a direct and positive effect on the supply of P to plants;

$$\text{Uptake} = D_e * b * (dCl/dr) + v_0 * Cl \qquad (4)$$

where D_e is the effective diffusion coefficient, b is the buffer capacity, Cl is the concentration of P in soil solution, r is the radial distance from the root, and v_0 is the rate of water flux into the root. Because the effective diffusion coefficient includes the inverse of the P buffer capacity (page 12, above), and the diffusion coefficient and the buffer capacity are multiplied in the calculation of plant P uptake, the effect of soil P buffer capacity cancels out.

However, P buffer capacity also appears in Barber's model in the calculation of the concentration gradient radial to the root's surface (dCl/dr). Here, increasing the P buffer capacity increases the gradient, which increases the diffusion of P to the root. The comprehensive effect of P buffer capacity on plant growth, in Barber's model, is to increase the supply of P to the plant.

Soil Phosphorus Tests: Introduction

Crop management practices should improve yields enough to pay for the practices and to provide a profit. Because crop growth is strongly influenced by the soil's capacity to supply nutrients, agriculturists need to know the nutrient status of soils in order to predict plant responses to alternative management strategies. People test soils to assess plant nutrient availability, predict crop performance, and estimate fertilizer requirements (White and Haydock 1968).

Soil testing has come to include both the chemical laboratory procedures, in which nutrients are extracted from soil and quantified, and the correlation of the results of these procedures with plant growth responses. Often, the goal of soil tests is to make fertilizer recommendations (Melsted and Peck 1973). Laboratory methods for extracting and quantifying a soil nutrient should be fast and accurate, and the results should correlate well with crop responses in a variety of soils (Bray 1948).

A chemical method that accurately determines the quantity (Q) of labile P, or the soil solution intensity (I), may not provide a good

assessment of the availability of P to plants (Barrow 1967; Rajan 1973; Holford 1976; Holford and Mattingly 1976b; Meyer 1980). This is because the supply of phosphorus to plants depends not only on Q and I, but on the soil's P buffer capacity (C), which determines how the soil solution P concentration will change (rapidly or slowly) as P is removed from the soil by a crop.

To counteract this inadequacy of I and Q estimates in predicting plant response, many of the standard methods of analyzing soils for phosphorus have been designed to generate an index of phosphate availability which is supposed to be balanced between I and Q (White and Haydock 1968; Kamprath and Watson 1980). These indices can be adjusted to favor either the intensity or the quantity parameter by adjusting any or all of the following: the ratio of solution to soil; the ionic strength of the solvent; and the period of contact between the soil and the extractant (Olsen et al. 1954; Barrow and Shaw 1976a, b, c, 1979).

Capacity can be measured either by adding P to soil (adsorption) or by removing P from soil (desorption). These two methods do not give identical results because sorption of phosphate by soils is not a completely reversible reaction (Henry 1961; Barrow 1983b). Adsorption data may be used to identify the desorption properties of soils because desorption is proportional to adsorption (Barrow 1973, 1980, 1983b). The extraction of P from soils by plants may be more closely correlated with adsorption than desorption measurements (Bowman and Olsen 1985).

Characterization of the P-retention properties of soils can be accomplished by measuring Q and I at several points and describing the

resultant Q/I plots using mechanistic models (Barrow 1978; Holford 1979). Some work has also been done with P sorption indices, which require only a single addition of P (Henry 1961; Bache and Williams 1971; Moody 1979).

Soil Phosphorus Tests and Fertilizer Recommendations

Because of their different chemical compositions, soil extracting solutions vary in the effectiveness with which they remove different forms of soil phophorus (Kamprath and Watson 1980; Bromfield 1967). Because pools of labile phosphate vary among soils, both in size and in chemical composition, estimates of labile phosphate obtained by laboratory methods depend on both the extractant and on the soil (John 1972; White and Haydock 1967; Ahmed and Islam 1975; Maida 1978; Holford and Mattingly 1979; Holford 1980a, 1980b, 1983; Holford et al. 1985; Holford and Cullis 1985). The calibration of fertilizer applications to soil test results is best done within groups of soils that are similar chemically and mineralogically (Kamprath and Watson 1980).

Soils that contain similar levels of extractable P can require different amounts of fertilizer P to obtain maximum yields (Barrow 1967b; Salmon 1973; Holford 1976; Meyer 1980; Holford and Cullis 1985). Where P is deficient, soils with higher P retention characteristics require greater amounts of P fertilizer than do soils with lower P retention (Reeve and Sumner 1970; Barrow 1976b; Moody 1979; Meyer 1980; Holford and Cullis 1985). Critical levels of soil test P from a heterogenous group of soils can be estimated better if a

measurement of C is included in the equation along with another extractable P measurements (Olsen and Watanabe 1970; Reeve and Sumner 1970; Rajan 1973; Salmon 1973; Barrow 1976b; Holford and Mattingly 1976b; Moody 1979; Schenk and Barber 1979; Holford 1980b; Meyer 1980; Nair and Mengel 1984; Holford and Cullis 1985).

Some soil nutrient extractants may be affected by C, and compensate for its effect on soil P requirements by removing a smaller quantity of P from soils of greater buffer capacity (Holford and Mattingly 1979; Holford 1980a, 1980b; Mendoza and Barrow 1987b). However, these conclusions may result from the homogeneity of the soils used in these experiments. With more heterogenous groups of soils, there may be little or no correlation between extractable phosphorus values and soil P buffering capacity (Reeve and Sumner 1970; Thomas and Peaslee 1973; Holford and Mattingly 1976b; Meyer 1980; Dandy et al. 1982).

Mechanisms of Plant Growth Enhancement by VAM

VAM apparently absorb phosphorus from the same pool of labile P that is available to nonmycorrhizal plants (Sanders and Tinker 1971; Hayman and Mosse 1972; Mosse et al. 1973; Powell 1975; Bolan et al. 1984). VAM improve the P supply of the host plants by exploring and exploiting the soil mass more thoroughly than do unaided plant roots (Sanders and Tinker, 1971; Hayman and Mosse, 1972), and perhaps by absorbing P from the soil solution at lower concentrations than can unaided roots (Mosse et al., 1973). Mycorrhizal fungi then transfer

some of the P to the host plant, probably in the form of polyphosphate granules (Ling et al. 1975).

VAM mycelia have smaller diameters than do root hairs, and are much longer. The fungal mycelium may consequently be able to extend into volumes of soil that are inaccessible to plant roots due to small pore size, soil compaction, or distance from the main root (Huang 1987).

The small diameter of fungal hyphae has yet another consequence; the flux per unit surface area may increase as diameter decreases (Bouldin 1961), improving the efficiency of uptake by mycorrhizal hyphae with respect to plant roots. This increased efficiency is accentuated if one considers uptake per unit volume relative to plant roots.

The extension of the depletion zone (DZ) around roots with time can be calculated (Schenk and Barber 1979), using the formula:

$$DZ = \mathrm{sqrt}(2*De*t/b), \qquad (5)$$

where De is the effective diffusion coefficient in soil, which takes into account the volumetric water content of the soil, the tortuosity factor, and the diffusion of phosphate ions in water; t is time; and b is the P buffer capacity of the soil, dQ/dI.

The distance P can travel in soil to reach plant roots decreases with increasing soil P buffer capacity (Table 1). The mycelia of mycorrhizal fungi need only extend a millimeter from plant roots to gain access to P that would not otherwise be accessible to their host.

Table 1. -- Calculated P depletion zone (mm) extension around plant roots with increasing time (t, days) and soil P buffer capacity (b = dQ/dI).

	time, days			
	1	3	10	30
b	-----Depletion Zone in mm---			
100	0.240	0.416	0.759	1.315
300	0.139	0.240	0.438	0.759
1000	0.076	0.132	0.240	0.416
3000	0.044	0.076	0.139	0.240
10000	0.024	0.042	0.076	0.132

From Schenk and Barber, 1979.

If we represent the length of VAM mycelium by L, and its radius by R, the additional volume of soil V that is made available by mycorrhizal mycelium for P supply is

$$V = (L-DZ)*pi*[(R+DZ)^2-R^2] \quad (6)$$

The depletion zone is subtracted from the length of mycelium because the P in that volume of soil is already available to the plant. For plant roots, the volume of soil available to supply P is

$$V = L*pi*[(R+DZ)^2-R^2] \quad (7)$$

Table 2 shows, for soils of different P buffer capacities, the volume of soil that can supply P to mycorrhizal mycelia and plant roots over a 10-day period, and the ratio of the additional soil volume available to mycorrhizal roots to that available to roots alone. These

Table 2. -- P depletion zone, volume of soil supplying P to 1 cm of mycelium or leucaena root, and ratio of soil volumes (VAM/root) for a range of P buffer capacities after 10 days.
Radii: mycelium = 0.005mm, root = 0.15mm.

b	DZ	VAM	root	VAM/root
dQ/dI	mm	mm^3	mm^3	mm^3/mm^3
100	0.759	17.00	25.32	0.67
300	0.438	5.92	10.19	0.58
1000	0.240	1.85	4.08	0.45
3000	0.139	0.64	1.91	0.33
10000	0.076	0.20	0.90	0.23

calculations assume that the mycorrhizal hypha and the plant root have different radii (0.005 and 0.15 mm, respectively) but equal lengths (1 cm), and that the hypha grows perpendicular to the root.

The crucial point is that, for each additional cm of hypha added per cm of plant root, the increase in soil volume available to supply P to the host plant can be about 25 to 67 percent.

Plants that do not depend on mycorrhizae for supplying their needs for P often have root systems composed of a fibrous mass of many fine roots (Baylis 1967, 1970) and thus would receive little increase in soil exploration capabilities from mycorrhizal infection.

Mycorrhizal infections of plant roots can reduce the Q or I of soil P required for good crop yields when mycorrhiza-dependent crops are grown (Yost and Fox 1979) and thereby both improve plant growth and reduce P fertilizer requirements.

Effects of Soil Characteristics on the VAM-Plant Symbiosis

The main benefit to plants of mycorrhizal colonization is improved P nutrition. Increased yields ensue only where the supply of P would otherwise restrict growth. In general, plant responses to VAM infection decrease as quantities of P applied increase (Abbott and Robson 1977; Hall 1978; Stribley et al. 1980). Where high levels of P are readily available to plants, VAM-infected plants have been observed to be smaller than their noninfected counterparts (Baylis 1967; Bethlenfalvay et al. 1983). VAM also can reduce plant growth at very low levels of soil P (Baylis 1967).

Plants may not respond to VAM or to P if another growth factor, such as nitrogen or water, is limiting (Koncheki and Read 1976; Hepper 1983). Soil properties can affect the plant or the fungus directly; effects on either of the partners can have repercussions on the functioning of the symbiosis (Mosse 1972. Lambert et al. 1980).

The Problem

Inoculation of soils with vesicular-arbuscular mycorrhizae can, in some circumstances, augment plant growth and reduce P fertilizer needs. However, inoculation with VAM is costly when done on a large scale. For inoculation to be profitable, gains resulting from inoculation must exceed the costs of the inoculum and its application. People proposing to inoculate plants or fields with mycorrhizal fungi will have to show that inoculation is likely to increase plant growth

significantly, profitably, and more effectively than P fertilizer additions alone, or that inoculation reduces the risk of financial loss.

The effect of P fertilization on plant growth depends on many factors, among which are the quantity of soil P that is available to the plant (Q), the soil solution P concentration (I), and the relationship between these attributes, the soil phosphate buffering capacity (C). Even where P is the only factor limiting plant growth, none of these measures alone can accurately predict plant response to P fertilizer additions, except within a group of soils that is relatively homogenous as to buffer capacity and within which plant response to P is known.

Ideally, C describes how Q or I can be expected to change (rapidly or slowly) as P is removed from the soil by a crop. Where the supply of P is a major limit to plant growth, an estimate of soil P buffer capacity, together with an estimate of either the intensity or the quantity of soil P, can provide enough information on soil P availability to permit a fairly accurate prediction of crop responses to P fertilizer applications, even across soils with a wide range of P sorption characteristics (Reeve and Sumner 1970; Holford and Mattingly 1976b; Meyer 1980; Ojala et al. 1983; Nair and Mengel 1984).

Because measures of I, Q, and C can be used to predict plant response to P fertilization, and since mycorrhizal inoculation can substitute to some degree for P fertilization, there is a good possibility that plant response to VAM inoculation could be partially predicted from measures of soil I, Q, and C.

Plants might respond to mycorrhizal inoculation as they would to P fertilizer additions. Responses to VAM inoculation, as to P additions, might be higher in soils of low phosphorus Q and I. The threshold I for growth enhancement of plants by VAM might be lower at higher C. The critical I for near-maximum yield with VAM might also be lower at higher C.

Testing these ideas requires that plants be grown both with and without mycorrhizal infections in conditions of equal Q with unequal I and C; equal I with unequal Q and C; and equal C with unequal Q and I. All other factors affecting plant growth should be standardized. Correlation of plant response to mycorrhizal inoculation with the results of standard laboratory soil tests might allow the results of this study to be applied beyond the present experiments.

MATERIALS AND METHODS

Soils

The soils used in these experiments were selected to provide a wide range of phosphate buffer capacities (C). They belong to four Orders: Vertisols, Oxisols, Ultisols, and Inceptisols. All of the soils are used for agriculture in Hawaii.

Lualualei soil is a very fine, montmorillonitic, Typic Chromustert. The dominant minerals in this soil are 2:1 and 1:1 clays (smectite, kaolinite, halloysite). Some $CaCO_3$ particles derived from coral were present in the sample used in the experiment.

Wahiawa soil is a clayey, kaolinitic, Tropeptic Eutrustox. Although the dominant minerals are 1:1 clays, gibbsite and goethite each make up about 10% of the soil by weight.

Kapaa soil is a clayey, oxidic, Typic Gibbsiorthox. The dominant minerals are gibbsite and goethite. The soil used for the experiment contained foreign particles of $CaCO_3$ and had an unusually high pH for this series.

Paaloa soil is a clayey, oxidic, isothermic Humoxic Tropohumult. Dominant minerals in the surface horizons are gibbsite and 2:1 clays, with appreciable amounts of quartz, goethite, hematite, and kaolinite. The soil collected for this experiment had previously been planted to sugarcane.

Honokaa soil is a thixotropic Typic Hydrandept. The dominant minerals are amorphous colloids of extremely high surface area, about

Table 3. -- Mineralogy and other characteristics of soils from the same series as the experimental soils.

	units	CHROM-USTERT	EUTR-USTOX	TROPO-HUMULT	GIBBSI-ORTHOX	HYDR-ANDEPT
2:1 clays	%	30	0	30	0	0
1:1 clays	%	60	70	10	0	0
Gibbsite	%	0	10	25	40	0
Goethite	%	0	10	10	50	0
Amorphous	%	0	0	0	0	100
oxides	%	10	10	25	10	0
pH(H2O)	---	7.0	5.0	4.8	5.8	5.5
pH(KCl)	---	6.0	4.4	4.4	6.0	4.9
CEC	cmol/kg	100	25	20	15	40
B.S.	%	100	20	10	10	20

Mineralogical data from R. C. Jones and R. Gavenda, Dept. Agron. Soil Sci., U. Hawaii: unpublished. Other data from USDA 1976.

30% of which are Fe-oxides, 50% Al-oxides, and 20% Si-oxides. The soil collected for this experiment was from a pasture that had never been fertilized. (USDA 1972, 1973, 1976; R. Jones and R. Gavenda, University of Hawaii, unpublished data).

The Test Plant and Fungus

The test plant used was Leucaena leucocephala (Lam.) de Wit ("leucaena"). Variety 565 was used in experiment 1 and variety K-8 (Hawaiian Giant) was used in experiment 2, both obtained from James L. Brewbaker at the University of Hawaii.

The vesicular-arbuscular mycorrhizal fungus used was an accession of Glomus aggregatum selected at the University of Hawaii for its

superior enhancement of growth of several species of plants, including Leucaena leucocephala (Huang Ruey-Shang, personal communication).

Design of the Experiments

All experiments were conducted by growing plants in 2-kg pots in the greenhouses in Manoa Valley belonging to the Department of Agronomy and Soil Science at the University of Hawaii.

Experiment 1

The first experiment was devised to explore the effects of native and introduced mycorrhizal populations on plant growth in different soils at a range of phosphorus levels. It was designed as a complete factorial experiment, laid out in a randomized complete block with three replications. Treatment variables were: fumigation with methyl bromide and no fumigation, inoculation with VAM and no inoculation, two soils (Wahiawa and Paaloa), and 3 P levels (P added, based on P sorption curves, to create solution concentrations of 0.025, 0.1, and 0.4 mg P/l). There were 24 treatments using 72 pots, each containing 1.5 kg soil.

Experiment 2

The second experiment was conducted in order to assess the effect of soil P buffering capacity on mycorrhizal enhancement of plant growth. Four soils of differing P buffer capacity were used. Differences among soils in properties not related to P supply were

minimized by liming, adding nutrients, irrigating 3 times per day, and by mixing all soils with coarse blasting sand in a ratio of 15:85 soil:sand (300 g soil and 1700 g sand per pot). Control treatments contained 100 percent blasting sand. All treatments were fumigated with methyl bromide. The experiment was laid out in a completely randomized design with two replications. Treatment variables were: 4 soils (Lualualei, Wahiawa, Kapaa, and Honokaa), 9 P levels (from about 0.003 to 0.3 mg P/l), and with and without mycorrhizal inoculation, making a total of 72 treatments.

Soil Preparation

The soil material used in these experiments were collected from three of the major islands of the Hawaiian chain; the Lualualei, Wahiawa, and Paaloa soils were collected on the island of Oahu, the Kapaa soil was obtained from the island of Kauai, and the Honokaa soil from the Island of Hawaii.

Sifting and Mixing. About 30 kg of each soil was stored in the greenhouse in plastic bags. Each soil was sieved (6 mm mesh), organic materials and large stones were removed, and soils were mixed to achieve a homogenous composition. The Lualualei soil was necessarily sieved in a slightly moist condition, and aggregates were broken by hand. The Hydrandept, which is thixotropic, was also kept moist during storage and preparation to avoid irreversible drying. The Oxisols and the Ultisol were stored and handled air-dry.

Table 4. -- Lime (g $CaCO_3$/kg soil) and P (mg P/kg soil) added for each soil at the targeted $CaCl_2$-P concentrations. Experiments 1 and 2.

EXPERIMENT 1

SOIL SERIES	$CaCO_3$ added (g/kg)	Targeted [P] 0.025	0.1	0.4
		P added (mg P/kg soil)		
Paaloa	7.92	55	177	378
Wahiawa	2.52	42	148	308

EXPERIMENT 2

SOIL SERIES	$CaCO_3$ added (g/kg)	Targeted [P] 0.01	0.02	0.04	0.08	0.16	0.32	0.64	1.28	2.56
		P added (mg P/kg soil)								
Lualualei	0.00	20	37	57	82	111	146	189	239	300
Wahiawa	2.52	3	41	89	147	220	309	421	558	729
Kapaa	0.00	0	164	380	638	945	1312	1749	2270	2892
Honokaa	5.00	209	643	1172	1814	2594	3543	4695	6096	7798

Liming. After determination of the liming response of each soil, precipitated laboratory $CaCO_3$ was mixed thoroughly with weighed portions of the soils to bring the soil pH above 6.0 (Table 4), after which the soils were placed in pots or plastic bags, watered, and allowed to equilibrate for at least 2 weeks.

Adding Nutrients. Macro- and micro-nutrients, including P, were added in solution by weighing the soil for one planting pot at a time, spreading it on a plastic sheet, and spraying it with measured volumes of solutions of known concentrations while mixing thoroughly. Phosphate was added as dissolved monocalcium phosphate where possible; when larger quantities of P were needed, monobasic potassium phosphate was also used to avoid adding too much water to the soil, making it

unworkable. Rates of P added are shown in Table 4. KNO_3 was added at 513 mg/kg soil. Magnesium (100 mg/kg), copper (20 mg/kg) and zinc (50 mg/kg) were added to the soil as sulfates, molybdenum (17 mg/kg) was added as ammonium molbdate, and boron (2.3 mg/kg) was added as an acid. After this preparation, soils were placed in pots or plastic bags to equilibrate for at least two weeks at or near field water capacity before further treatment.

Fumigation. The soils were fumigated in vats 75 cm deep and 2 m in diameter set into the ground. Pots of soil at about field capacity were placed into the vats, stacking pots on the rims of the pots below to allow for free air movement. One canister containing 48 g of methyl bromide and 1 g of chloropicrin was used for each 150 kg soil. The soils were kept for 3 days in this chamber, after which the plastic covers of the vats were removed and the soils allowed to air for several hours. The pots were then moved to the greenhouse, where they were allowed to dry for at least one week before planting to allow the methyl bromide to dissipate from the soil. For the second experiment, soils were equilibrated with lime and fertilizer for 6 weeks, then mixed with blasting sand, put in pots, and the pots of sand-soil mixture were fumigated as described above.

Preparation of Planting Material and Planting

Seeds of Leucaena leucocephala var. 565 (experiment 1) or K-8 (experiment 2) were inspected visually; and small, cracked, broken, or malformed seeds were removed. The remaining seeds were placed in

concentrated H_2SO_4 and stirred with a magnetic stirrer for 20 minutes, after which the seeds were rinsed thoroughly and kept immersed in deionized water for 24 hours. The water was changed several times during this soaking period. The seeds were then placed on a metal sieve and kept in shade for another 24 hours, during which time they were soaked several times for 30 minutes in deionized water. When the radicles began to emerge from the seedcoat, seedlings were selected for uniform size and length of the emerging radicle. Scarification and germination were timed so that seeds could be planted while the radicles, though emerging, were as short as possible. In the first experiment, depressions were made in the soil and the sprouted seeds were planted with the root pointing down. In the second experiment this effort was not made, and some seeds pushed roots into the air, others extended their roots horizontally several centimeters before pushing them down into the soil, while the other seeds grew normally. The loss of planting material in pots was compensated by planting six seeds per pot, and thinning to 3 to 5 plants per pot.

Inoculation

Two methods of inoculation were used in the experiments. In the first experiment, about 1 cc of inoculum, consisting of a mixture of ground basaltic rock, root fragments, hyphae, and spores, was put into each planting hole. In the second experiment, germinated seeds were placed on the soil surface, and the soil was inoculated by pipetting two ml of a slurry composed of Glomus aggregatum spores and root

fragments containing hyphae onto the soil around each seed. Seeds were then covered with 1 cm of blasting sand.

In the second experiment Rhizobium sp. strain # 1145SR, obtained from M. Habte (University of Hawaii), was also introduced into the soil at a rate of $2*10^6$ bacteria/pot, included in the slurry containing the mycorrhizal inoculum.

Management of the Experiments

Watering. For the first experiment watering was done once a day by hand, saturating the soils in the pots and allowing them to drain. Thus all plants had equal access to water. As the plants grew, pots containing larger plants were watered twice a day. For the second experiment, in which the rooting medium consisted of 85 percent blasting sand and 15 percent soil, an irrigation system was built that delivered 60 ml of water 3 times per day, at 10:00 AM, 1:00 PM, and 3:00 PM.

Fertilization. In both experiments, nitrogen and potassium were added to the soils during soil preparation as KNO_3. Additional nitrogen and potassium was applied during the course of the experiments by watering with a solution of KNO_3 that contained 100 mg N/L. In the first experiment, a total of 600 mg of N was provided. Because pots were watered to saturation, excess water drained from the pots, and N was probably lost by leaching; therefore, in the second experiment, fertilization with these elements and with calcium was done two or three times per week by turning off the irrigation system and watering

by hand, alternating solutions of KNO_3 and $CaNO_3$ that were 200 mg N/L. This was intended to maintain a soil solution concentration of N above 50 mg/L.

Insect Control. For the first experiment, Psillid insects were treated by physical removal and by introducing a natural predator of the psillids into the greenhouse. For the second experiment, plants were sprayed weekly with Cygon regardless of psillid infestation; this seemed to strike about an equal balance between insect damage and pesticide damage to the plants, both of which were slight.

Pinnule Sampling

Pinnules were collected periodically from the plants during the course of the experiments, starting at 10 days after planting (DAP). One pinnule per pot was harvested; generally, the fourth pinnule counting from the base of a pinna of the most recently fully expanded leaf. These pinnules were weighed and tested for phosphorus as described below.

Harvest Measurements and Sampling

Experiment 1. Stem heights were measured, plant tops were harvested, and leaves were separated from stems, dried, and weighed.

Experiment 2. The number of plants per pot was counted, as this varied between three and five. Stem diameters and heights were

recorded. One plant per pot, intermediate in height, was selected for further measurement.

On the selected plant, leaves were numbered, pinnae and pinnules were counted, and leaf areas were measured. Leaves were weighed and analyzed for P. The stem was cut at the cotyledon leaf scar, and everything below that was counted as root. The stems were dried, weighed, ground, and tested for P content.

Roots and soil were dumped out of the pots onto a plastic sheet, and with slight shaking a soil sample of about 200 g was removed. This was analyzed for P. The roots were washed and dried, weighed, and analyzed for percent mycorrhizal infection and P content.

Determination of Phosphorus

Solutions containing phosphorus, whether soil extracts or plant digests, were reacted with Reagent B (Olsen and Watanabe 1963), a solution containing ammonium molybdate, potassium antimony tartrate, sulfuric acid, ascorbic acid, and water. After allowing time for the color to develop, samples were analyzed spectrophotometrically (colorimetrically) on a Hitachi model 100-40 spectrophotometer.

Soil samples weighing from 3.0 to 7.0 g (depending on the soil test performed) were placed in 50 ml centrifuge tubes, and solutions were added that contained phosphorus (when P sorption was measured) or extractant solutions as described in Soil Analysis Methods below. Samples were then shaken longitudinally on a Eberbach mechanical back-and-forth shaker, except where otherwise noted. Soil was separated

from the solution by centrifugation in a DuPont Sorvall SS-3 centrifuge, and the supernatant solution was tested for P.

Ground plant samples (10 to 25 mg) were placed in Pyrex test tubes and heated at 500° C until the ash turned white or gray. This ash was dissolved in a 1:4 solution of Reagent B and water and tested for phosphorus.

Reagent A was prepared by dissolving 19.2 g ammonium molybdate [$(NH_4)_6Mo_7O_{24}*4H_2O$] and 0.466 g potassium antimony tartrate ($KSbO*C_4H_4O_6$) in about 1 L of deionized water, adding 141 ml concentrated H_2SO_4 (5 moles), and bringing the final solution volume to 2.0 L.

Reagent B was prepared by dissolving ascorbic acid in Reagent A, using 0.428 g ascorbic acid for each 100 ml of the reagent. This was done on the same day that the analysis was made (Reagent B does not keep more than 1 day). All solutions and extracts were allowed to react with Reagent B for at least 10 minutes for full color development.

Phosphorus in extracts was determined by mixing Reagent B with extract solutions in a ratio of 1:4 and measuring the amount of light absorbed at 840 nm on the spectrophotometer. The spectrophotometer was calibrated before and while readings were taken, using solutions of known P concentration (including blank solutions) mixed 4:1 with Reagent B.

Soil Analysis Methods

Soil pH was tested on a Fisher Accumet model 805 MP pH meter in a 1:2 soil:water paste with a 30 minute equilibration time. The slurry was mixed once with a glass rod when the water was added, then mixed again immediately before the pH electrode was inserted into the slurry.

Liming curves. The response of soil pH to liming was estimated in two ways; a short-term method using 250 g of soil, 5 levels of $Ca(OH)_2$, and an equilibration time of several days at 50° C, and a longer-term method in which several pots, each containing 2 kg of soil, were amended with different amounts of lime and incubated at field capacity in the greenhouse for several weeks.

P sorption curves. 3.0 g of soil and 30 ml of 0.01 M $CaCl_2$ solutions containing known amounts of monocalcium phosphate were added to a 50 ml centrifuge tube. Two drops of toluene were added to suppress microbial activity and the tubes were stoppered firmly and shaken for 30 minutes twice a day for 6 days. The tubes were then centrifuged at 10,000 rpm for 10 minutes. A 10.0 ml aliquot of the supernatant solution was withdrawn, mixed with Reagent B, and the resulting solution was analyzed for P using a spectrophotometer (Fox and Kamprath 1970).

When the 15:85 soil:blasting sand mix was tested for P, 20.0 g of the mix (on an oven-dry basis) was used. This provided 3.0 g of soil in the sample and reduced the variance in the actual quantity of soil in the sample that might result from uneven mixing were 3.0 g of soil:sand mixture to be used. Results (e.g. mg P/kg soil) will be

reported on the basis of the 3.0 g of soil in the mixture, unless otherwise stated. When soils were not mixed with sand, a sample weighing 3.0 g was used. All weights are calculated on an oven-dry basis, although the soils were tested in a state between air-dry and field capacity.

$CaCl_2$ extract P test (Ozanne and Shaw 1967). 3.0 g of soil was combined with 10.0 ml of 0.03 M $CaCl_2$ solution and sufficient deionized water was added to bring the total volume of solution in the tube, including the water contained in the soil, to 30 ml. Two drops of toluene were added to suppress microbial activity and the tube was firmly stoppered. The tubes were inverted gently by hand several times per day to achieve a complete mixing of the soil with the solution without destroying the soil aggregates (a consideration important in the testing of soils that were mixed with sand). After 40 hours, the tubes were shaken one last time and then centrifuged at 10,000 rpm for 10 minutes, after which a 10.0 ml aliquot of the supernatant solution was withdrawn and tested for P. Results were calculated as mg P/L of solution, and reflect the soil solution P concentration (Dalal and Hallsworth 1976, 1977), although they are not identical with it (Olsen and Watanabe 1970; Soltanpour et al. 1974)

Olsen ($NaHCO_3$) P test (Olsen et al. 1954). 30 ml of 0.5 M $NaHCO_3$, adjusted to pH 8.5 with NaOH, was added to 3.0 g of soil in a 50 ml centrifuge tube. The tube was stoppered firmly and shaken for 30 minutes, then promptly removed, unstoppered, and centrifuged at 5,000 rpm for 5 minutes. 5.0 ml of the supernatant was drawn off and tested for P. Results were calculated as mg P/kg soil.

Mehlich (double acid) P test (Sabbe and Breland 1974). 12.0 ml of an extracting solution (0.05 M HCl and 0.05 M H_2SO_4) was added to 3.0 g of soil in a 50 ml centrifuge tube. This was shaken for 5 minutes and immediately removed, unstoppered, and centrifuged for 5 minutes at 5,000 rpm. A 1.00 ml aliquot was withdrawn and the P content of the solution was determined. Results are expressed as mg P/kg soil.

Bray # 1 (acid fluoride) P test (Bray and Kurtz 1945). 20.0 ml of solution (0.025 M HCl and 0.03 M NH_4F) was added to 3.0 g of soil in a 50 ml centrifuge tube. From this point, treatment of the samples was exactly as in the double acid test with respect to time of shaking, centrifugation, and sample and reagent volumes. Results are expressed as mg P/kg soil.

Plant Analysis

Leucaena leucocephala pinnules were sampled at intervals of 3 to 13 days over the course of the experiments (see Pinnule Sampling, above). Pinnules were dried in a convection oven at 50° C for one to several days, then weighed to the nearest 0.1 mg. Harvest samples (leaves, stems, roots) were also dried at 50° C, weights were determined to the nearest mg.

For determination of P, pinnules were placed in a Pyrex test tube and ashed in a muffle furnace at 500° C until the residue was white or light gray, which usually took about two hours. After cooling, 10.0 ml of a solution containing 4 parts deionized water to 1 part Reagent B was added to the ash, the solution was mixed thoroughly, and the P

content determined colorimetrically. Results are expressed as total P per pinnule and as percent P of oven-dry weight (before ashing).

Stem heights were measured from the cotyledon leaf scar to the meristem. This was done to both standardize the measurements and to avoid measurement differences due to different planting depths. All plant parts below the cotyledon leaf scars were included in root weights.

When leaves were counted, in the second experiment, the cotyledons (rarely present) or cotyledon leaf scars were assigned leaf number zero, and the rest of the leaves and leaf scars were numbered in ascending order toward the meristem. The presence or absence of each leaf was noted and the pinnae and pinnules were counted on leaves that were present.

Leaf area was measured by removing the pinnules from a leaf, laying them between two sheets of clear plastic, and running this through a LI-COR Model 3050-A portable area meter. On larger leaves bearing many pinnules, only 1/4 of the pinnules were used for this measurement and the measured area was multiplied by 4. The use of only 1/4 of the pinnules was warranted by the fact that the leaves of L. leucocephala are bilaterally symmetrical in two ways; the pinnae are symmetrical on each side of a leaf, and the pinnules are symmetrical on each side of a pinna.

Other plant materials (roots, stems, and leaves) were ground with a Bodine NSI-55 plant grinder using a 20-mesh sieve, and subsamples weighing 10.0 to 20.0 mg were transferred into Pyrex test tubes, ashed,

and analyzed for P as above. For each plant fraction, results were calculated both on a plant and a pot basis.

After the dry root weights were recorded, a sample of the finer roots was composited from several parts of the root mass for assessment of mycorrhizal infection. The rest of the root mass was ground and P content determined.

Roots for evaluation of mycorrhizal infection were soaked for 48 hours in 10 % KOH, then rinsed in deionized water, soaked for 3 minutes in 10 % HCl, rinsed again with deionized water, then placed in a solution of lactophenol (1750 ml), glycerine (126 ml), acid fuchsin (3.0 g), and deionized water (126 ml) for staining (Phillips and Hayman 1970). After the roots had absorbed the stain, they were rinsed three times in a solution identical to the above except that it contained no acid fuchsin. The roots were then inspected for percent mycorrhizal infection using the line-intercept method (Giovannetti and Mosse 1980) with a grid marked off in 1 cm squares. 100 root-line intersections were counted, and the number of intersections at which the roots were infected was divided by 100 (the number of intersections counted). Root-line intercepts were recorded as infected if fungal hyphae were seen within 1 cm of the intersection point. Results are expressed as percent infection.

Statistical Methods

In the first experiment, soil P levels were not determined except as to amount of P added or target P level in $CaCl_2$, and thus were

treated as class variables. Soils, fumigation of soil, and inoculation with mycorrhizal fungi were also treated as class variables. Therefore, analysis of the biweekly pinnule sampling data from the first experiment was based on analysis of variance. Effects of treatments on pinnule weight and P content over time were followed by graphing the data and by noting the probability value for each main effect and interaction over the course of plant growth. Harvest data was also analyzed using analysis of variance. Both untransformed and log-transformed data were analyzed in this manner. The log transformation, while equalizing variance over the data range, carries with it the property of testing for multiplicative effects (addition of logarithms) rather than additive effects of treatments, and additive effects show as interactions. Conversely, where treatments act in a synergistic (multiplicative) fashion, analysis of variance of untransformed data will show an interaction .

The second experiment was designed to be analyzed by regression. Soil P was measured in the original soils to which P had been added and in soils that had reacted with P for the duration of the experiment. This permitted analysis of plant response with regard to soil test P levels. The soils were also treated as continuous variables for some analyses by using a measure of their respective P sorption capacities as an independent variable in the equation, so that inoculation with mycorrhizae (or lack of it) was the only treatment variable that was not treated as a continuous variable. Estimates of the parameters of nonlinear models were made using the NONLIN module of the SYSTAT statistical analysis program.

RESULTS AND DISCUSSION

Experiment 1

This experiment was designed to investigate the effect of native and introduced mycorrhizal fungi on leucaena growth and P uptake across a range of levels of soil P supply. Some of the factors addressed were: the effect of mycorrhizal inoculation in soils with and without native mycorrhizal populations; effects of soil P levels on mycorrhizal enhancement of leucaena growth; and effects of soil series on the plant-fungus symbiosis.

Pinnule Sampling Results

Effect of Inoculation. The mean quantity of P was higher in the pinnules of plants growing in soils that were inoculated with VAM than in the plants growing in noninoculated soils on every sampling day (Table 5, Figure 2).

Effect of Fumigation. From 38 DAP on, plants growing in fumigated soils had less P per pinnule than plants in nonfumigated soils (Table 5, Figure 3).

Effect of Fumigation + Inoculation. In the soils that were inoculated, fumigation decreased the amount of P in pinnules on days 21 and 24 after planting (Figure 3). For plants growing in noninoculated soils, fumigation decreased pinnule P contents from 42 DAP, when plants growing in nonfumigated, noninoculated ("natural") soils had larger quantities of pinnule P than plants grown in fumigated, noninoculated

Table 5. -- Treatment effects on pinnule P, experiment 1. Results of analyses of variance of ln(μgP per pinnule) for each sampling day. Probabilities of type I error.

	Days After Planting												
Effect	10	14	17	21	24	31	35	42	45	49	52	56	59
CONSTANT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R[a]	0.74	0.57	0.20	0.10	0.19	0.31	0.30	0.40	0.96	0.99	0.42	0.38	0.70
I	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	0.24	0.02	0.52	0.11	0.01	0.31	0.87	0.07	0.01	0.01	0.00	0.00	0.00
P	0.47	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
S	0.41	0.27	0.04	0.20	0.08	0.07	0.08	0.05	0.02	0.00	0.05	0.03	0.01
I*F	0.79	0.91	0.41	0.04	0.00	0.26	0.98	0.76	0.03	0.00	0.00	0.00	0.00
I*P	0.19	0.94	0.75	0.44	0.09	0.00	0.00	0.00	0.00	0.03	0.05	0.00	0.00
I*S	0.58	0.76	0.36	0.42	0.38	0.09	0.00	0.01	0.00	0.00	0.07	0.00	0.00
F*P	0.29	0.92	0.76	0.69	0.68	0.01	0.25	0.80	0.67	0.98	0.01	0.00	0.01
F*S	0.51	0.34	0.76	0.06	0.00	0.05	0.83	0.31	0.81	0.27	0.66	0.00	0.50
P*S	0.96	0.50	0.78	0.63	0.69	0.95	0.49	0.65	0.32	0.11	0.32	0.15	0.47
I*F*P	0.12	0.32	0.88	0.26	0.09	0.53	0.38	0.25	0.36	0.73	0.19	0.00	0.00
I*F*S	0.06	0.13	0.68	0.01	0.00	0.19	0.46	0.43	0.40	0.74	0.66	0.01	0.29
I*P*S	0.07	0.19	0.39	0.53	0.20	0.30	0.82	0.91	0.16	0.03	0.01	0.93	0.63
F*P*S	0.47	0.23	0.04	0.39	0.80	0.25	0.49	0.61	0.42	0.58	0.68	0.35	0.04
I*F*P*S	0.79	0.19	0.11	0.97	0.96	0.88	0.67	0.67	0.80	0.26	0.19	0.03	0.00
R^2	0.47	0.62	0.73	0.75	0.83	0.87	0.90	0.87	0.88	0.85	0.85	0.91	0.86

[a] Effects are denoted as follows: R = Replication, I = Inoculation with VAM, F = Fumigation, P = Phosphate fertilization levels, S = Soils. Interaction terms have asterisks, e.g. I*F = Inoculation by Fumigation.

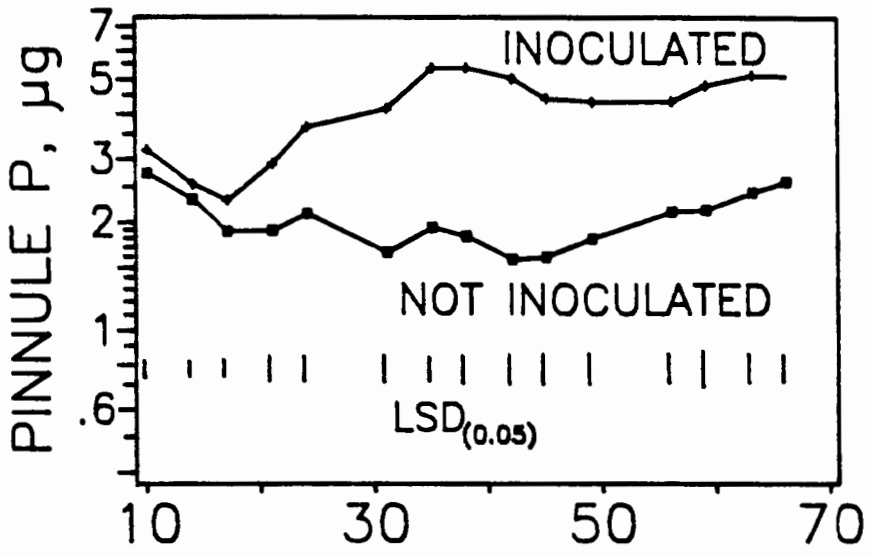

Figure 2. Inoculation of soils with Glomus aggregatum: effects on pinnule P content over time.

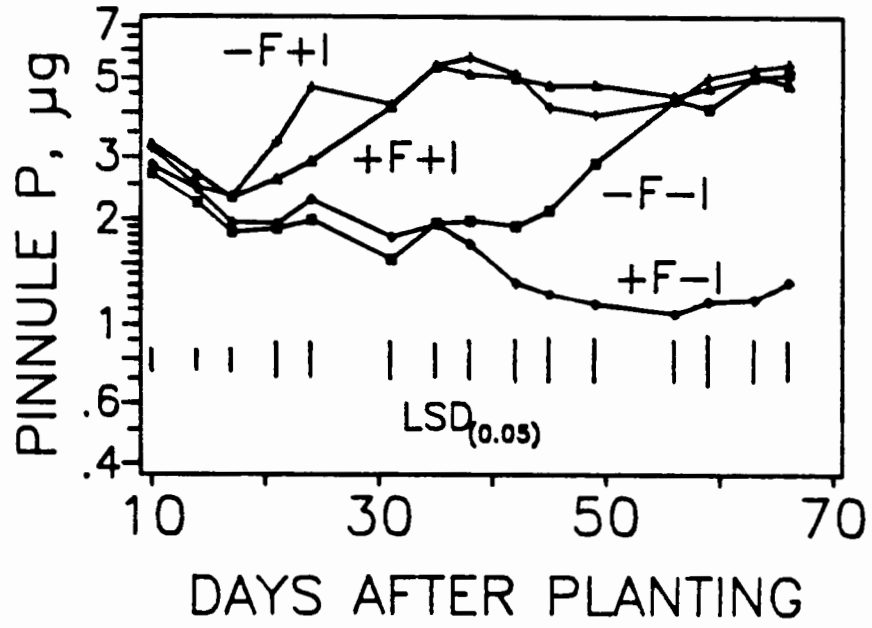

Figure 3. Combined fumigation and inoculation of soils: effects on pinnule P content over time. +F and -F depict fumigated and nonfumigated; +I and -I depict inoculated and noninoculated.

("sterile") soils (Figure 3). From 42 DAP until the harvest, the plants growing in soils that were fumigated and were not inoculated had, of all treatments, the lowest amounts of P in the pinnules.

Effect of Phosphorus Levels. From 14 DAP on, the quantity of P in pinnules increased with increasing soil P levels (Figure 4).

Effect of Inoculation + P Levels. Increases in pinnule P status in response to increasing soil P levels were larger in the noninoculated treatments than in the soils inoculated with Glomus aggregatum (Figure 5). The quantity of P in pinnules was affected from 24 DAP, when the effect that increasing soil P had of elevating pinnule P was nullified in soils inoculated with Glomus aggregatum.

Effect of Fumigation + P Levels. Plant responses to increases in soil P levels were larger in fumigated soils than in nonfumigated soils (Figure 6). From 49 DAP through harvest, plants growing in nonfumigated soils at all P levels had similar quantities of P in their pinnules (Figure 6B), while plants in fumigated soils had increased amounts of P in their pinnules in response to increasing levels of soil P (Figure 6A).

Effect of Inoculation, Fumigation, and P Levels. By the end of the growing period, the plants growing in soils that were fumigated and not inoculated with Glomus aggregatum (in "sterile" soils) had the smallest pinnules and the lowest percentages and quantities of P in their pinnules. These were the only plants that still responded to increasing soil P levels (Figure 7). Other combinations of fumigation and inoculation treatments were not sensitive to differences in soil P availability.

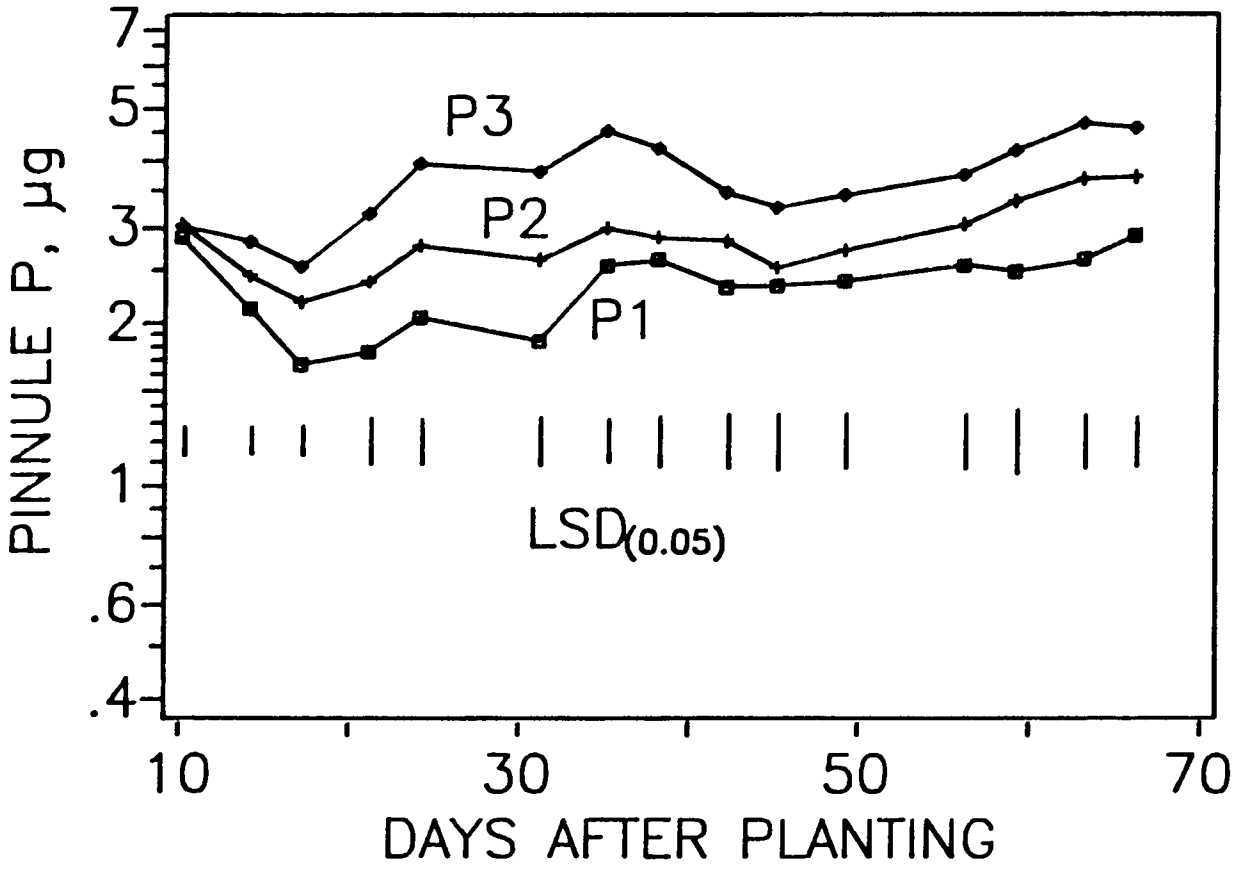

Figure 4. Differences in soil P availability: effects on pinnule P content over time.

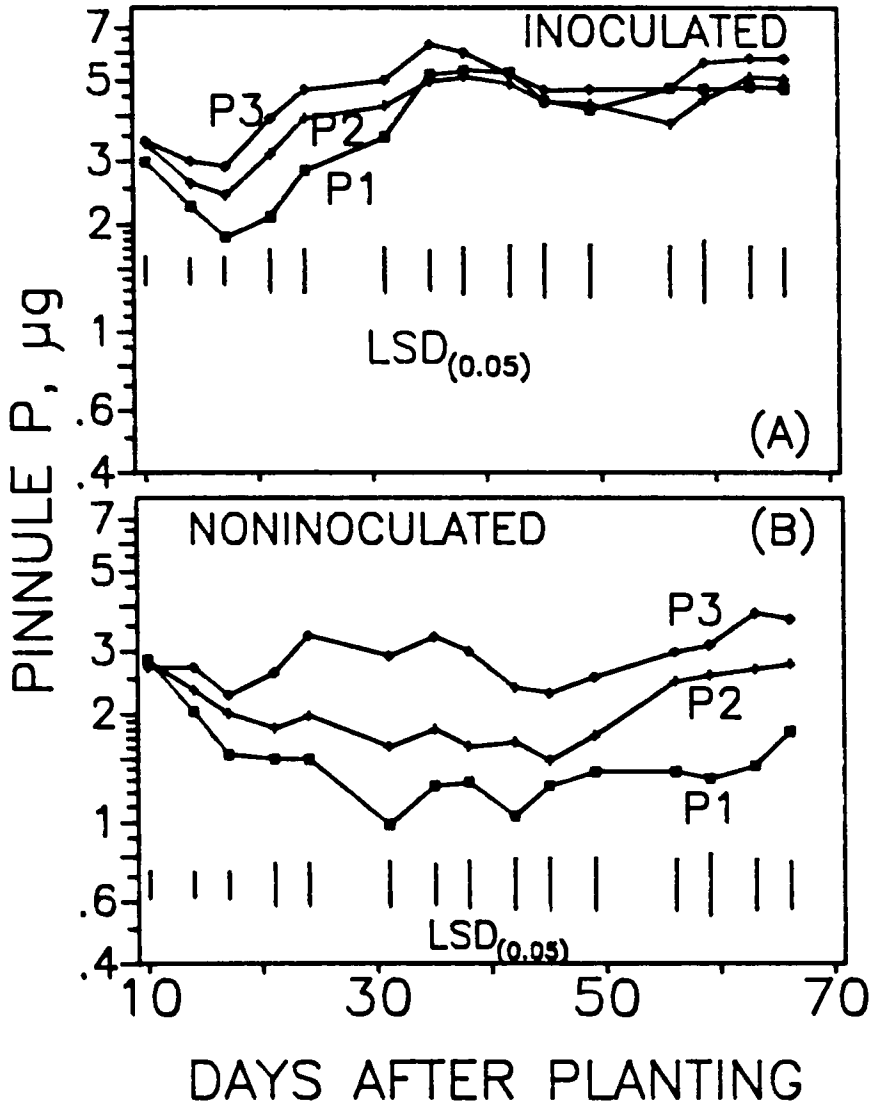

Figure 5. Inoculation with VAM and differences in soil P availability: combined effects on pinnule P content over time. P1, P2, P3 show increasing P. (A) noninoculated, and (B) inoculated.

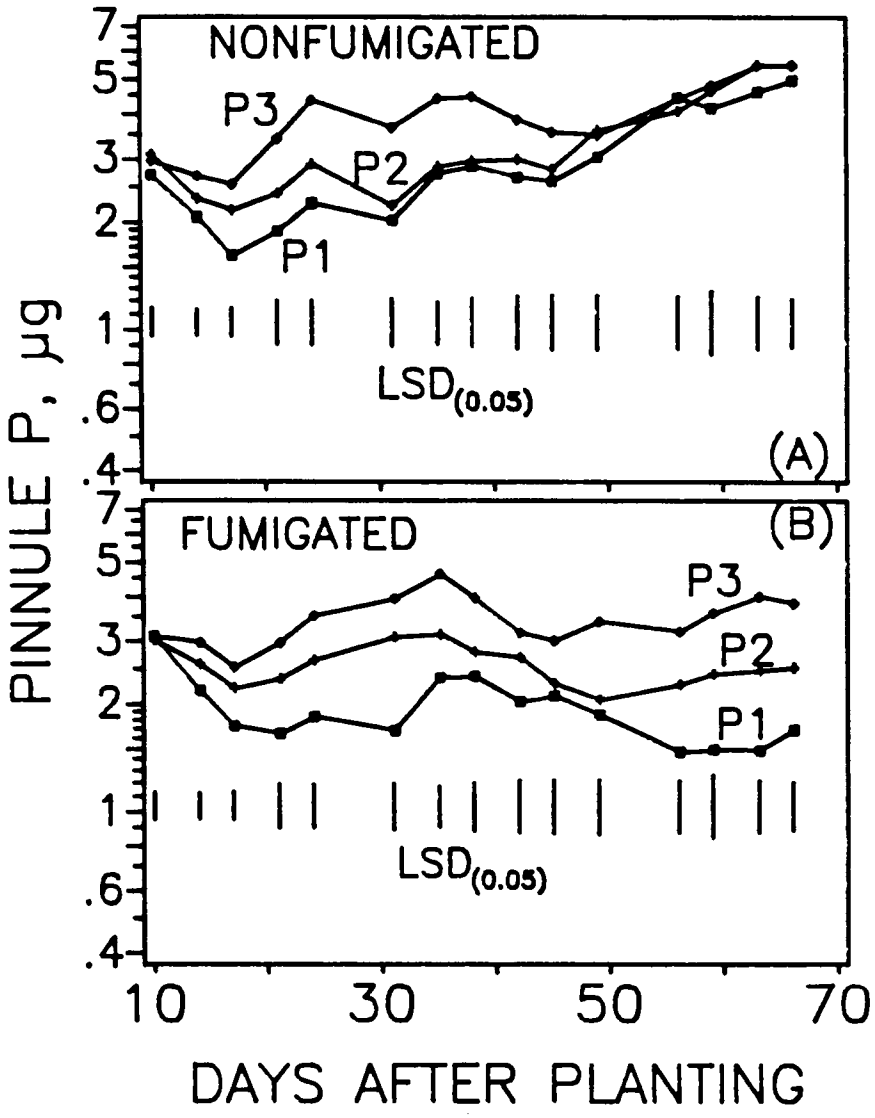

Figure 6. Fumigation with methyl bromide and differences in soil P availability: combined effects on pinnule P content over time. P1, P2, P3 show increasing P. (A) fumigated, and (B) nonfumigated.

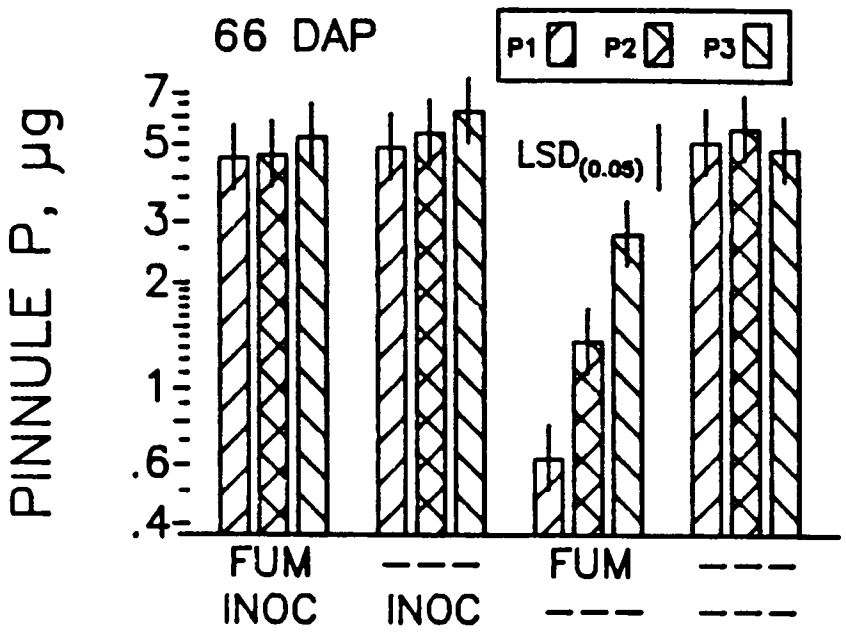

Figure 7. Fumigation, inoculation, and soil P availability: combined effects on pinnule P content at 66 days after planting.

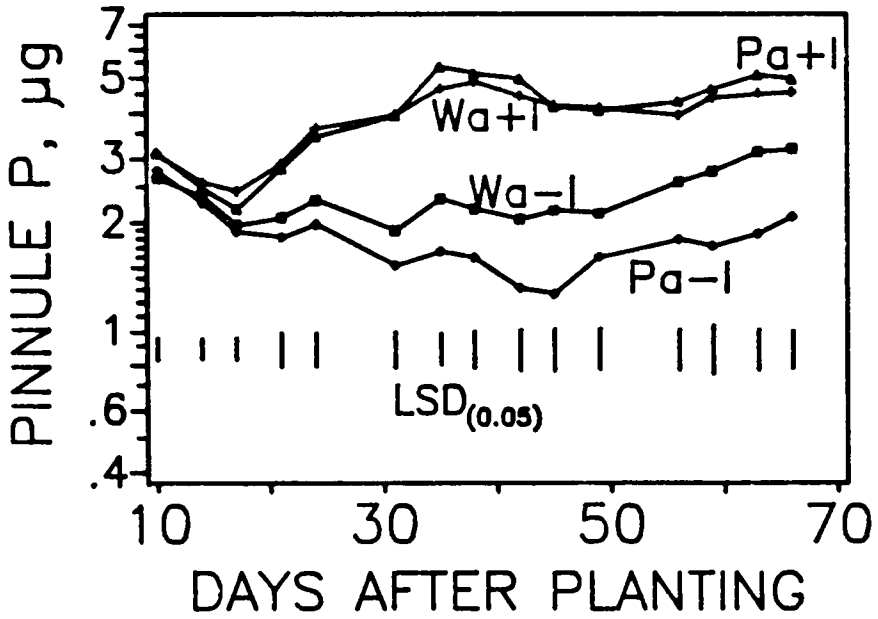

Figure 8. Soil series and inoculation: combined effects on pinnule P content over time. Soils: Wa = Wahiawa, Pa = Paaloa. +I and -I depict inoculated and noninoculated treatments.

Effect of Soils. The plants growing in the Paaloa soil consistently had less total P per pinnule than did the plants growing in the Wahiawa soil. There was no significant interaction between soils and P levels at any time, indicating that both of these treatments acted on plants in a similar manner. Probably the Paaloa soil provided a lower level of available P than the Wahiawa soil at the same target P level.

Effect of Inoculation + Soil. At 31 DAP, differences in pinnule P contents between inoculated and noninoculated treatments were larger in the Paaloa soil than in the Wahiawa soil (Figure 8), the treatments inoculated with Glomus aggregatum having the higher values. These differences persisted through harvest.

Effect of Inoculation, Fumigation, and Soil. Pinnule P contents of plants growing in the Paaloa soil increased much more slowly in response to inoculation with VAM when the soil was fumigated than when the soil was not fumigated (Figure 9). Plant response to inoculation did not differ between fumigation treatments in the Wahiawa soil (Figure 9).

Toward the end of the growing period, in the soils that were fumigated and not inoculated, the pinnules of plants growing in the Paaloa soil were lower in P than were those in the Wahiawa soil.

Pinnule P Content, P concentration, and Weight: Relationships

Overall, the treatments affected all response variables in a similar fashion (see the Appendix for pinnule weight and P concentration data). That is, inoculation of soils with Glomus aggregatum increased pinnule weights and P percentages, as it did

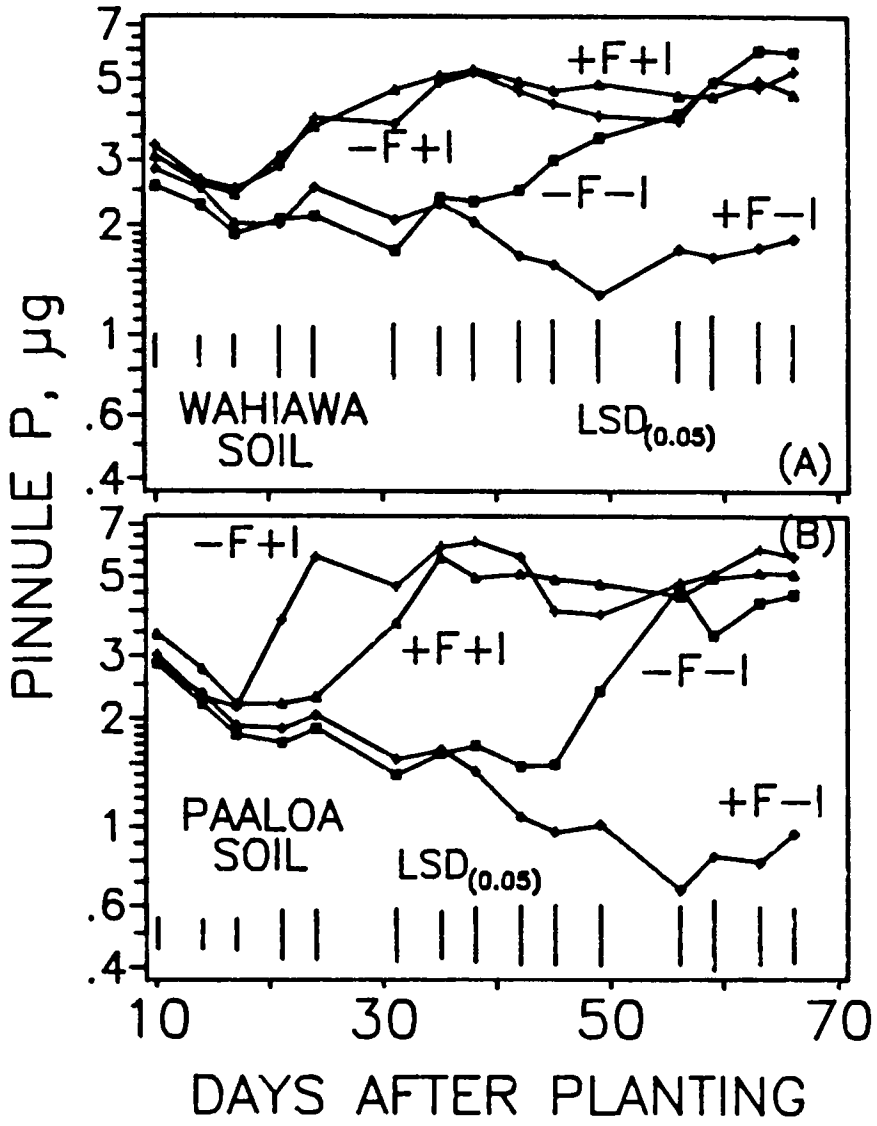

Figure 9. Soil series, fumigation, and inoculation: combined effects on pinnule P content over time. +F and -F depict fumigated and non-fumigated; +I and -I depict inoculated and noninoculated. (A) Wahiawa, and (B) Paaloa soil.

Table 6. — Percent of variation accounted for ($100*R^2$) from analyses of variance of pinnule P content (μg), percent P, and pinnule weight, for each day after planting. Analyses of log-transformed data.

	DAYS AFTER PLANTING														
	10	14	17	21	24	31	35	38	42	45	49	56	59	63	66
PINNULE RESPONSE	PERCENT OF VARIATION ACCOUNTED FOR ($100*R^2$)														
P (μg)	47	62	73	75	83	87	90	87	88	85	85	91	86	91	90
P (%)	41	52	64	64	89	91	90	85	86	71	69	88	78	89	82
weight	39	58	58	32	45	47	48	50	48	51	73	82	71	83	78

pinnule P contents as discussed above. Fumigation of soils decreased pinnule weights and P percentages in a manner similar to that observed in pinnule P content.

The variable that was most strongly influenced by the treatments was the quantity of P per pinnule, followed by percent P and finally by pinnule weight (Table 6. The R^2 for P content is larger than or equal to that for percent P on all but two days; the R^2 for percent P is larger than that for pinnule weight on all but two days).

It seems likely that the direct effect of mycorrhizal infections on plant development was to increase the supply of P to the plant, which then increased plant's internal P concentrations and, eventually, their growth. These ideas will be discussed more fully in the following section.

Summary of Pinnule Sampling Results

Inoculation of soils with Glomus aggregatum augmented the quantity of P in leucaena pinnules by 10 days after planting (Figure 2,

Table 5). Pinnule P percentages were consistently higher in inoculated treatments as of 21 DAP (Appendix A Table 13; Appendix B Figure 26), and likewise pinnule weights after 35 DAP (Appendix A Table 14; Appendix B Figure 27). Increasing soil P consistently increased pinnule P contents (Figure 4, Table 5) and concentrations (Appendix A Table 13; Appendix B Figure 28) from 14 DAP, but pinnule weights were not so strongly or consistently affected (Appendix A Table 14; Appendix B Figure 29). Because increases in pinnule P contents (whether by increasing soil P availability or by mycorrhizal colonization) occurred before increases in pinnule weight, it seems likely that P supply controlled the growth of Leucaena leucocephala.

The coefficient of determination (unadjusted R^2) for the quantity of P per pinnule was above 0.83 from 24 DAP through harvest (Table 6), showing that the treatments accounted for most of the plant P uptake. Treatment variables accounted for a slightly smaller proportion of the variation in pinnule P percentages (Table 6). But treatment variables accounted for less than 60 % of the variation in leaf weights until 49 DAP, after which the R^2 remained above 0.70 for the duration of the experiment. Deformation of pinnules by psillid insects may have contributed to the increased variability in pinnule weights.

The P percentages and P content of Leucaena leucocephala in nonfumigated and noninoculated ("natural") soils rose above those of plants grown in fumigated and noninoculated ("sterile") soils as of 38 DAP; by harvest, pinnules of the plants in the "natural" soils were about equal in P content (Figures 7 and 9), P concentration (Appendix A Table 14; Appendix B Figure 30), and weight (Appendix A Table 13;

Appendix B Figure 31) to pinnules of plants grown in soils inoculated with Glomus aggregatum. Parallels between the P uptake of plants grown in "natural" soils after 38 DAP and that of plants grown in inoculated soils after 17 DAP (Figure 3) suggest that the plants in the "natural" soils became infected with indigenous mycorrhizal fungi after about one month.

If all the plants grown in the VAM-inoculated and the "natural" soils formed effective mycorrhizal associations, then only the plants growing in "sterile" soils were left without the benefit of mycorrhizal symbioses. These were the only plants still responding to soil P availability by the end of the experiment (Figure 7).

There may have been differences between the two soils in the quality or density of the native mycorrhizal populations. The plants growing in the inoculated Paaloa soil increased pinnule P earlier and faster when the soil was not fumigated than they did when the soil was fumigated, while there were no differences in response to inoculation with regard to fumigation in the Wahiawa soil. In the noninoculated treatments, the response to the eventual infection of plant roots by indigenous mycorrhizal fungi, around 45 DAP, was much more rapid in the Paaloa soil than in the Wahiawa soil (Figure 9).

Treatment Effects on Leaf Production and Plant Height

Inoculation of soils with Glomus aggregatum increased, and fumigation of soils with methyl bromide decreased, leaf yields (Figure 10). These two treatments had by far the largest effects on leucaena growth (Table 7). Increasing soil P availability increased growth

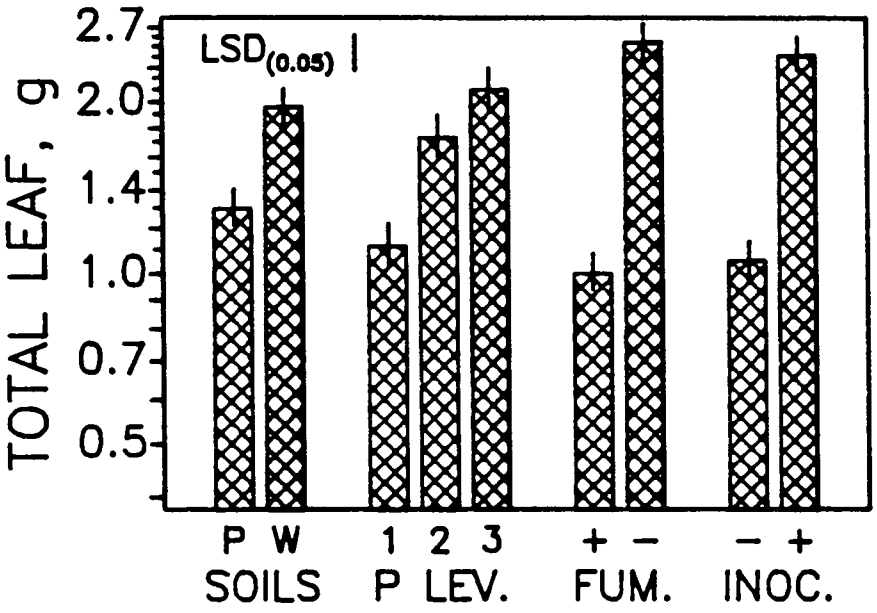

Figure 10. Leaf yield; main treatment effects. Soils: P = Paaloa, W = Wahiawa. P levels 1, 2, 3 show increasing P. Fumigation (FUM.) and Inoculation (INOC.): + = yes, - = no.

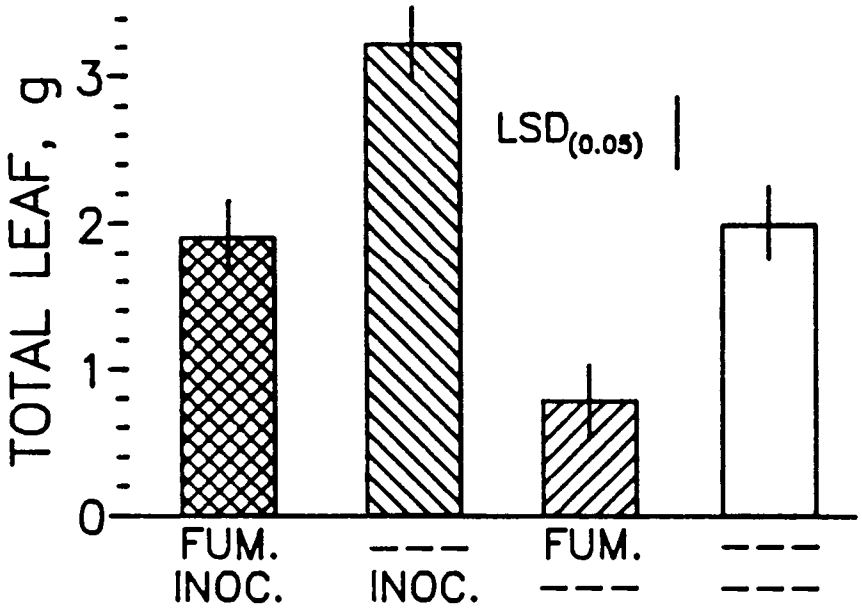

Figure 11. Fumigation and inoculation: combined effects on leaf yield.

Table 7. — Percentage of variation (%) in harvest data that each treatment variable accounted for, and probabilities (P) that independent variables are not significant.

	DATA ANALYZED							
	WT		HT		LNWT		LNHT	
SOURCE	%	P	%	P	%	P	%	P
Replication	0.1	0.829	0.7	0.563	0.0	0.867	0.4	0.620
Fumigation	31.4	0.000	28.5	0.000	29.9	0.000	37.1	0.000
Inoculation	27.1	0.000	15.6	0.000	23.8	0.000	20.3	0.000
Soils	4.3	0.002	4.1	0.010	5.8	0.000	6.6	0.000
P Levels	6.3	0.001	5.7	0.010	9.5	0.000	7.3	0.001
F*I[a]	0.1	0.697	0.4	0.390	4.3	0.000	3.4	0.007
F*S	2.3	0.017	3.5	0.016	3.0	0.000	5.4	0.001
F*P	0.1	0.907	0.9	0.448	2.6	0.000	0.3	0.748
I*S	0.5	0.268	0.5	0.366	1.5	0.001	0.8	0.181
I*P	0.7	0.407	2.2	0.159	6.9	0.000	6.4	0.002
S*P	0.9	0.306	1.4	0.303	0.1	0.704	0.6	0.500
F*I*S	2.6	0.012	2.9	0.028	0.0	0.999	0.8	0.175
F*I*P	1.4	0.175	2.3	0.140	6.1	0.000	5.2	0.004
F*S*P	2.8	0.033	2.1	0.166	0.8	0.026	1.7	0.141
I*S*P	1.2	0.215	1.8	0.223	0.5	0.125	1.8	0.120
F*I*S*P	1.3	0.185	1.8	0.216	0.9	0.025	2.0	0.117
R^2 (%)	82.8		74.1		95.0		83.6	

[a] Symbols are as follows: F = fumigation, I = inoculation, P = soil P levels, S = different soils; the asterisk (*) shows an interaction term.

(Figure 10). Plants growing in the Paaloa soil were smaller than those growing in the Wahiawa soil.

The effects of fumigation and inoculation of soils on leaf production were additive, and nearly equal. That is, fumigation reduced leaf yield by about the same amount that inoculation increased it (Figure 11).

Inoculation of soils eliminated the effect of soil P levels on leaf yield; but plants in uninoculated soils grew larger with increasing soil P (Figure 12).

Combining P availability with fumigation and inoculation treatments, it was again the plants growing in "sterile" soils that responded most to increased soil P. Within the inoculated treatments, the plants growing in the nonfumigated soils responded to increased soil P by increasing leaf production, while in the fumigated soils plants showed no response to increasing P levels (Figure 13).

It is likely that some factor other than P was limiting plant growth in the fumigated-inoculated treatment; quite possibly nitrogen. Soils in this experiment were not inoculated with Rhizobium. Fumigation of soils should have eliminated native Rhizobium bacteria as well as vesicular-arbuscular mycorrhizal fungi. Except at the lowest level of soil P availability, the plants growing in the "natural" soils (neither fumigated nor inoculated) were larger and taller than the plants grown in soils that were both fumigated and inoculated.

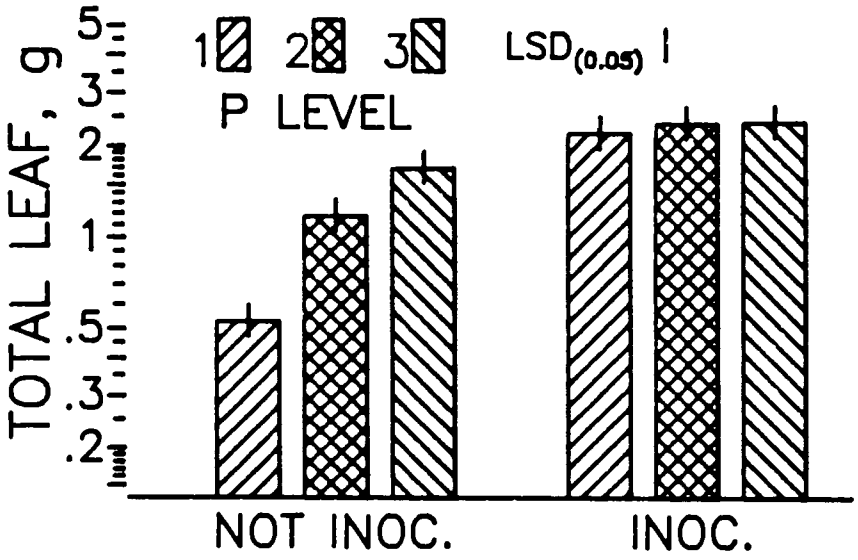

Figure 12. Inoculation with VAM and differences in soil P availability: combined effects on leaf yield. P1, P2, P3 show increasing P.

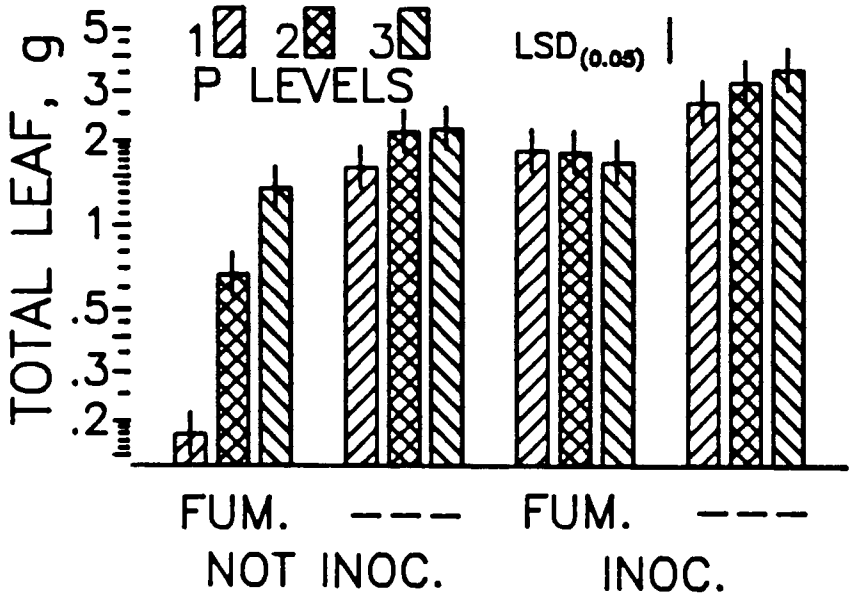

Figure 13. Fumigation, inoculation, and soil P availability: combined effects on leaf yield. P1, P2, P3 show increasing P. p 59

Experiment 2

This experiment was designed to clarify the effect of soil P buffer capacity on enhancement of leucaena growth and P uptake by Glomus aggregatum. Four soils were adjusted to 9 levels of P supply; all soils were fumigated with methyl bromide and mixed 15:85 with blasting sand. Soil P was measured by 6 different methods.

Results of Soil P Sorption Tests

Results of soil P sorption tests of the soils selected for this experiment, using the method of Fox and Kamprath (1970), are presented in Figure 14.

A curve of the form

$$\log(CaCl_2\text{-}P) = \alpha + \beta * \log(\Gamma + P \text{ added}) \qquad (8)$$

was fit to the data for each soil. α and β were determined by standard least squares procedures while adjusting Γ to minimize the error sum of squares. This curve seems to fit the data quite well (Figure 14). The α and β coefficients provide the slope and intercept. The term $\log(\Gamma + P \text{ added})$ produces a line whose curvature decreases with increasing amounts of P sorbed. This conforms with a model of P sorption in which the energy of sorption decreases as the amount of P sorbed increases (Barrow, 1978). The rate of decrease of curvature is described by the coefficient Γ.

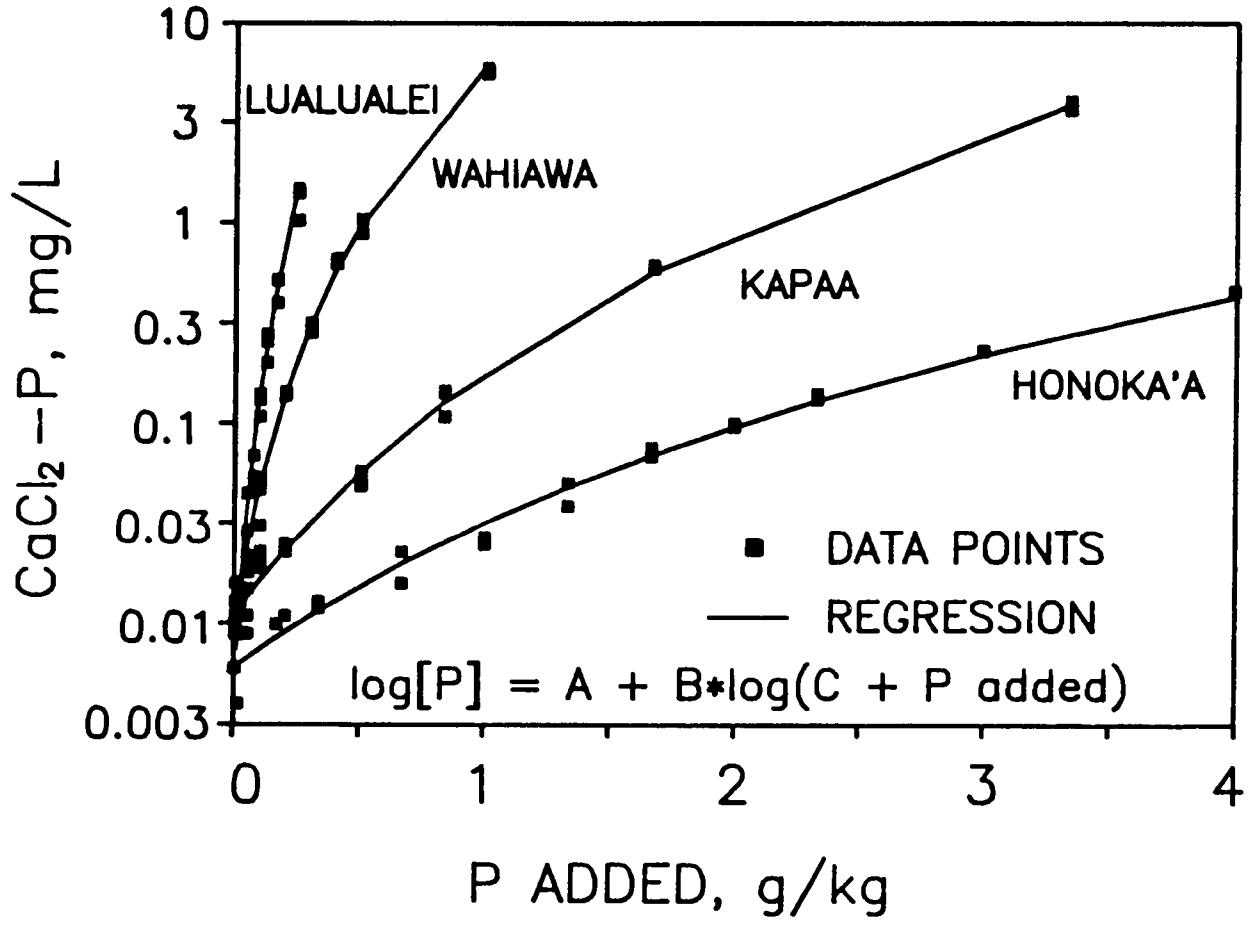

Figure 14. P sorption data and fitted curves for soils of experiment 2. 6-day equilibration procedure.

The values for α, β, and Γ have no meaning in themselves; they are closely correlated, and are very sensitive to the specific range of data used. However, once the values for α, β, and Γ have been determined, the quantity of P necessary to attain a given P concentration can be calculated;

$$P \text{ to add} = ([P]/10^{\alpha})^{1/\beta} - \Gamma \qquad (9)$$

this value is fairly insensitive to the exact range of data used in its calculation. That is, excluding the highest values from the data set during the calculation of the coefficients had only a small affect on the estimate of the quantity of P to add.

The slope, d(P added)/d($CaCl_2$ P), can be calculated;

$$dQ/dI = [CaCl_2\text{-}P]^{(1/\beta)-1} * [10^{(-\alpha/\beta)}]/\beta \qquad (10)$$

dQ/dI is the soil's P sorption capacity. It is also fairly insensitive to variations in the data range, and can be calculated for any $CaCl_2$-P concentration or amount of added P within the range of the data. Table 8 shows the slope of the Q/I relationship and the P necessary (in mg P/kg) to bring each soil to each of the 9 P levels ($CaCl_2$-extract P concentrations). Because the P sorption capacity of a soil changes with the solution P concentration (Table 8), comparisons of P sorption capacities among soils should be at one concentration.

The ratios of P sorption capacities, calculated for all combinations of the 4 soils at each targeted P intensity, vary more among

Table 8. -- The slope of the Q/I relationship and P necessary (in mg P/kg soil) for each soil at the targeted $CaCl_2$-P concentrations.

SOIL	Targeted [P]	0.01	0.02	0.04	0.08	0.16	0.32	0.64	1.28	2.56
Lualualei	dQ/dI	2234	1340	804	482	289	174	104	63	38
	P needed	20	37	57	82	111	146	189	239	300
Wahiawa	dQ/dI	4940	3059	1894	1173	726	450	278	172	107
	P needed	3	41	89	147	220	309	421	558	729
Kapaa	dQ/dI	23919	14261	8503	5070	3023	1802	1075	641	382
	P needed	-17	164	380	638	945	1312	1749	2270	2892
Honokaa	dQ/dI	56809	34520	20976	12746	7745	4706	2860	1738	1056
	P needed	209	643	1172	1814	2594	3543	4695	6096	7798

Table 9. -- Ratios of soil P capacity estimates among soils at targeted $CaCl_2$-P concentrations. First column shows soils compared: Lu = Lualualei, Wa = Wahiawa, Ka = Kapaa, and Ho = Honokaa. Wa/Lu = Wahiawa/Lualualei ratio, etc.

	Targeted $CaCl_2$-P Concentration									
SOILS	0.01	0.02	0.04	0.08	0.16	0.32	0.64	1.28	2.56	c.v. (%)
Wa/Lu	2.2	2.3	2.4	2.4	2.5	2.6	2.7	2.8	2.8	8.6
Ka/Lu	10.7	10.6	10.6	10.5	10.4	10.4	10.3	10.2	10.2	1.7
Ho/Lu	25.4	25.8	26.1	26.4	26.8	27.1	27.4	27.8	28.2	3.5
Ka/Wa	4.8	4.7	4.5	4.3	4.2	4.0	3.9	3.7	3.6	10.3
Ho/Wa	11.5	11.3	11.1	10.9	10.7	10.5	10.3	10.1	9.9	5.2
Ho/Ka	2.4	2.4	2.5	2.5	2.6	2.6	2.7	2.7	2.8	5.2
cv(%)	92	93	95	96	97	98	99	100	102	

soils at a targeted P concentration than they do within any 2-soil comparison across the range of P levels (Table 9). It might be practicable to use a single value to represent the P sorption capacity of each soil across all P levels.

Long-Term Soil P Sorption Measurement

After soil amendments were added, a sample of each soil by P level combination was set aside and maintained at field capacity and room temperature for 90 days, then tested for P intensity by equilibration with 0.01 M $CaCl_2$ (Figure 15).

At measured solution P concentrations above 0.01 mg P/l, the general pattern of Q/I relationships predicted by the P sorption curves was maintained, with the Lualualei soil sorbing the least P at a given soil solution P level, followed in order by the Wahiawa soil, the Kapaa soil, and the Honokaa soil. Below 0.01 mg P/l, the Kapaa soil showed the lowest solution P concentration in relation to P sorbed.

Comparison of P Sorption Measurement Methods

$CaCl_2$ solution P concentrations attained after the 90-day incubation were (except in one case) lower than predicted by the 6-day equilibration procedure (Figure 16). The ratio of measured $CaCl_2$-P to targeted $CaCl_2$-P concentration was smallest at the highest P levels. The Kapaa soil had the lowest ratio of measured to targeted P concentration at all P levels, followed by the Honokaa, Wahiawa, and Lualualei soils.

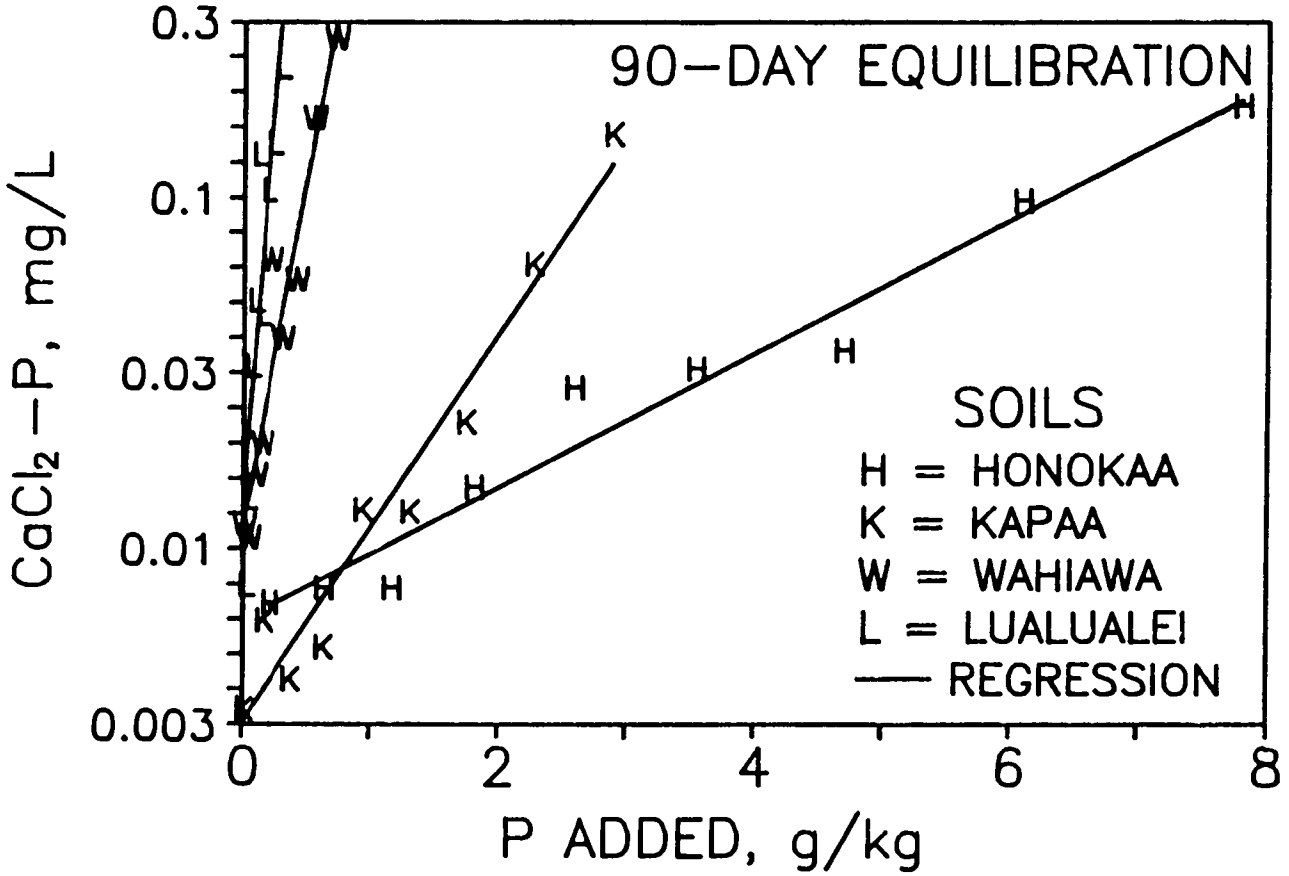

Figure 15. P sorption data and fitted curves for soils of experiment 2. 90-day equilibration procedure.

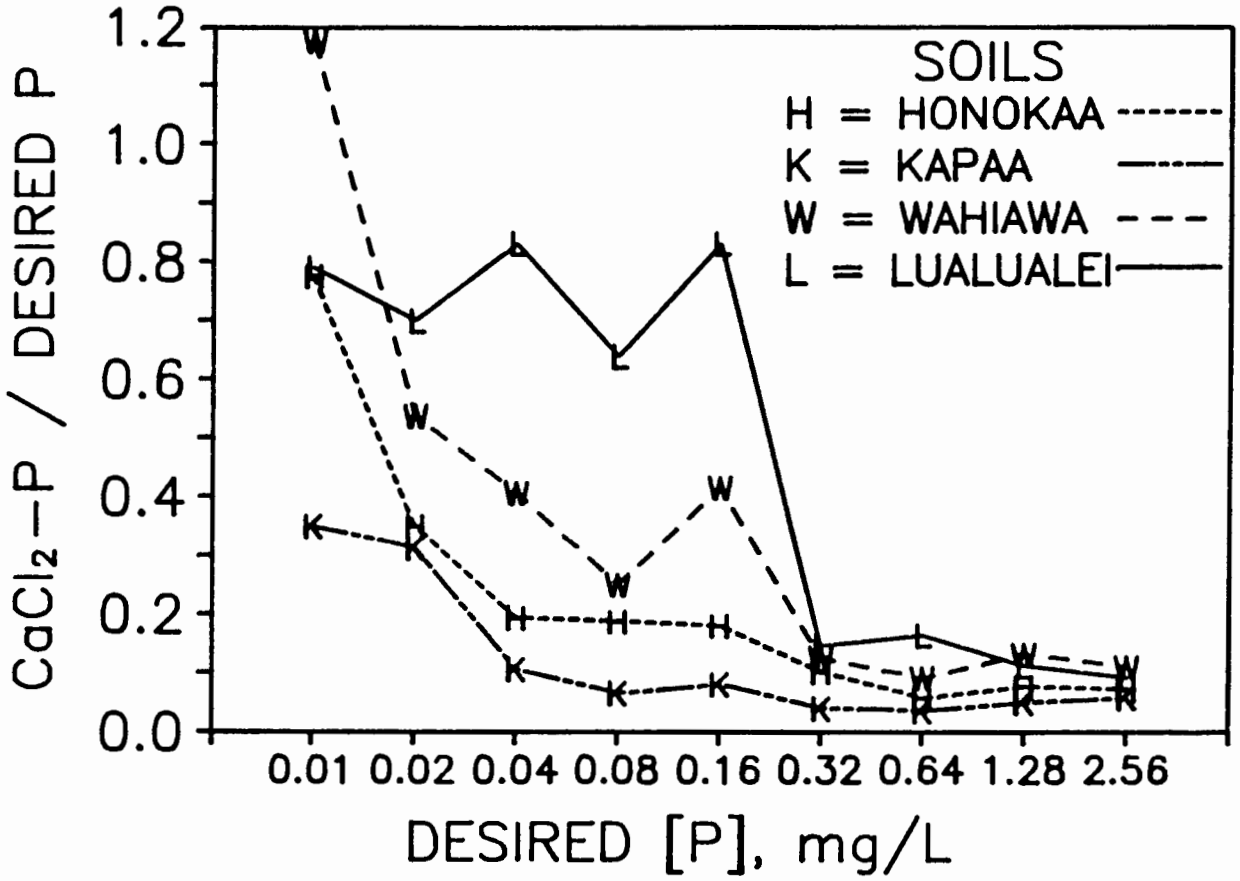

Figure 16. Ratio of $CaCl_2$-P concentration attained to desired P concentration. Soils of experiment 2.

In general, then, the 6-day equilibration procedure underestimated the quantity of P to add to attain a given $CaCl_2$-P concentration. The soils with lower P sorption capacities were closer to "equilibrium" after the 6-day equilibration procedure than were the soils with higher P sorption capacities. But the Honokaa soil was closer to equilibrium after the 6-day P-sorption test than was the Kapaa soil, which had a lower measured P sorption capacity.

Soil P Availability Measurements

The amended soils were tested for P availability after the 90-day equilibration, using three common P-extracting solutions. Plots of $CaCl_2$-P versus "available" P are shown in Figure 17.

While the HCl-H_2SO_4-extractable P values for the Honokaa soil were lower than those for all three other soils, the $NaHCO_3$-extractable P values for the Honokaa soil were above those of the Lualualei soil and below those of the two Oxisols. The NH_4F-HCl-extractable P values for the Honokaa soil were about the same as those for the Wahiawa soil, above those for the Lualualei soil and below those for the Kapaa soil (Figure 17).

At comparable $CaCl_2$-P concentrations, all three of the soil P availability indexes gave extractable P values for the Kapaa soil and Wahiawa soil that are above those for the Lualualei soil. However, the Kapaa soil released more P than the Wahiawa soil when extracted with $NaHCO_3$ and NH_4F-HCl, but less than the Wahiawa soil when extracted with HCl-H_2SO_4. These three soil P availability indexes may extract soil P

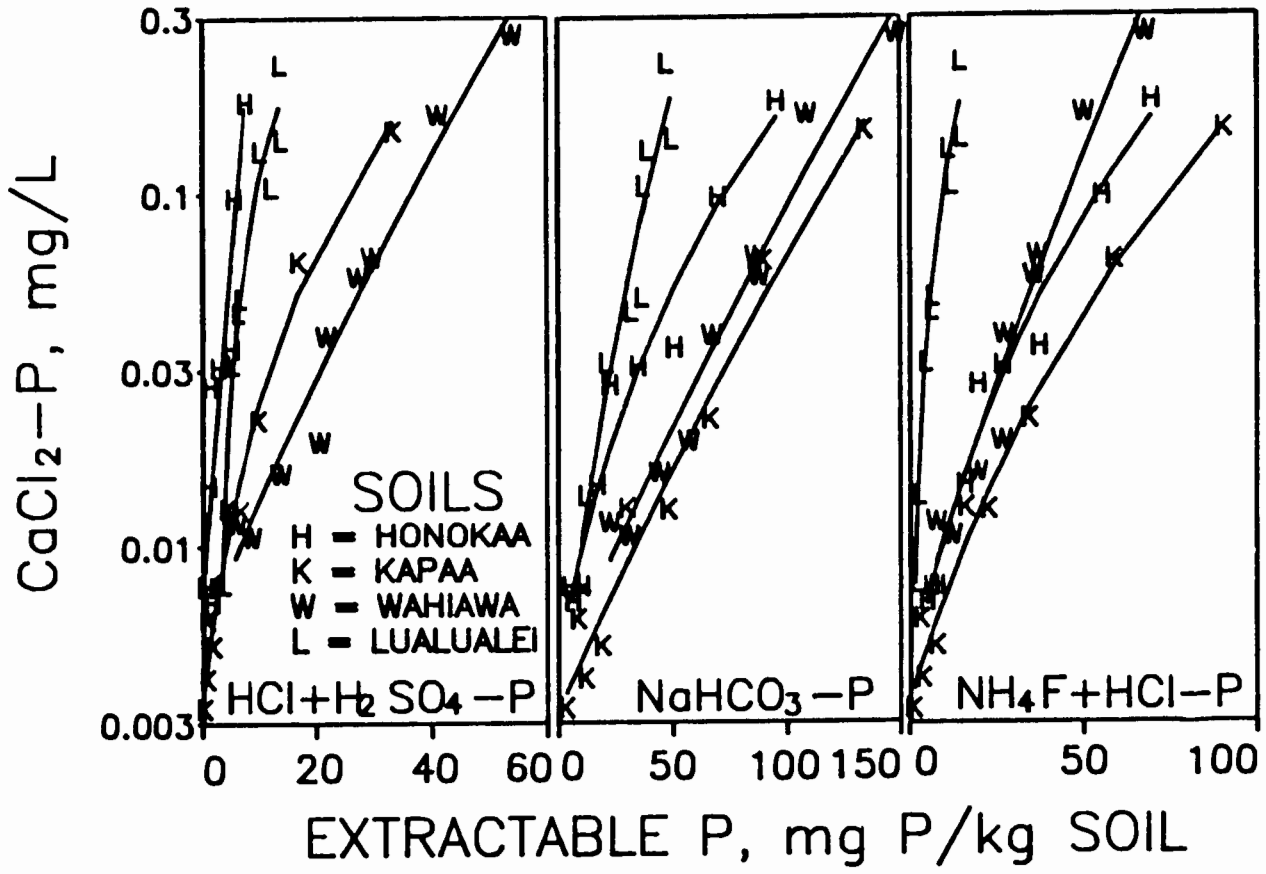

Figure 17. $CaCl_2$-P versus extractable P. Soils of experiment 2.

in different proportions from the different pools of P in the soil (Thomas and Peaslee 1973).

Because the three availability indices gave different results, we can conclude that at least one of them is not appropriate for assessing plant-available P across the range of soils used in the present experiment. We will try to determine which index was most useful in describing plant response to soil P and to mycorrhizal inoculation.

Plant P Status During Growth

Increases in solution P (as measured by $CaCl_2$ equilibration) caused increased plant P, as measured by the P content of pinnules, through most of the experiment (Table 10). Inoculation of soils with Glomus aggregatum did not affect plant P through the first 38 days after planting (Table 10, Figure 18), but by 41 DAP, plants growing in some inoculated soils had larger quantities of P in pinnules than plants in noninoculated soils. From 52 DAP through harvest, all plants in inoculated soils had larger quantities of P per pinnule than did plants grown without VAM. So for about 20 to 30 days, plants growing in soils inoculated with G. aggregatum had increased access to P over that of plants growing without the fungus.

Effect of VAM Inoculation on Root Colonization by Mycorrhizal Fungi

Plants grown in soils inoculated with G. aggregatum had higher percentages of their root length colonized by fungi than did plants grown in soils that were not inoculated ($P < 10^{-9}$; Figure 19). Colonization of plant roots by the fungus indicates that differences in

Table 10. -- Pinnule P response to solution P concentrations and VAM inoculation during plant growth. Probabilities of type I error.

EFFECT OF SOIL SOLUTION P CONCENTRATION
Days after planting

	12	16	19	25	38	41	45	52	59	62	72
	Probability Level										
SOILS											
Lualualei	0.16	0.01	0.00	0.00	0.00	0.00	0.02	0.41	0.15	0.14	0.73
Wahiawa	0.00	0.85	0.00	0.00	0.00	0.00	0.00	0.24	0.16	0.88	0.90
Kapaa	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.01	0.21	0.37
Honokaa	0.00	0.02	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.27	0.38

EFFECT OF INOCULATION WITH VAM FUNGI
Days after planting

	12	16	19	25	38	41	45	52	59	62	72
	Probability Level										
SOILS											
Lualualei	0.85	0.28	0.26	0.20	0.08	0.01	0.00	0.00	0.00	0.00	0.00
Wahiawa	0.55	0.33	0.40	0.96	0.32	0.00	0.00	0.00	0.00	0.00	0.00
Kapaa	0.95	0.51	0.00	0.58	0.07	0.27	0.17	0.00	0.00	0.00	0.00
Honokaa	0.03	0.16	0.69	0.92	0.91	0.07	0.00	0.00	0.00	0.00	0.00

leucaena growth and P uptake resulting from inoculation of soils with G. aggregatum could have resulted from a symbiosis between the plant and the fungus.

Soil P Measurements and Plant P Uptake

When mycorrhizal and nonmycorrhizal treatments were pooled, total plant P uptake was more highly correlated with inoculation of soils with VAM fungi (inoculation coded as +1 and -1; $r = 0.70$) than by soil P as measured by any of the extractants (see POOLED DATA in Table 11).

As levels of extractable soil P increased, plant P also increased (all correlation coefficients are positive; Table 11). NH_4F+HCl-P

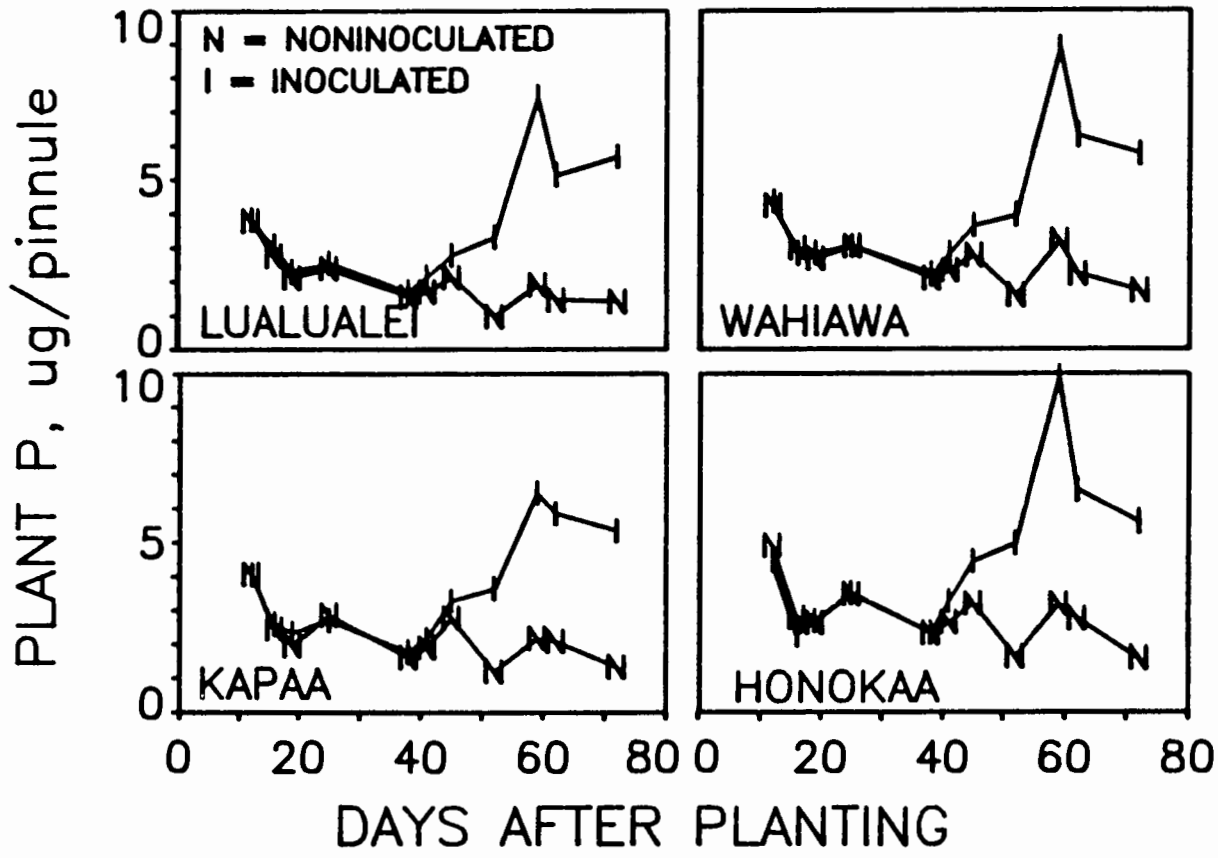

Figure 18. Soil series and inoculation: combined effects on pinnule P content over time. Experiment 2.

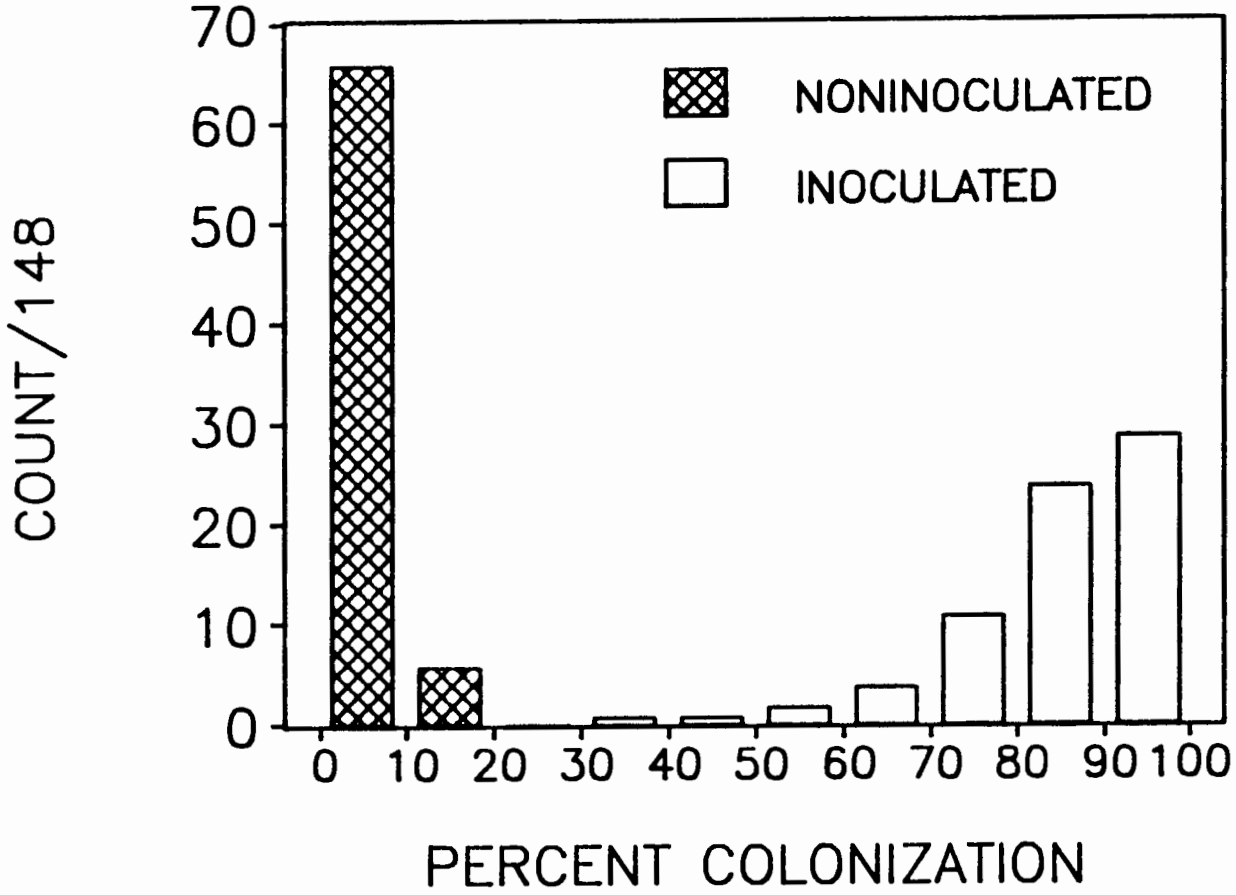

Figure 19. Inoculation of soils with Glomus aggregatum: effects on percentage of root length colonized by fungi.

Table 11. -- Correlation coefficients of total plant P with several measures of extractable P. The column of means is for the analyses within soils and inoculation classes.

		INOCULATION		WITHIN SOILS AND INOCULATION CLASSES								
	POOLED	CLASSES		Lualualei		Wahiawa		Kapaa		Honokaa		
SOIL TEST	DATA	-VAM	+VAM	-VAM	+VAM	-VAM	+VAM	-VAM	+VAM	-VAM	+VAM	mean
CaCl2-P	0.30	0.61	0.41	0.91	0.60	0.85	0.59	0.91	0.62	0.90	0.74	0.76
NaHCO3-P	0.40	0.76	0.59	0.84	0.86	0.97	0.65	0.95	0.79	0.99	0.84	0.86
HCl+H2SO4-P	0.22	0.46	0.31	0.92	0.73	0.97	0.66	0.94	0.71	0.97	0.81	0.84
HCl+NH4F-P	0.48	0.86	0.70	0.91	0.77	0.97	0.66	0.97	0.77	0.98	0.84	0.86
logCaCl2	0.38	0.69	0.57	0.84	0.81	0.97	0.64	0.95	0.84	0.95	0.87	0.86
logNaHCO3	0.43	0.70	0.66	0.76	0.87	0.97	0.65	0.78	0.89	0.92	0.90	0.84
logHCl+H2SO4	0.27	0.48	0.43	0.89	0.80	0.97	0.66	0.77	0.90	0.89	0.82	0.83
logHCl+NH4F	0.50	0.78	0.79	0.82	0.85	0.96	0.65	0.79	0.90	0.92	0.89	0.85
MEANS	0.39	0.64	0.54	0.86	0.78	0.95	0.63	0.88	0.81	0.94	0.85	0.84
Prob. (5%)	0.20	0.23	0.23	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47	0.47

correlated best with plant P in the pooled data, followed in order by $NaHCO_3$-P, $CaCl_2$-P, and $HCl+H_2SO_4$-P. By analyzing inoculated and noninoculated treatments separately (see INOCULATION CLASSES in Table 11), correlations of extractable soil P measurements with plant P uptake were greatly improved, demonstrating that mycorrhizal and nonmycorrhizal plants respond differently to differences in soil P availability. The ranking of the soil P extraction methods remained the same as with the pooled data, with $HCl+NH_4F$-P again performing the best.

P uptake by nonmycorrhizal plants paralleled measured soil P more closely (higher coefficients of correlation) than did the P uptake by mycorrhizal plants. Some possible explanations of this are the following:

(1) Differences in time (and hence degree) of colonization of mycorrhizal plants may have introduced a source of variability that was absent with the -VAM plants, and increased the variation among +VAM plants. The variation among mycorrhizal plants was higher than that among nonmycorrhizal plants, partly because the mycorrhizal plants were larger.

(2) Correlation coefficients can reflect the range of differences within measured variables (in this case, soil P and plant P uptake) with respect to the variance of the measurements. That is, if the range is small relative to the variance, it is not possible to get a high correlation. Among the noninoculated treatments there were plants that took up very little P, as well as some that took up a fair amount. This provided a wide range of plant P uptake values, and we find high correlations with soil P. In inoculated Wahiawa soil, which had the

lowest correlation between soil test P and plant P, we also find the smallest difference between low and high plant P content (17 to 34 mg; Figure 20). Mycorrhizal plants in Honokaa soil took up between 10 and 54 mg P per plant, and the correlation is higher.

(3) Nonmycorrhizal leucaena was highly responsive in the range of soil P availability presented, while mycorrhizal leucaena might be most responsive over a smaller (and lower) range of P availability.

Within soil and inoculation classes, the relationship between plant P uptake and "available" soil P was fairly close (mean of 0.84; Table 11). In the Lualualei, Kapaa, and Honokaa soils, P uptake by nonmycorrhizal plants seemed to be more closely related to the untransformed measurements of "available" soil P than to the logarithm of soil P, while P uptake by mycorrhizal plants was more closely related to the logarithm of the soil P measures. This may indicate that P uptake of nonmycorrhizal plants increased in response to increasing soil P over its entire range, while P uptake of mycorrhizal plants increased rapidly at low soil P before approaching an asymptote. Taking the logarithm of the extractable P values improved the relation of $CaCl_2$-P to plant P uptake in all but one of the soil-by-inoculation classes. A linear relationship between plant P uptake and log(soil P) implies a model in which doubling soil P adds a constant increment to plant P uptake.

Although some P extractant solutions performed quite well with specific soils in extracting a quantity of soil P proportional to that taken up by plants, there was not much difference in overall effectiveness of one extractant over another across the range of soils and

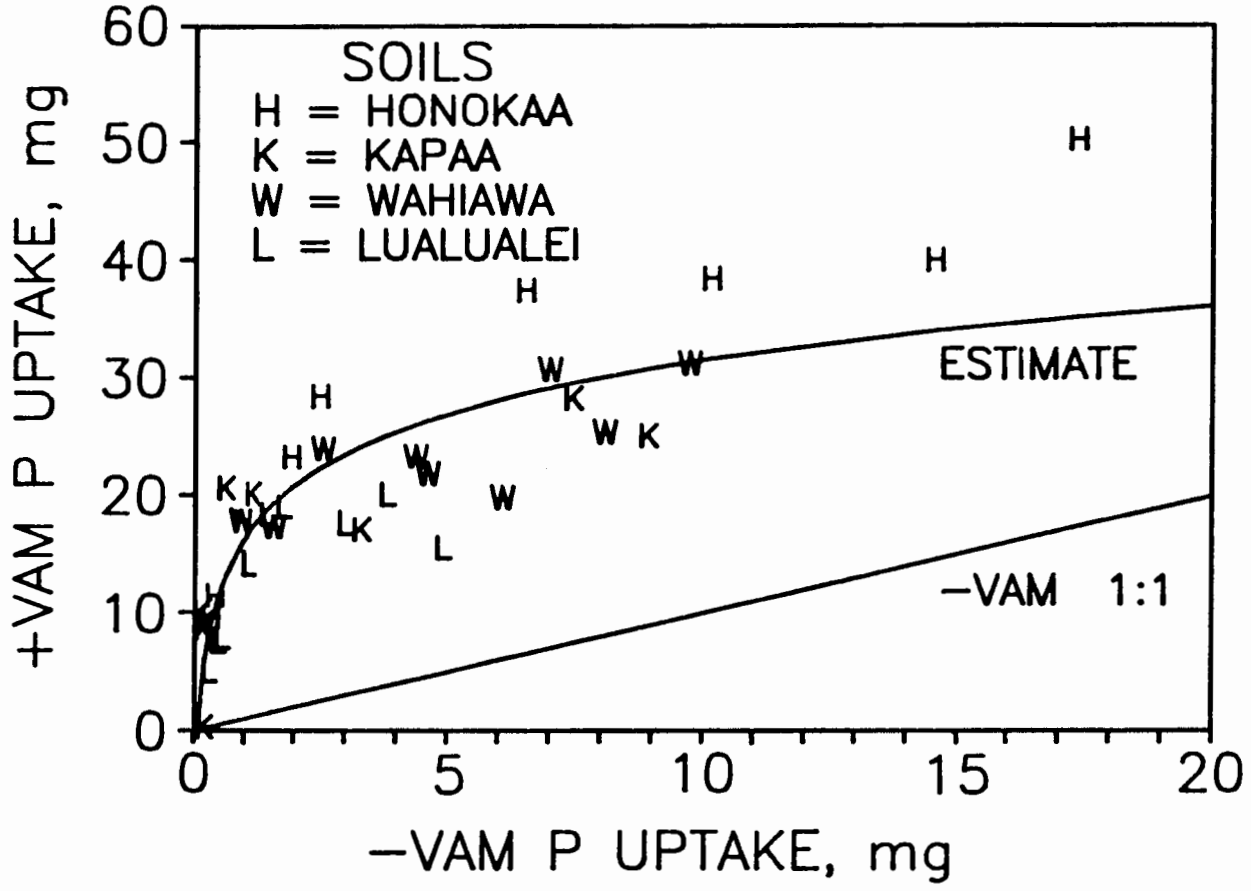

Figure 20. P uptake, mycorrhizal versus nonmycorrhizal.

inoculation classes. An ideal soil P extractant would extract a quantity of soil P proportional to that taken up by plants, and would do so across a wide range of soils. The best extractant in this sense was the HCl+NH_4F (see INOCULATION CLASSES, Table 11).

VAM Fungi and Plant P Uptake

Plants that developed mycorrhizal root systems absorbed more P from the soil than did plants without mycorrhizae (Fig 20). The relation between the total P uptake (mg P in leaves and stems) of the two treatments was

$$-\text{VAM P uptake} = 0.087 * 10^{0.065*(+\text{VAM P uptake})} \quad (11)$$

Except at the very lowest levels of soil P availability (corresponding to the lowest -VAM plant P uptake), mycorrhizal associations increased total plant P. At the lowest values of plant P among nonmycorrhizal treatments, the advantage conferred by the VAM fungus was eliminated; there was no difference between the total P content of inoculated and noninoculated treatments in the control treatment (100% sand) and in the Kapaa soil at the lowest $CaCl_2$-P concentration (about 0.003 mg P l^{-1}). The supply of P from these soils was apparently below a threshold at which mycorrhizal roots could absorb P.

The largest increases in the quantity of P taken up by mycorrhizal plants relative to nonmycorrhizal plants were in the range of 30 mg (Figure 21), at which the ratio of (+VAM P uptake)/(-VAM P uptake)

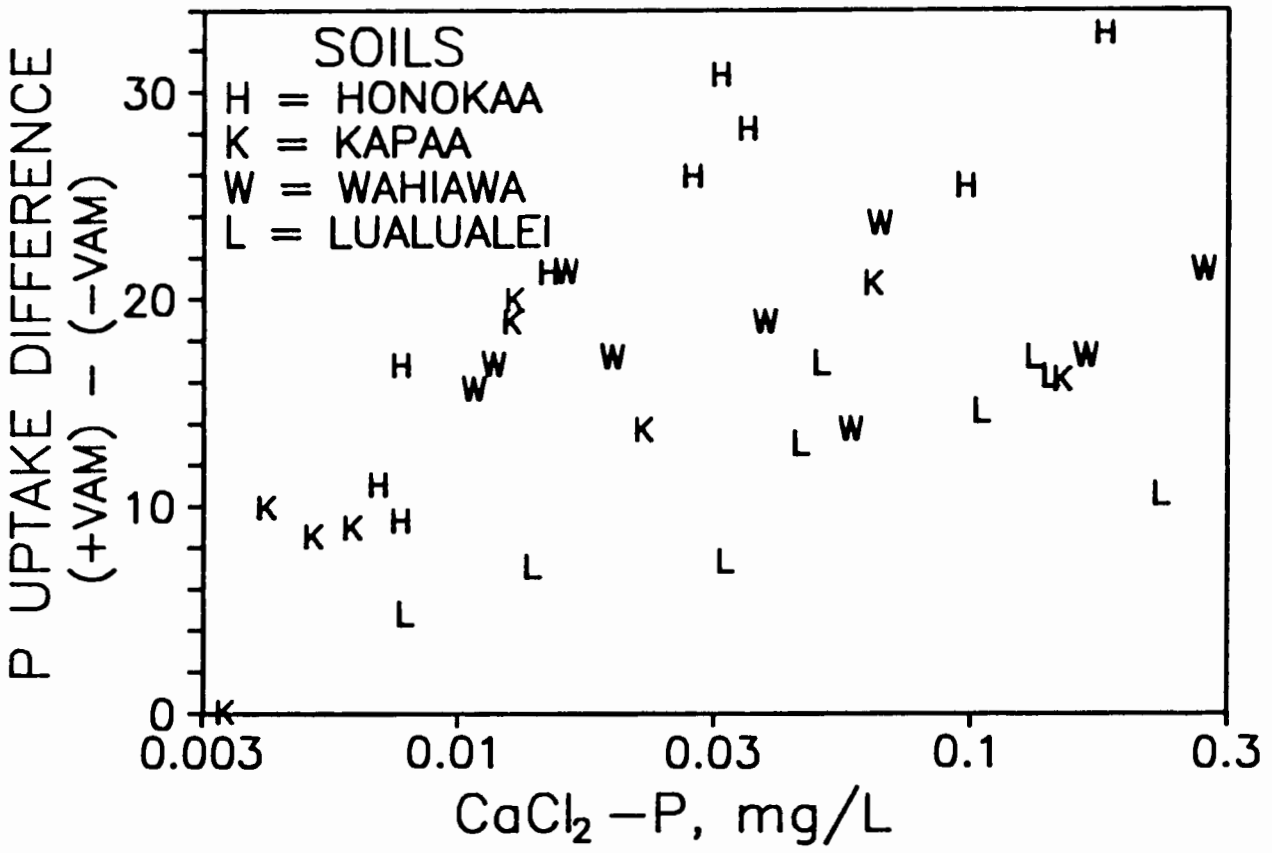

Figure 21. P uptake difference, mycorrhizal minus nonmycorrhizal, versus $CaCl_2$-P concentration.

was about 2.8. When the solution P concentration was above the threshold of about 0.003 mg P l^{-1}, the difference between mycorrhizal and nonmycorrhizal P uptake increased as soil P availability increased (Figure 21), while the ratio of mycorrhizal to nonmycorrhizal P uptake decreased with increasing soil P availability. The largest ratios of mycorrhizal to nonmycorrhizal plant P uptake occurred just above the threshold, where mycorrhizal plants contained 40 to 60 times the amount of P of nonmycorrhizal plants. The actual increase in quantity of P from -VAM to +VAM between these plants was about 10 mg, so the magnitude of the ratio was largely produced by the small quantity of P in the nonmycorrhizal plants.

Increases of mycorrhizal over nonmycorrhizal plant P uptake might be produced by differences between leucaena roots and mycorrhizal hyphae (or mycorrhizal roots) in threshold P concentration, response to increasing solution P concentrations above the threshold, and maximum rate of P uptake, as well as by increases in the volume of soil exploited as noted earlier. These topics will be addressed below, with the discussion of the relationship between solution P concentration and plant P uptake.

Solution P Concentration and Plant P Uptake

While $CaCl_2$-extractable P is not identical with soil solution P, it is a useful index of soil solution P intensity (Olsen and Watanabe 1970; Soltanpour et al. 1974), and will be used as such in the following discussion. Where "solution P" is stated, it will be $CaCl_2$-P that is reported unless otherwise noted. Increasing solution P

concentrations increased plant P for both +VAM and -VAM treatments (Table 11, Figures 22 and 23). There apparently was a solution P concentration where nonmycorrhizal leucaena could not obtain P from the soil solution, but where leucaena with the help of VAM fungi could (compare Figures 22 and 23, especially Kapaa soil). This suggests that at least a part of the enhancement of leucaena growth by the fungus was due to a lower threshold P concentration for mycorrhizal than for nonmycorrhizal roots.

Immediately above the threshold, P uptake increased in response to increasing solution P concentrations much more rapidly in mycorrhizal than nonmycorrhizal leucaena (Figure 23). But the responses of the +VAM and -VAM plants to increasing P concentrations beyond 0.1 mg P/l are not much different; both mycorrhizal and nonmycorrhizal plants appear to have increased P uptake at about the same rate.

Mycorrhizal leucaena may have reached its maximum uptake within each soil (Figure 21); the difference between mycorrhizal and nonmycorrhizal plant P uptake, and therefore the P uptake attributable to the VAM fungus, appears to plateau between 0.02 and 0.05 mgP/l. But nonmycorrhizal leucaena seems not to have achieved a maximum of P uptake, because total plant P continued to increase with increasing solution P concentrations in all soils (Figure 22).

Soil P Buffer Capacity and Plant P Uptake

At comparable $CaCl_2$-P concentrations, increasing P buffer capacity increased plant P contents in both mycorrhizal and nonmycorrhizal treatments (Figures 22, 23). The soil with the highest P sorption

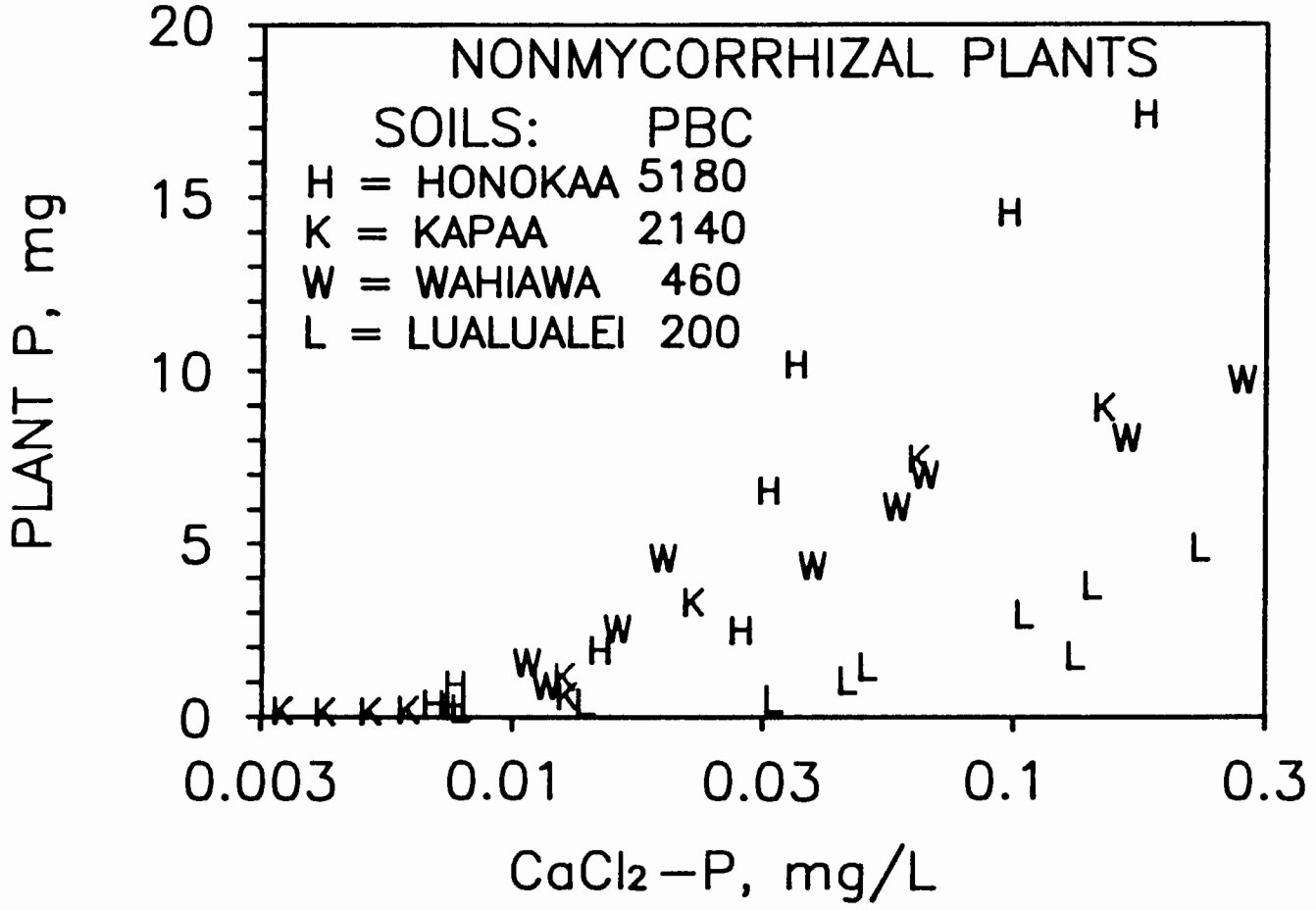

Figure 22. P uptake by nonmycorrhizal plants versus $CaCl_2$-P concentration.

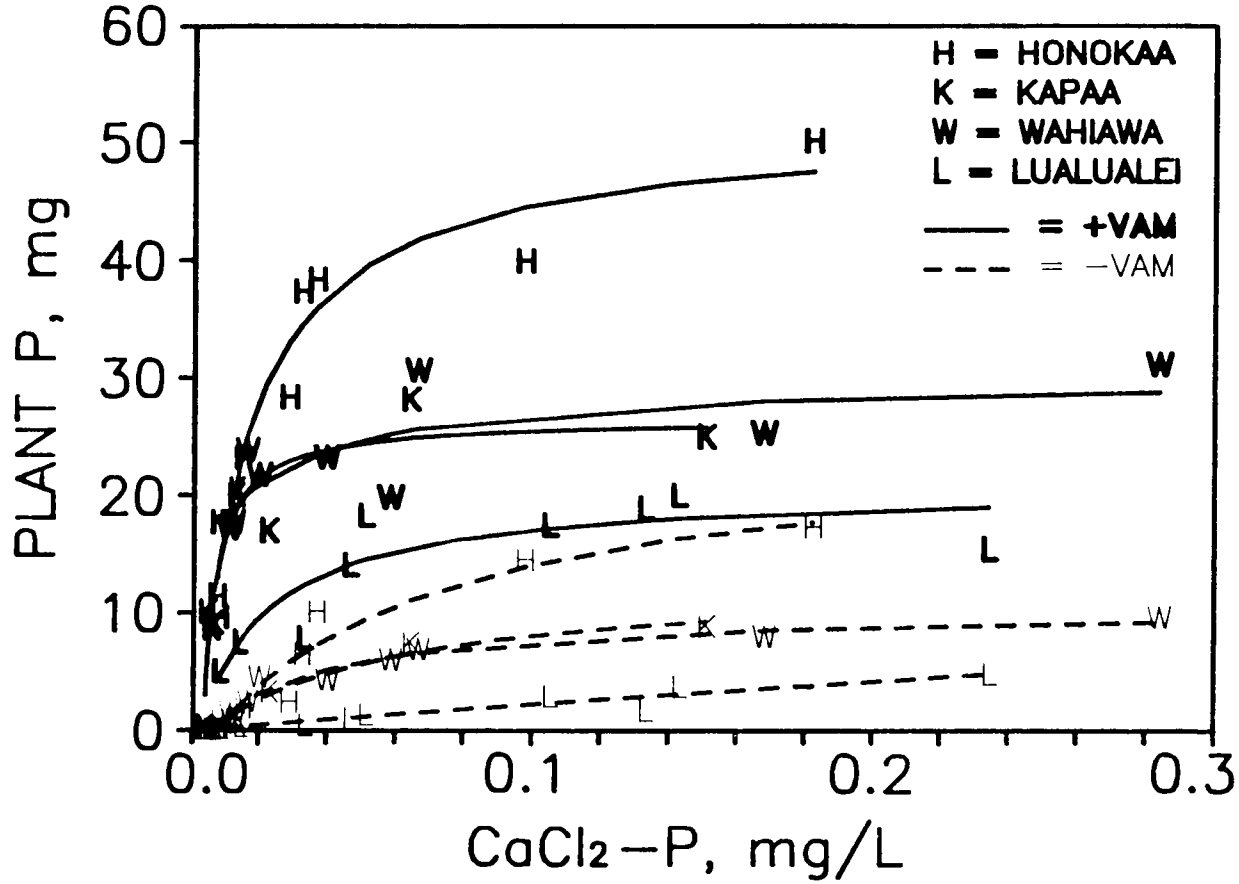

Figure 23. P uptake by mycorrhizal and nonmycorrhizal plants versus $CaCl_2$-P concentration.

capacity (Honokaa) supplied larger quantities P to plants than did soils with lower buffer capacities. This is especially evident when the total P uptake of plants grown in the Honokaa soil is compared with that of plants grown in the Lualualei soil, which had the lowest P buffer capacity.

This relationship between P buffer capacity and plant P uptake is to be expected from the definition of P buffer capacity: the power of a soil to resist changes in soil solution P concentration as P is removed from the soil. Thus, soils having the same initial solution P concentration but different P buffer capacities should initially provide P to plants growing in them at the same rate. As plants absorb P, solution P concentrations would decrease faster in a soil with a lower buffer capacity than they would in a soil with a higher P buffer capacity, resulting in higher solution P concentrations in the soil having the higher buffer capacity than in the soil with the lower buffer capacity.

Combined Effects of VAM and Soil P Availability on Plant P Uptake

In comparing the differences in plant P uptake between Honokaa and Lualualei soils (Figure 23), it seems apparent that increasing P buffer capacity increased plant P uptake more for mycorrhizal plants than for those without VAM fungi; or, from the opposite standpoint, the P supply to plants was impaired more by low P buffer capacity when mycorrhizal plants removed larger quantities of P from the soil. Plants that were colonized by VAM fungi extracted more P from the soil than did plants that were not mycorrhizal (Figure 23); this would have

increased the stress on the soil's ability to maintain solution P concentrations. Increasing P buffer capacity would have decreased the rate at which the solution concentration dropped as P was withdrawn, and thus maintained a better supply of P.

While it appears that mycorrhizal plants growing in the Honokaa soil continued to increase P uptake with increasing solution P concentrations, mycorrhizal plants in the other three soils seem to have reached maxima of P content (Figure 23). Increasing solution P concentrations may have increased plant P uptake more when the P buffer capacity was high than when it was low. Perhaps the increased plant P uptake obtained when P concentrations were high was only maintained when P buffer capacity was also high, and therefore able to sustain those high P concentrations.

Because P buffer capacity decreases with increasing solution P (Figure 1, Table 8), differences among soils in ability to buffer solution P concentrations as P is removed might be accentuated at higher solution P levels; the decrease in solution P concentration resulting from a given withdrawal of P would be largest in a soil of low P buffer capacity at high solution P levels. This would affect plant P uptake where plants are still responding to increasing solution P.

It seems likely that leucaena roots in symbiosis with Glomus aggregatum can obtain P from solution at lower concentrations than can leucaena roots alone. Increased P uptake by mycorrhizal plants may not simply be due to a more extensive exploitation of the soil volume by the fungi, but also to a physiological difference between mycorrhizal

and nonmycorrhizal roots in threshold concentrations for P uptake. This would produce a more intensive exploitation of the soil P resources.

Because soil P buffer capacity (dQ/dI) increases as soil solution concentrations decrease (Table 8), a larger quantity of P would be released for a given decrease in P concentration (for example, a decrease of 0.005 mg P/L) from a soil that is at a lower initial P concentration. A lower threshold concentration for mycorrhizal as compared to nonmycorrhizal roots would thus have two complementary effects.

First, it would increase P uptake for mycorrhizal plants at solution concentrations at which plants without mycorrhizae would be deficient in P. This might explain the high ratio of +VAM/-VAM P uptake at low solution P concentrations.

Second, it would increase P uptake by mycorrhizal plants relative to nonmycorrhizal plants at the concentrations at which such increases would provide the most benefit (where dQ/dI is largest); this might account for the steep slope of the P uptake response curve for mycorrhizal leucaena at low solution P concentrations.

A lower threshold P concentration for G. aggregatum hyphae as compared with leucaena roots implies that the fungus must have a more efficient uptake mechanism than the plant; the specific P absorption sites of the fungus have a stronger affinity for P than do those of the plant. This would also create a steeper response to increasing solution P concentrations.

Plant Weight and P Uptake

Plant weights followed the general pattern of P uptake (Figure 24); leucaena weight and P uptake were increased by increasing solution P concentrations, increasing P buffer capacity, and mycorrhizal symbioses. However, plants with high P contents had higher P percentages than plants with lower P contents (Figure 24). This resulted in smaller differences between treatments when comparisons were made on the basis of weight rather than P content.

Development of the P Uptake Equation

Thus far, much of the discussion of the data has been qualitative. To make quantitative estimates of the effects of solution P concentrations, P buffer capacity, and mycorrhizal symbioses on P uptake by leucaena, an equation was formulated that described leucaena P uptake in terms of these treatment variables. The equation included the treatment variables in forms that could depict mechanisms of the P supply and uptake system, in order to provide insight into the physical, chemical, and biological processes operating in the soil-root-fungus system.

Concepts upon which the equation was based include the following:

(1) There was a minimum P concentration below which plants could not absorb P from the soil solution. Results support this stipulation (Figures 22 and 23).

(2) The threshold concentration differed between mycorrhizal and nonmycorrhizal plants.

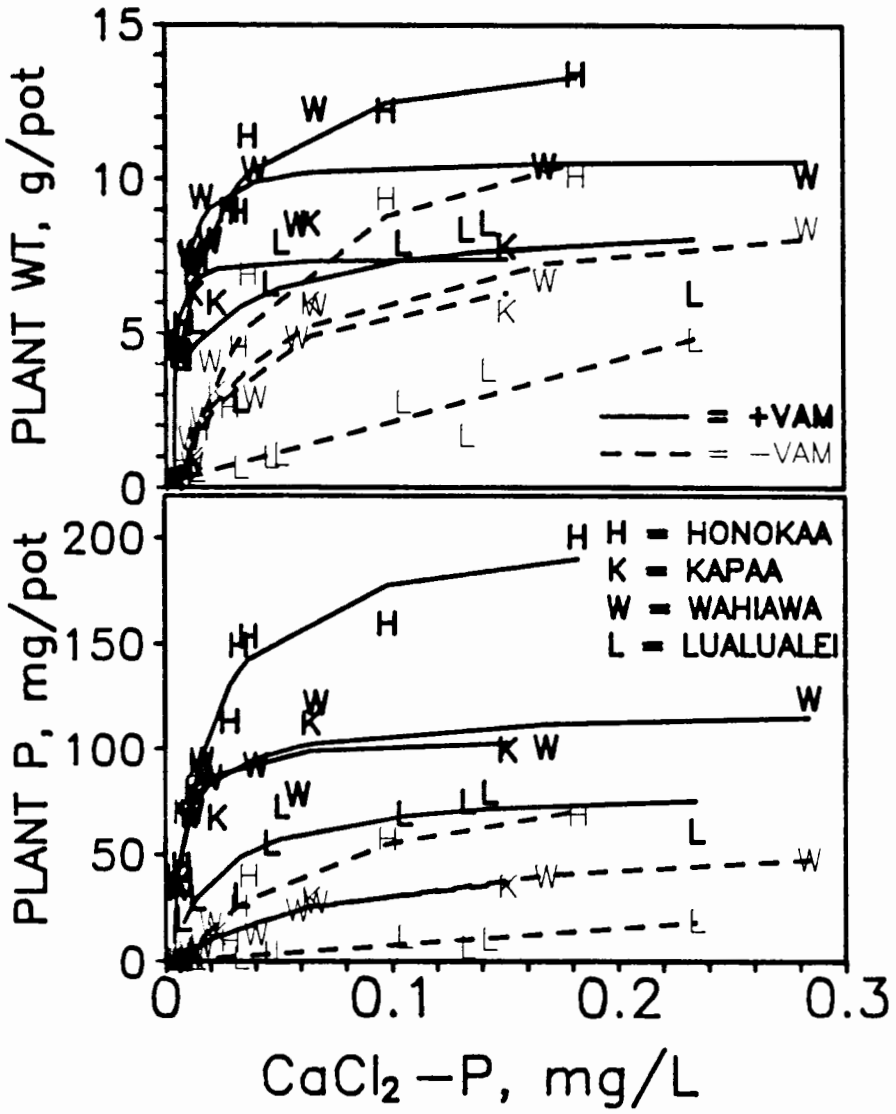

Figure 24. Plant weight and P uptake versus $CaCl_2$-P. Per pot basis. Regression calculated separately for each combination of soil series and inoculation class.

(3) As solution P concentrations increased above their respective thresholds, the initial responses (slopes of the response curves) of mycorrhizal and nonmycorrhizal plants to increasing P concentrations differed markedly (Figure 23)

(4) There was a maximum potential for P uptake, determined in part by a maximum P uptake rate and a maximum rate of new root growth (which would increase the P uptake rate). It appears from Figure 23 that in three of the inoculated soils (Lualualei, Wahiawa, and Kapaa) plant P uptake reached a plateau at about 0.1 mg P l^{-1}.

(5) The maximum potential for P uptake differed between mycorrhizal and nonmycorrhizal plants. Even at the highest P concentrations, mycorrhizal plants took up considerably more P than comparable nonmycorrhizal plants (Figure 23). It might be that solution P was not high enough to support maximum uptake by nonmycorrhizal plants; this idea will be tested when estimating the model parameters.

(6) The maximum P uptake potential was also influenced by the P-supplying capacity of the soil in which the plants were grown; that is, the soil, the root, and the fungal symbiont were an integrated system that supplied P to plants. As discussed above, even in soils with high initial solution P concentrations, P buffer capacity affects the rate of delivery of P to roots once the plant begins to withdraw P from the system.

The equation used to describe the relationship of leucaena P uptake to soil solution P concentration, soil P buffer capacity, and

mycorrhizal colonization was adapted from the Michaelis-Menten model of enzyme kinetics.

The Michaelis-Menten equation,

$$V = Vmax*M/(Km+M) \tag{12}$$

describes the rate of an enzyme-catalyzed reaction (V) in relation to the enzyme concentration (Vmax), substrate concentration (M), and the concentration of substrate (Km) at which the rate of the reaction is one-half of the maximum (Km = M where V = 0.5*Vmax) (Barber 1984). Km has been interpreted as expressing the affinity of the enzyme for the substrate (Salisbury and Ross 1985).

Claassen and Barber (1976) modified the equation to describe nutrient uptake by plants, writing it as

$$In = (Imax*Cl_0/(Km+Cl_0))-E \tag{13}$$

where In is the rate of nutrient uptake per unit area, Imax is the uptake rate at "infinite concentration", Cl_0 is the solution concentration of nutrient at the root surface, and E is the rate of loss of the nutrient from the root through leakage.

The form of the equation used by Barber (1984), in his model of soil nutrient availability, further modified the Michaelis-Menten equation to

$$In = Imax*(Cl-Cmin)/(Km+Cl-Cmin) \tag{14}$$

substituting Cl for Cl_0, and introducing a term (Cmin) for the solution concentration at which there is no nutrient influx (Nielsen 1976). When this equation is used, Km+Cmin (and not Km alone) is the solution concentration at which the rate of nutrient uptake is one-half of the maximum. Cmin can be interpreted as the concentration at which influx to the root equals efflux from the root ("net influx reaches zero"; Barber, 1984). Because total plant nutrient uptake integrates many rate processes over time, the application of this rate equation to account for P uptake by leucaena during a 72-day period may be appropriate.

In the final equation, a term was added to account for the effect of soil P buffer capacity on plant P uptake, and notation was altered to emphasize that the situation described is different from both the Michaelis-Menten model of enzyme kinetics and the Barber model of root nutrient uptake. The equation used was

$$Up = (B_0+B_1*PBC)*Umax*(Cl-Cthr)/(Kr+(Cl-Cthr)) \qquad (15)$$

where Up is total plant P uptake, B_0 and B_1 are parameters, PBC is the square root of the estimated P buffer capacity (dQ/dI) of a soil at 0.02 mg P l^{-1}, Umax estimates the maximum P uptake potential, Cl is the initial solution P concentration, Cthr estimates the threshold P concentration, and Kr estimates the solution P concentration where Up = 0.5*Umax. The equation was fit separately for mycorrhizal and non-mycorrhizal plants.

Table 12. -- Parameter estimates for P uptake model (equation 14)[a], estimated separately for mycorrhizal (+VAM) and nonmycorrhizal (-VAM) plants.

PARAMETER[b]	+VAM	-VAM	units
B_0	0.523	0.275	---
B_1	0.013	0.026	---
Umax	148	61.6	mg P pot^{-1}
Cthr	0.0026	0.0046	mg P l^{-1}
Kr	0.0103	0.0916	mg P l^{-1}

[a] $Up = (B_0+B_1*PBC)*Umax*(Cl-Cthr)/(Kr+(Cl-Cthr))$

[b] Up = plant P uptake pot^{-1}, B_0 and B_1 are coefficients, Umax = maximum potential P uptake, $PBC = (PBC_{0.02})^{0.5}$, Cl = initial solution P concentration, Cthr = threshold P concentration, Kr = Cl-Cthr where Up = 0.5*Umax.

Parameter Estimates of the P Uptake Equation

The parameters of the model were estimated using data from experiment 2, in which both mycorrhizal and nonmycorrhizal leucaena were grown with two replications in four soils, each adjusted to nine levels of soil P availability (144 data points). Parameters, estimated separately for mycorrhizal and nonmycorrhizal plants, are presented in Table 12.

The equation accounted for 91% of the variation in the data. Estimated values are shown in figure 25, with the data points.

The estimate of Umax was larger, and estimates of Cthr and Kr were lower, for mycorrhizal than for nonmycorrhizal plants. It appears that the main differences between mycorrhizal and nonmycorrhizal plants was in their respective maxima of P uptake (Umax) and in the affinity (Kr) of their P absorption sites for P in solution. The large

difference between the estimates of Umax might indicate the degree to which leucaena depends on mycorrhizae for its supply of P, and suggests that nonmycorrhizal leucaena roots can not absorb P from solution at the same rate as mycorrhizal leucaena, even at very high solution P concentrations. Because the duration of mycorrhizal P uptake was probably less than half that of nonmycorrhizal P uptake (see above, Plant P Status During Growth), the actual value of Umax (if it is regarded as a rate constant) could be more than double that shown here. Umax might represent the absorption sites contributed by the mycorrhizae to the total P uptake capacity of the plant.

The solution P concentration at which uptake is one-half the maximum is Kr+Cthr (see above). This equals about 0.013 and 0.096 for mycorrhizal and nonmycorrhizal plants, respectively. Thus, not only did mycorrhizal roots absorb P at a faster rate (higher Umax) than did nonmycorrhizal roots, but they did so at lower solution concentrations.

The difference between the estimates of Cthr is very small. The estimated Cthr for nonmycorrhizal plants (0.005 mg P l^{-1}) was much smaller than the apparent threshold P concentration of about 0.008 mg P l^{-1} estimated from Figure 22.

The parameter estimates, B_0 and B_1 (Table 12), describing the effect of soil P buffer capacity on plant P uptake, suggest that higher buffer capacity increased P uptake for both mycorrhizal and nonmycorrhizal plants. The percentage increase in P uptake attributable to P buffer capacity was probably greater on nonmycorrhizal plants (B_1 is larger for the -VAM plants); however, because mycorrhizal plants took

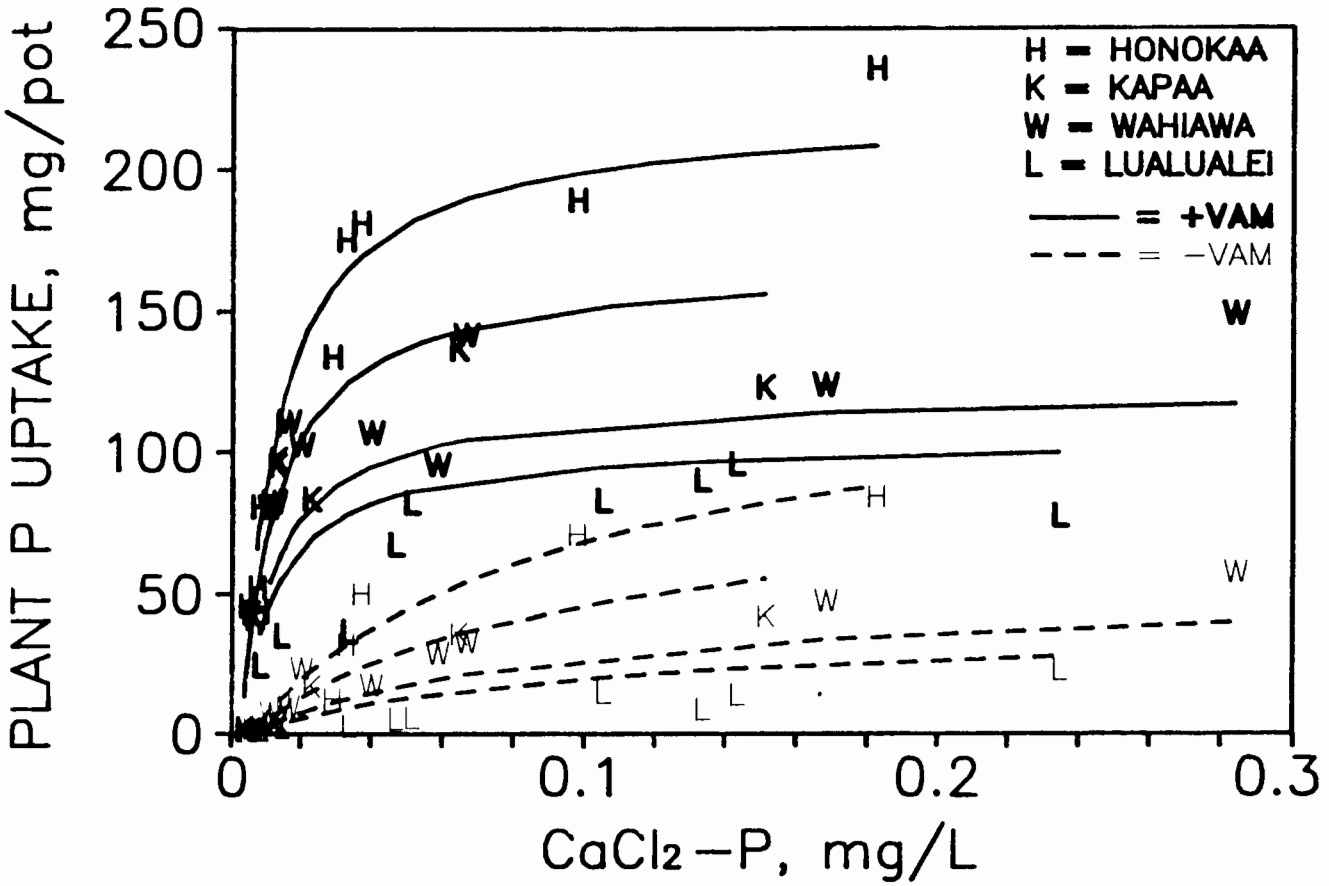

Figure 25. Plant P uptake per pot. Estimates calculated from model (Eq. 14).

up more total P, the increase in quantity of P taken up was greater among the mycorrhizal plants (Figure 25).

Even though this equation accounted for 91% of the variation in the experimental data while using reasonable assumptions, there were several discrepancies between model predictions and data points.

While the Wahiawa and Kapaa soils had different P sorption characteristics (Table 8, Figures 14, 15), P uptake by plants was nearly identical in response to solution P concentration and mycorrhizal status in these soils (Figures 22, 23). Two possible explanations are the following:

The Wahiawa and Honokaa soils broke into smaller aggregates when mixed with the blasting sand than did the Kapaa and Lualualei soils (personal observation). Thus, while the roots in all soils should have had similar environments with respect to aeration, water supply, and penetrability, plants growing in the Wahiawa and Honokaa soils probably had a larger portion of their root surface in contact with soil particles than did plants growing in the Kapaa and Lualualei soils. Because soil particles were the source of P in this system, plants growing in the Wahiawa and Honokaa soils might have had enhanced access to P over those growing in the Kapaa and Lualualei soils due to increased contact with the soils.

A second possibility is that soil solution P concentrations were other than indicated by the $CaCl_2$-P extraction. Because solution P concentrations decrease as calcium concentrations increase (Olsen and Watanabe 1970; Soltanpour et al. 1974), the P concentration of a soil solution that is lower than 0.01 M in Ca may be underestimated by the

$CaCl_2$-P extractant, because the soil solution P could be depressed by the Ca in the extractant. Where soil solution Ca is higher than 0.01 M, the extractant could decrease the concentration of Ca, and soil solution P would be overestimated. A 0.01 M $CaCl_2$ extract might accurately reflect soil solution P concentrations if the soil solution Ca concentration is also near 0.01 M (Olsen and Watanabe 1970). Because both the Lualualei and the Kapaa soil used in this experiment had high pHs and contained fragments of $CaCO_3$, soil solution P concentrations in these soils may have been overestimated in the $CaCl_2$-P extract, while plants growing in the Wahiawa and Honokaa soils may have been in contact with soil solution P concentrations higher than were indicated by the $CaCl_2$-P extraction.

With these discrepancies in mind, the following observations were made. Leucaena roots in symbiosis with Glomus aggregatum extracted P from solution at lower concentrations than did nonmycorrhizal leucaena roots. This may have been a result of higher affinity for P by specific P absorption sites on the fungal mycelium as compared to the leucaena root. By extracting P from the soil solution at lower concentrations, and by extracting more P from soil at the same initial P concentration, mycorrhizal roots used the soil P supply more intensively than did nonmycorrhizal roots.

Increases in soil P buffering capacity increased the supply of P to plants. At comparable solution P concentrations, increases in P buffer capacity should lead to greater P uptake by plants that exploit soil P resources more intensively, such as mycorrhizal leucaena.

The relationship between soil P buffering capacity and P supply to plants is complex. P buffering capacity affects not only the rate at which solution P decreases as P is absorbed from soil solution by plants, but also the rate that P diffuses in soil and the slope of the concentration gradient that drives the diffusion process. The square root of the P buffer capacity (Olsen et al. 1962) at 0.02 mg P l^{-1} ($CaCl_2$-extractable P) proved useful in fitting P sorption measurements to plant growth response. However, a more mechanistic equation, integrating the different effects of soil P buffer capacity to create an index that would correspond to the effect of P buffer capacity on soil P supply to plants, would be more desirable.

SUMMARY

Phosphate uptake by plants is strongly influenced by soil solution P concentration, structure and physiology of roots, and soil microorganisms, most notably mycorrhizal fungi.

Inoculating soils with an appropriate species of mycorrhizal fungus can increase growth and P uptake of leucaena. If native mycorrhizal fungi are present, and if nutrients other than P limit plant growth, there may be no advantage to mycorrhizal inoculation.

The six-day equilibration procedure for estimating soil P buffer capacity (Fox and Kamprath 1970) underestimated the quantity of P needed to attain selected solution P concentrations. The square root of the slope of the P sorption curve (dQ/dI) at 0.02 mg P l^{-1} proved

useful in estimating the effect of P buffer capacity of soils on plant P uptake.

$CaCl_2$-extractable P can be a useful index of soil solution P intensity. Using the logarithm of the concentration of P in $CaCl_2$ extract greatly increased the correlation between soil P measurements and plant P uptake. The predictive power of $CaCl_2$-extractable P across a range of soils was improved by combining it with soil P buffer capacity. Increasing P buffer capacity increased P supply to plants at comparable concentrations of P extracted in $CaCl_2$.

The soil P extractant that correlated best with plant P uptake across the range of soils (Oxisols, an Andisol, and a Vertisol) and plants (mycorrhizal and nonmycorrhizal leucaena) was NH_4F+HCl (Bray and Kurtz 1945). Where stress on the P-supplying capacities of the soils was low (with nonmycorrhizal leucaena), the direct measures of extractable P provided the best correlations with plant P uptake. Where the P-supplying capacities of the soils were stressed (with mycorrhizal leucaena), taking the logarithm of the extractable P measurements improved correlations with plant P uptake.

Leucaena leucocephala growing symbiotically with Glomus aggregatum extracted P from soil solution at concentrations too low for leucaena to do so alone. Mycorrhizal leucaena also absorbed larger amounts of P than did nonmycorrhizal leucaena at a given P concentration. The ability of the symbionts to absorb P at very low concentrations (around 0.003 mg P/l) probably reflects a higher affinity for P of the P absorption sites of the fungus as compared to those of the plant. Because soil P buffer capacity increases as soil solution P

concentration decreases, relatively large quantities of P become available to organisms that can absorb P from soil solution at low concentrations.

Mycorrhizal leucaena increased total P uptake more in response to increasing P buffer capacity than did nonmycorrhizal leucaena.

Table 13. — Treatment effects on pinnule P, experiment 1. Results of analyses of variance of ln(% P per pinnule) for each sampling day. Probabilities of type I error.

	Days After Planting														
Effect	10	14	17	21	24	31	35	42	45	49	52	56	59	63	66
CONSTANT	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R[a]	0.05	0.51	0.08	0.44	0.02	0.01	0.38	0.05	0.11	0.34	0.07	0.47	0.33	0.03	0.27
I	0.29	0.00	0.50	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	0.79	0.35	0.14	0.56	0.00	0.07	0.04	0.06	0.10	0.00	0.00	0.00	0.00	0.00	0.00
P	0.97	0.02	0.00	0.00	0.00	0.00	0.00	0.04	0.00	0.01	0.01	0.00	0.00	0.00	0.00
S	0.86	0.13	0.03	0.02	0.00	0.05	0.31	0.06	0.02	0.06	0.12	0.01	0.36	0.17	0.01
I*F	0.01	0.40	0.02	0.29	0.01	0.83	0.23	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
I*P	0.81	0.74	0.24	0.57	0.20	0.00	0.00	0.00	0.00	0.05	0.43	0.10	0.13	0.53	0.02
I*S	0.12	0.43	0.53	0.99	0.98	0.08	0.01	0.00	0.01	0.36	0.02	0.00	0.07	0.00	0.00
F*P	0.55	0.04	0.09	0.01	0.07	0.34	0.03	0.00	0.72	0.32	0.39	0.03	0.26	0.04	0.06
S*F	0.36	0.01	0.77	0.21	0.00	0.79	0.16	0.96	0.91	0.51	0.07	0.25	0.93	0.68	0.88
P*S	0.11	0.89	0.50	0.80	0.51	0.05	0.31	0.35	0.43	0.61	0.54	0.07	0.72	0.19	0.91
I*F*P	0.74	0.11	0.28	0.09	0.30	0.92	0.26	0.18	0.32	0.51	0.04	0.00	0.00	0.00	0.11
I*F*S	0.07	0.71	0.02	0.09	0.00	0.76	0.00	0.03	0.28	0.65	0.01	0.00	0.21	0.00	0.05
I*P*S	0.95	0.52	0.32	0.46	0.17	0.04	0.99	0.94	0.13	0.18	0.36	0.03	0.93	0.54	0.50
F*P*S	0.55	0.64	0.15	0.47	0.08	0.86	0.61	0.44	0.05	0.30	0.08	0.04	1.00	0.17	0.01
I*F*P*S	0.52	0.46	0.57	0.49	0.72	0.23	0.03	0.21	0.08	0.77	0.36	0.02	0.38	0.27	0.34
R^2	0.41	0.52	0.64	0.64	0.89	0.91	0.90	0.85	0.86	0.71	0.69	0.88	0.78	0.89	0.82

[a] Effects are denoted as follows: R = Replication, I = Inoculation with VAM, F = Fumigation, P = Phosphate fertilization levels, S = Soils. Interaction terms have asterisks, e.g. I*F = Inoculation by Fumigation.

Table 14. -- Treatment effects on pinnule weight, experiment 1. Results of analyses of variance of ln(pinnule weight) for each sampling day. Probabilities of type I error.

	Days After Planting														
Effect	10	14	17	21	24	31	35	42	45	49	52	56	59	63	66
CONSTANT	0.12	0.06	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
R[a]	0.14	0.46	0.02	0.17	0.06	0.73	0.61	0.82	0.44	0.56	0.58	0.38	0.88	0.18	0.49
I	0.10	0.00	0.00	0.21	0.89	0.77	0.07	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00
F	0.58	0.03	0.13	0.53	0.57	0.73	0.21	0.01	0.27	0.44	0.21	0.00	0.00	0.00	0.00
P	0.82	0.01	0.02	0.26	0.59	0.00	0.00	0.01	0.35	0.43	0.01	0.20	0.20	0.01	0.03
S	0.54	0.49	0.89	0.35	0.06	0.73	0.51	0.79	0.64	0.15	0.66	0.39	0.04	0.02	0.32
I*F	0.08	0.51	0.03	0.56	0.01	0.22	0.49	0.12	0.09	0.79	0.01	0.00	0.00	0.00	0.00
I*P	0.65	0.69	0.76	0.57	0.62	0.99	0.66	0.76	0.78	0.55	0.17	0.00	0.00	0.00	0.00
I*S	0.54	0.61	0.85	0.60	0.40	0.71	0.13	0.91	0.14	0.04	0.58	0.07	0.04	0.01	0.25
F*P	0.29	0.11	0.18	0.03	0.05	0.03	0.05	0.04	0.44	0.66	0.18	0.01	0.02	0.00	0.02
F*S	0.88	0.08	0.98	0.76	0.45	0.04	0.53	0.43	0.78	0.71	0.04	0.00	0.51	0.05	0.23
P*S	0.35	0.63	0.48	0.66	0.25	0.17	0.58	0.31	0.12	0.19	0.06	0.42	0.65	0.10	0.34
I*F*P	0.23	0.10	0.60	0.84	0.50	0.69	0.77	0.99	0.87	0.96	0.19	0.00	0.11	0.00	0.01
I*F*S	0.02	0.23	0.05	0.57	0.23	0.15	0.03	0.05	0.14	0.57	0.00	0.81	0.82	0.06	0.84
I*P*S	0.28	0.20	0.14	0.87	0.78	0.75	0.86	0.97	0.91	0.28	0.00	0.14	0.68	0.96	0.96
F*P*S	0.83	0.20	0.02	0.84	0.26	0.16	0.87	1.00	0.78	0.62	0.15	0.03	0.02	0.00	0.04
I*F*P*S	0.73	0.34	0.53	0.66	0.82	0.37	0.11	0.28	0.43	0.66	0.20	0.33	0.02	0.02	0.17
R^2	0.39	0.58	0.58	0.32	0.45	0.47	0.48	0.50	0.48	0.51	0.73	0.82	0.71	0.83	0.78

[a] Effects are denoted as follows: R = Replication, I = Inoculation with VAM, F = Fumigation, P = Phosphate fertilization levels, S = Soils. Interaction terms have asterisks, e.g. I*F = Inoculation by Fumigation.

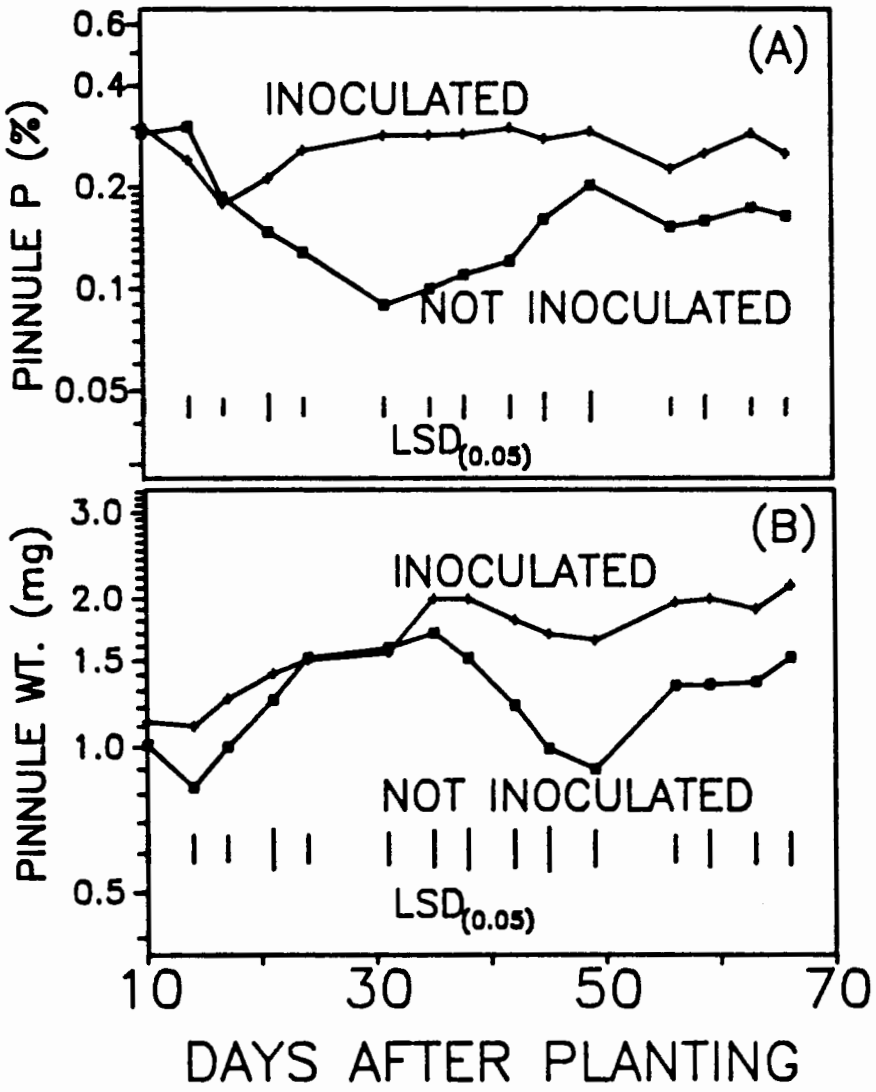

Figure 26. Inoculation of soils with Glomus aggregatum: effects on (A) pinnule P percentages, and (B) pinnule weights, over time.

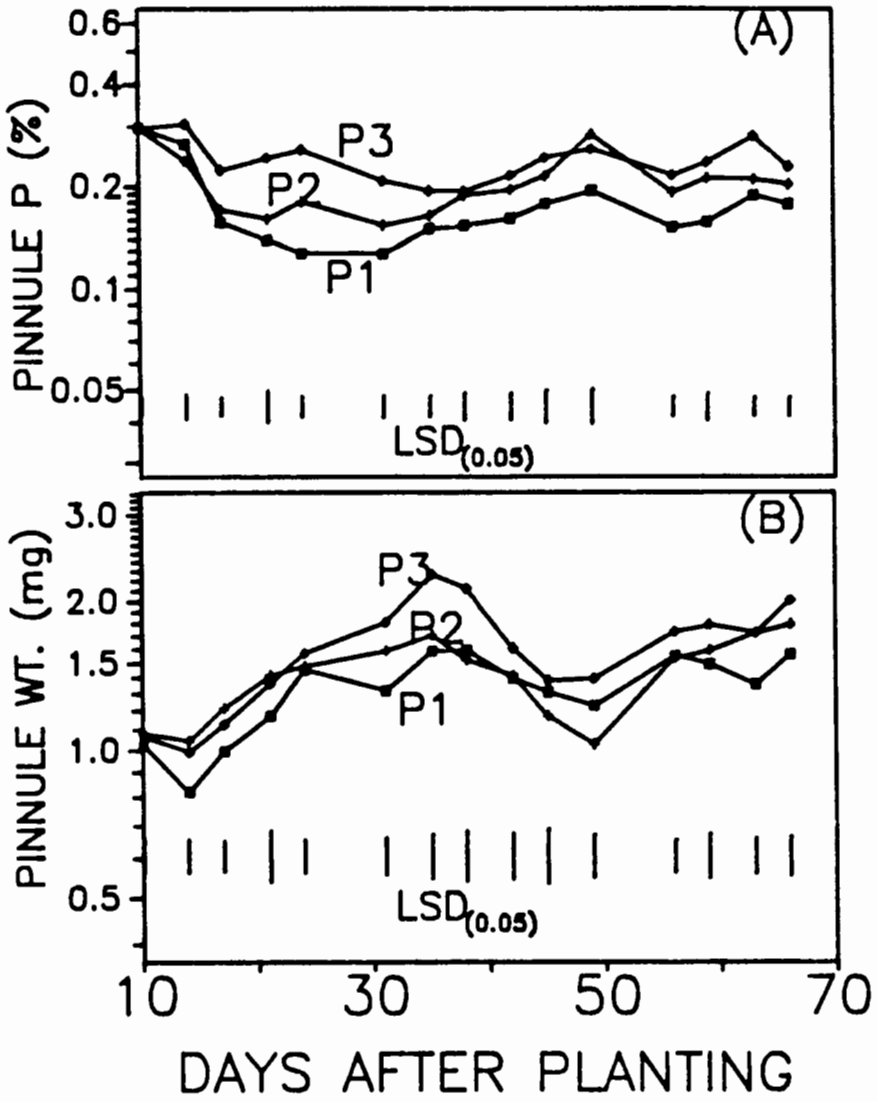

Figure 27. Differences in soil P availability: effects on (A) pinnule P percentages, and (B) pinnule weights, over time. P1, P2, P3 show increasing soil P levels.

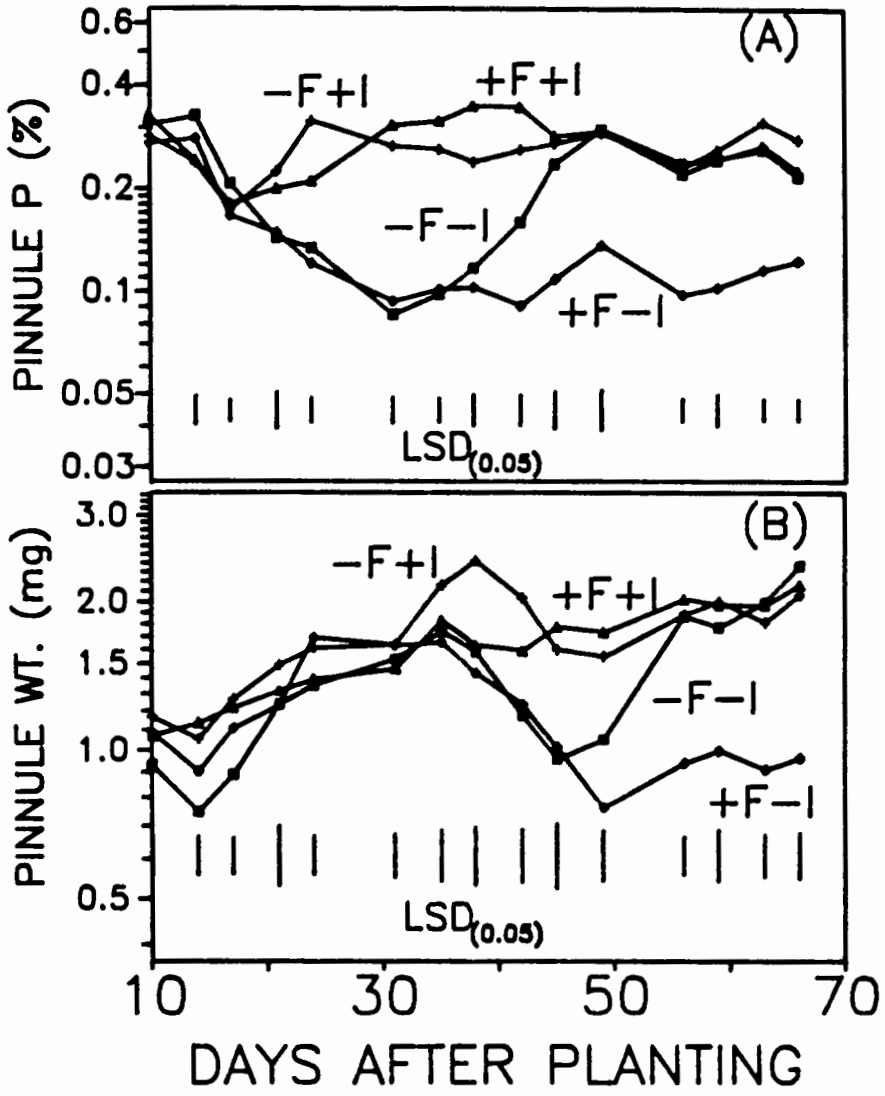

Figure 28. Combined fumigation and inoculation of soils: effects on (A) pinnule P percentages, and (B) pinnule weights, over time. +F and -F depict fumigated and nonfumigated treatments; +I and -I depict inoculated and noninoculated treatments.

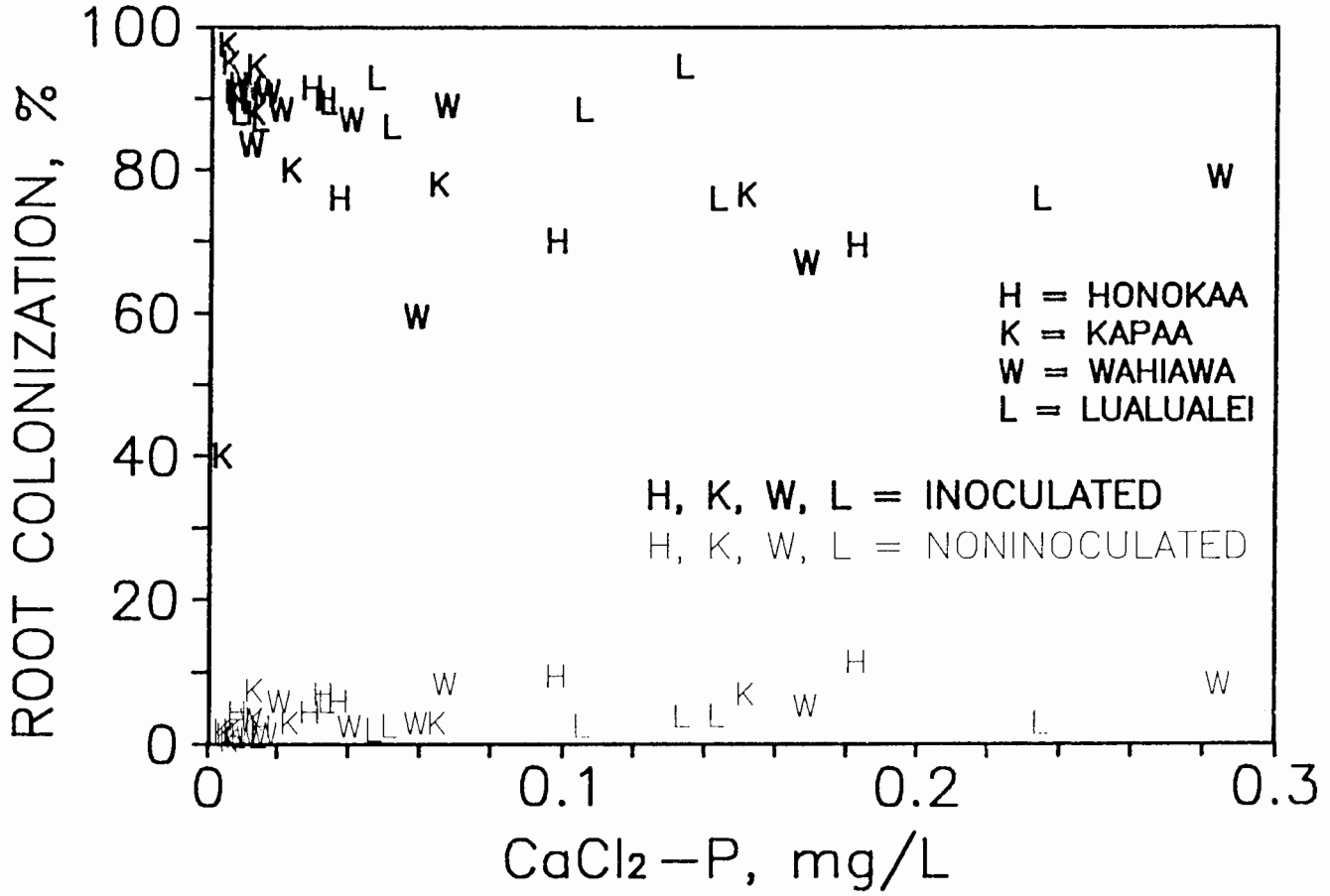

Figure 29. Percent root mycorrhizal colonization. Effects of inoculation, soil, and $CaCl_2$-P concentration.

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