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Contribution of Mycorrhizae to P Nutrition of Crops Growing on an Oxisol[1]

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ABSTRACT

Most plant species, including the major crops, form symbiotic mycorrhizal associations between their roots and certain fungi which influence nutrient uptake, especially P, from infertile soils. How well-supplied the phosphate must be before mycorrhizae cease to enhance P uptake is not known; nor have the effects of mycorrhizae on external P requirements of crops been adequately determined in the field. This study examined the influence of mycorrhizae on the P requirements of crops in a tropical field environment on a Tropeptic Eutrustox. Plant growth and P uptake by non-mycorrhizal and mycorrhizal plants (methyl bromide-fumigated and non-fumigated soil) were measured at 10 levels of soil P using seven plant species. *Brassica chinensis*, which does not form symbiotic mycorrhizal associations, consistently grew better and took up more P from the fumigated than from the non-fumigated soil. All other species growing in non-fumigated plots formed associations with mycorrhizae. In general, plants growing on fumigated soil did not become infected with mycorrhizae. In P-deficient situations, plant concentration of P was enhanced by the mycorrhizal associations. The levels of soil P at which fumigation ceased to make a difference in the P percentage in plants of the various species were as follows: *Glycine max* (L.) Merr. (0.1 μg P/ml), *Vigna unguiculata* L. (0.2 μg P/ml), *Allium cepa* L. (0.8 μg P/ml), *Leucaena leucocephala* (1.6 μg P/ml), *Stylosanthes hamata* and *Manihot esculenta* (1.6 + μg P/ml). We suggest that this listing may be the order in which these species depend on mycorrhizae in P-deficient soils. As a mean of six species growing on the two lowest soil P levels, P uptake by mycorrhizal plants was 25 times greater than by plants without mycorrhizal associations. Thus, some crops appear to be quite dependent upon a mycorrhizal association for P absorption from a soil of high sorption capacity.

Additional index words: Cowpea, Cabbage, Cassava, Leucaena, Onion, Soybean, Stylosanthes, P requirement.

PLANTS which develop symbiotic associations with mycorrhizal fungi frequently absorb more mineral nutrients, especially P, from soils than plants without mycorrhizae (Mosse, 1973, 1977). One explanation for increased P uptake by mycorrhizal plants is that the P used is not otherwise physically accessible to plant roots. Phosphorus absorption is thought to be enhanced by the extension of hyphae beyond the zones where P has been depleted by diffusion to roots (Tinker, 1975). For some soil-plant systems, similar L values (a measure of the pool of available P) were obtained with mycorrhizal and non-mycorrhizal plants (Sanders and Tinker, 1971). Barrow et al. (1977) concluded that mycorrhizae did not give onion (*Allium cepa* L.) or subterranean clover (*Trifolium subterraneum* L.) any special access to firmly held P. Nevertheless, this study and that of Murdoch et al. (1967) suggest that mycorrhizal plants use sparingly soluble P more readily than non-mycorrhizal plants. The possibility of using sparingly soluble P is of special interest to those who work with high P-sorbing soils, because the initial P requirements of some crop species growing on such soils may be very high. Soils of tropical areas are often P deficient, and some of them sorb much P (Fox et al., 1974; Fox and Searle, 1979).

Numerous pot trials and other short-term experiments have shown sizeable responses to mycorrhizal infection. Such effects should now be evaluated by field studies to better assess the potential benefits for crop production. The soils involved in such studies should be classified and the P-sorption properties and P levels should be characterized so that results will be transferable.

Objectives of the experiment were to: (a) evaluate the effects of mycorrhizae on the P requirement of some food and forage crops in a field environment; and (b) evaluate the effects of mycorrhizae on supplying P to plants in a soil with large amounts of sorbed P of low availability.

MATERIALS AND METHODS

The experiment was located on the Poamoho Experimental Farm, College of Tropical Agriculture, Island of Oahu, Hawaii, on plots of Wahiawa soil (Tropeptic Eutrustox) that were initiated in 1971 as part of a program to evaluate the external P requirement of important tropical crops. Some experimental results pertaining to this long-range study have been published (Fox, 1979; Nishimoto et al., 1977; Fox et al., 1974). Ten levels of P were established in 1971 and were subsequently re-established for each crop, so that P concentrations in solutions equilibrated with soil from the plots were approximately as presented in Table 1. Treatments of 0.012, 0.025, 0.05, and 0.1 μg P/ml were replicated three times while the remaining were single replicates as prescribed by an augmented block design (Federer, 1956). In practice, rates of P fertilizer were determined from P sorption curves prepared as described by Fox and Kamprath (1970). The P, as concentrated superphosphate, was broadcast on the surface of the soil and thoroughly tilled into the surface 15 cm of soil.

Soil P was extracted by three procedures: 0.5 *M* $NaHCO_3$ (Banderis, 1976); 0.3 *N* NH_4F + 0.025 *N* HCl; and 0.05 *N* NCl + 0.025 *N* H_2SO_4 (Council on Soil Testing and Plant Analysis, 1974). Extractable P-levels, as determined at the beginning of this experiment, are presented in Table 1. Although large amounts of fertilizer P were required to initially establish the soil P-levels, maintenance applications required for near-maximum yields were not much greater than estimated plant removal of P.

Soil pH had been adjusted with precipitated $CaCO_3$ to about 5.8. Potassium was added to provide 1 meq K/100 g soil. Nitrogen (100 kg N/ha as urea) was supplied to the non-legumes chinese cabbage, onion, and cassava. All tillage, including making irrigation furrows, was completed before the plots were fumigated. Instead of simulating natural infectivity by fumigating the whole area and inoculating half, we chose to compare the natural infectivity of the soil with that of fumigated soil. This choice was partly based on difficulties some investigators have experienced in obtaining rapid infection of plants growing in previously sterilized soils. The differences due to treatments observed in this study seem to justify the choice in methodology.

Half of each plot was fumigated with 48 g methyl bromide and 1 g chloropicrin/m^2. The material was applied under a

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Table 1. Intended levels of P maintained in the Wahiawa soil, quantities of P required for establishment and maintenance, and soil phosphate measured by three widely used extractants.

P level maintained in soil	Quantities of P† added to soil: Establishment	Maintenance: 10 crops	Maintenance: Last three crops	Phosphate extracted from soil by 3 extractants: 0.5 *N* $NaHCO_3$	0.025 *N* HCl + 0.03 *N* NH_4F	0.05 *N* HCl + 0.025 *N* H_2SO_4
μg P/ml	kg/ha	kg/ha/crop		μg P/g soil		
0.003	35	5	0	12	3	6
0.006	88	9	0	15	5	9
0.012	190	29	8	30	14	20
0.025	300	51	42	44	28	35
0.05	440	80	34	72	55	57
0.1	600	103	40	93	72	86
0.2	700	145	58	164	144	158
0.4	700	182	73	160	156	209
0.8‡	770	–	–	152	132	174
1.6	1,440	281	173	295	339	337

† Phosphate fertilizer requirements were estimated from phosphate sorption curves (Fox and Kamprath, 1970). The maintenance applications were made in installments during 7 years. ‡ This treatment was a No P check for the first 5 years. Contamination with P from an adjacent plot led to its discontinuence as a check and conversion into a P treated plot.

Table 2. Plant species and cultivars grown, time of sampling, and plant material sampled in the experiment on soil P-mycorrhizae-plant interaction.

Crop	Cultivar	Harvest information: Plant age	Plant part	Sample size
Brassica chinensis (chinese cabbage)	China King	Mature heads	Entire top (yield)	3.8 m row
		70	Wrapper leaf (P content)	
Vigna unguiculata L. (cowpea)	TVu 3563	66	Leaf blades,	20 leaves
		76	entire top	4 plants
Glycine max L. Merr. (soybean)	Kailua	52	Leaf blades	20 leaves
		86	green pods	2 m row
		112	grain	1 m row
			entire top	4 plants
Manihot esculenta Crantz (cassava)	Ceiba	80	Leaf blades	9 leaves
		118	Entire top	5 plants
Leucaena leucocephala	S72	91	Entire top	10 plants
Allium cepa L.	Yellow Granite	91	Entire plant	3.8 m row
Stylosanthes hamata	Verano	91	Entire top	10 plants

plastic cover which was dug into the soil around the perimeter of the plot. The plastic cover was removed from the plots 1 week after the fumigant was applied. The experiment was planted one month after fumigation. Plastic bags were used as shoe covers during plot work to avoid contaminating the plots.

The crop species, listed in Table 2 were planted in single rows 3.8 m long, except for *Stylosanthes* and *Leucaena* which were in rows 1.9 m long. Row spacing was 0.91 m. Planting was during early March 1978.

It was assumed that the non-fumigated soil would contain ample mycorrhizal spores for adequate infection of roots. Chinese cabbage was included as a control plant because, as a member of the *Cruciferae* family, it generally does not form mycorrhizal associations (Gerdemann, 1968). Therefore, any influence of fumigation, as indicated by the growth and P content of chinese cabbage, could be ascribed to non-mycorrhizal effects.

Mycorrhizal infection was evaluated by collecting fine roots (<2 mm diam) from 3 to 4 plants per treatment. After washing and staining (Phillips and Hayman, 1970), roots were examined in an open petri dish with a dissecting microscope. Mycorrhizal infection was rated on a scale of 0 to 5. This was done by one person in an attempt to apply a uniform standard across all treatments and species.

Table 3. Influence of soil fumigation on relative growth of seven crop species.

Crop	Growth parameter evaluated	Mycorrhiza† infection rating	Weight fumigated‡ / Weight non-fumigated
Chinese cabbage	Fresh top weight	0	1.78
Cassava	Dry weight tops	+	0.10
Soybean	Grain yield	+	0.05
Cowpea	Dry weight of plant tops	+	0.01
Onion	Dry weight of plant tops	+	0.05
Leucaena	Dry weight of plant tops	+	0.03
Stylosanthes	Dry weight of plant tops	+	0.03

† 0 absent, + = present. ‡ Weight of the specified plant growth parameter from fumigated plots divided by the same measure from non-fumigated plots. Data are from the two lowest levels of soil P (0.003 and 0.006 μg P/ml).

Plant materials were digested with nitric-perchloric acid. Phosphorus and several nutrient cations were determined in the digest. Only P uptake will be presented in this report.

RESULTS AND DISCUSSION

Side Effects of Fumigation

Soil fumigation can result in numerous effects in addition to elimination of fungi. However, in our experiment, means of $NaHCO_3$-extractable P in the fumigated and non-fumigated subplots were not significantly different, being 58.4 and 58.9 μg P/g respectively. Our data agree with results reported by Lopes and Wollum (1976). Means for NH_4^+ and NO_3^- were higher in the fumigated plots but the effect was small and not consistent. Rovira (1976) and Lopes and Wollum (1976) also observed more NH_4^+ and NO_3^- in methylbromide-fumigated soil.

Although the legumes were inoculated with Rhizobia, nodulation of cowpea and soybean [*Glycine max* (L.) Merr.] plants was consistently lower in fumigated than in non-fumigated plots. A regression of number of nodules on various factors suggested that the differences in nodulation was due more to fumigation ($P < 0.001$) than to NH_4^+ and NO_3^- level ($P = 0.20$) or soil P levels ($P = 0.90$). It is possible that residual chloropicrin may have adversely affected the

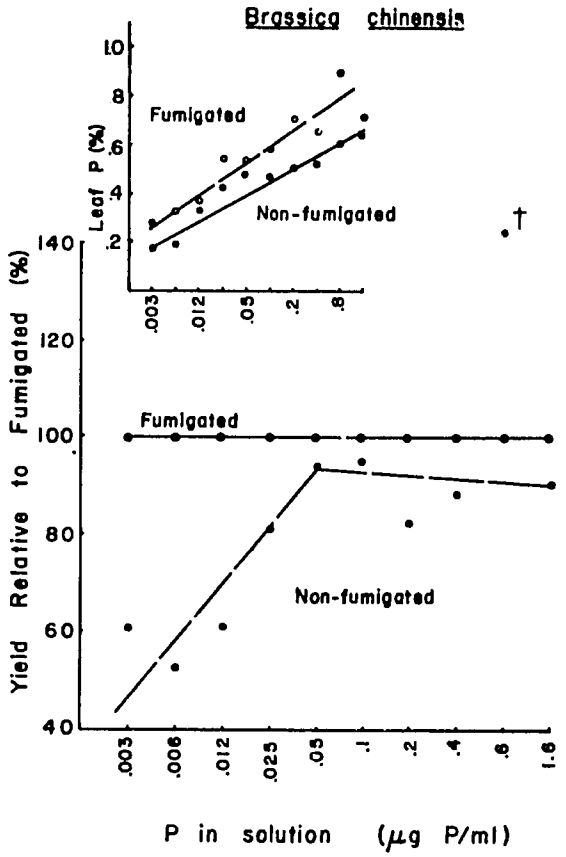

Fig. 1. Relative fresh weight of chinese cabbage and P percentage (dry weight basis) of wrapper leaves as affected by fumigation and levels of P in the soil. † This value is exceptionally high because the yield of the corresponding fumigated treatment was low. The low yield of the fumigated treatment was associated with exceptionally high P content (see insert graph).

rhizobia and probable that inoculum alone in fumigated soils was not as effective as inoculum plus indigenous Rhizobia in the non-fumigated soils.

The confounding effects of nematodes may be a more serious problem. This should have been a positive effect of fumigation and will be added to any effects which might be attributed to mycorrhizae.

Effects of Fumigation on Growth of Test Crops

With one exception, the crops used for this investigation formed symbiotic associations with vesicular, arbuscular mycorrhizae. The exception was chinese cabbage which, because it does not form mycorrhizal associations, was included in this investigation to indicate the non-mycorrhizal effects of fumigation on crop growth, especially at low soil P levels (Fig. 1). Phosphorus percentage of chinese cabbage was increased by fumigation across the range of soil P investigated. Fresh weight of chinese cabbage tops averaged over the two lowest P treatments was increased 78% by fumigation. Fumigation depressed growth of all other species in P deficient soil (Table 3). It appears that fumigation had at least two major influences on growth: first, which was unrelated to mycorrhizal infection, resulted in increased growth, presumably due to control of soil-borne pathogens; and the second, which was related to a reduction in mycorrhizal infection, decreased growth by reducing nutrient uptake. In the case of the six species which developed mycorrhizal associations (Table 3), the net effect of fumigation was depressed growth in those plots which were very low in soluble or extractable P. That the depressed growth could be mitigated by increased soil P levels strongly suggests that the effects were, in fact, due to differences in P nutrition.

Some species did not progress beyond the seedling stage in the fumigated, low-P soil. This was especially true of onion and the small-seeded legumes *Stylosanthes hamata* and *Leucaena leucocephala*. Although cowpea plants continued to develop new leaves, they did so at the expense of older leaves, which dropped. Each succeeding new leaf became smaller than the preceding leaf, suggesting that the plants were redistributing P from older tissue rather than absorbing additional P from the soil. This suggests that the lowest level of P (about 0.003 μg P/ml) was near the threshold concentration (the concentration at which net P uptake is zero) for P uptake by these non-mycorrhizal plants.

There was some evidence that the ability of the species to make at least some growth at the lowest P level was directly related to the size of the seed or seed piece. The major exception to this generalization was chinese cabbage, which grows well at levels of soil P as low as 0.05 μg P/ml (Fox et al., 1974). According to the results of previous studies on these plots, the expected leaf P content associated with 95% of maximum yield of chinese cabbage is 0.7% (Nishimoto et al., 1977). It appears from Fig. 1 that P content may have been a limiting growth factor for chinese cabbage in non-fumigated plots at all levels of soil P.

Effects of Fumigation and Soil P on P Uptake by Test Crops

The general pattern of P absorption for these species which were capable of forming a symbiotic mycorrhizal association (species other than chinese cabbage) was reversed from that observed with chinese cabbage. Plants growing in fumigated, low-P soil were exceedingly P deficient. The contrasting treatment, non-fumigated soil of the same P status, provided improved P nutrition. An example involving cowpea is presented in Fig. 2.

The net effect of fumigation on P uptake was obvious at low levels of solution P. Fumigation sharply reduced P uptake by the cowpea from the plot fertilized to give 0.003 μg P/ml in solution. Phosphorus uptake from that plot was 1 mg per plant, approximately 50% of which could be accounted for by the P in one seed. Fumigation ceased to make a difference in P uptake when soil levels reached 0.1 μg P/ml in solution, a concentration which indicates a high level of available soil P.

Curves presenting data on yield and P content of soybean are shown in Fig. 3. The net effect of fumigation on P uptake and yield was zero at about 0.025 μg P/ml. Assuming that the positive effects of fumi-

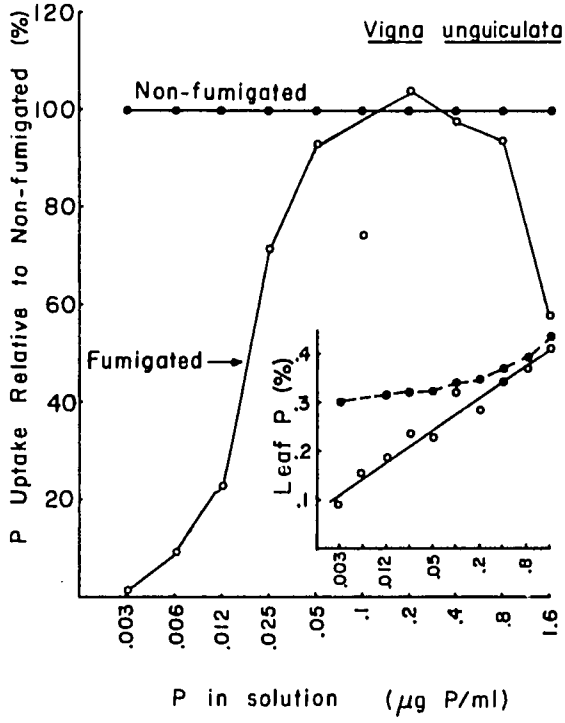

Fig. 2. Phosphorus percentage (dry weight basis) of cowpea leaves as affected by soil fumigation and soil P status, and the uptake of P by plants growing in fumigated soil in relation to uptake by plants growing in non-fumigated soil.

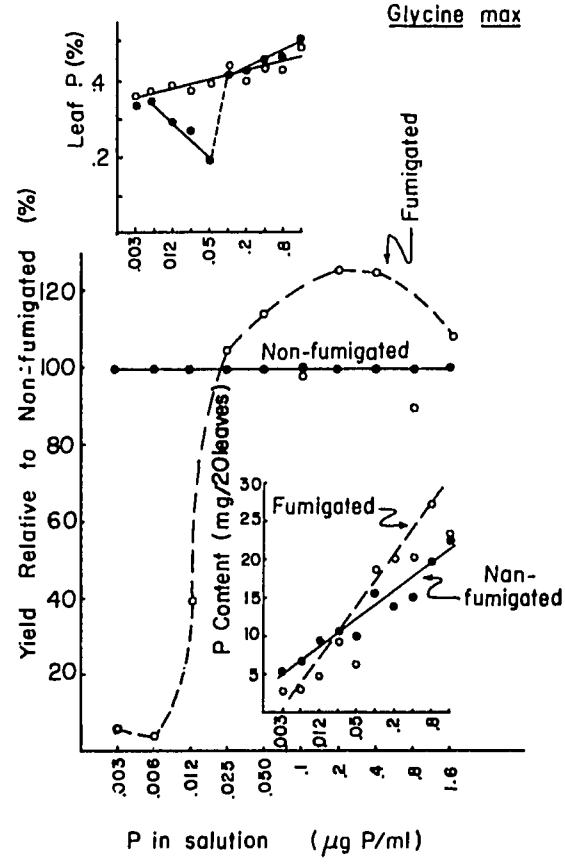

Fig. 3. Yield of fresh soybean pods in fumigated plots relative to yield of pods in non-fumigated plots, and as insert graphs, P percentage and P content of 20 soybean leaflets as affected by soil fumigation and P status of the soil.

gation on P uptake (effects not related to mycorrhizal associations) were the same for cowpea and soybean, it appears that cowpea was dependent on a symbiotic mycorrhizal association for P uptake to a greater extent and over a wider range of solution P concentration, than was soybean. Various estimates place the external P requirement of soybean in the range 0.02 to 0.2 μg P/ml in solution (Peaslee and Fox, in press; Nishimoto et al., 1977). A plot of green pod yields, on an absolute scale, vs. P in solution (data not presented) suggest an external P requirement of 0.08 μg P/ml. It seems, for soybean at least, that a mycorrhizal association will not materially influence the external P requirement for maximum yields. However, for less than the maximum yield, the presence of mycorrhizal associations can be more influential than a twofold change in soil P level (Fig. 3). When the availability of soil P is being assessed, or when the external P requirements of crops are being determined, the presence or absence of mycorrhizal associations should be ascertained.

The pattern of response for *Allium, Stylosanthes, Leucaena,* and *Manihot* (Figs. 4A-4D) was similar in four respects: 1) P percentage was much greater in non-fumigated, mycorrhizal plants than in fumigated, non-mycorrhizal ones; 2) there was little tendency for curves showing P percentage in plants to converge until high concentrations of soil P were imposed; 3) growth and P uptake by the non-mycorrhizal plants did not increase much as soil solution P increased over the low range (0.003 to 0.012 μg P/ml); and 4) for the non-fumigated (mycorrhizal) plants, curves showing P uptake, and P percentage (Figs. 4A-D) tended to reach a plateau or even to decline somewhere in the intermediate soil P concentration range. Based on the data presented in Fig. 2 through 4, we think the following statements are justified. First, it was evident that *Manihot* and *Stylosanthes,* which are noted for their ability to grow in infertile soils, were quite inefficient at using soil P unless mycorrhizae were present. According to the data on P percentage in Fig. 2 through 4, cassava and *Stylosanthes* were among the most dependent on a mycorrhizal association for adequate P nutrition. In this study, onion was less dependent on a mycorrhizal association than were *Stylosanthes* and *Leucaena.* Depressed onion growth (data not shown) appeared to be associated with 0.4% P in the tissues. This condition obtained at 1.6 μg P/ml on fumigated soil. Mosse (1973) has reported that P toxicity develops in onion at P concentration > 0.3%. High P concentration in plants usually was associated with non-fumigated soils (mycorrhizal plants), but in our experiment, with onion, the highest P percentage observed was in nonmycorrhizal plants growing at the highest P soil. Onion roots from the non-fumigated plots were infested with rootknot nematodes which may have been responsible for the lower P uptake by plants at high P levels from the non-fumigated plots than by plants from fumigated plots.

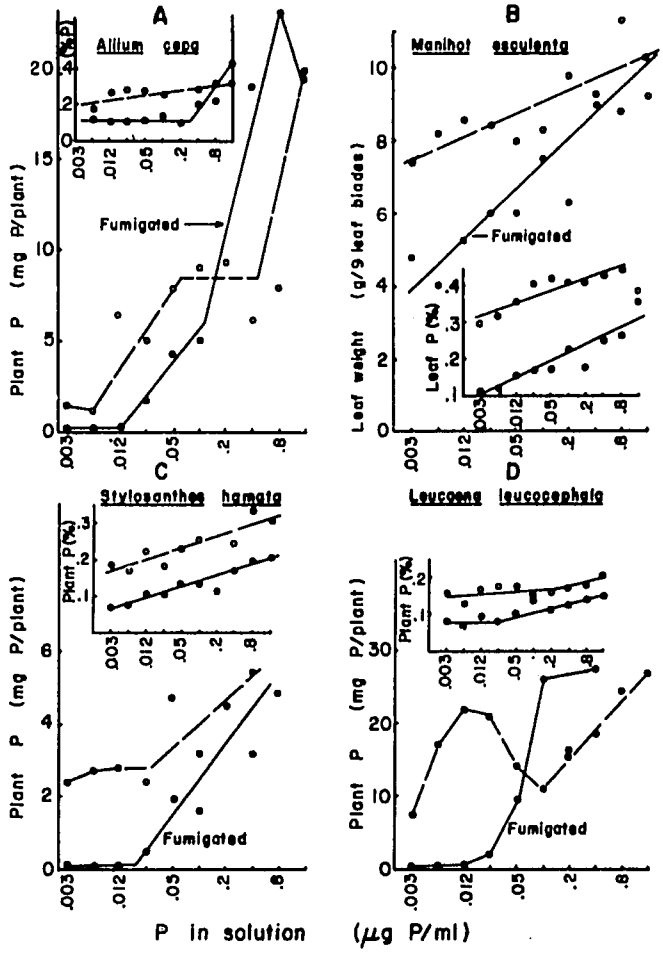

Fig. 4. Influence of soil fumigation and P level in the soil on the P nutrition of 4 plant species as indicated by P percentage, and growth or P uptake.

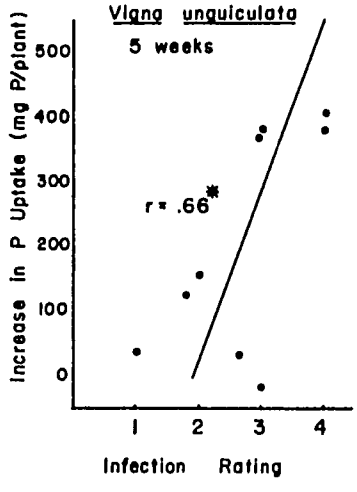

Fig. 5. Increased P uptake by cowpea growing on non-fumigated plots over P uptake from fumigated plots in relation to mycorrhiza infection ratings. Infection ratings were: 1) Detectable presence of hyphae, vesicles, or arbuscles. 2) Occasional occurrence of hyphae, vesicles, or arbuscles. 3) Frequent occurrence of hyphae, vesicles, or arbuscles (at least 50% of roots infected). 4) Very frequent occurrence (at least 75% of roots infected). 5) Almost no roots without extensive infection.

Secondly, the external P requirement of mycorrhizal plants can be exaggerated by basing these requirements on non-mycorrhizal plants, as is probably the case, in most solution and sand cultures. For example, the curves for cassava leaf weight (Fig. 4B) intersect at about 2 µg P/ml in the soil solution. This values is in general agreement with results of flowing nutrient solution experiments of Edwards et al. (1976) in which the requirement for young cassava was in the range 1.5 to 3.9 µg P/ml. These values are about two orders of magnitude greater than the requirements for cassava grown in some soils (Vander Zaag et al., in press).

Thirdly, intermediate soil P levels can be associated with decreased P uptake if, with increasing levels of soil P, the effectiveness of vesicular arbuscular mycorrhiza diminishes faster than P uptake by unaided plant roots increases. The data for *Leucaena* suggest this relationship. Phosphorus percentage of the plant and P uptake per plant decreased with increasing P in the soil in the concentration range 0.012 to 0.1 µg P/ml. Similar trends in the data can be seen for other species as well. Mycorrhizal infection diminishes when P is adequately supplied by the soil (Hayman and Mosse, 1971), but Sanders (1975) and Menge et al. (1978) have shown that it is P in the plant that matters most. One could speculate that the mycorrhizal contribution of P may have decreased prior to attainment of optimal internal P for plant growth. This possibility is supported by Azcón et al. (1978) who reported that mycorrhizal infection of lettuce roots was reduced by P levels which were inadequate for maximum yields. This type of relationship provides a possible explanation for sigmoidal-shaped yield curves sometimes obtained in P rate studies.

Effects of Fumigation on Vesicular Arbuscular Mycorrhizae Formation

Fumigation essentially eliminated mycorrhizae formation, as indicated by the root staining technique, during the first 2 to 3 months of crop growth. However, after 3 months, some individually infected plants could be noted in fumigated plots. Onions were among the first to become contaminated, probably by spore transmission to the shallow rooted plants by irrigation water. Most of the infected plants were near plot boundaries, an association suggesting that infection also was related to the extension of plant roots into non-fumigated soil or perhaps to inefficient fumigation near plot boundaries. Cassava was especially susceptible to this kind of infection because of its very extensive root system. A characteristic consequence of the infection of non-mycorrhizal plants was their sudden recovery from severe P deficiency. Leguminous plants, especially *Stylosanthes*, remained in the first true leaf stage for several weeks. At first they have all of the symptoms of extreme P-deficiency—very small, dark bluish-green leaves, with leaflets folded inward and and held in an upright posture. Later they abruptly took on the typical lighter green color and, within 1 or 2 weeks, increased in size several-fold more than neighboring non-mycorrhizal plants. Similar observations were made on cassava, although the symptoms of deficiency were different.

The rating of infection intensity was much less quantitative than desired. Procedures which would

improve the precision of this measurement would be welcome. Nevertheless, the infection ratings were positively correlated with yield or P uptake by all crop species which formed mycorrhizal association. An example of this relationship is shown in Fig. 5. For cowpea and soybean, infection ratings were taken twice in order to determine the most appropriate evaluation time. Soybean green pod weight (86 days) and P uptake by cowpea tops (76 days) was correlated with the early ratings, but not with the later ratings as shown by the following:

Soybean	
(green pod weight)	Correlation coefficient
7 weeks	0.80 (significant at 0.05 level)
12 weeks	0.06
Cowpea	
(P uptake of tops)	
5 weeks	0.66 (significant at 0.05 level)
11 weeks	0.26

Soybean and cowpea dry matter yields measured at the time of the earlier ratings were not significantly affected by fumigation even though the infection ratings were very different. Shortly thereafter, growth differences were observed. This suggests that infection ratings determined early in the growing season were more useful in estimating overall growth and P uptake response to mycorrhizae than ratings determined at harvest and that differences in infection rating preceded growth differences.

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