EVALUATING MYCORRHIZAL INOCULUM LEVELS IN SOIL AND QUANTIFYING THEIR CONTRIBUTION TO THE PHOSPHORUS NUTRITION OF COWPEA

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INTRODUCTION

Most crop species form endosymbiotic associations with soil fungi of the family Endogonaceae. Typically, fungal spores germinate, infect fine roots of host plants, and form characteristic structures, vesicles and arbuscules, inside the roots. Outside the roots, mycelia spread profusely in the soil. The fungus-root structure is known as vesiculararbuscular (VA) mycorrhizae, or endomycorrhizae.

Although mycorrhizae have been known to biological scientists for 100 years, agronomists and soil scientists have only recently focused their attention on mycorrhizae. Several factors were responsible for the lack of interest heretofore and the current surge of interest. Major obstacles inhibited studies of practical aspects of mycorrhizae. These were: (1) difficulties of identification and classification of the fungi and (2) inability to culture the fungi on synthetic media in the absence of host plants. The first problem new has a workable solution while the second appears to be one researchers must live with at least temporarily. Another hindrance was one of philosophy; since mycorrhizal fungi were known to be widely distributed in soil it was assumed that plants were already deriving maximum benefit from the symbiotic association. Recent research has called this view into question.

Basic research has indicated that VA mycorrhizae play a central role in the phosphorus nutrition of higher plants, particularly when soil phosphorus levels are low, as is often the situation in soils in the tropics. Enhanced nutrient uptake is not limited to phosophorus; VA mycorrhizae also enhance Zn, S, K, and Sr uptake.

In the tropics the human population continues to grow at a rate which increases pressures on an already insufficient food supply. New

emphasis is being directed toward increasing food production from tropical soils. Parallel with needs for accelerated food production is the high cost of fossil fuels which in turn has increased fertilizer costs. These events have placed a burden on many small farmers who need fertilizer to increase yields. A search for ways to increase the economy of phosphate fertilizer has intensified interest in mycorrhizae.

Agronomists and soil scientist are challenged to understand the ecology of vesicular-arbuscular (VA) mycorrhizae to such an extent that they can be utilized to increase crop yields. Soils in which the mycorrhizal fungal inoculum level is suboptimal for maximum effectiveness should be identified and the inoculum level correlated with the benefits bestowed upon the host by the mycorrhizal association. The genetic variability within the VA fungi should be quantified and tested so that inoculation technology may be developed. Such understanding would be helpful in evaluating strain effectiveness, as well as identifying conditions where insufficient or inefficient mycorrhizae may be limiting factors for plant growth.

The objectives of this study are:

(1) To develop a biological method, to assess the VA mycorrhizae inoculum level and infectivity potential of soils.

(2) To quantify the mycorrhizae contribution to the phosphorus nutrition of cowpea growing in three soils thought to have different levels of mycorrhizae inoculum.

LITERATURE REVIEW

The term 'mycorrhiza' (Gr: fungus root) was coined by Frank to describe associations between certain non-pathogenic fungi and roots of higher plants. Peyronel et al. (1969) proposed grouping mycorrhizae into three broad categories: ectomycorrhizae, endomycorrhizae, and ectendomycorrhizae. In ectomycorrhizae, the fungus forms a compact mantle over the root surface from which the hyphae arise and grow into the cortex intercellularly. Endomycorrhizae have external hyphae which are not aggregated to any great extent and there is extensive growth within the root cortex. Ectendomycorrhizae are similar to ectomycorrhizae but have both intercellular and intracellular hyphae. Lewis (1973) suggested grouping ectotrophic and endotrophic mycorrhizae together and classified them as 'sheathing', 'ericaceous', 'orchidaceous', 'vesicular-arbuscular', or 'miscellaneous'. Over the past twenty years it has become evident that the most common and widespread mycorrhizal infections are the vesicular-arbuscular (VA) type caused by the Phycomycete group (Nicolson, 1967). Despite their wide occurrence and ecological importance, only recently have agronomists and soil scientists begun to recognize the importance of VA mycorrhizae to the nutrition of crops; such importance is underscored by the statement of Wilhelm (1966) '... under agricultural field conditions, crops do not, strictly speaking, have roots, they have mycorrhizae.'

Gerdemann and Trappe (1975) reviewed the history of the taxonomy of the genus Endogone. The genus was first described by Link in 1809 and later revised by Thaxter (1922). According to this classification, all species form sporocarps and are distinguished by the structure of the sporocarps and the spores they contain. Peyronel first suggested in 1923 that Endogone spp. produce VA mycorrhizae, but it was not until the work of

Mosse in 1962 that this was generally accepted (Gerdemann, 1968). The genus Endogone was revised by Nicolson and Gerdemann (1968) to include species that produce ectocarpic resting spores. Mosse and Bowen (1968a; 1968b) surveyed 250 samples of Australian and New Zealand soils and some Rothamsted field soils. They described nine types of spores and devised a key for the identification of Endogone spores using several diagnostic features: spore attachment, spore contents, spore wall, spore color, and spore size and shape. Gerdemann and Trappe (1974) surveyed the Pacific Northwest USA and proposed a new classification of Endogonaceae that divides the family into five genera: Glomus, Gigaspora, Acaulospora, Sclerocystis, and Endogone. Endogone is a zygosporic genus and the only one that does not contain VA endophytes. The two classification systems just described, Mosse and Bowen (1968a) and Gerdemann and Trappe (1974), are the most widely used. Hall and Fish (1979) have recently proposed a new key to the Endogonaceae which was compiled using a computer program. The program assigns weights to the diagnostic characteristics, assigning high weights to characteristics which vary least and are easily observed.

As other surveys are completed, new species are being described. The taxonomy may undergo further revision to accommodate these additions (Gerdemann, 1976; Redhead, 1977). It should be stressed that the present taxonomy of Endogonaceae is tentative. Walker (1979) suggested that researchers place specimens of fungi in herbaria so that the identification can be checked as taxonomic knowledge increases. Undoubtably new methods will be developed which will facilitate the identification of the endophytes. One promising method is the fluorescent antibody technique which is being tested on fungi (Malajczuk et al., 1978).

During the past several years there has been an interest in surveying soils around the world to determine the presence of VA

endophytes. The resulting information from several continents, encompassing a wide variety of natural and agricultural ecosystems, has provided insight regarding the ecological significance of VA mycorrhizae. Recently two papers have reported the occurrence of VA mycorrhizae in aquatic plants (Sondergaard and Laegaard, 1977; Bagyaraj et al., 1979a), an environment where mycorrhizae were previously thought to be absent (Harley, 1969).

When studying the niche mycorrhizal fungi occupy in a given ecosystem, it is important to understand the adaptations of the endophytes to that ecosystem. If more is known about the behavior of mycorrhizal fungi in different environments, then more sensitive methods can be employed to enumerate the fungi. Three methods have been used to characterize the inoculum level: 1. extracting and counting spores; 2. direct observation of infection levels in the plant population; and 3. measuring the rate that test seedlings become infected (Mosse, 1979). As Mosse has pointed out, the method selected depends upon the objectives of the inquiry. Unfortunately no single method satisfactorily assesses the inoculum level of soils. This deficiency is being filled by modifying serial dilution techniques and most probable number (MPN) methods in order to enumerate the viable propagules in the soil (Moorman and Reeves, 1979; Porter, 1979).

Mycocrhizal spores are usually extracted from soil by wet sieving and decanting (Gerdemann and Nicolson, 1963). This method leads to variable results when duplicate samples are handled by two individuals and besides, the method is laborious when large samples are handled. The method preferentially selects for spores that are easily extracted (Harley, 969). Modified procedures involve adding agents to disperse heavy-textured soils (Sanders, 1976). Differences between replicate samples analyzed by the wet sieving method may be so great that statistical comparisons are futile

(Nicolson, 1967; Crush, 1973); furthermore, some VA endophytes produce Spores so small that extraction and counting are difficult. A flotation-adhesion method (Sutton and Barron, 1972) resulted in 94-98% efficiency in the recovery of spores. This method has the advantage of recovering spores regardless of size, but results in the recovery of organic debris which interferes with spore counting. Separating spores from organic debris, either by centrifuging in a sucrose solution (Ohms, 1957) or by differential sedimentation on gelatin columns (Mosse and Jones, 1968), permits a more quantitative measure of the spore population. Smith and Skipper (1979) compared several spore extraction methods and described a new plating method. Their study points out sources of error in each of the methods studied and suggests conditions under which one method may be preferred over another. These methods do not distinguish between viable and nonviable spores and it is this distinction that is necessary for a practical assessment of the soil inoculum level.

Mosse (1973a) and Tinker (1975a) have reviewed the work on factors which influence spore populations in soils. Spore populations are dynamic, being influenced by season, soil type, soil moisture, light intensity, nutrient availability and land usage. Whether these factors influence the fungi directly or indirectly through effects on the host plant is largely unknown. The system is complex. Interactions between the strain of fungi, the host plant, and soil-environment conditions make generalizations difficult. As one might expect, the correlation between spore population and infection is strong under certain conditions and weak under others. Daft and Nicolson (1972) evaluated three methods for estimating infection levels in plants and concluded that counting spores produced on external mycelia was the most accurate and convenient. However, Mosse (1979) has pointed out that correlations between number of spores and infection are usually good in experimental situations but much

less reliable in situations involving various soils and various strains of fungi. Owusu-Bennoah and Mosse (in press) suggested that spore number was determined by inherent characteristics of the fungi and specific interactions between the fungi and the soil. In a field inoculation trial involving two fungal species and three crop plants, they concluded that spore number was not a good index of infection.

Another difficulty in relying on spore numbers as a measure of soil infectivity is that spores are not the only infecting propagules in the soil. Powell (1976a) showed that hyphae from infected root segments cause infection. Read et al. (1976) surveyed the major vegetation types in east-central England and concluded that the major source of inoculum was infected roots or mycelia. While testing systemic fungicides, Boatman et al. (1978) found that new roots are infected from mycelia in the soil. Observations such as these point to an additional difficulty: fungi may have distinctly different life cycles in cultivated and in fallow or noncultivated soil. Mason (1964) observed increased spore numbers in a cultivated field as new root growth ceased and old roots senescenced. Mosse and Bowen (1968b)
suggested that spores were formed where root growth is intermittent. In a lowland rainforest in Nigeria, seedlings were heavily infected while the soil contained no spores at all (Redhead, 1977). Hayman and Stovold (1979) surveyed 73 sites in New South Wales and found great variability in spore numbers. Spore population varied for the same crop at different sites; they found more spores in agricultural soils than in native grassland-bush soils. Thus agricultural field conditions may select sporulating endophytes, while natural fallow soil conditions may select non-sporulating endophytes.

Soil infectivity can be semi-quantitatively estimated by examining the extent of infection in sample plants. Soil infectivity may be

defined as a property of the soil which determines the rate and extent plants form mycorrhizae. A quantitative measure of soil infectivity may be possible provided the same host plant is used and careful, thorough sampling procedures are followed. The semi-quantitative visual evaluation of infection in the plant roots needs to be standardized, and even then duplication of results will be difficult. There are, however, differences in the ease and the degree in which different host species become infected. There is also variation in the amount of vesicles, arbuscules, and hyphae formed by different strains of fungi. Precisely what to look for when evaluating infection is a problem. Hayman (1974) suggested that the total arbuscular formation may be more important than total infection per se; unfavorable light and temperature conditions resulting in slow growth of onion was associated with a deficiency of arbuscules. Nicolson (1960) developed a root slide technique to quantitatively measure infection. He cut the roots into small segments and collected the following data: percentage of infection; the number of infected and noninfected segments; percentage of moribund roots (roots which showed loss of cortical cells); percentage of external mycelia; the proportion of all roots which showed mycelia; and the percentage of roots with brown septate mycelia. Researchers have since used this technique, often with modifications, to inspect roots for both extent and intensity of infection. Read et al. (1976) used the root slide technique to estimate the percent of VA infection by the expression:

$$\% \text{ VA infection} = \frac{\text{No. infected segments}}{\text{total No. segments examined}} \times 100.$$

They noted that this procedure describes the distribution of mycelia throughout the root system but does not describe the intensity of infection in the system. Hayman (1970) attempted to measure both parameters of infection by recording length of infected root in each

segment, percent root segments with infection, and percent root segments with attached Endogone hyphae, spores, or vesicles. Not only are these techniques time consuming but the relationship of the results to soil inoculum levels is difficult to determine. Strzemska (1974) noted that the occurrence of VA infection in a given species varied considerably from year to year. If we accept the concept of a dynamic population of mycorrhizal fungi, then we must concern ourselves with the implications regarding the infectivity of the soil. The relation of soil inoculum to variation in infection needs to be investigated.

Giovannetti and Mosse (in press) compared four methods for evaluating root infection. They compared: 1. the gridline intersect method; 2. visual estimate of percentage cortex occupied by fungi; 3. estimate of length of cortex infected from a sample mounted on a slide; and 4. recording presence or absence of infection on a sample mounted on a slide. They indicated that the visual estimate of infection, although subjective, can give reliable results. All methods probably overestimate the extent of infection; because after clearing and staining, roots appear as two dimensional rather than three dimensional objects.

Measuring the rate a test seedling becomes infected is rarely reported, although this approach is a promising method for evaluating soil infectivity. Hayman and Stovold (1979) measured the rate of mycorrhizal development in clover seedlings in soils from 23 sites. Infectivity of the VA population was not well correlated with spore population, especially in the native grassland-bush soils. Moorman and Reeves (1979) made 1/4 and 1/40 dilutions of disturbed and nondisturbed soils. After thirty days corn roots were 77% infected on the nondisturbed soil but were only 1% infected on the disturbed soil. The effect of dilution was to reduce the amount of infection accordingly; however in the disturbed soil this effect was not apparent until 90 days because of the low inoculum

density in the soil. Porter (1979) adopted a most probable number (MPN) technique to estimate the infective propagules of VA mycorrhizal fungi. Clover and medic seedlings were planted in sterile soil which contained serial dilution of sterile and non-sterile soil. The MPN method was designed to be used with aqueous solutions where the distribution of the organism to be enumerated is assumed to be spatially uniform and random. This assumption may not apply in soil where severe clumping of propagules occurs. The ability of this technique to generate reproducible results remains to be tested. Nevertheless a bioassay is needed that detects only the viable propagules in the soil. Such a method would avoid the obvious difficulties in relying on spore numbers as an estimate of soil inoculum level or soil infectivity.

The evaluation of variations in soil inoculum level interests agronomists. Although VA endophytes are present in most soils, there is evidence that the level is suboptimal under certain conditions. Further research is needed to identify these conditions. Ross (1979) has observed that colonization of soybean roots by naturally-occurring mycorrhizal fungi is lower compared with inoculated soybeans which are grown in sterile soil. He concluded that low sporulation of these fungi in field soil probably results in low inoculum level for subsequent crops. The inoculum level in some Nigerian soils was so low that Stylosanthes guyanensis seedlings did not become infected during the course of the experiment (Mosse, 1977). However S. guyanensis does not appear to be very mycotrophic, thus it is probably a poor indicator of soil inoculum levels.

The standing vegetation or the preceding crop may have an impact on the soil inoculum level. Khan (1972) made use of this fact and transplanted infected and noninfected maize seedlings into unfertilized plots which had previously been occupied by weeds of the Chenopodiaceae

family, reported to be non-mycorrhizal (Gerdemann, 1968). P uptake and dry weight of mycorrhizal plants were much greater than the controls; grain weight was almost 12 times greater on mycorrhizal plants. In a study designed to measure the rate of spread of an introduced VA fungi, the effect of growing nonmycorrhizal plants in the soil was to reduce the vigor of the indigenous fungi thereby enhancing the spread of the introduced species (Powell, 1979b). Kruckelmann (1975) found that fertilizers, soil tillage, and crop rotations affected the number of spores in arable soil. His results showed spores were more frequent in loamy soils than in sandy soils. Spore populaticn correlated better with pH than with K, carbon,
or nitrogen content of the soils. Spore numbers increased with higher pH values and decreased with increasing phosphate contents. It certainly would be desirable to know what effect, if any, flooding the soil has on the soil infectivity.

In citrus culture, and some other perennial plantation crops, it is a common practice to fumigate soil or use sterilized growth media to grow seedlings. Heavy P fertilization is necessary to relieve stress resulting from the lack of mycorrhizae (Kleinschmidt and Gerdemann, 1972). Under these conditions inoculation with mycorrhizal fungi can partially substitute for P fertilization (Menge et al., 1978).

The importance of mycorrhizae for eroded lands has not been experimentally determined. However there are several reports on the vertical distribution of mycorrhizal spores in the soil. Sutton and Barron (1972) found that the number of spores changed little with soil depth to 16-24 cm, but declined with further increase in depth. Spores occurred mostly in the top 15 cm of soil in Nigeria (Redhead, 1977). The mean number of spores per 500 cm^3 at various depths were 2 cm, 748; 7.5 cm, 1946; 15 cm, 1064; 30 cm, 55. Spore numbers in eroded soils were 25%

of adjacent non-eroded sites, and a response to inoculation was obtained in 8 out of 10 eroded soils (Hall and Armstrong, 1979). In soils disturbed by strip mining operations the inoculum level was suboptimal (Reeves et al.,1979). Daft et al.(1975) postulated that a mycorrhizal association may be essential for the survival of most herbaceous plants growing in coal spoils. They obtained a significant response to inoculation. The implication is that where the surface soil has been removed the inoculum level of exposed soil may be suboptimal for plant growth.

Effect of VA Mycorrhizae on the Host Plant

Increased phosphate absorption by plants infected with VA mycorrhizae when compared with noninfected plants and the increase in P concentration in plant tissue has been well established (Mosse, 1973a; Tinker, 1975a). Although reports of increased uptake of other nutrients and increased water absorption possibly indicate multiple effects of mycorrhizae on plant nutrition, nearly all host growth responses have been attributed to improved phosphorous nutrition.

Experiments using insoluble phosphates have demonstrated that enhanced growth and P uptake was associated with mycorrhizal plants (Murdoch et al., 1967). It has been inferred by same investigators that mycorrhizal fungi may possess P-solubilizing mechanism by which mycorrhizal plants utilize forms of P unavailable to nonmycorrhizal plants. Although no such mechanisms have been demonstrated there is data to suggest that mycorrhizal plants absorb sparingly soluble P more readily than nonmycorrhizal plants. Working with a high P sorbing soil in Hawaii, Yost and Fox (1979) indicated that the threshold concentration for P uptake (the concentration of P in the soil solution below which no

P is absorbed) is lower for mycorrhizal plants. Data from Cress et al.(1979) raises the possibility that a major factor contributing to the increased uptake of phosphorus by mycorrhizal plants is a greater ion affinity by the mycorrhizal absorbing sites. The relative importance of this increased ion affinity in soil situations where diffusion of phosphorus is rate limiting can not be determined from their study. Jackson et al.(1972) studied utilization of rock phosphate and did not observe a response to inoculation with VA fungi unless the rock phosphate were mixed in the soil, indicating the importance of the spatial proximity of the association and the nutrient source.

To identify the source of P for mycorrhizal and nonmycorrhizal plants, soil was labeled with ^{32}P and the specific activity of absorbed P in infected and noninfected plants was determined. The results indicated that mycorrhizal and nonmycorrhizal plants obtain phosphorus from the same source (Sanders and Tinker, 1971). Such data support the idea that the effect of mycorrhizae results from the hyphae forming a better distributed surface for absorbing phosphorus than roots alone.

Hattingh et al.(1973) provided direct evidence of hyphal uptake and translocation of phosphorus. ^{32}P-labeled phosphate which had been placed 27 mm from the root surface was absorbed when the roots were mycorrhizal; when the hyphae were severed mycorrhizal roots did not differ significantly in content of ^{32}P-labeled phosphate content from nonmycorrhizal roots. Growth chamber results such as these should be interpreted with caution. It is probable that hyphal growth is more profuse because of conditions on the soil plane (Hattingh, 1975). Owusu-Bennoah and Wild (1979) used autoradiography to demonstrate phosphate depletion zones around mycorrhizal and nonmycorrhizal roots. They concluded that the main increase of phosphate uptake by mycorrhizae was from soil within 2 mm of the root surface. However the experimental

conditions of such work demand a closer look before extrapolations are made to other soil-plant systems. Finely crushed soil with small pore spaces which may be water saturated probably restricts hyphal growth. The increased absorbing; surface of fungal hyphae is important as well as the distribution of absorbing surface in the soil. The specific interaction between the fungal strain and the soil properties will affect the relative importance of these two parameters.

The diffusion of phosphorus in soil and uptake by plants has been studied in detail. Bhat and Nye (1974) indicated that a phosphorus depletion zone surrounds the active absorbing root; hence the value of external mycelia may be that they extend beyond the depletion zone and absorb phosphorus in non-depleted soil. Realization of this prompted Rhodes (1979) to write "...nutrients most likely to be involved in plant growth responses to VA mycorrhizal infection are those for which the rate-limiting step for uptake by plants is movement to roots through soil by diffusion."

Properties of mycorrhizal hyphae are becoming better understood. Pearson and Tinker (1975) demonstrated P transport and measured a mean steady state flux of P in the external hyphae of 0.3-1.0 x 10^{-9} moles $cm^{-2}s^{-1}$. They did not determine a value for absorbing power of the hyphae per unit length. Cooper and Tinker (1978) studied the uptake and translocation of P, Zn, and S. In clover external hyphae translocated molar amounts of P, Zn, and S in the ratio of 35:5:1 and the mean fluxes in the ratio of 50:8:1 which suggests high relative efficiency in the uptake and translocation mechanisms for P. Their results also indicated that the phosphorus demand of the host affected the flow of P in the hyphae, and that the amount of external hyphae in the soil (i.e. the total hyphae length) was secondary in importance.

From an ecological perspective, Baylis (1975) suggested that mycorrhizal fungi have exercised a controlling influence on the evolution of roots. He submitted that magnolioid roots are more dependent on mycorrhizae for P uptake in low P soils than are graminoid roots. Magnolioid roots are coarsely branched and the ultimate roots are rarely less than 0.5 mm in diameter. The roots have a compact stele and normally do not have root hairs. Graminoid roots are finely divided, with ultimate branches often less than 0.1 mm in diameter. They are densely covered with root hairs 1-2 mm in length. Data from Yost and Fox (1979) lend support to this hypothesis. Tinker (1975b) went a step further by pointing out that root hairs may be less effective than hyphae because root hairs have short lives and inter-hair competition is keen; hyphae are more dispersed and hence will have fewer overlapping P depletion volumes.

Evaluating the mycorrhizae-legume symbiosis is particularly challenging. Legumes may play a central role in increasing food production in tropical soils. Because of their ability to obtain nitrogen through symbiotic association with Rhizobia, it may be possible for farmers to obtain good yields with a minimum of expensive chemical fertilizers. Symbiotic nitrogen fixation by legumes may have a high P requirement (Munns, 1977). Phosphorus content of nodules may be 2-3 times more than the P content of the roots on which they are formed (Mosse et al., 1976). Also other micronutrients, notably Cu and Zn, have been shown to enhance or be necessary for nodulation (Hallsworth, 1958; McIlveen et al., 1975). It is not surprising that the, literature reports instances of legumes halving better nodulation, higher nitrogen percentage, and greater nitrogenase activity when they were inoculated with VA mircorrhizal fungi

(Abbott and Robson, 1977; Mosse, 1977; Daft and El-Giahmi, 1976). Bagyaraj et al. (1979b) attempted to directly test the effect of VA fungi on N-fixation and plant growth. Four inoculation treatments were used: 1. uninoculated control, 2. inoculated with Rhizobium japonicum, 3. inoculated with a VA fungus Glomus fasciculatus, and 4. inoculated with Rhizobium and Glomus. After 60 days nodule mass and nodule nitrogen content from treatment 4 were double that from treatment 2. Shoot dry weight from treatment 4 was increased 64% over treatment 2; however the increase in grain yield was not significant (at $P = 0.05$). Waidyanatha et al. (1979) found that inoculation with VA fungi stimulated nodule weights and nitrogenase activity far more than plant growth. They believed that this effect on N fixation may be the most important effect of mycorrhizae on legumes.

The relative progression of Rhizobium nodulation and mycorrhizal infection is not known. The first infection units in soybean seedlings appeared 10-12 days after planting which corresponded with the appearance of root nodules (Carling et al., 1979a). Cox and Sanders (1974) defined an infection unit to include the internal mycelia relating to a single entry point. In another study Carling et al. (1979b) worked with nodulating and non-nodulating isolines of soybeans. Total plant and nodule dry weight and nitrate reductaset and nitrogenase activities were increased significantly in mycorrhizal, nodulating plants as compared to nonmycorzhizal nodulating plants. When phosphorus was substituted for mycorrhizae, similar growth and enzyme activities were observed. They concluded that the effects resulted from an improved nutritional environment for the plant rather than a direct interaction between the fungus and the bacterium. These results emphasize that our knowledge about the mycorrhizosphere is limited. The competition and the synergism

among mycorrhizae, Rhizobia, and the host plant apparently are complex and are not adequately understood at present.

The significance of mycorrhizae in plant nutrition is probably greatest when fertility is low. Generalizations about the role of mycorrhizae in high fertility situations are more difficult to make. There appears to be a critical value of soil phosphorus for each species in certain growth conditions above which plants will grow well without mycorrhizae. This critical value may be determined in part by the diffusion rate of phosphorus in a particular soil (Cooper, 1975). However it appears that it is the concentration of P in the plant that regulates the mycorrhizal association rather than the concentration of P in the soil. Sanders (1975) induced high P concentrations in the plant by foliar feeding which inhibited infection. Menge et al. (1978a) used a 'split root' technique to demonstrate that number of spores, vesicles, arbuscules, and hyphae were not influenced by high soil P levels but were negatively influenced by high concentrations of P in the root.

The physiology of the root is likely to change as the percentage phosphorus content increases. This may be a self-regulatory mechanism of the plant. Ratnayake et al. (1978) examined root exudates, phospholipid content of root tissue, root P content, and membrane permeability. They concluded that a major consequence of low P nutrition is a decline in membrane phospholipids, an increase in membrane permeability, and increased exudation of metabolites. With increased P nutrition membrane permeability decreases as does the exudation of metabolites.

Growth depressions resulting from inoculation with VA mycorrhizae have been reported. Mosse (1973b) attributed this to phosphorus toxicity and specific interactions between the soil and the endophyte. However there are reports of growth suppresions when P toxicity was clearly not a problem. Cooper (1975) suggests the suppresions were related to the P

status of the soil: at high phosphorus levels little infection develops and there is no growth response to mycorrhizae; at low levels of soil P a large response to fungi occurs because of an overriding improvement in the P nutrition of the plant; at intermediate levels, fungal infection is infrequent and transient reductions in growth occur which are followed by increases in growth response to the fungi with time as infection increases and P demand increases. A similar line of reasoning was pursued by Yost and Fox (1979) who suggested that at intermediate soil P levels decreased P uptake can occur if, with increasing levels of soil P, effectiveness of the endophyte decreases faster than P uptake increases by the uninfected root. Sparling and Tinker (1978) reported a decrease in shoot weight of three grasses which had been inoculated with VA mycorrhizae; they thought the decrease was understandable in light of the branched root system with many root hairs and relatively small P requirement.

Although there are many aspects of VA mycorrhizae that are not understood, research is moving ahead rapidly-toward developing inoculation techniques, testing relative efficiency of fungal strains, evaluating field inoculation trials, and attempting to define soil and plant conditions when a response to inoculation may be expected. The objective of this study was to characterize the soil inoculum level of some soils and to relate this to the quantity of phosphorus contributed by mycorrhizae to plants.

MATERIALS AND METHODS

Cowpea (Vigna unguiculata L. TVu 3563) was grown in 10 cm pots (525 g oven dry soil) in a glass house. The Wahiawa soil used (a Tropeptic Eutrustox) was collected from three sites on the Poamoho Experiment Station. The Wahiawa soil is deficient in available phosphorus for most crop species. I was presumed to have different levels of native mycorrhizal fungi. Cultivated surface soil, 0-10 cm depth, was collect from a field which has been cultivated intermittently for at least ten years. Subsoil was collected at 120 cm dept from the same site. Surface soil, 0-10 cm depth, was collected from a site that has not been cultivated for at leas fifteen years. This site had been occupied by various perennial grasses.

The soils were characterized by the following measurements: pH (1:1 soil to distilled water ratio), organic carbon (Walkley-Black method, 1935), Bray-1 P, NO_3^- and NH_4^+ (1N KCl soil extraction with distillation on Micro-Kjeldahl apparatus), and exchangeable cations (1N NH_4OAc soil extraction with Ca, K, and Na determined by flame emission spectrophotometry and Mg determined by atomic absorption spectrophotometry. The results are presented in Table 1.

Experiment 1: Soil Inoculum Bioassay

All soil materials were passed through a 3/16 in. screen. Phosphorus was added to each soil in amounts that were determined by P sorption curves (Fox and Kamprath, 1970). The P sorption curves are presented in Fig. 1. Phosphorus was added as KH_2PO_4 to each soil to bring the level of P in the soil solution to .025 mg/liter. This level of soil P was chosen so that most soils could be brought to a standard P level

Table 1. Soil pH, organic carbon, Bray-1 P, NO_3^--N and NH_4^+-N, and exchangeable cations in cultivated, noncultivated, and subsoil materials.

Soil	pH	Organic carbon	Bray-1 P	NO_3^--N and NH_4^+-N	Exchangeable cations
		%	ug/g	ug/g	meq/100g
Cultivated soil material	5.85	1.54	1.8	32.6	8.3
Noncultivated soil material	4.78	3.18	6.6	18.6	3.6
Subsoil material	6.39	.40	.1	12.5	7.6

and thus the test plant growth and infection would more closely reflect the inoculum level. Soil pH was adjusted with $CaC0_3$ to about 6.5. Zinc (10 kgZn/ha as $ZnS0_4 \cdot 7H_20$) was added to each soil.

A portion of each soil was sterilized by Y-irradiation (1.5 Mrad) from a ^{60}Co source. This exposure is approximately twice the dose of irradiation reported by other researchers, and was used to ensure complete sterilization (Pearson and Tinker, 1975). The non-sterile soil was 'diluted' by mixing with various amounts of sterile soil. The proportions of non-sterile to sterile soil were 1/0, 1/3, 1/9, 1/27, 1/81, 1/243, and 1/729. There were four pots for each treatment. The dilutions were made by weighing the appropriate amount of sterile and non-sterile soil and mixing the soil in a mechanical soil mixer for 5 minutes. The highest dilutions were mixed first to avoid contamination from less diluted soil materials. After mixing each treatment the soil mixture was divided into four pots.

Three seeds were planted per pot which were later thinned to 1 plant per pot. The plants were harvested 22 (Phillips and Hayman, 1970) and examined in an open petri dish with a dissecting microscope for the presence or absence of mycorrhizal infection. Stained preparations for roots of plants grown in cultivated and subsoil materials were derived from the entire root system. However, due to a greater root mass, only half of the root system of plants in the noncultivated soil was adequate for this purpose. The samples were evaluated by enumerating the incidence of mycorrhizal infection. An incidence of infection was defined as a continuous area along the root where vesicles, hyphae, or arbuscules were observed.

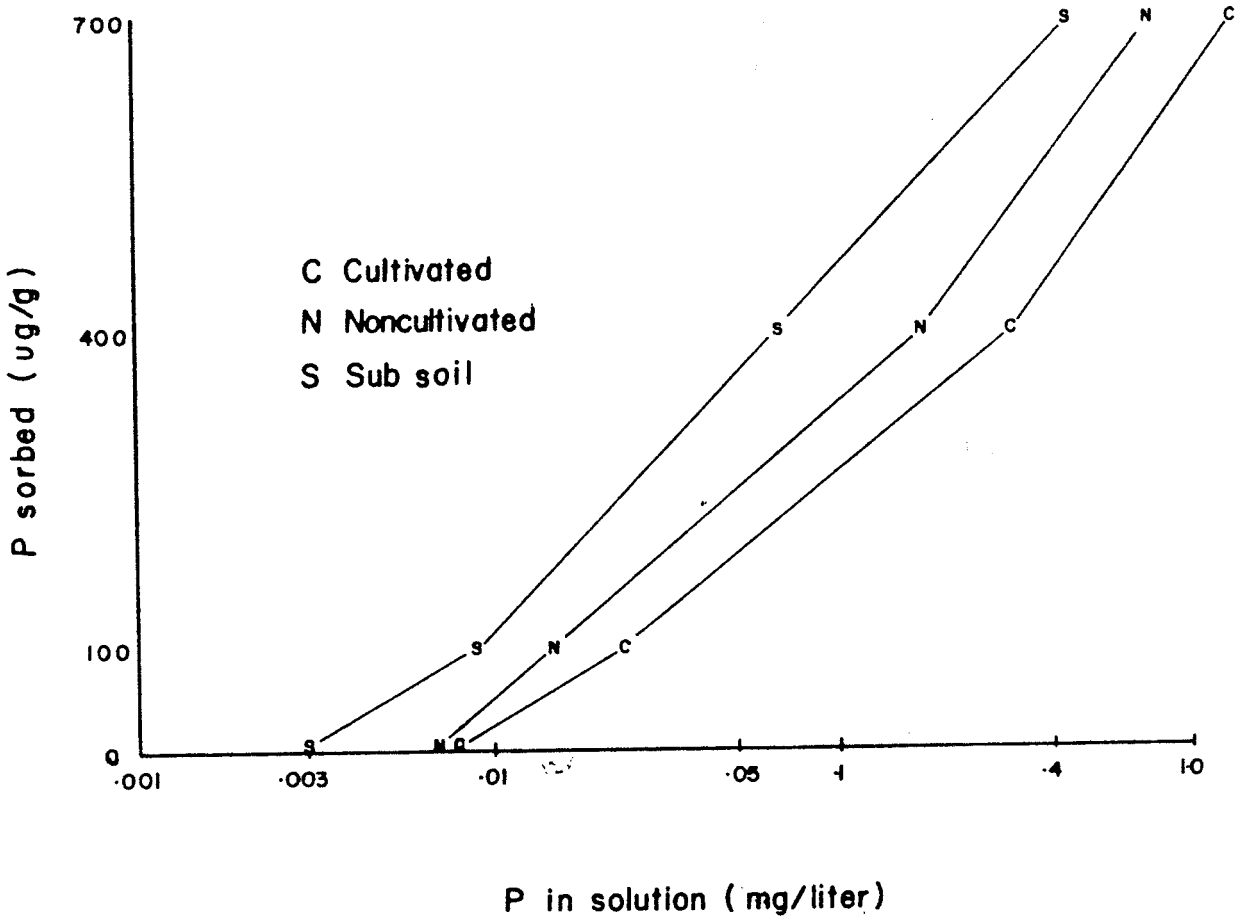

Figure 1. Phosphate sorption isotherms for cultivated, noncultivated and sub soil.

Experiment 2: Quantifying the P Contribution by Mycorrhizae

Cowpea (Vigna unguiculata L. TVu 3563) was grown in 2½ gallon plastic pots (9 kg OD soil) in the glasshouse. The three soils used in this experiment were collected from the same sites as in experiment 1. Soils were passed through a ¼ in. screen. Rates of P added to each soil were determined from the P sorption curves presented in Fig. 1. The following levels of P in the soil solution were established for each soil:

		Cultivated soil	Noncultivated soil	Subsoil
		P in solution (mg/liter)		
P level:	1	.008	.007	.003
	2	.015	.009	.008
	3	.028	.012	.017
	4	.062	.029	.03
	5	.14	.09	.052
	6	.52	.44	.23

Phosphorus was added as KH_2P0_4. Potassium (as KCl) was added to each soil in varying amounts to equalize the amount of K in each pot. Soil pH was adjusted with $CaC0_3$ to about 6.5. Zinc (10 kgZn/ha as $ZnS0_4 \cdot 7H_20$) was added to each soil. Nitrogen (as NH_4NO_3) was added to each soil to bring extractable NH_4^+ and NO_3^--N levels to 35 mg/liter for all soil materials in order to provide for equal N availability among the three soils.

Six levels of P were established in each lot of soil that remained non-sterile, and in each lot of sterilized soil (Y-irradiated with 1.5 Mrad). In addition to the nonsterile and sterile treatments there was one mycorrhizal inoculation treatment in the subsoil. In the pots containing non-sterile subsoil, one-gram samples of fresh mycorrhizal

cowpea roots were placed approximately 1 inch below the seed. The inoculum was obtained by growing cowpea in soil material collected from the small plots at the Mauka campus research facility. There were three replicates for each treatment, giving a total of 126 pots.

Pots were arranged on benches in the glasshouse in a completely randomized design. Seven seeds were planted per pot; these were later thinned to three plants per pot. Seeds were inoculated with Rhizobium strain Nit: 176 A22 of the Cowpea group. Pots were placed in plastic basins containing water and the plants were watered by capillary rise. This method of watering was chosen to avoid contaminating the sterile soil by splashing water from one pot to another.

Plants were harvested 38 days after planting. Whole tops were oven dried, weighed, and the nutrient composition was determined by x-ray emission spectroscopy. Mycorrhizal infection was evaluated on samples of fine roots. The soil was washed from the entire root system of the plants in each pot. Four samples, approximately 8 cm long and 2 cm wide, were cut from the root system; two samples were cut from either side of the root system 4 cm below the root crown, one sample was cut from the center of the root system 10 cm below the root crown, and one sample 16 cm below the root crown. The root samples were cleared and stained as in experiment 1, and examined in an open petri dish with a dissecting microscope. Mycorrhizal infection was semi-quantitatively rated on a scale of 0-100%.

RESULTS AND DISCUSSION

I. Soil Inoculum Bioassay

A serious limitation to research with VA mycorrhizae is the lack of methods to determine the infectivity potential of soils. Using the soil dilution method described, differences were detected among the mycorrhizae inoculum levels of various soil materials (Fig. 2). Incidence of infection was greatest in the undiluted (1/0), noncultivated soil and least in the subsoil. As the non-sterile soil was diluted with sterile soil, the incidence of infection declined to a level that was not affected by further additions of sterile soil material. In general, for all soil materials, an absolute extinction point, a dilution with sterile soil until no infection occurred, was not observed. The proportion of non-sterile soil at which there was no further decrease in infectiveness was 1/3, 1/9,and 1/27 for the subsoil, cultivated, and noncultivated soil material respectively. The reciprocals of these proportions give the following indexes of infectivity for the respective soil material: 3, 9, and 27. The significance of such 'base levels' is not clear. This observation should be verified and then studied in greater detail.

The concept of 'incidence of infection' is introduced here to differentiate from 'infection unit' which was described by Cox and Sanders (1974) as the internal mycelia relating to a single entry point. Using this bioassay procedure the identification of the source of infection (i.e. the infecting propagule or propagules) was not critical to detecting differences in soil inoculum levels; contrary to the enumeration of infection units where points of entry must be determined to differentiate each infection unit. What was observed in the roots of the test plants was the initial penetration and early development within the roots of hyphae; extensive development of infection within the root had not

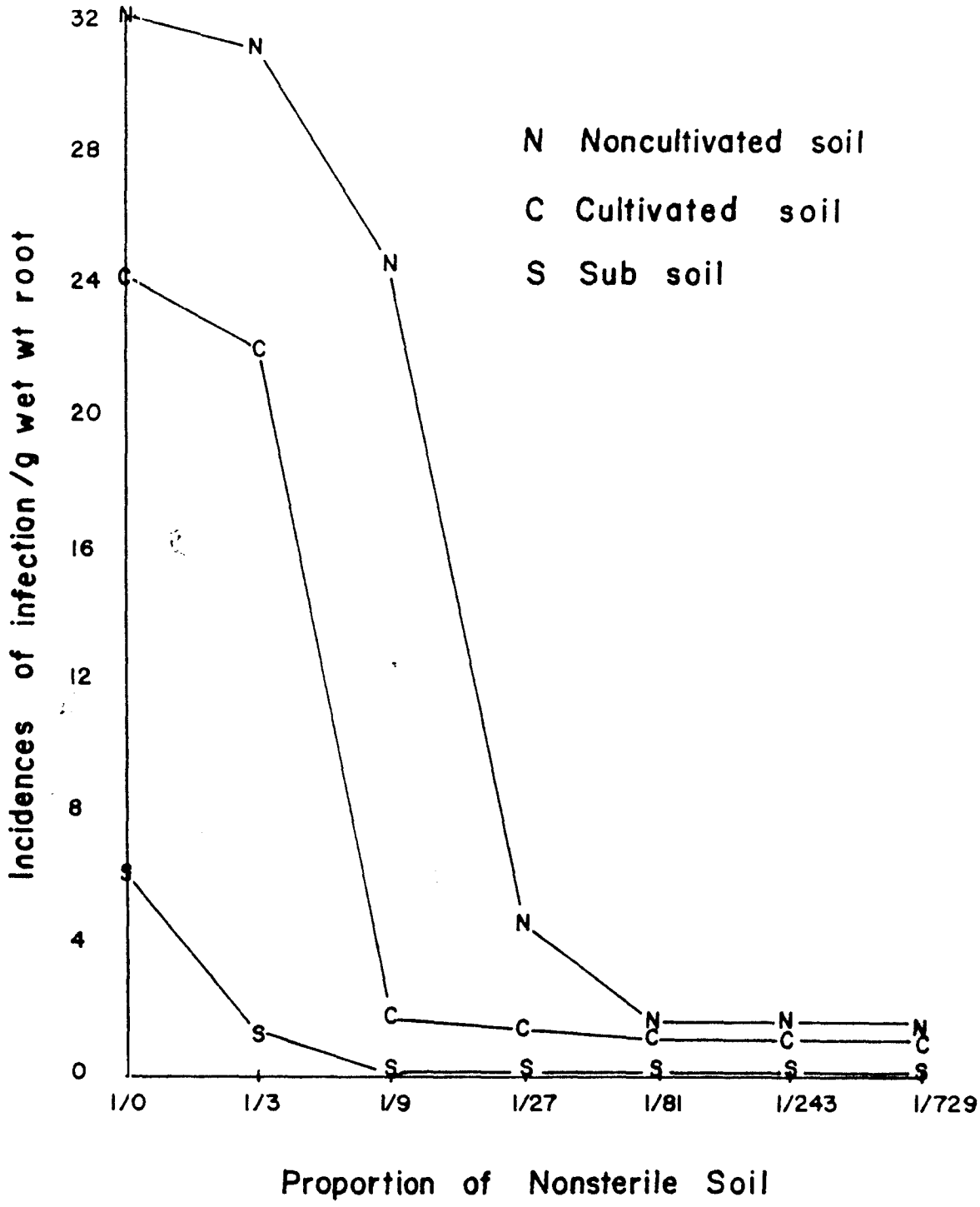

Figure 2. Incidence of mycorrhizal infection in cultivated, noncultivated, and sub soil materials as affected by dilutions of nonsterile soil material.

occurred. Incidences of infection were observed in discrete areas in the root; most often infections were observed as either a piece of penetrating hyphae, arbuscules, or vesicles. Enumeration of incidence of infection in diluted soil materials where infection was less developed was more accurate than in undiluted soil materials where the spread of infection was more extensive.

In a bioassay of this type it is important that the growth of the indicator plants be as uniform as possible so that results are comparable. It was apparent at the end of the growing period that plant growth in the three soils was not equal. Root development, and to a lesser extent shoot development, were greatest in the noncultivated soil material. As a result, a greater volume of soil was explored in the noncultivated soil. To some extent this difference was mitigated by expressing incidence of infection on a per gram root weight basis.

The timing of harvest was critical. Soil systems are dynamic; roots are growing, spreading through the soil, encountering viable propagules and becoming infected. Not only do the number of viable propagules determine the final extent of infection, but also the abundance of susceptible roots. For this reason it is important to have an equal production of roots in all soils being tested. Methods should be developed that minimize differences in root growth. Stanford and DeMent (1957) devised a method for measuring nutrient absorption using predeveloped standard root mats. Seeds were planted in sand cultures in bottomless cardboard cartons which were nested in a second carton with the bottom intact. At the end of the initial growing period a mat of roots had formed at the bottom of the carton. The bottomless cartons were then removed and the roots were placed on the soil materials for fertility evaluation. The relevance of this method to the inoculum bioassay is that differences in root biomass were minimized. Unfortunately in this bioassay there were unexpected differences in the growth of the host plant in the

three soil materials. Differences began to appear ten days after planting. Seedlings in the noncultivated soil were taller, and first true leaves began to appear earlier than in the other soil materials. Growth of seedlings in the cultivated and subsoils was similar. Reasons for these differences will be discussed later, but one thing is apparent the chemical and physical properties of test soils should be normalized to the degree possible.

The first adaptation of the principles of serial dilution to soils was reported by Tsao (1960) who attempted to estimate the infectivity of soil with respect to Phytophthora fungi. His rational of the method was that serial dilutions of infected soil, with sterile soil as the dilutent, would eventually reduce the disease potential to zero. One aspect of the method used in this thesis which could be improved is the method of dilution. Because of the relatively large volume of soil used (525 g 0D soil/pot) it was believed that mixing and preparation must start with the most dilute mixture to avoid contamination. For this reason serial dilutions of soil were not made. An interesting approach to soil dilution was recently reported by Porter (1979). Using a smaller volume of soil he serially diluted non-sterile soil with sterile soil and then placed the mixtures into the center of pots containing sterile soil. Two seeds of clover were planted over each soil mixture and after six weeks the roots were examined for the presence or absence of mycorrhizae. The number of infective propagules was estimated using standard microbiological most probable number (MPN) techniques (Cochrane, 1950). The estimates using MPN tables were greater than estimates using the wet sieve method, particularly for endophytes with hyphal diameters less than 3 um. The comparisons of these methods were made using two soils, a sandy clay and a sandy loam. This is significant because the wet sieving method is well adapted for use on coarse textured soils where spores are more readily extracted. Also, serial dilutions, as used in soil investigations, have a greater chance of success where clods and strong aggregation are not

factors. It needs to be determined whether the dilution method can give reliable results in high clay soils where spore extraction is difficult and clumping may interfere with the assumption about the random distribution of the test organism.

II. Quantifying the P Contribution of Mycorrhizae

Effect of VA Mycorrhizae and Soil P Level

The response of cowpea to soil phosphorus levels was strongly influenced by whether or not plants grew in sterile or non-sterile soil. The P concentration of whole tops and P uptake by plants growing in cultivated, noncultivated, and subsoil are shown in Fig. 3-5. As soil P levels increased, the concentration of P in plant tissue and total P uptake increased (Table 2). The relative advantage of native mycorrhizae versus no mycorrhizae was greatest in the noncultivated soil, and least in the subsoil. This was manifested at the lowest soil P levels where total P uptake in nonmycorrhizal plants was 1, 9, and 56% of the naturally infected plants in the noncultivated, cultivated, and subsoil respectively. Total P uptake in the nonmycorrhizal plants in the subsoil was only 22% as much as the inoculated plants. It is tempting to explain these results in terms of differences in soil inoculum density; however inoculum density was only one of the contributing factors, albeit an important one.

Growth, P concentration, and P uptake by nonmycorrhizal plants were greater when compared with mycorrhizal plants at higher soil P levels with two notable exceptions: 1. the P concentration in mycorrhizal plants in the noncultivated soil material remained higher than in the nonmycorrhizal plants at all levels of soil P; and 2. the P concentrations of inoculated plants growing in the subsoil material were higher than plants growing in sterile or non-sterile subsoil material at all levels of soil P.

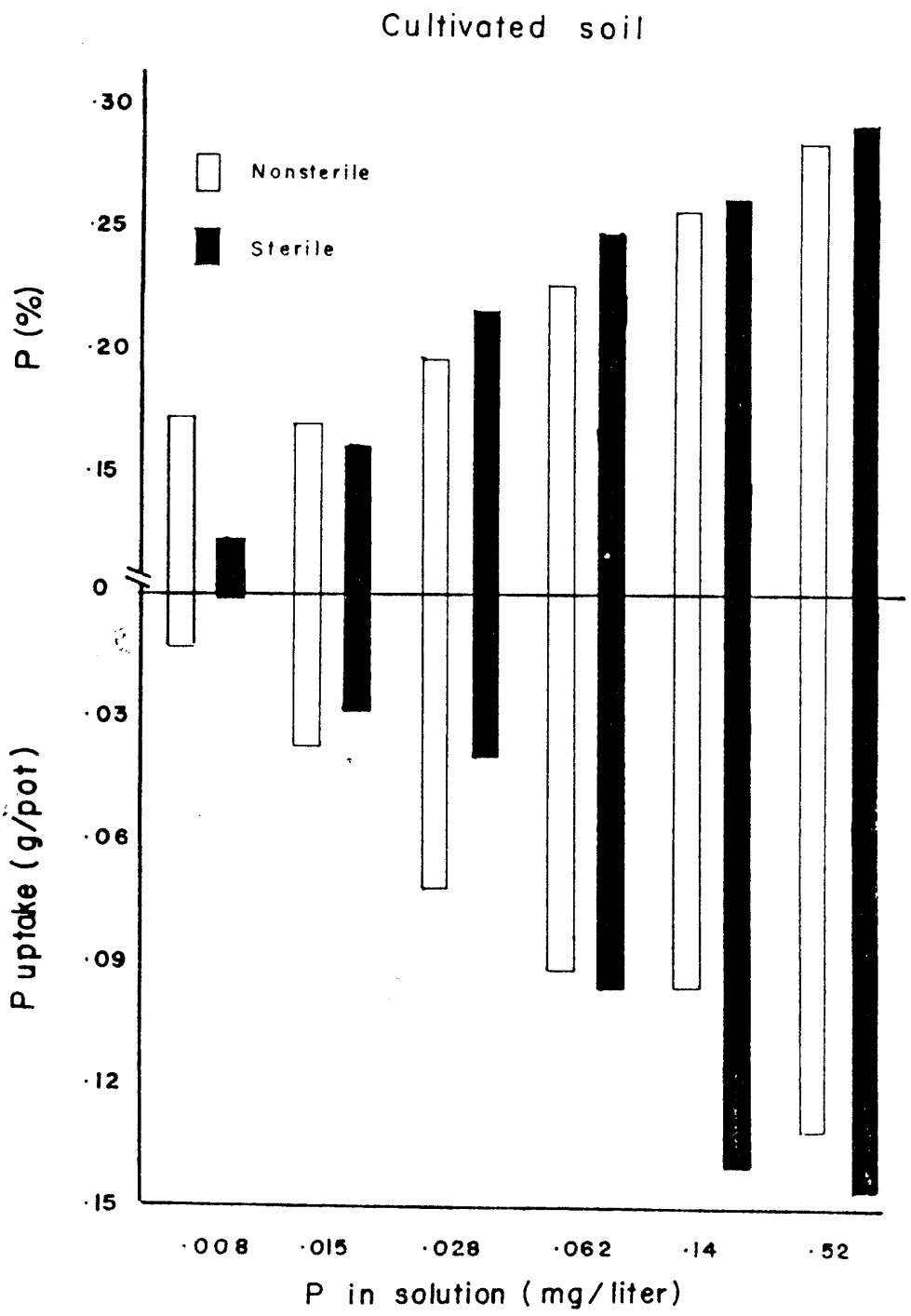

Figure 3. P percentages and total P uptake by cowpea growing in cultivated soil material as affected by soil sterilization and soil P status.

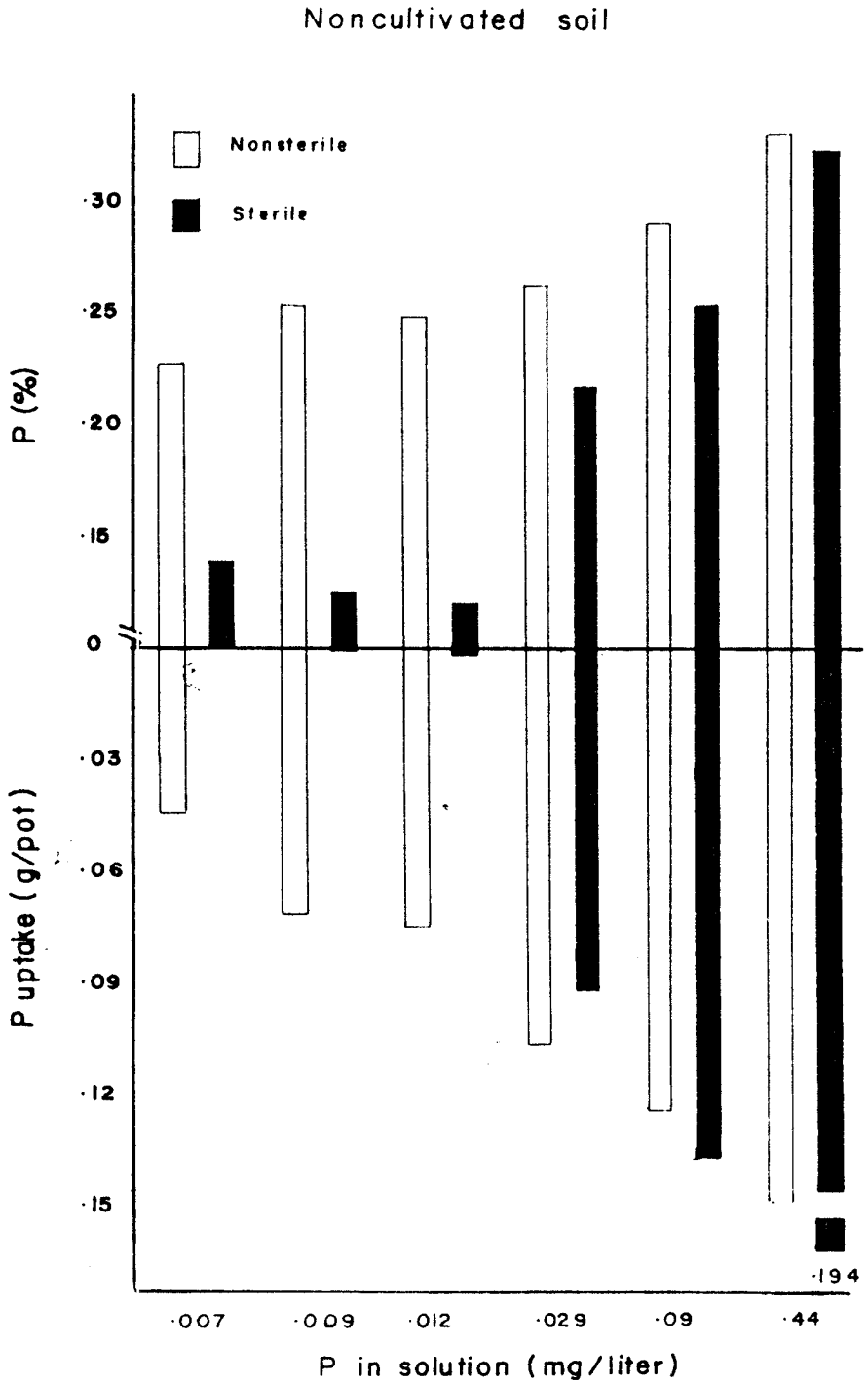

Figure 4. P percentages and total P uptake by cowpea growing in noncultivated soil material as affected by soil sterilization and soil P status.

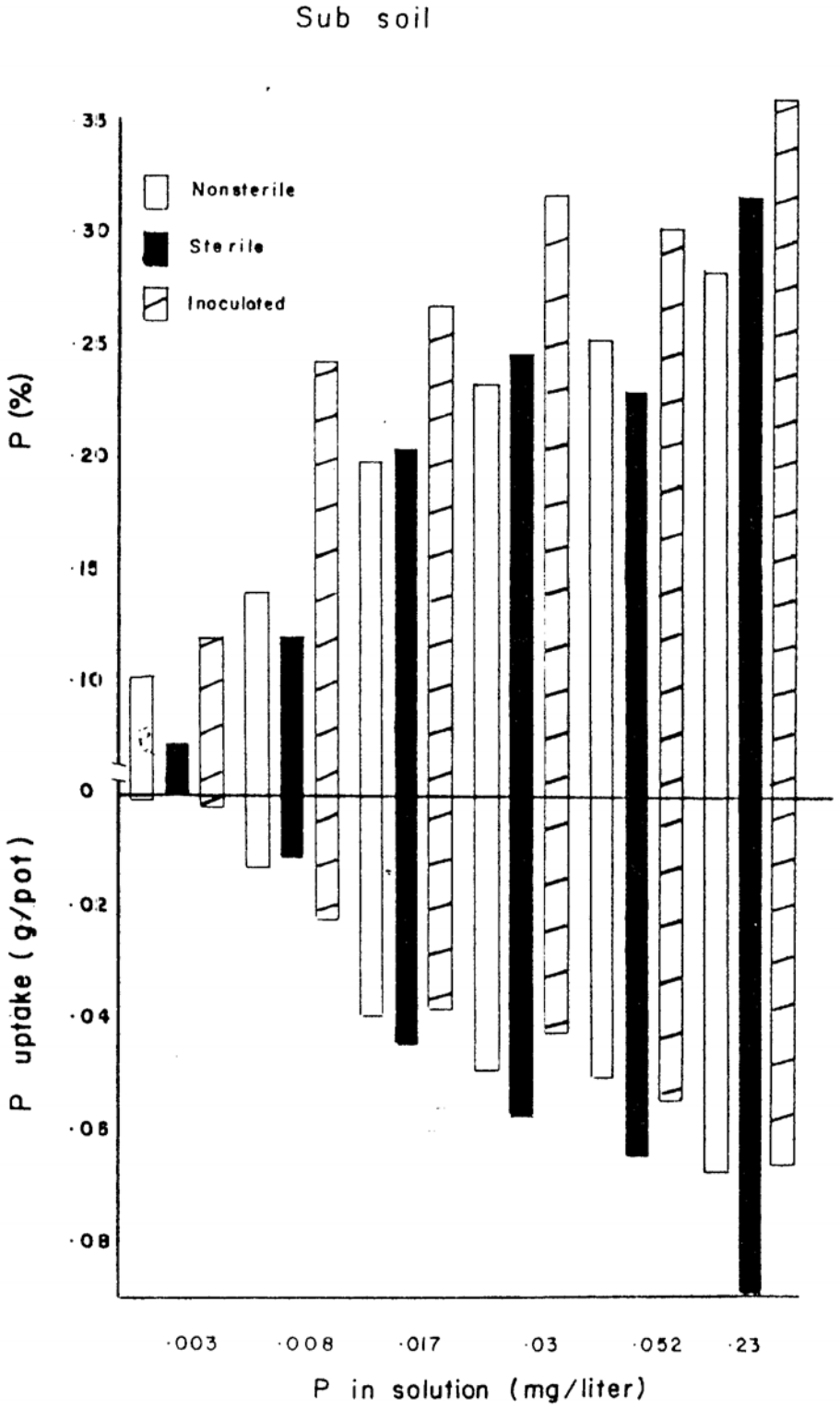

Figure 5. P percentages and total P uptake by cowpea growing in sub soil material as affected by soil sterilization and soil P status.

Table 2. Effect of P fertilizer addition on the P concentration and P uptake by cowpea growing in cultivated, noncultivated, and subsoil materials.

Soil P	P added	Mean P uptake	Mean P concentration
µgP/liter	kg/ha	mg/pot	%
		Cultivated soil	
.008	0	7	.148
.015	120	34	.165
.028	242	71	.207
.062	424	95	.237
.14	596	119	.260
.52	1030	141	.290
		Noncultivated soil	
.007	0	23	.182
.009	70	43	.202
.012	140	39	.183
.029	356	99	.240
.09	644	131	.272
.44	1168	172	.327
		Sub soil	
.003	0	1	.096
.008	190	16	.168
.017	400	40	.222
.03	552	49	.265
.052	716	56	.262
.23	1180	73	.317

Ultimately agronomists and soil scientists are interested in the yield response as affected by mycorrhizae (Fig. 6-8). The response curves resemble the hypothetical growth response curves for mycorrhizal and nonmycorrhizal plants discussed by Mosse (1979). The relative advantage of mycorrhizal plants over nonmycorrhizal plants disappeared at approximately .062 mg P/liter, .029 mg P/liter, and .008 mg P/liter for the cultivated, noncultivated, and subsoil materials respectively. In the subsoil, the differences in yield among all plants, those inoculated and those in sterile and non-sterile soil, are small. The trends, if any, are not distinct. When soils are irradiated, as in this experiment, not only are the mycorrhizal fungi eliminated from the soil, but so are all micro-organisms. The intersection of the response curves for plants grown in non-sterile and sterile soil probably also reflects the degree to which pathogenic organisms are inhibiting growth. If there were a high population of nematodes, for example, yield in non-sterile soil may be depressed and the curves may intersect at a lower level of soil P. In this experiment, pathogens did not appear to be a factor in nonsterile soil materials. The soil P level where the growth response curves intersect, termed the critical P level for mycotrophy (Cooper, 1975), reflects several soil characteristics, inoculum density being only one of them.

The significance of differences in inoculum densities are not easily determined. Are the differences between the noncultivated and cultivated soils, for example, significant to the growth of a crop? The answer to that question may depend upon the soil as well as the inoculum. Daft and Nicolson (1969) conducted an inoculum density experiment in pots and found that even low levels of inoculum, 3 spores per plant, were able to effect complete colonization of roots. No significant difference in growth response occurred among the various inoculated treatments. However because of the confining conditions in pots, the relevance of these findings to

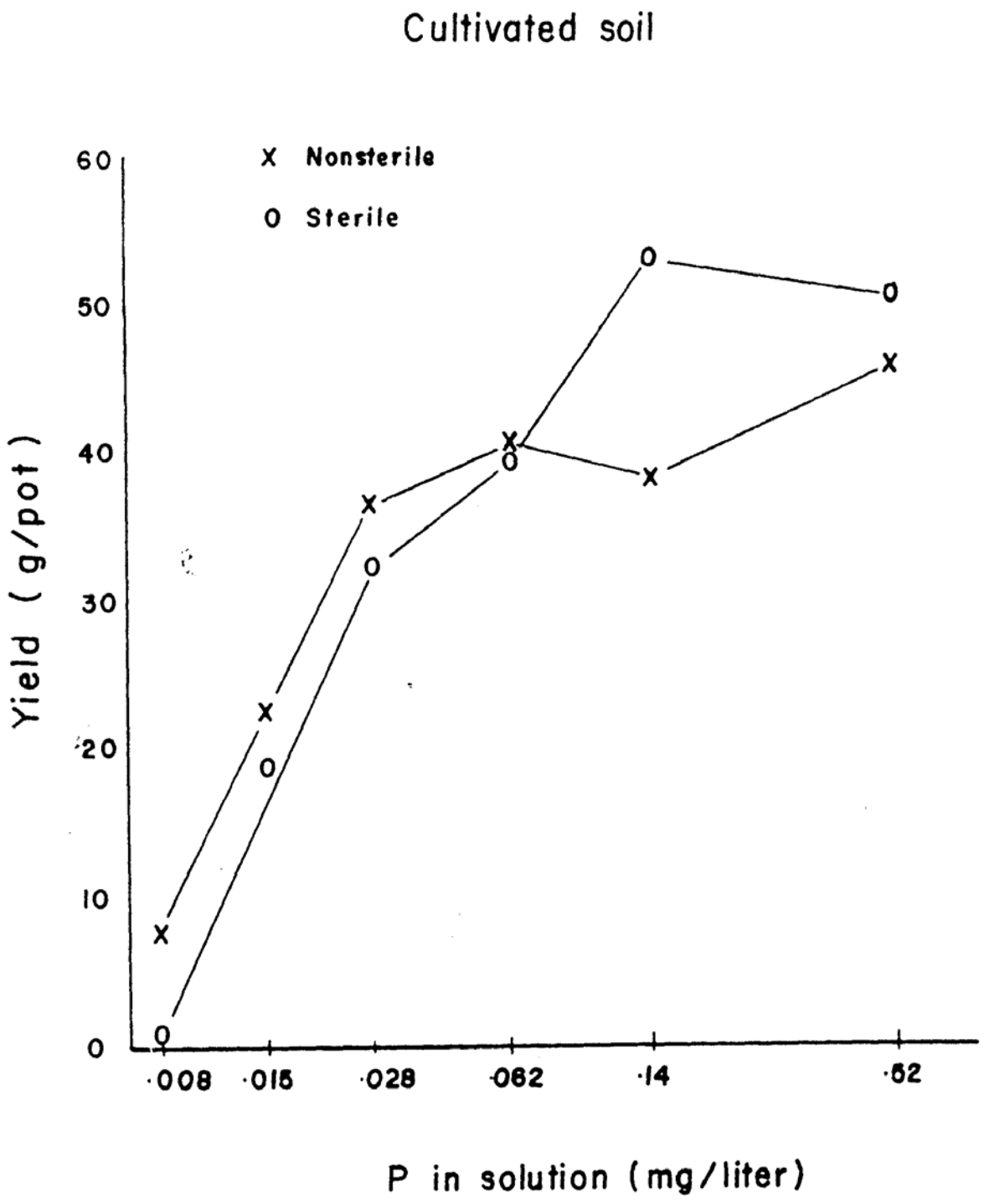

Figure 6. Yield of cowpea growing in cultivated soil material as affected by soil sterilization and soil P status.

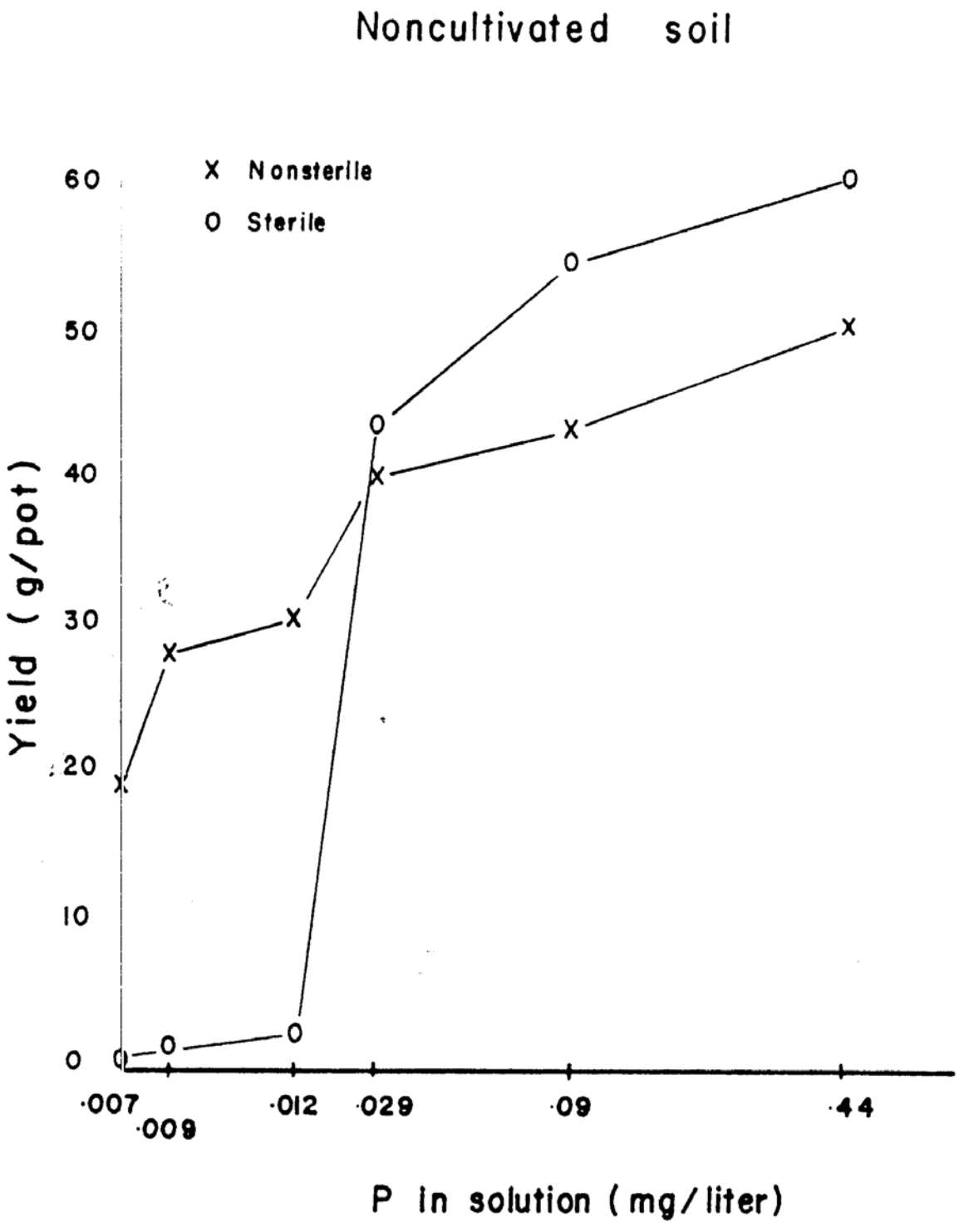

Figure 7. Yield of cowpea growing in noncultivated soil material as affected by soil sterilization and soil P status.

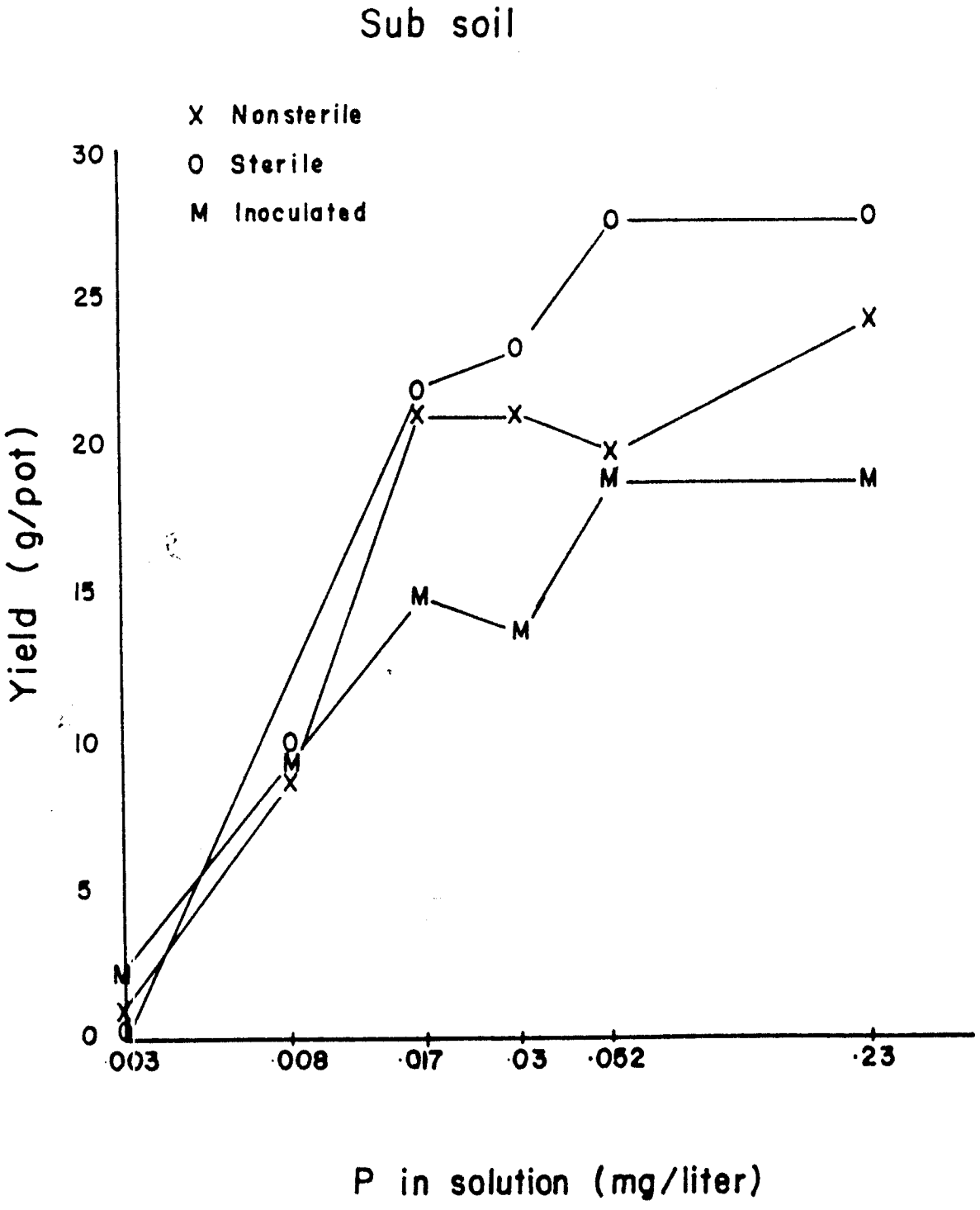

Figure 8. Yield of cowpea growing in sub soil material as affected by soil sterilization, inoculation with VA mycorrhizae, and soil P status.

field conditions cannot be assumed. It is quite probable that in field situations a higher inoculum density is needed for maximum growth response. Working with pot cultures, Carling et al. (1979a) found that the number of infection units in 21 day-old soybean seedlings was dependent upon inoculum density. This suggests that inoculum density of mycorrhizae-forming fungi may be particularly important in early seedling establishment. This may account for the improved establishment of clover in pastures after inoculation with VA fungi (Powell, 1977; 1979). The slow development of mycorrhizae in seedlings may account for the observation that the nutritional requirement of seedlings for P is much greater than after the plants are established. The forage legume Desmodium aparines required about 0.2 ppm P in solution for establishment, but .01 ppm P was adequate for regrowth after harvest (Fox et al., 1974). It is evident that conditions under which differences in inoculum density will be significant to the ultimate growth response of a crop need to be defined. It is not known whether high infection levels in the seedling stage are a requisite for maximum growth responses.

The amount of phosphorus required to bring the level of soil solution P to the critical level of mycotrophy was different for the three soil materials. A farmer who must add phosphate fertilizer to the soil in order to sustain yields might well ask of what value are the mycorrhizae? In Table 3, yield, P concentration, and P uptake are presented relative to the amount of P fertilizer added to the soil. The amount of phosphate fertilizer needed to compensate for the lack of mycorrhizae can be estimated from this data. For example, in non-sterile cultivated soil with 0 P added (.008 mg P/liter), yield was 7.7 g/pot. When mycorrhizae were eliminated from the soil, similiar yields were theoretically possible (Fig. 6) if the soil solution P was increased to approximately .01 mg P/liter. From the P sorption curve, the soil requires an addition of approximately 40 kg P/ha to raise the level of P in the soil solution to .01 mg P/liter. Similar calculations were made for all

three soils using P% and P uptake as the indicators of the mycorrhizal effect and the estimates are presented in Table 3. The range of estimates were 40-140 kg P/ha, 220-440 kg P/ha, and 10-100 kg P/ha for the cultivated, noncultivated, and subsoil materials respectively. When the inoculum density was increased by inoculation with mycorrhizal fungi, the estimate of the amount of P necessary to compensate for the lack of mycorrhizae increased (Table 3).

This method can also be used to estimate the amount of fertilizer P for which mycorrizae can substitute (Menge, 1978). Methods such as these, though imperfect, represent an attempt to quantify the mycorrhizal benefit to the host. In addition such methods may contribute in the evaluation of different strains of mycorrhizal-forming fungi, particularly strains that are morphologically similar. At present, there are no sure methods that can measure the effects of native mycorrhizae to the host plant.

The differences in inoculum level in the three soils were reflected in the extent of colonization of the roots by mycorrhizal fungi (Fig. 9). Because of the semi-quantitative nature of the evaluation of infection, the trends in Fig. 9 are more important than the actual percentages. In plants growing in the subsoil, the native mycorrhizal fungi did not colonize the roots to the same extent as roots were colonized in the cultivated and noncultivated soils. Infection was also somewhat less in the cultivated than in the noncultivated soil materials. With increasing levels of soil P the extent of infection declined, with the exception of plants inoculated with VA mycorrhizae. The extent of infection in the inoculated plants remained high in spite of increasing levels of soil and plant P. The fact that the plants continued to increase P uptake even though the yield response to P had ceased indicates that the mycorrhizae were still active. It is interesting to observe that mycorrhizae infection in naturally infected plants did not cease altogether at high soil P levels. What effects, if any, mycorrhizae have on plants with adequate P nutrition warrants further study.

Table 3. Yield, P concentration, and P uptake by cowpea as affected by the addition of phosphate fertilizer in sterile soil and estimates of P fertilizer required to compensate for the lack of mycorrhizae.

	P added	Yield	P concentration	P uptake
	kg/ha	g/pot	%	mg/pot
		Cultivated soil		
Nonsterile	0	7.7	.17	13.3
Sterile	0	.96	.12	1.1
	120	19.1	.16	29.7
	242	32.3	.22	69.9
	424	39.9	.25	97.7
	596	53.8	.26	141
	1030	51.0	.29	147
Estimate of compensatory P	----	----	---	----
		40 kgP/ha	140 kgP/ha	50 kgP/ha
		Noncultivated soil		
Nonsterile	0	19.6	.23	44.2
Sterile	0	.70	.14	.93
	70	1.27	.12	1.6
	140	2.15	.12	2.6
	356	43.5	.22	92.7
	644	54.0	.25	137
	1168	60.3	.32	194
Estimate of compensatory P	----	----	---	----
		220 kgP/ha	440 kgP/ha	250 kgP/ha
		Sub soil		
Nonsterile	0	.85	.10	.85
Sterile	0	.62	.07	.47
	190	9.3	.12	11.4
	400	21.7	.20	44.1
	552	23.2	.25	56.8
	716	27.7	.23	64.1
	1180	27.6	.32	87.8
Nonsterile inoculated with VA mycorrhizae	0	1.8	.12	2.2
	----	----	----	----
Estimate of compensatory P (based on natural inoculum level in nonsterile soil)		10 kgP/ha	100 kgP/ha	10 kgP/ha
Estimate of compensatory P (based on inoculated soil)		20 kgP/ha	120 kgP/ha	30 kgP/ha

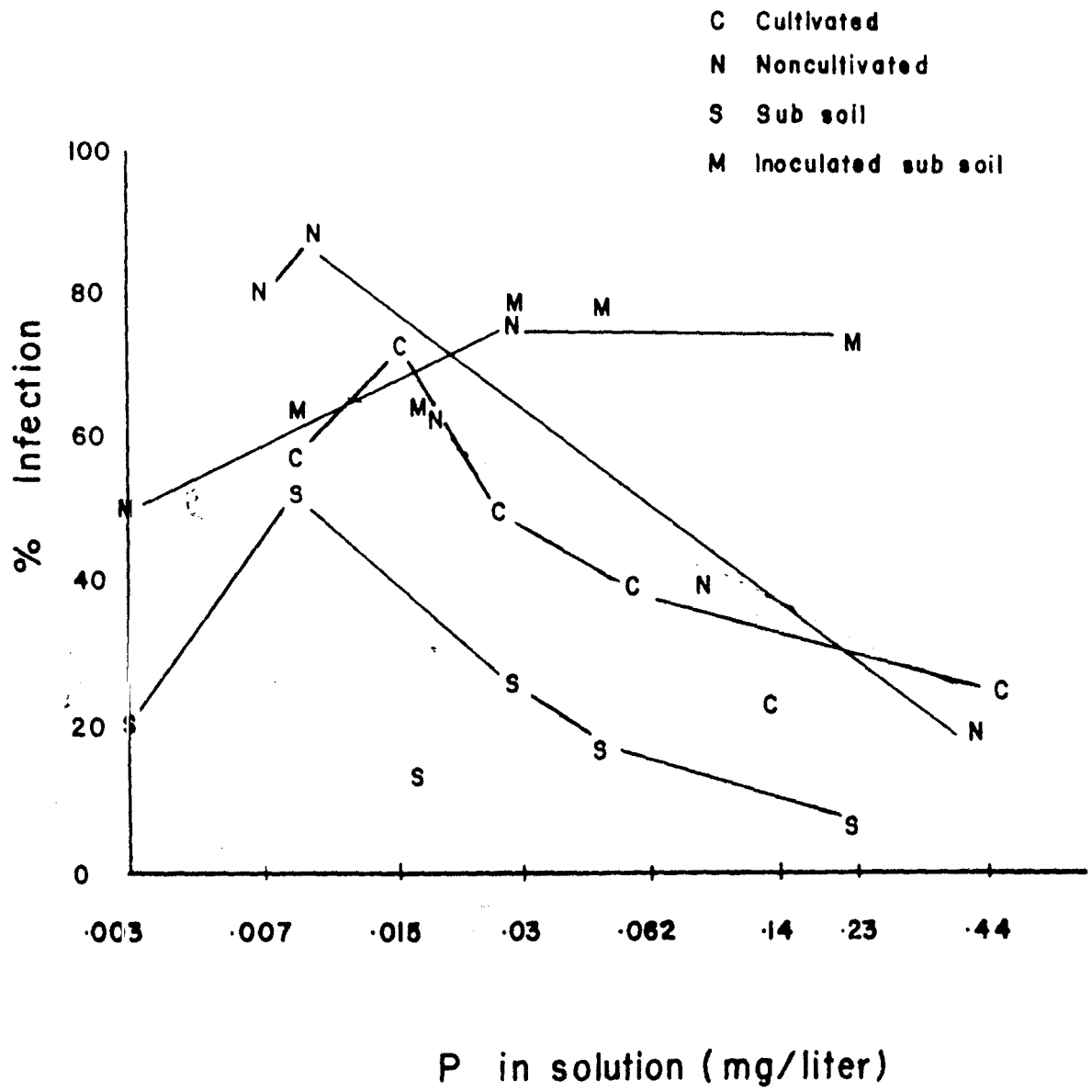

Figure 9. Percent mycorrhizae infection in roots of plants growing in cultivated, noncultivated, and sub soil material as affected by soil P status.

Previous work exploring the mechanisms which regulate the mycorrhizae have established that mycorrhizal infection is attuned to the phosphorus nutrition in the plant, rather than the phosphorus level in the soil (Sanders and Tinker, 1975; Menge et al. 1978). The hypothesis that phosphorus inhibition of mycorrhizae is associated with a decrease in root exudation and associated changes in root membrane permeability is particularly interesting in light of this study (Ratnayke et al., 1978). During early autotrophic growth, seedlings are dependent upon seed reserves for nutrition and relatively independent of the nutrient status of the soil. During this stage, seedlings may have the same susceptibility to infection, regardless of soil nutrient levels. As the phosphorus concentration in the plant increases, presumably the control mechanism in the plant exerts some unspecified influence which inhibits development of mycorrhizae. It may be that the mycorrhizae-forming fungi in the inoculum was a different strain than the native fungi in the soil materials used in this experiment, and that this strain responded differently than the native fungi. The native fungi was inhibited by high P concentrations in the plant, whereas the mycorrhizae in the inoculated plants maintained a heavy colonization despite high P concentrations in the plant. Mycorrhizae are known to differ in their response to fertilization. The data from this experiment suggests the feasibility of selecting mycorrhizae that tolerate high levels of plant P. These data may also suggest another intriguing possibility: the mycorrhizae control mechanism in the plant may act to inhibit development of new infection rather than inactivate the functioning mycorrhizae. If roots are treated with highly infective inoculum, such as infected root segments appear to be, then a more rapid, heavy colonization of the root may develop. If spores are used as inoculum, the time required for spore germination and infection may be greater than that required for the plant to begin regulating the mycorrhizae.

Increased P uptake was not the only benefit to the host by mycorrhizae. This study was not designed to quantify the mycorrhizal effects on the uptake of other elements because K, Zn, and Ca were added to the soil in liberal amounts to ensure they would not limit growth. The concentrations of K, Zn, S, and Ca in plant tops are presented in Fig. 10-13. For K, Zn, and Ca the concentrations of mycorrhizal plants were significantly higher (P=.05) than the nonmycorrhizal plants in the cultivated and noncultivated soil materials. The concentrations of K, Zn, and Ca in the inoculated plants growing in the subsoil were significantly greater than in the plants growing in sterile and non-sterile subsoil materials. The fact that there were no significant differences among plants growing in sterile and non-sterile subsoil further indicates the low inoculum density in the subsoil. The Ca data do not support the hypothesis of resistance to Ca transport in fungi (Rhodes and Gerdemann, 1978). Yost and Fox (in press) reported higher Ca concentrations in mycorrhizal cowpea growing in the field as compared to nonmycorrhizal cowpea, although Vander Zaag et al. (1979) did not observe an increase in Ca concentration in mycorrhizal cassava as compared to nonmycorrhizal cassava which may indicate that mycorrhizal uptake of Ca is more influenced by plant species than by an affinity of the fungi for Ca.

With respect to S, the concentration in the inoculated plants in the subsoil was significantly higher than in plants grown in sterile or nonsterile subsoil materials; the S concentration in mycorrhizal plants was significantly higher than in nonmycorrhizal plants growing in the cultivated soil material. The reverse was true for plants growing in the noncultivated soil material. However if the extremely stunted plants growing in sterile soil with low P are discounted, and comparisons are made among the plants of similar size, then S percentages were greater in mycorrhizal plants for all soil materials. Uptake of S has been demonstrated in mycorrhizae in onions

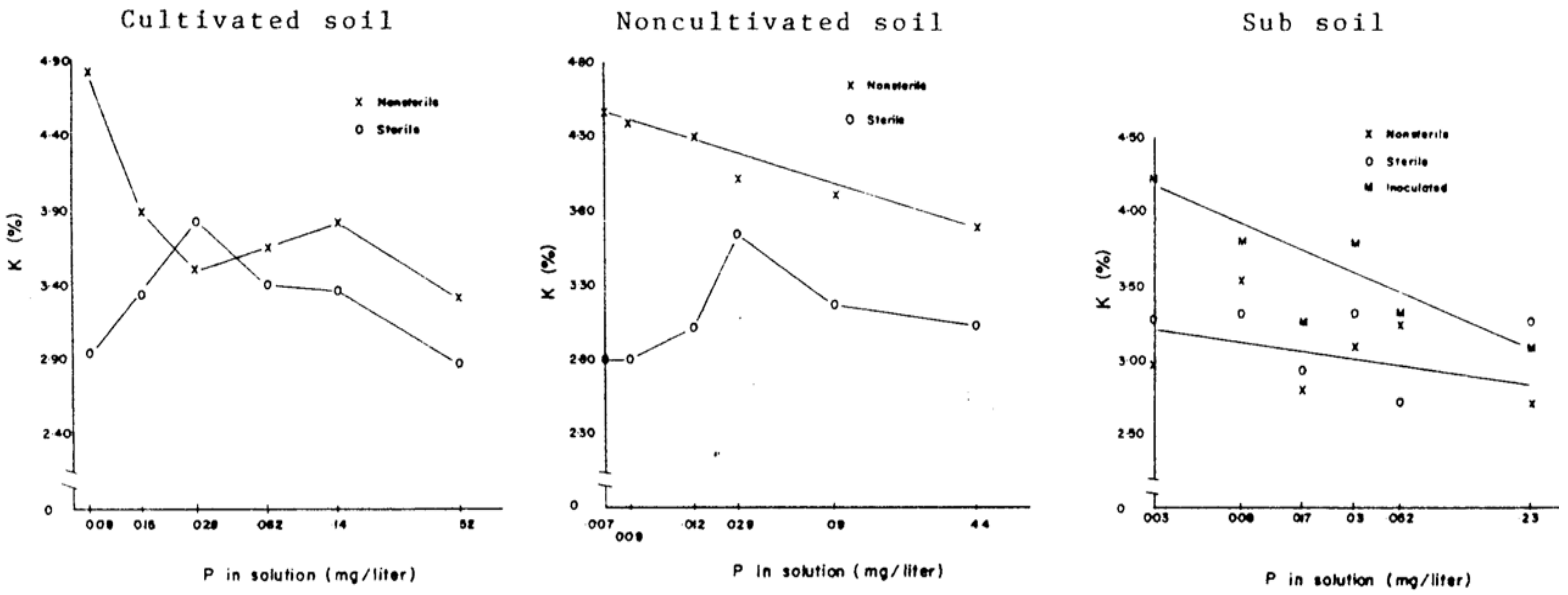

Figure 10. Potassium percentages in cowpea tops as affected by soil sterilization and soil P status.

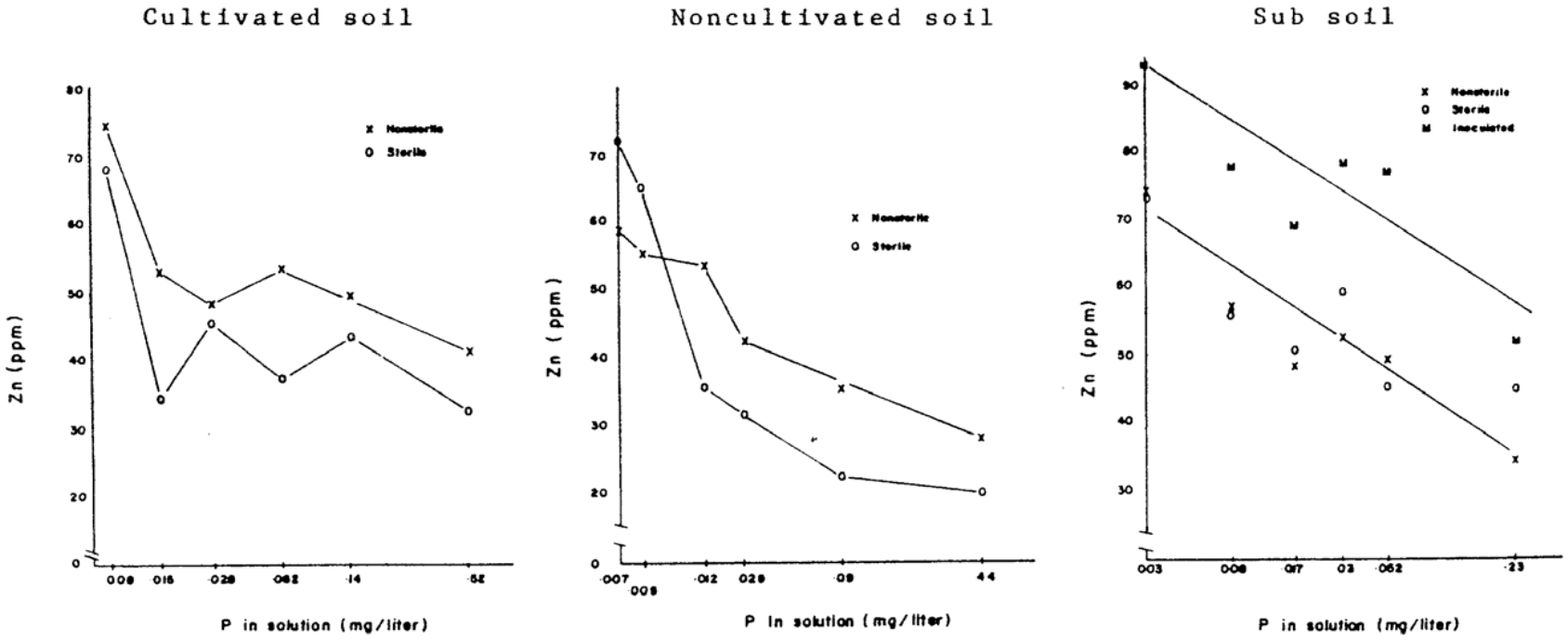

Figure 11. Zinc percentages in cowpea tops as affected by soil sterilization and soil P status.

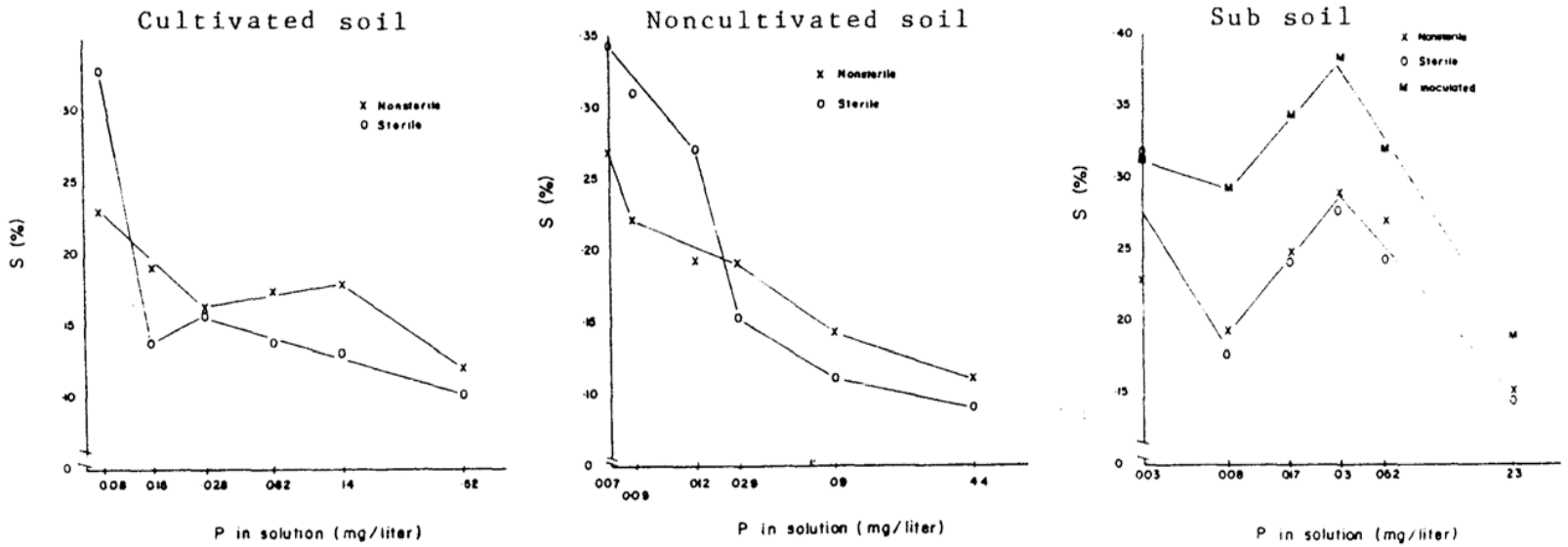

Figure 12. Sulphur percentages in cowpea tops as affected by soil sterilization and soil P status.

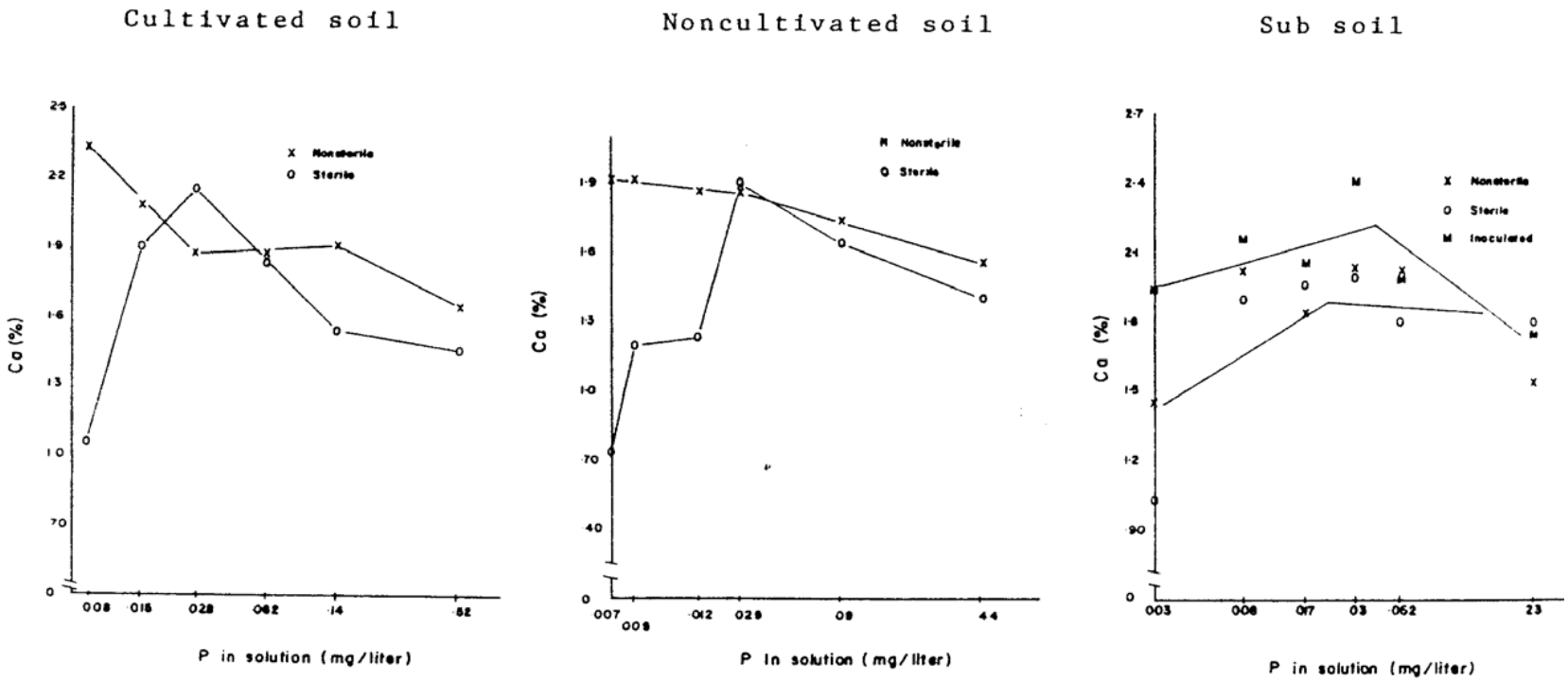

Figure 13. Calcium percentages in cowpea tops as affected by soil sterilization and soil P status.

(Gray and Gerdemann, 1973) but the significance of the effect has yet to be determined (Rhodes and Gerdemann, 1978). In the case of Cu, contamination of samples precluded a detailed consideration of the data. In general Cu concentrations in mycorrhizal plants were higher as compared to nonmycorrhizal plants in the cultivated and noncultivated soil; Cu concentrations were higher in the inoculated plants than in plants growing in sterile or non-sterile subsoil. In all soils the effect of sterilization was significant (Table 4).

The reports in the literature are not consistent regarding the role of mycorrhizae in the uptake of these nutrients, although it is generally accepted that mycorrhizae affect the uptake of nutrients other than phosphorus. Apparent inconsistencies in the literature may in part be related to differences among plant species and experimental conditions. The observation that mycorrhizae enhance Si uptake in soybean and not cowpea is an example of the interaction between plant species and mycorrhizae (Yost and Fox, in press). The conflicting reports on K uptake by mycorrhizae may also be related to plant species (Powell, 1975; Gerdemann, 1964).

The influence of P level in the plant may also exert effects which confound the effects of mycorrhizae. Phosphorus fertilization results in increased P levels in the plant which in turn may inhibit the development of mycorrhizae. P fertilization may also inhibit nutrient uptake due to antagonisms between the nutrient and phosphorus; P fertilization may also enhance the uptake of a nutrient as a result of better plant nutrition and a larger root system.

The literature concerning the antagonisms of Zn and Cu by phosphorus have been reviewed by Olsen (1972). When evaluating the effects of mycorrhizae on Zn and Cu uptake two simultaneous processes should be considered: 1. the suppression of mycorrhizal uptake of other nutrients by phosphorus because of reduced infection; and 2. P antagonistic effects

Table 4. Nutrient analyses and statistical data for Cowpea growing in three soils with six levels of phosphorus fertilization.

	Intended P in solution	Yield	P	P uptake	N	N uptake	K	Ca	S	Cu	Zn
	mg/liter	g/pot	%	mg/pot	%	mg/pot	------%		---------	ppm	ppm
Cultivated soil											
Nonsterile	.008	7.68	.17	13.3	2.88	221	4.84	2.34	.23	19.7	74.7
	.015	22.8	.17	38.7	2.90	661	3.90	2.10	.19	18.0	53.0
	.028	36.4	.20	72.0	2.67	979	3.50	1.88	.16	17.7	48.3
	.062	40.9	.23	92.4	2.42	982	3.65	1.86	.17	*	53.5
	.14	38.1	.26	97.6	2.76	1048	3.83	1.91	.18	*	49.3
	.52	46.4	.29	132	2.32	1078	3.30	1.65	.12	13.5	41.0
Sterile	.008	.95	.12	1.1	7.43	70.5	2.94	1.06	.33	19.0	68.0
	.015	19.1	.16	29.8	2.48	472	3.32	1.89	.14	12.3	34.7
	.028	32.3	.22	69.9	2.83	913	3.82	2.17	.16	*	46.3
	.062	39.9	.25	97.8	2.34	930	3.41	1.84	.14	13.7	37.0
	.14	53.8	.26	141	1.95	1051	3.37	1.54	.13	*	43.7
	.52	51.0	.29	147	1.80	906	2.86	1.46	.10	11.3	32.3
ANOVA	------	-----	----	----	----	----	----	----	----	----	----
Sterilization (Ster.)+		n.s.++	n.s.	.0001	.0001	.022	.0006	.0001	n.s.	.0013	.0001
P level		.0001	.0001	.0001	.0001	.0001	n.s.	.0015	.0001	.0005	.0001
Ster.*P		.0027	n.s.	.0001	.0001	n.s.	.0091	.0001	.0025	---	n.s.
Noncultivated soil											
Nonsterile	.007	19.6	.23	44.2	2.83	553	4.48	1.91	.27	26.7	58.7
	.009	28.3	.25	71.0	2.89	814	4.38	1.91	.22	26.3	55.0
	.012	30.5	.25	74.8	2.96	897	4.28	1.86	.19	23.4	53.0
	.029	40.3	.26	106	2.98	1202	4.01	1.86	.19	*	42.3
	.09	43.1	.29	125	2.87	1237	3.94	1.74	.14	*	35.3
	.44	45.2	.33	149	2.87	1300	3.68	1.57	.11	14.0	28.0
Sterile	.007	.70	.14	.94	8.98	62	2.77	.73	.34	28.7	72.0
	.009	1.27	.12	1.6	8.15	103	2.76	1.12	.31	25.0	65.0
	.012	2.15	.12	2.6	6.55	140	3.07	1.22	.27	17.0	35.7
	.029	43.5	.22	92.7	2.52	1079	3.63	1.87	.15	16.0	31.3
	.09	54.0	.25	137	2.30	1240	3.19	1.65	.11	13.3	22.3
	.44	60.3	.32	194	1.87	1210	2.97	1.39	.09	12.7	20.7
ANOVA	----	----	----	----	----	----	----	----	----	----	----
Ster.		.0001	.0001	.0001	.0001	.0001	.0001	.0001	.0094	.0001	n.s.
P level		.0001	.0001	.0001	.0001	.0001	n.s.	.0001	.0001	.0001	.0001
Ster.*P		.0001	.0011	.0001	.0001	.0001	.0185	.0001	.0001	---	n.s.
Sub soil											
Nonsterile	.003	.85	.10	.85	3.07	26	2.97	1.44	.23	29.7	73.7
	.008	9.6	.14	13.4	1.81	171	3.52	2.02	.19	19.7	57.0
	.017	20.1	.20	39.5	1.35	272	2.81	1.84	.25	21.0	48.3
	.03	21.1	.23	49.1	1.53	321	3.38	2.02	.29	21.0	52.7
	.052	19.8	.25	50.1	1.74	345	3.27	2.02	.27	17.3	49.3
	.23	24.3	.27	66.8	1.60	390	2.70	1.54	.15	*	34.3
Sterile	.003	.62	.07	.48	3.47	21	3.28	1.03	.32	25.0	73.0
	.008	9.3	.12	11.4	2.04	186	3.32	1.89	.18	18.3	56.7
	.017	21.7	.20	44.1	1.50	325	2.94	1.96	.24	17.3	50.3
	.03	23.2	.25	56.8	1.73	394	3.32	2.02	.28	18.7	59.0
	.052	27.7	.23	64.0	1.49	414	2.71	1.80	.24	17.0	45.0
	.23	27.6	.32	87.8	2.17	601	3.25	1.79	.15	7.3	44.3
Inoculated	.003	1.83	.12	2.2	3.21	59	4.19	1.93	.31	25.5	92.5
with VA	.008	9.34	.24	22.4	1.75	161	3.77	2.13	.29	21.0	77.3
mycorrhizae	.017	14.8	.27	37.9	1.59	227	3.23	2.03	.34	19.7	68.7
	.03	13.6	.32	42.4	1.94	257	3.79	2.39	.38	19.0	77.7
	.052	17.9	.30	53.7	1.67	297	3.27	1.98	.32	17.7	65.3
	.23	18.6	.36	65.3	2.03	373	3.08	1.75	.19	7.0	51.3
ANOVA	----	----	---	----	----	---	----	----	---	----	----
Ster.		.0001	.0001	.0181	.0247	.0001	.0043	.0001	.0001	.0001	.0001
P level		.0001	.0001	.0001	.0001	.0001	.0061	.0001	.0001	.0001	.0001
Ster.*P		.0054	---	.0324	n.s.	.0047	n.s.	.0263	n.s.	---	n.s.

* Missing values due to contamination of sample
\+ Source of variation
++ Significance levels are expressed as probabilities. Levels greater than .05 are not significant.

toward Zn and Cu uptake.

P fertilization can enhance the uptake of a nutrient due to better plant nutrition and a larger root system, and can result in a nutrient becoming 'diluted'. The concentration of a nutrient in plant tissue will be diluted when the rate of plant growth exceeds the rate of nutrient uptake. When evaluating the effect of mycorrhizae, the rate of plant growth and nutrient uptake should be considered. Only recently have researchers begun to look at the rate of both of these processes (Post and Fox, in press; Lambert et al., 1979).

Effect of Soil

Three criteria were used as a basis for selecting the soils for this experiment: 1. the soils should have similar mineralogy; 2. the soils should be low in phosphorus; and 3. there should be suspected differences in mycorrhizal inoculum densities. Before plants were grown, soils were limed and fertilized according to requirements predicted by soil analysis. Results of soil analysis are presented in Table 1. It is now apparent that physical properties of the soil materials should have been determined.

During the experiment it was evident that each soil had its own potential for growth. A comparison of growth potentials among the soil materials is most valid when there are no differences in inoculum density. In Fig. 14 the yield response to soil P is presented for plants growing in sterile cultivated, noncultivated, and subsoil materials. The growth response was dependent upon the soil as well as the soil P level.

Water movement in the soils was another indication of differences among the soils. After the initial wetting of the soil, the surface of the noncultivated soil dried out. Capillary rise was not sufficient to wet the entire soil section. In the subsoil, the surface portion remained moist

throughout the experiment. The moisture situation in the cultivated soil was intermediate between the noncultivated and subsoil.

A small experiment was conducted to test the hypothesis that water movement and retention was different in the three soils. An attempt was made to simulate the experimental conditions. Three 30 cm plexiglass tubes (6.8 cm diameter) were cut into six 5 cm sections and taped together to make the soil column. A known amount of soil which had passed a 1/4 in. sieve was placed in the columns. A piece of cheese cloth was placed over the bottom of the column to hold the soils in place. The soil materials were saturated and equilibrated for 24 hours. The columns were then placed upright in a dish containing water so that water was able to move up through the soils by capillary movement. After 24 hours the columns were removed from the dishes and allowed to drain for 12 hours. The columns were covered so water loss by evaporation was minimized. After 12 hours the columns were replaced in the dishes for 24 hours after which the soils were allowed to drain for 12 hours. The columns were then disassembled and the percent water saturation by volume was determined for each section of the column. Water percentage by volume in the first section, where soil water tension was least, was considered to be water content at saturation. Water retention in the other sections was calculated relative to the water content at saturation. The water retention curves are presented in Fig. 15. The results confirm the hypothesis that there were large differences among the soil materials. At the top of the column, the percent saturation was approximately 25% lower in the noncultivated than in the subsoil. The percent saturation changed relatively little with increasing soil water tension in the subsoil, but in the noncultivated soil it decreased markedly. These results undoubtedly underestimate the differences which existed in the pots in the glasshouse. These curves were obtained under optimum conditions of wetting and drying, but in the glasshouse evaporation and water uptake by

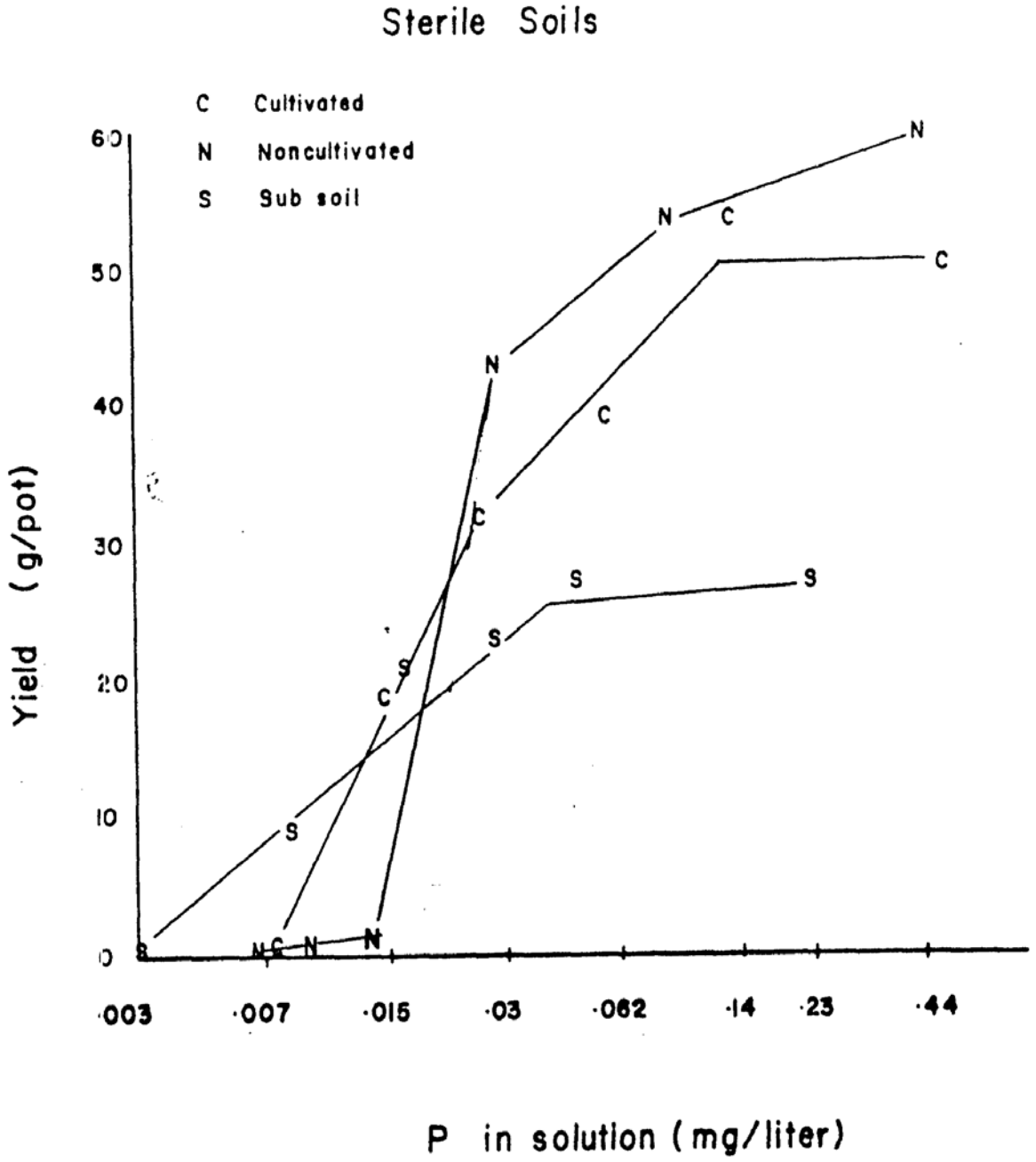

Figure 14. Yield of cowpea growing in sterile cultivated, noncultivated, and sub soil materials as affected by soil P status.

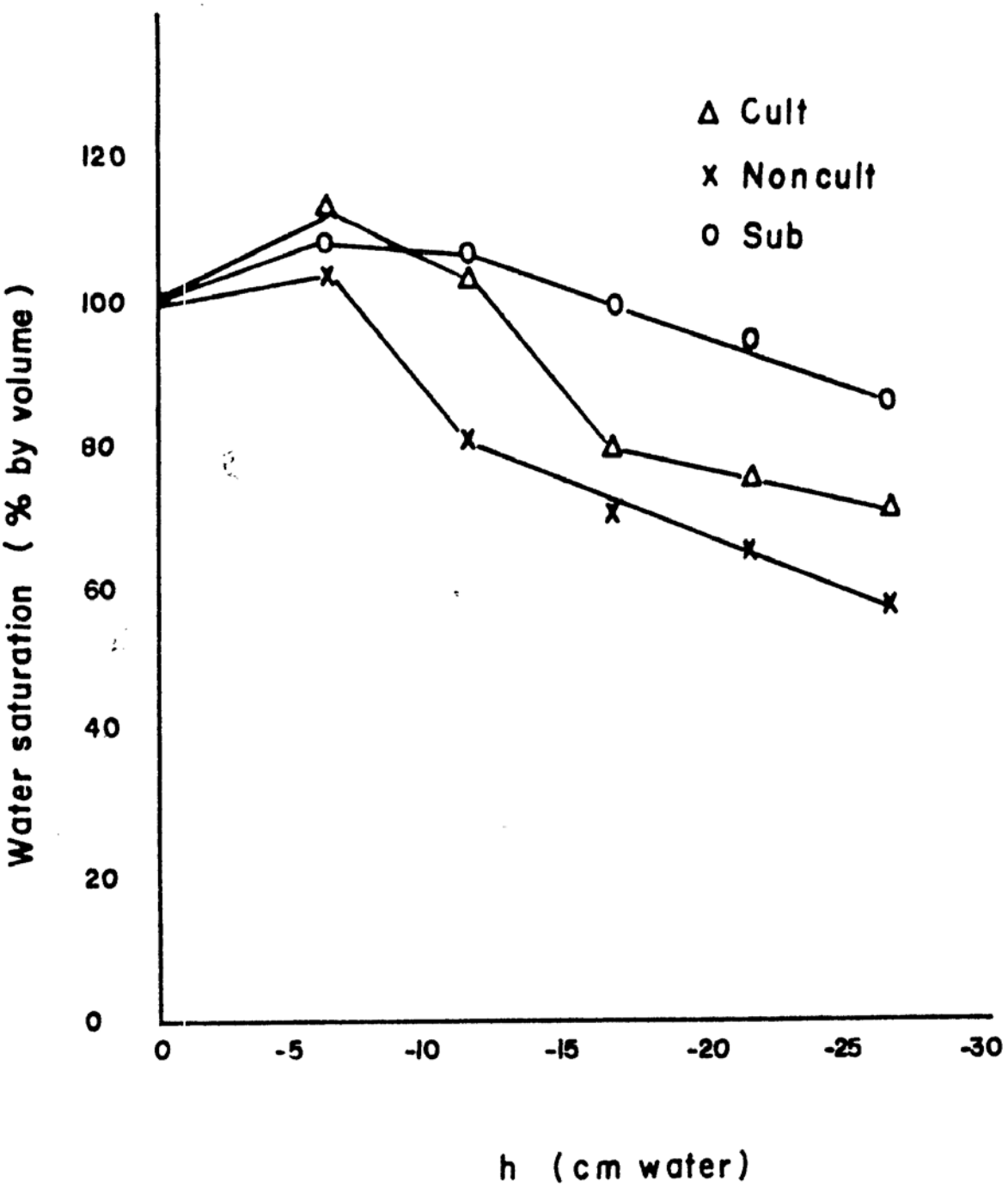

Figure 15. Water retention curves for cultivated, noncultivated, and sub soil materials.

the plants would further accentuate the differences.

With the soil columns the bulk densities of the soil materials were calculated to be 0.76, 0.88, and 1.09 for the noncultivated, cultivated, and subsoil respectively. The combination of increased bulk density and poor aeration created conditions for plant growth that were very different in the three soil materials. The source of these differences may not be solely attributed to bulk density. Soils containing volcanic ash materials and high amounts of organic matter may exhibit 'hard to wet' properties. Capillary rise is not as great in soils exhibiting such properties; and is related to the contact angle at the water-solid interface. Where soil surfaces are coated with volcanic ash and organic surfactants, the contact angle can be greater than 90°, in which case the surfaces will resist wetting. The Wahiawa soil has exhibited these properties (Fox, personal communication), and it is possible that surface soil materials are inherently different from subsoil materials with respect to the ability of water to move by capillary rise.

The differences among the soils are likely to have an effect on the diffusion of solutes in the soil. Nye and Tinker (1977) defined the diffusion coefficient of nonvolatile solutes as $F = D_1 \theta f_1 dC_1/dx + F_e$ where

D_1 is the diffusion coefficient of the solute in free solution

θ is the fraction of soil volume occupied by solution

f_1 is an impedence factor

C_1 is the concentration of solute in the soil solution

F_E is the excess flux created by surface diffusion

The water content of the soil thus exerts a major effect upon the diffusion coefficient of a solute in soil. With the information from the water retention curves, we may speculate that the diffusion

coefficient for phosphorus was greatest in the subsoil, and least in the noncultivated soil.

The effect of diffusion coefficients on the ability of plants to absorb phosphorus will be greatest when soil P levels are low. Nonmycorrhizal plants growing in noncultivated soil were less able to absorb phosphorus than comparable plants in cultivated and subsoils when the soil P levels were low (Fig, 14). The threshold value of soil P for a particular soil, that value below which the plant is unable to extract phosphorus from the soil, may be determined by both the P diffusion coefficient in that soil as well as the plant species. Yost and Fox (1979) reported similar threshold values of .012 mg P/liter for nonmycorrhizal Allium cepa, Stylosanthes, and Leuceaena leucocephala growing in the field. If the roots affinity for phosphorus is similar among plant species, then the predominant factor influencing the threshold value for P absorption may be the P diffusion coefficient.

The principle advantage of mycorrhizae is increased P uptake. This is possible because the hyphae extend into the soil beyond the volume of P depletion by the root. The relative advantage of mycorrhizae should be greatest in soils where the P diffusion coefficients are least and the volume of P depletion is the smallest. For example, in the subsoil where the diffusion coefficient should have been relatively high, mycorrhizae may not be as advantageous to the host as in the noncultivated soil where the diffusion of P should be relatively less. The concept of specific interactions between the soil and the fungi has not received the attention it deserves. Cooper (1975) noted that the effects of soil properties need to be considered when evaluating plant response to mycorrhizae, including P diffusion rates which are not reflected in extractable P values.

The critical P level for mycotrophy may have been influenced by the P diffusion rate in the soil. In the subsoil this critical P level was approximately .008 mg P/liter, and in the cultivated soil it was .062 mg P/liter. Given the more similar physical characteristics of these two soils the relative difference in their critical P levels for mycotrophy may also reflect different inoculum densities. In the noncultivated soil, with a relatively high inoculum density and relatively low P diffusion coefficient, the lower critical level for mycotrophy is unexpected. However soil structure has other effects besides affecting water retention and diffusion rates. Root development can be impeded in soils with high bulk densities. In the noncultivated soil, aeration was undoubtedly more favorable for root development relative to the cultivated and subsoils. The lower P levels for mycotrophy together with the higher growth potential may indicate that mycorrhizae are not a substitute for a well developed root system. The unfavorable soil structure for root proliferatioa may have been the limiting factor in plant growth in the subsoil material. The lack of response to inoculation in an environment not favorable for root growth is understandable.

Another factor which may have contributed to different growth potentials in the soils was the available nitrogen supply. During early growth, leaves of plants growing in sterile soils were a dark green color, while leaves of plants in non-sterile soils were a pale green color. Differences in nitrogen nutrition were suspected, perhaps due to the competition-free environment in sterile soil which allowed for rapid growth of introduced Rhizobia. Other contributing factors may have been the release of NH_4^+ caused by y-irradiation (Singh and Kanehiro, 1970) and the Birch effect. After 22 days the differences in color among the plants disappeared. By the end of the

experiment total N uptake was greater in plants in non-sterile soil than sterile soil (Table 4). The warm moist conditions in the pots may have promoted mineralization of organic matter relatively more in non-sterile soils. The differences in organic carbon in the soils (Table 1) should be considered a factor in the different growth potentials of the soil materials. Organic matter may not only have contributed to the 'hard to wet' properties of the noncultivated soil material, but also to the greater uptake of N by plants relative to plants in the other soil materials.

Conclusions

1. A soil dilution bioassay method can detect differences in the mycorrhizal inoculum level in soil.

2. Soil materials should be brought to standard conditions to ensure the accuracy of the bioassay. Soil physical properties should be considered when the standard conditions are defined. Plant material should also be standardized.

3. The significance of differences in mycorrhizal inoculum level is not easily interpreted. Attention must be given to the interaction between the soil and the fungi in order to determine the effect of inoculum level on the growth of the host plant.

4. Two factors are involved when considering the amount of phosphate fertilizer required to replace mycorrhizae: l. the effectiveness of the mycorrhizal association; and 2. the P requirement of the soil.

5. Mycorrhizae enhance the uptake of nutrients other than phosphorus. Potassium, Zn, S, and Ca are also transported through the hyphae. The extent to which the nutrition of the host improves as a result of infection depends upon specific interactions among the fungi, the host plant, and the soil.

APPENDIX A

Table 5. Additional nutrient analyses and statistical data for Cowpea growing in three soils with six levels of phosphorus fertilization.

	Intended P in solution	Mg	Si	Na	Mn	Fe
	mg/liter	---------%----------			ppm	ppm
		Cultivated soil				
Nonsterile	.008	.31	.13	.07	653	133
	.015	.31	.14	.07	751	161
	.028	.29	.12	.06	852	150
	.062	.31	.13	.07	889	144
	.14	.33	.13	.07	1006	146
	.52	.29	.15	.06	822	152
Sterile	.008	.46	.22	.08	759	*
	.015	.32	.11	.07	766	167
	.028	.35	.13	.06	1023	146
	.062	.33	.12	.06	1044	155
	.14	.33	.15	.06	1193	156
	.52	.33	.15	.06	831	156
ANOVA	---	---	---	---	---	---
Sterilization	(Ster.)+	.0002++	n.s.	n.s.	.0211	n.s.
P level		.0042	n.s.	.0249	.0002	n.s.
Ster.*P		.0019	n.s.	n.s.	n.s.	n.s.
		Noncultivated soil				
Nonsterile	.007	.30	.07	.07	170	141
	.009	.31	.07	.06	206	154
	.012	.31	.08	.06	246	150
	.029	.31	.07	.06	266	154
	.09	.32	.07	.05	205	152
	.44	.31	.07	.05	152	161
Sterile	.007	.35	.05	.06	328	171
	.009	.36	.04	.06	342	166
	.012	.41	.07	.05	310	184
	.029	.38	.09	.06	468	150
	.09	.34	.08	.05	548	150
	.44	.34	.08	.05	554	146
ANOVA	---	---	---	---	---	---
Ster.		.0001	n.s.	n.s.	.0001	n.s.
P level		n.s.	.008	n.s.	n.s.	n.s.
Ster.*P		n.s.	.0019	n.s.	n.s.	.0339
		Sub soil				
Nonsterile	.003	.45	.11	.06	265	184
	.008	.36	.14	.06	456	150
	.017	.29	.15	.06	468	154
	.03	.34	.17	.06	499	134
	.052	.34	.20	.06	570	156
	.23	.29	.21	.05	460	161
Sterile	.003	.44	.10	.08	362	*
	.008	.35	.13	.06	634	161
	.017	.32	.16	.06	607	180
	.03	.34	.18	.06	595	173
	.052	.30	.15	.06	455	141
	.23	.34	.22	.06	564	167
Inoculated	.003	.38	.12	.06	496	134
with VA	.008	.38	.14	.06	482	138
mycorrhizae	.017	.34	.16	.06	426	138
	.03	.41	.22	.06	686	163
	.052	.35	.20	.06	575	159
	.23	.34	.23	.06	535	164
ANOVA	---	---	---	---	---	---
Ster.		n.s.	.0062	n.s.	.0364	n.s.
P level		.0001	.0001	n.s.	.0028	n.s.
Ster.*P		.006	n.s.	n.s.	n.s.	n.s.

* Missing values due to contamination of sample
\+ Source of variation
++ Significance levels are expressed as probabilities. Levels greater than .05 are not significant.

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