SOME FACTORS RESPONSIBLE FOR DIFFERENCES BETWEEN PLANT SPECIES IN ABSORPTION AND UTILIZATION OF PHOSPHATE

by

M.G. Temple-Smith, B. Agr. Sc. (Hons), Tas.

Submitted in partial fulfilment of the requirements for the Degree of Doctor of Philosophy.

UNIVERSITY OF TASMANIA

HOBART

MAY 1973

This thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge contains no copy or paraphrase of material previously published or written by any other person except where due reference is made in the text of the thesis.

M.G. Temple-Smith

University of Tasmania,
Hobart.

May, 1973.

TABLE OF CONTENTS

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my supervisor, Dr. R.C. Menary, Senior Lecturer in Horticultural Science of the Faculty of Agricultural Science, for his guidance, constructive criticism and continual encouragement throughout the course of this study. His efforts attracted the support of the Rural Credits Development Fund of the Reserve Bank of Australia to the project and this financial backing is acknowledged and appreciated.

I express my thanks to Dr. K.C. Marshall and Professor G.C. Wade, Reader in Microbiology and Dean respectively, the Faculty of Agricultural Science, for beneficial discussion and criticism of the work in this thesis. I also thank Dr. J.A. Beattie, Senior Lecturer in Soil Science, the Faculty of Agricultural Science, for helpful advice during the course of this study.

Many other people contributed to the work of this thesis. In particular I wish to thank, Mr. G. McPherson, Department of Mathematics, University of Tasmania, for assistance with experimental design, Mr. R. Ford, Department of Geology, University of Tasmania for undertaking the X-ray diffraction analyses and the technical staff of the Faculty of Agricultural Science, especially Mr. W. Peterson, for technical advice and assistance.

Finally, to my wife Anne I extend my appreciation for her continual support and willing assistance throughout the course of the project.

SUMMARY

The ability of plants to absorb phosphate and grow in a soil system depends upon a number of soil and environmental factors as well as a number of plant factors determined by the particular plant species involved. The purpose of this study was to compare the growth and phosphate nutrition of four plant species, two grasses and two vegetables, and to identify the plant factors which control the responses of these species to applied phosphate.

Glasshouse pot experiments using a krasnozem soil with a high capacity to fix phosphate showed that the four species differed in both their relative growth and phosphate uptake in unfertilized soil and also in their responses to applied fertilizer phosphate. Cabbage grew relatively better than the other species at all levels of applied phosphate and produced near maximum yield at a lower level of applied phosphate. Of the four species, lettuce was found to produce the lowest relative yields at each phosphate treatment. Despite species differences in both the absolute and relative amounts of phosphate absorbed, the yield differences between species were largely the result of differences in the efficiency with which absorbed phosphate was utilized in plant growth. This appeared to be partly due to differences in the nitrogen status of the four species at harvest. In addition, fractionation of the phosphate in shoots of lettuce and cabbage grown under phosphate deficient conditions showed a difference in the relative amounts of inorganic orthophosphate in the shoot. Cabbage was found to have a greater proportion of its phosphate in organic form.

Sand culture experiments using organic phosphates and inorganic phosphate-fertilizer reaction products as sources of phosphate showed that interspecific differences in the utilization of these compounds were apparently not involved in any differential species response to phosphate in the soil. However, some reservations were expressed concerning the extrapolation of these results to the soil situation. Depletion zone studies using ^{32}P demonstrated that the diameter of the zones of phosphate depletion around roots of these species were significantly different between two soil types but no differences were observed between different species in either soil.

Plant growth rates and rates of phosphate absorption in continuous-flow nutrient cultures of low phosphate concentration were found to differ widely between species especially at below optimum concentrations. These results suggested that the poor growth of lettuce in krasnozem soil was partly due to its limited rate of phosphate absorption from solutions of low concentration.

The results of the continuous-flow nutrient culture experiments were confirmed in short-term phosphate uptake studies using excised roots. Kinetic analysis of the ^{32}P absorption data for non-sterile excised roots revealed that all species possessed two separate absorption mechanisms. For the vegetables the relative contribution of each uptake mechanism to the total phosphate absorbed was similar, but the rates of phosphate uptake by both the "a" and "b" mechanisms were always greater for cabbage than for lettuce.

Differences in phosphate uptake between grass and vegetable excised roots were due to differences in both the rates of absorption and the relative magnitudes of the two uptake mechanisms.

Phosphate uptake by sterile, excised roots of cabbage and lettuce was also characterized by the operation of dual absorption mechanisms. In comparison with rates of phosphate uptake under non-sterile conditions the absence of micro-organisms had no significant effect on the rate of phosphate absorption by lettuce. However, phosphate uptake by excised roots of cabbage was reduced under sterile conditions indicating a positive interaction between cabbage roots and the microflora, with respect to phosphate absorption under non-sterile conditions.

I INTRODUCTION

Studies conducted under glasshouse or laboratory conditions have indicated that a large number of soil and plant factors may operate in controlling the rate and pattern of phosphate uptake by the roots and the subsequent utilization of phosphate within the plant. These include plant factors such as the amount and type of root tissue, the physiological and nutritional status of the plant, the bio-chemical make-up of the plant and the presence of rhizosphere micro-organisms as well as soil factors such as soil pH, the soil's chemical characteristics and also its physical structure. Presumably some of the plant factors controlling the absorption and utilization of phosphate, may operate differently in different plant species and may therefore account for differences in phosphate nutrition commonly observed between plant species.

Although previous studies comparing the phosphate nutrition of different plant species have often concluded that a single factor is responsible for the differential effect, few studies have investigated and attempted to relate the various factors that may be operating simultaneously. At the same time, there appears to be, in some cases, a basic lack of knowledge concerning the physiological or bio-chemical mechanisms inherent in these factors.

Differences between plant species in phosphate absorption and relative growth rates are usually most obvious when plants are grown in soils containing a low level of

available phosphate but which are not deficient in phosphate *per se*. Under these conditions in the red loam (krasnozem) soils of south east Queensland, Menary (private communication) has observed apparently normal growth of grasses while tomatoes and certain other vegetables show severe phosphate deficiency symptoms associated with marked depressions in plant growth. Such interspecific differences in response to phosphate in these soils could be due to differences between species in one or any combination of the following factors:

1. Plant requirement for, and internal utilization of phosphate.
2. Rate of phosphate absorption per unit of root.
3. Amount, structure and type of root system.
4. Root exudates and root microbial populations (both of which may influence the utilization and absorption of soil inorganic and organic phosphates).
5. Other factors such as optimum pH for plant growth and the toxicity threshold for heavy metals (e.g. Al, Fe, Mn).

The aim of the present study was to examine the growth and phosphate nutrition of a number of plant species and to determine and compare the relative importance of the various factors that may operate in controlling the phosphate nutrition of plants. To simplify interspecific comparisons of phosphate nutrition, an attempt was made to select species

with similar tissue phosphate requirements for maximum growth, but which gave differential yield responses to applied phosphate. From reports in the literature and from the results of a short, preliminary pot experiment in which shoot phosphate concentrations and yield responses to phosphate applied to a krasnozem soil were measured, four plant species (two vegetables, lettuce and cabbage and two grasses, phalaris and perennial ryegrass) were selected for study. Of these four species, cabbage and perennial ryegrass appeared to be able to utilize soil phosphate more effectively than did lettuce and phalaris. Tomato was not included as one of the vegetable species because its simultaneous vegetative and reproductive growth may have caused problems of interpretion in comparisons made with a "vegetative type" species.

The initial approach taken in this project was to compare plant growth, absorption and utilization of both soil and fertilizer phosphate by the four species when growing in a krasnozem soil of low available phosphate status. The role of a number of factors of possible importance in phosphate nutrition of plants was then studied in subsequent experiments. These included studies of the phosphate depletion zones around plant roots grown in two soil types, investigation of the ability of plants to utilize phosphate from organic phosphates and inorganic soil-fertilizer reaction products, and determination of the rate of phosphate absorption by plant roots grown in solution cultures. The latter study was made using excised roots and radioactive phosphate in short term uptake

experiments in addition to longer-term studies with whole plants grown in large volume, continuous-flow, nutrient solution cultures. In some cases the experiments, mentioned above, were conducted under both sterile and non-sterile conditions to show how micro-organisms influence the phosphate nutrition of plants.

It was hoped that these experiments would enable the factors of prime importance in determining interspecific differences in plant phosphate nutrition to be identified so that further investigation could elucidate the basic physiological and bio-chemical mechanisms involved. Comparative studies of this type are of potential practical importance since identification and subsequent explanation of the mechanisms controlling efficient plant absorption and utilization of phosphate would facilitate selection for these characters in plant breeding programmes. Species with such characteristics would be particularly important in soils having a high capacity to fix phosphate.

II LITERATURE REVIEW

TABLE OF CONTENTS

C REPORTED DIFFERENCES BETWEEN PLANT SPECIES IN RESPONSE TO PHOSPHATE

II A FORMS OF PHOSPHATE IN ACID SOILS

II A 1 INTRODUCTION

Apart from the presence of condensed phosphates, which are readily hydrolysed to orthophosphate by micro-organisms (Sutton and Larsen, 1964), phosphorus in soil occurs exclusively in the orthophosphate form.

In this review the emphasis will be placed on the forms of orthophosphate in acid soils and a distinction will be made between forms present in recently fertilized soils (phosphate reaction products), a non-equilibrium condition, and those found in virgin soils or soil unfertilized for some years where a position nearer to phosphate equilibrium has been attained.

II A 2 FORMS IN VIRGIN AND UNFERTILIZED SOILS

For convenience of discussion phosphorus in soils will be considered as either solution phase or solid phase phosphates both of which will be further subdivided into inorganic and organic forms.

II A 2.1 Phosphate in the soil solution

II A 2.1(a) Inorganic forms

Orthosphate may exist in solution as H_3PO_4, $H_2PO_4^-$, HPO_4^{2-} and PO_4^{3-}, the proportions present in any solution depending primarily upon the pH. Olsen (1953) has calculated that in normal soils only $H_2PO_4^-$ and HPO_4^{2-} are important, the proportions at the following pH's being:-

pH	5	7.2	9.0
% as $H_2PO_4^-$	99.3	50.0	1.5
% as HPO_4^{2-}	0.6	50.0	98.4

Larsen (1967) has pointed out that at least some of the phosphate ions in the soil solution may be complexed with metallic ions. From stability constants of various ion-pairs he predicted that soluble phosphate complexes of iron and aluminium would be those most likely to occur in soil solutions, with calcium phosphate complexes of lesser importance, except in calcareous soils. Larsen (1967) could find no information in the literature concerning phosphate complexes in the soil solution, thus substantiating the claim of Adams (1971) that the ionic composition of the true soil solution remained a neglected area in soil chemistry.

II A 2.1(b) Organic forms

The importance of organic phosphates in soil solutions was first shown by the work of Pierre and Parker (1927) who found that the average concentration of organic phosphates in displaced soil solutions from twenty soils was 0.47 ug/ml, representing 84% of the total phosphorus. However, the forms of organic phosphates in soil solutions have only recently been investigated.

Hannapel et al. (1964) showed that movement of phosphate in a calcareous soil occurred almost completely in the organic form and in a further paper, Hannapel, Fuller

and Fox (1964), they suggested that a large fraction of the organic phosphorus in the soil solution was particulate in nature being associated with microbial cells and cellular debris in the colloidal size fraction. Martin (1970) investigated the compounds in water extracts of ten soils after incubation of soil with ^{32}P labelled orthophosphate under aerobic conditions. More than thirty ^{32}P labelled compounds were identified in these extracts, from 2 to 40% of the extracted activity being in the organic form. However, only one organic component could be detected by two-dimensional thin-layer chromatography using conventional staining with molybdate reagent and this was not identified fully although it was found to have properties distinct from the simple phosphate esters expected in soils. Further experiments under partially or completely sterile conditions showed that the ^{32}P compounds extracted were derived largely from growing microbial cells and a significant proportion of the intra-cellular organic phosphate pool was liberated into solution from damaged cells with the phosphate ester bond intact.

II A 2.2 Phosphate in the solid phase

II A 2.2(a) Inorganic forms

Inorganic solid phase phosphate is present in a number of forms, as crystalline phosphate compounds, as phosphate adsorbed to soil constituents and as phosphate present in the lattice of silicate minerals. The latter form is of very minor importance and will not be discussed here.

II A 2.2(a)(i) Crystalline compounds

Crystalline phosphates have been identified in soils usually as the result of petrographic examination of sand and silt fractions or where concentrations permitted, by x-ray diffraction and differential thermal analysis of clay sized fractions. Evidence of the presence of various crystalline phosphates has also been obtained by the application of solubility criteria to the soil as a whole.

Because high phosphate mineral concentrations are necessary for direct examination by the petrographic microscope only apatite (unit cell formula $M_{10}(PO_4)_6 x_2$), vivianite ($Fe(PO_4)_6 \cdot 8H_2O$), and wavellite ($Al_8(OH)_3(PO_4)_2 \cdot 5H_2O$) have been identified by this method (Black, 1968). Of these only apatite is of general importance, since it is the most common primary phosphate mineral. However, it does not usually occur in acid soils (Russell, 1961).

Norrish (1968) identified a number of secondary phosphate minerals in Australian soils using x-ray diffraction analysis of various particle-size fractions. These minerals, members of the plumbogummite group, were concentrated in the 0.5-5.0 μm size fraction and in some cases accounted for up to 60% of the total soil phosphorus. All these minerals contain phosphorus and aluminium in two structural positions with a third position containing one of a number of cations, e.g. plumbogummite, $PbAl_3(PO_4)_2OH_5H_2O$ and crandallite where Ca

occupies the position of Pb. These minerals are believed to be formed during weathering and soil formation and their occurrence in highly leached soils is indicative of their insolubility. Norrish (1968) has identified these minerals in 40 soils, including 10 krasnozems, most of which had higher than average phosphate contents and he considered that because of detection difficulties in soils of low phosphorus content these minerals may be more common in soils generally, than is at present indicated in the literature.

Methods based on the solubility product principle have also been used to predict the crystalline forms of phosphate likely to be found in soils. This involves a comparison of the ion activities in "equilibrium" soil solutions with the corresponding ion activities of known phosphate minerals, the basic concept being that at "equilibrium" the soil solution ion activities are controlled by the solubility of the least stable soil phosphate mineral. Using this method many calcium, aluminium and iron phosphates have been claimed to occur in soils. The method, however, is open to a number of objections. First the solubility product of calcium phosphate minerals has been shown to vary due to impurities in the equilibrium system (Ericsson, 1949) and depending upon whether the equilibrium position was reached via precipitation or dissolution of the compound (Bjerrum, 1949). In the soil situation both of these factors will operate together and

further complicate the system. Secondly, Murrman and Peech (1969) have shown that insoluble minerals such as varascite ($AlPO4.2H_2O$) and fluorapatite ($Ca_{10}(PO4)_6F_2$) dissolve very slowly in soil and require considerable periods of time to reach equilibrium. Despite these difficulties the presence in soils of fluorapatite (Murrman and Peech, 1968), varascite (Wright and Peech, 1960) and strengite (Chakravarti and Talibudeen, 1962) have been inferred on the basis of solubility criteria. However, studies by Raupach (1963) and Bache (1963) concluded that the correspondence between ion activity products in soils and those of pure varascite and strengite could also be explained as surface reactions of phosphate with aluminium oxy-hydroxides and iron hydroxides.

Recently Murrman and Peech (1969) concluded that crystalline phosphates and labile phosphate (i.e. isotopically exchangeable soil phosphate - usually considered to be phosphate sorbed onto soil constituents) both determine the concentration of phosphate in the soil solution according to the equation:-

$$\text{labile P} \rightleftharpoons \text{solution P} \rightleftharpoons \text{crystalline P}$$

Since equilibrium between solution and crystalline phosphate is attained so slowly they considered that in the short term the labile phosphate determines the concentration of phosphate in the soil solution. Thus, only when labile and crystalline phosphate are in equilibrium will the concentration of phosphate in the soil solution be controlled by the solubility product of a crystalline phosphate compound.

II A 2.2(a)(ii) Phosphate adsorbed to soil constituents

In acid soils the main constituents responsible for the sorption of phosphorus are compounds of aluminium and iron (Hemwall, 1957; Smith, 1965). The importance of soil aluminium and iron in phosphorus adsorption has been shown by a large number of workers. Leaver and Russell (1957) found that both phosphate fixing soils and hydrated aluminium and iron oxides had a decreased capacity for phosphate sorption after treatment with blocking agents which react with aluminium and iron. Bromfield (1965) was able to correlate phosphate adsorption by 47 acid surface soils from eastern Australia with the amounts of iron and aluminium extracted from them by Tamms acid oxalate reagent. The correlation coefficients, when all soils were considered, were highest for sorption and extractable aluminium but with certain soils the extractable iron values or iron plus aluminium gave higher coefficients than did aluminium alone. Chemical removal of various amounts of iron and aluminium from eleven of these soils generally resulted in a decrease in phosphate adsorption. Other workers, Williams, Scott and McDonald, (1958), Coleman, Thorup and Jackson (1960) and Franklin and Reisenauer (1960), have also found a high correlation between phosphate sorption and soil aluminium but in each case the capacity of soil iron to sorb phosphate was found to be of minor importance in comparison with aluminium.

Clay minerals and oxides or hydrous oxides of iron and aluminium are the principle compounds in soil responsible for the adsorption of phosphate (Hemwall, 1957; Hsu, 1965).

Hsu (1965) believes that the mechanism of adsorption involves breakage of part of the Si-O-Al or Al-OH linkages leaving aluminium (or iron) still in the crystal lattice, and that the same process occurs in both clays and sesquioxides. It is now generally believed that anion adsorption can occur in one of two ways. One mechanism involves the retention of anions as counter ions in the diffuse layer opposite a net positively charged surface and this has been called non specific anion adsorption by Hingston et al. (1967, 1968). Hsu (1965), however, believes that physical adsorption should not be considered as a fixation process of any significance. The second mechanism involves co-ordination of the anion with an oxide metal ion causing simultaneous displacement of another anion or ligand. This type of adsorption by ligand exchange has been termed specific anion adsorption, (Hingston et al., 1967, 1968), and always results in the charge on the surface becoming more negative. This theory, although originally developed from a study of anion adsorption by geothite, appears to have wider implications and recently has been shown to be equally valid for adsorption of phosphate and silicate by four strongly, phosphate adsorbing soils, (Obihara and Russell, 1972).

II A 2.2(b) Organic forms

The organic phosphate content of soils expressed as a percentage of the total soil phosphate ranges from very small values up to figures as high as 70-80% (Williams and Steinbergs, 1958). The characterization of the organic phosphates in soils was for many years hampered by the lack of suitable techniques to separate and identify the heterogeneous and complex products isolated from soils. Only in the last decade or so with the advent of advanced chromatographic techniques has any real progress been made. Even now the chemical nature of a large proportion of the soil organic phosphate remains obscure (Cosgrove 1967).

According to Anderson (1967) the organic phosphate compounds so far identified in soils belong to or are derived from three classes of phosphate esters, the inositol phosphates, the nucleic acids and the phospholipids. The latter two classes are of minor importance and rarely account for more than 5-10% of the total soil organic phosphate (Anderson, 1957, 1961; and Hance and Anderson, 1963).

The most important class of phosphate esters occurring in soil are the inositol phosphates which contribute up to 52% of the total organic phosphate (Anderson, 1967). These phosphates occur in a number of stereoisomeric forms of which the myo-configurations, especially myo-inositol hexaphosphate, has received the greatest attention. The higher esters of inositol, the hexa- and penta-phosphates have been studied in greater detail than the lower ones because these account for the major fraction of the total organic phosphate present in

soil (Anderson, 1956; Martin and Wicken, 1966; Dormaar, 1967; Omotoso and Wild, 1970). The amounts of inositol phosphate in soil vary widely and also account for a highly variable percentage of the soil organic phosphorus (Anderson, 1967; McKercher and Anderson, 1968a, 1968b; Williams and Anderson, 1968; Omotoso and Wild, 1970). The reasons for this variability between soils remains obscure and Williams and Anderson (1968) have found that neither the absolute content of inositol phosphate nor the proportion of the total organic phosphate present in the form of inositol phosphates could be closely associated with any particular soil property.

In the soil situation little is known of the factors responsible for adsorbing and stabilizing the inositol phosphates. In general it appears that inositol phosphates react with inorganic soil constituents to form insoluble complexes or alternatively may react with other organic constituents to form larger organic complexes. Apart from the alkali metal salts most other salts of inositol polyphosphates are only slightly soluble in water. Jackman and Black (1951) found the iron and aluminium salts were very insoluble in acid solutions above pH3 while the alkali metal salts were insoluble in alkaline media. Adsorption of inositol phosphates to clay minerals, soil clays and hydrated sesquioxides has been reported by Anderson and Arlidge (1962). In general the greater the number of phosphate groups in the molecule the greater was the degree of adsorption. The adsorption activity of clay minerals and soil clays was attributed to the active iron and aluminium present in/on these clays.

II A 3 Reactions of fertilizer phosphate with soil

A knowledge of the chemical reactions that occur in soil on addition of fertilizer phosphate and the identification of the phosphate reaction products produced is essential in fully defining the phosphate status of soils and in determining the likely availability to plants of phosphate added as fertilizer.

Much of the extensive knowledge now available on this subject has been contributed by workers of the Tennessee Valley Authority at Muscle Shoals, U.S.A. This work has been reviewed by Huffman (1962, 1970). The early work of Haseman, et al. (1950, 1951) and Low and Black (1950) provided data suggesting the types of reaction products that may be formed in soils, but it was left to later workers using fertilizer granules in the soil situation to clarify these reactions and identify the products.

Lehr, Brown and Brown (1959) placed tablets of monocalcium phosphate (MCP) in soil and concluded that the main factor controlling rate of tablet dissolution was vapour transport of water to the tablet, although factors such as soil moisture content, soil capillarity and temperature were also involved. They found that a considerable part (21-34%) of the applied phosphate remained at the tablet site, mostly as dicalcium phosphate. Lindsay and Stephenson (1959a) found that the chemical nature of the solution leaving a band of MCP in soil closely approximated the composition of the metastable triple point solution of MCP (i.e. MTPS - the solution saturated with respect to both $Ca(H_2PO_4)_2 \cdot H_2O$ and $CaHPO_4.2H_2O$). Because

the solution leaving the band was acidic (pH = 1.48), it caused considerable amounts of aluminium, iron and manganese to be brought into solution and the concentration of calcium and phosphate in solution decreased with increasing distance from the band, reflecting the effects of dilution and reaction with the soil. In later work, Lindsay, Lehr and Stephenson (1959) and Lindsay and Stephenson (1959b) found that the dissolution of further, more basic soil components caused a rise in pH and supersaturation of the solution with some ions dissolved from soil constituents. This resulted in precipitation of phosphates and the simultaneous removal from solution of aluminium, iron, calcium and potassium ions. Filtrates obtained from these reaction sequences yielded crystalline precipitates upon standing, the most important initial reaction product of MCP in both acid and calcareous soils being dicalcium phosphate dihydrate.

Lindsay, Frazer and Stephenson (1962) made a more detailed study of the solid phase reactions products formed in three soils by a variety of phosphate fertilizers and identified about thirty crystalline phosphate compounds. The initial reaction products formed depended upon the soil pH, the fertilizer used and the presence of reactive ions (i.e., soil type). For MCP at least fifteen reaction products were identified mostly after addition to the reaction filtrate of reactive ions likely to occur in soils. This procedure may be criticised as being artificial and relatively simple in comparison to the much more complex situation occurring in soils. Nevertheless, even without addition of reactive ions a number of reaction products were identified. Colloidal ferric

and aluminium phosphates of indefinite composition were the main products after reaction of MTPS in acid soil for three days. Addition of iron or aluminium to the filtrates caused precipitation of various calcium, iron and aluminium phosphates while addition of potassium ions precipitated $CaHPO_4 \cdot 2H_2O$ and $K(AlFe)_3\ H8(PO4)_6.6H_2O$ with potassium taranakite being formed at slightly higher pH values. Further work by Taylor and Gurney (1965) established the importance of potassium taranakite ($H_6K_3Al_5(PO_4)_8.18H_2O$) as a major reaction product of MCP in non-calcareous soils containing potassium. Where potassium was present in only small amounts or available only from the soil, rather than fertilizers, all the potassium was precipitated at taranakite the remaining phosphate forming amorphous aluminium phosphate and calcium aluminium phosphate. Taylor and Gurney (loc. cit.) also found that aluminium hydroxides were much more reactive than ferric oxides in the precipitation of phosphate from acidic fertilizer solutions and they concluded that significant precipitation of iron phosphate would only occur in soils deficient in reactive forms of aluminium or in those containing very finely divided and highly reactive forms of iron oxides.

Das and Datta (1966, 1967, 1968), working on the forms of phosphate fertilizer reaction products in a number of Indian soils, have confirmed the results of the American workers. They found that for MCP the major reaction products were brushite ($CaHPO_4 \cdot 2H_2O$), monetite ($CaHPO_4$), potassium taranakite, calcium aluminium phosphates and amorphous iron and aluminium phosphates. These products, however, are only the initial ones and being thermodynamically unstable, further chemical change within the soil will occur (Huffman, 1962).

All of the crystalline reaction products so far discussed have been identified after precipitation from filtrates obtained from contact of saturated water-soluble phosphate fertilizers with soil. These may be regarded as somewhat artificial and strictly speaking only a few crystalline reaction products have actually been identified in soil. Anhydrous and dehydrated forms of dicalcium phosphate as well as octacalcium phosphate have been identified in soils as reaction products of MCP (Bouldin et al., 1960; Lehr and Brown, 1958). Recently Bell and Black (1970) reported the formation of brushite and monetite in five alkaline to slightly acid soils, but although the pH close to the MCP source in the soil dropped to as low as 2.8 no evidence of iron or aluminium phosphate formation was found. Detection of these crystalline phosphates may however have been overlooked due to their small crystal size and the presence of only trace amounts. Thus, although crystalline iron and aluminium phosphates are thought to form in soils their presence in the soil proper has yet to be established.

The stabilities of the initial phosphate reaction products identified in soil filtrates have been studied by laboratory measurement of their rates of dissolution and also by examination of their alteration products or residues after incubation in soil. Huffman (1962) has stressed the importance of surface area in determining the rate of dissolution of slightly soluble reaction products and in general the larger the surface area of a particular product the more rapid the

rate of solution. Huffman, Cate, Deming and Elmore (1960) measured the initial rates of solution of a wide range of reaction products and taking strengite (crystalline ferric phosphate) as unity, the relative rates of solution, in water, per unit surface area were 1-3 for amorphous aluminium and iron phosphates, 10-165 for complex phosphates such as potassium taranakite and calcium aluminium phosphates and 250 for monetite. Dissolution of dicalcium phosphate was the only one controlled by diffusion through a liquid film the rates of the others being controlled by chemical reaction. The pH also had a major influence on dissolution, the rate of solution increasing with increasing pH for iron and aluminium phosphates and decreasing with increasing pH for calcium phosphates. They also found that the complex phosphates dissolved incongruently in water releasing phosphorus and cations (K^+, NH_4^+ Ca^{2+}) and at the same time forming amorphous iron and aluminium phosphates. Phosphorus from these tertiary products dissolved preferentially leaving residues coated with amorphous material resembling ferric and aluminium hydroxides.

Taylor, Gurney and Lehr (1963) also found the complex ferric phosphates, $H_4CaFe_2(PO4)_4 \cdot 5H_2O$ and $H8Fe_3(PO_4)_6 \cdot 6H_2O$, to dissolve incongruently releasing about half their phosphorus and leaving hulls of strengitic material after 313 days decay in an acid soil. Potassium taranakite on the other hand remained virtually unaltered and they concluded that its hydrolysis was impeded by surface coatings of amorphous aluminium phosphate that form in the early stages of dissolution. The amorphous iron and aluminium phosphates were similarly unchanged and no evidence of crystallization or alteration was observed after 313 days. More recently Juo

and Ellis (1968) have shown that under identical, artifically imposed conditions amorphous ferric phosphate crystallized at a much faster rate than amorphous aluminium phosphate. From this finding they argued that the ratio crystalline: amorphous forms in soil was likely to be greater for iron than for aluminium phosphate at any particular time after phosphate fertilization.

The stability of crystalline calcium phosphate reaction products have also been investigated by a number of workers. Bouldin and Sample (1959) showed that the relative rates of solution of dicalcium phosphate, both anhydrous and dihydrate, were proportional to the surface area of the fertilizer granules. They have found the dissolution of powdered fertilizers to be much faster than that of granules, granules of dicalcium phosphate larger than 60 mesh remaining partially intact in cropped soils for more than six months. Larsen et al. (1964) found no new phases present after incubation of brushite in four acid soils for 26 months, complete dissolution of these granules taking a further 13 months period.

From the published work, taken as a whole, it appears that the most important initial reactions products formed in acid soils after application of monocalcium phosphate are brushite ($CaHPO_4 \cdot 2H_2O$), monetite ($CaHPO_4$), potassium taranakite ($H_6K_3Al_5(PO_4)_8 \cdot 18H_2O$), amorphous aluminium and iron phosphates and calcium aluminium phosphates. Further alteration of at least some of these compounds is known to occur in soils with time and although conclusive evidence in the soil situation has not yet been obtained, due to the extremely slow rate of reaction,

it appears from artificial laboratory studies that strengite ($FePO_4.2H_2O$) and varascite ($AlPO_4.2H_2O$) may be the final reaction products in acid soils.

II B SOME FACTORS INFLUENCING THE AVAILABILITY OF PHOSPHATE AND ITS ABSORPTION BY PLANTS

II B 1 SOURCE AND METHOD OF APPLICATION OF PHOSPHATE

The chemical form of phosphate available for utilization and absorption by plants and the method of its application to the soil are both important factors in controlling the amounts of phosphate absorbed by plants.

II B 1.1 Inorganic phosphates

Since it is generally considered that the immediate source of phosphate for plants is as $H_2PO_4^-$ and HPO_4^{2-} in solution, the availability of any source is partly controlled by its solubility in the soil solution. However, in a soil situation where phosphate fertilizers react with the soil constituents the water solubility of the reaction products must also be considered.

Lindsay and Taylor (1960) reviewed the results of many glasshouse experiments and compared the abilities of various soil-phosphate reaction products to supply phosphate to plants grown in neutral soils. Huffman (1962) reported that the relative availabilities of these phosphate sources to plants closely paralleled their relative initial rates of solution. Where discrepancies occurred these could be at least partly explained by the large differences in surface areas that occurred between different phosphate sources. In general, phosphate availability to plants decreased in the order:- calcium phosphates > amorphous iron and aluminium phosphates > taranakites > crystalline iron and aluminium. Many other workers have also demon-

strated that dry matter yields and phosphate uptake were closely related to the degree of water solubility of the phosphate fertilizer applied to the soil (Lawton et al., 1956; Hagin and Berkovitis, 1961; Webb, Pesek and Eik, 1961; Beaton, Read and Hinman, 1962; Romsdal and Schmehl, 1964 and Terman and Allen, 1969). Engelstad and Moreno (1965) concluded that the main effect of water-soluble fertilizers was the increase in soil volume contacted by the fertilizer phosphate. In acid soils this may increase phosphate fixation and it is not surprising to find that Martin, Vlamis and Quirk (1953) and McLean and Logan (1970) reported no benefits from the use of water-soluble fertilizer phosphate in acid soils. In fact these workers found a decrease in plant phosphorus content as water solubility of the fertilizer increased and under these conditions they found banding the fertilizer or liming the soil increased phosphate uptake by the plant by reducing phosphate fixation. For the same reasons granulated water-soluble phosphates will be more efficient sources than liquid or finely powdered phosphates on acid soils, particularly those of high, reactive iron and aluminium content (Terman et al., 1960). With fertilizers of lower water solubility, such as dicalcium phosphates, Bouldin and Sample (1959) have correlated availability with the geometric surface of the granules and finely divided fertilizers of this type, mixed well with the soil, are usually more effective than when granulated or banded in soil (Terman et al., 1956; Hagin, 1958; Webb, Eik and Pesek, 1961).

From these reports it is obvious that the most efficient source of phosphate fertilizer and the best method of application to the soil is directly related to the properties of the particular soil in which plants are to be grown.

II B 1.2 Organic phosphates

These forms of phosphate must also be considered as likely sources of phosphate for plants. Although Pierre and Parker (1927) failed to show a decrease in the organic phosphate content of soil extracts and displaced soil solutions during a 24 hour absorption period by corn, soybean and buckwheat plants, later work by Rogers, Pearson and Pierre (1940) showed that corn and tomato roots under non-sterile conditions could utilize the pure organic phosphates, phytin, lecithin, nucleic acid and calcium glycerophosphate. Szember (1960) followed-up this work using sand and agar substrates containing lecithin and phytin as phosphate sources and was able to report a similar utilization by plants, this time under sterile conditions. More recently Wild and Oke (1966) have concentrated, purified and identified the organic phosphates in $CaCl_2$ extracts of dried soils. They identified three organic phosphate fractions all of which were at least partially utilized as sources of phosphate by clover plants grown under aseptic conditions. Most of the phosphate in each of the three fractions was identified as myo-inositol monophosphate.

Recently, Fardeau, Delille and Ambramovici (1968) have shown, using an inverse isotopic dilution procedure on an argillocalcareous soil, that at least some portion of the phosphate absorbed by ryegrass plants originated from sodium inositol hexaphosphate added to this soil. Different results were reported by Martin and Cartwright (1971) who compared ^{32}P labelled myo-inositol hexaphosphate and potassium dihydrogen orthophosphate as sources of phosphate for ryegrass grown on three soils differing in phosphate sorption capacity. Both sources were equally available to ryegrass in a coarse river sand but in a calcareous sand and a lateritic podzolic soil the availability of added myo-inositol hexaphosphate was essentially zero whereas uptake from inorganic orthophosphate was demonstrated in each case. It was concluded that the formation of insoluble salts and/or sorption reactions on sesquioxide and clay surfaces made inositol hexaphosphate completely unavailable to ryegrass grown in a soil situation. A similar resistance of inositol hexaphosphate to mineralization has been reported by Greaves and Webley (1969) using micro-organisms.

Thus although plants are able to utilize various organic phosphates as sources of phosphorus when grown in water, agar and sand cultures, they appear unable to do so in soil because the phosphate is rendered unavailable by chemical and physical reactions with soil constituents.

II B 2 SOIL FACTORS

II B 2.1 Soil physical properties

The physical properties of soils are known to affect both the rate of phosphate uptake and the rate of growth of plant roots (Lutz, 1952; Flocker and Nielsen, 1962; Menary and Kruger, 1966; Barley and Greacen, 1967).

The harmful effect of poor soil structure on the growth and rate of phosphate absorption of wheat plants has been reported by Murdock and Seay (1954). Later, the direct influence of coarseness of soil structure on plant absorption of phosphate and nitrate was studied by Wiersum (1962) using artificial aggregates of varying sizes. He found that nutrient absorption depended upon the density of rooting and the mobility of the nutrient in the system. Nitrate uptake remained fairly constant over the range of aggregate sizes but phosphate uptake was severely reduced in the coarse aggregate substrates. This result was attributed to the relative immobility of phosphate in comparison with nitrate and emphasises the importance of the degree of soil exploitation by plant roots when considering absorption of immobile nutrients such as phosphate. Gunary and Sutton (1967) have concluded that continued exploitation of fresh soil by root extension was required for plant absorption of phosphate over an extended time period.

Soil texture also has an important effect on phosphate availability. Phosphate adsorption by soil minerals is known to increase as soil particle size decreases due to the increased surface area and number of reactive sites on

lattice edges (Coleman, 1944; Perkins and King, 1944). Cole and Olsen (1959) explained differences in phosphate solubility behaviour between soils of different textures as the result of differences in measured soil surface area and the soil's capacity for monolayer phosphate adsorption. Olsen and Watanabe (1963) noted that diffusive transport of soil phosphate to a plant root depended upon three factors all of which were affected by changes in soil texture, viz. the phosphate diffusion coefficient, the concentration gradient between soil and root and the capacity of the soil to replenish soil solution phosphate absorbed by the roots. These workers were able to develop the following equation:-

$$\text{Rate of plant phosphate uptake} \propto \sqrt{bDp}$$

where Dp is the diffusion coefficient for phosphate in the soil and b is a measure of the soil's phosphate capacity. The rate of phosphorus absorption by corn plants grown in three soils of different texture was measured by Olsen and Watanabe (1963) and these values agreed closely with those calculated using their equation. In a clay soil the rate of phosphorus uptake by corn was found to be three times that in a fine sandy loam, both soils having equivalent concentrations of phosphate in the soil solution. Olsen and Watanabe (1970) extended this work and were able to calculate from their diffusion equation the amounts of fertilizer phosphate required to produce maximum yields of corn (i.e. optimum soil solution phosphate concentrations) for soils of three texture classes. These values agreed remarkably well with those obtained in a glasshouse

trial which measured the yield response to phosphate applied to these soils. The clay soil required only 3.5uMP in the soil solution for maximum yield while the fine sandy loam required 27.3 u MP. However, the clay soil required double the amount of fertilizer phosphate (40ugP/g soil) required by the sandy soil to establish the respective soil solution concentrations. This example illustrates the importance of soil texture when considering the phosphorus nutrition of soil-grown plants.

Soil bulk density is another physical factor affecting plant growth and phosphate absorption by the plant. In general high soil bulk densities reduce plant growth and phosphate uptake. Marked reductions in shoot and/or root growth have been observed as soil bulk density increased from about 1.0 to about 1.5 - 1.8 g cm^{-3}, (Flocker, Vomocil and Howard, 1959; Phillips and Kirkham, 1962; Menary and Kruger, 1966 and Mazurak and Pohlman, 1968). Flocker et al. (1959) and Menary and Kruger (1966) attribute the reduction in growth to a decrease in non-capillary porosity as bulk density increased, since sharp reductions in growth were observed below values of 25-30% for non-capillary porosity. Menary and Kruger (loc. cit.) also reported a decrease in phosphorus tissue concentrations and total uptake by tomato shoots as soil bulk density decreased, but the uptake of calcium and sodium, which were more mobile elements than phosphate in the soil system, were found to be independent of bulk density. This result highlights the role of root extension and exploration of the soil mass in controlling the uptake of phosphate by plants from soil.

The importance of soil strength as a factor influencing root growth in soil has been recognised within the last decade, (Phillips and Kirkham, 1962; Barley, 1963; Taylor and Burnett, 1964; Barley, Farrell and Greacen, 1965; and Mazurak and Pohlman, 1968). On the basis of these studies it appears that the uptake of phosphorus by crop plants grown in soils of high mechanical resistance would be reduced because of a restriction in root growth and therefore in soil exploitation.

II B 2.2 Soil temperature

Soil temperature may influence plant uptake of phosphate firstly by its effect on the solubility of phosphate within the soil system and secondly by its effects on plant growth and metabolic activity.

II B 2.2(a) Effect on the solubility of phosphates

Soil temperature affects the concentration of soluble soil or fertilizer phosphate in the soil system by determining the rate of mineralization or immobilization of organic phosphates and also the rate of solubilization or fixation of inorganic forms of phosphate.

Many workers have reported that the rate of mineralization of organic phosphate increases markedly with increases in soil temperature particularly above 25-30^{o}C (Thompson and Black, 1947; Van Diest and Black, 1959; Cunningham, 1963; Acquaye, 1963). For example, Acquaye (1963) found that incubation of Ghana soils at 50% water-holding capacity and at 27^{o}C for periods of from 14 to 70 days resulted in mineralization of organic

phosphate equivalent to the application of up to 54 lbs P/acre. More mineralization occurred at 50°C than at 27 or 40°C and from field experiments he was able to report that the yield response of cocoa was highly correlated with the organic phosphorus content of the 0.5 cm layer of the soil profiles.

Soil temperature can also affect the solubility of inorganic phosphates. Robinson (1942) and Beaton and Read (1963) found that the availability of water-soluble phosphate fertilizers to various plant species was greater after pre-incubation of the fertilized soil at low temperatures (15°C) than at higher temperatures. After incubation of MCP pellets in a calcareous soil for 14 days Hinman, Beaton and Read (1962) found significantly greater water-soluble phosphate in soil incubated at 5°C than in those incubated at 16 or 27°C. Beaton, Speer and Brown (1965) studied the dissolution of various phosphate fertilizers in two soils and discovered that the rate of dissolution of four of the five fertilizers increased markedly as soil temperature increased from 5 to 20 to 35°C. However, for each 15°C rise in temperature there was about a 33% reduction in the concentration of water-soluble phosphate in the soil-fertilizer reaction zone. These results were explained as an increase in the reversion of phosphate to less soluble forms as the temperature increased. Since none of the above workers considered the possible role of soil micro-organisms in immobilizing soluble phosphate the results obtained may also indicate that at higher soil temperatures a greater microbial

immobilization of soluble phosphate took place. However, the work of Van Diest (1968) suggests that this sequence is unlikely. He showed that increases in organic phosphate in fertilized, cultivated soils were due to the large quantities of plant remains, containing slowly mineralized organic phosphate, that were returned to the soil rather than to an increase in the soil microflora following fertilization and the subsequent increase in immobilization of soluble phosphate.

Obviously a number of different processes may be involved simultaneously and as noted by Power et al. (1964) the net result of a change in soil temperature on the solubility of soil and fertilizer phosphate will depend upon the relative rates at which these processes change with temperature. Factors such as biological activity, soil type, rate of phosphate addition and the temperature change involved will ultimately determine the extent and direction of the change in soil phosphate solubility.

II B 2.2(b) Effect on plant growth and phosphate uptake

The effects of soil temperature on plant growth and nutrition has received a great deal of study and has been thoroughly reviewed by Richards, Hagan and McCalla (1952), Went (1953) and more recently by Nielson and Humphries (1966). Less has been reported on plant phosphorus concentration and total phosphate uptake as affected by soil temperature although many studies have been made in the last decade.

Reductions in plant growth and phosphate absorption have been widely reported to occur at low soil temperatures (Apple and Butts, 1953; Ketcheson, 1957; Lingle and Davis, 1959; Wilcox, Martin and Langston, 1962; Knoll, Brady and Lathwell, 1964; Power et al. 1963, 1964). Knoll et al. (1964) for example, studying growth of corn in two soils found that dry weight, phosphorus content, and total phosphorus uptake of the shoot after 3 and 5 weeks growth all increased as soil temperature increased from 15-25°C. Power et al. (1967) analysed barley growth, as affected by soil temperature, and concluded that the rate of plant morphological development declined as the soil temperature was reduced from 22-9°C. A similar result was obtained by Beauchamp (1967) studying maize. In a later paper, Power et al. (1970) state that "in experiments in which plants of equal calender age are compared the effects of soil temperature on plant growth and nutrition are confounded by differences in physiological and morphological maturity. This confounding has often led to the erroneous conclusion that below optimum soil temperatures severely retard plant growth." Prior to this statement most studies compared plants at the same calender age and thus a reappraisal of the situation was required. Power et al. (1970) did this by growing barley to maturity at soil temperatures of 9, 15.5 and 22°C, comparing dry matter production and nutrient uptake at these temperatures for plants harvested at equal stages of morphological development. Their results indicate that soil temperatures per se are not detrimental to

growth and nutrition. In fact they found that at a given stage of plant growth dry matter production and nitrogen and phosphate uptake were usually lowest at the highest soil temperature of 22°C. Dry weights, grain yields and nutrient uptake at 9°C usually equalled or exceeded those at 15.5°C, the temperature considered optimum for barley production. Beauchamp (1967) has also reported that dry weights of maize roots and shoots harvested at identical growth stages decreased as the root-zone temperature increased. Power et al. (1970) did however report that at the lowest temperature (9°C) growth proceeded very slowly until the 4-leaf stage was reached, after which the growth rate approximated that at higher soil temperatures. These workers attributed this lag in early development to a reduced rate of nutrient transport from roots to shoots at low temperatures. Recent work by Patterson, Grunes and Lathwell (1972) has shown that under low temperature stress, incorporation of absorbed inorganic phosphorus into organic forms and polymerization of amino acids into proteins was restricted. These factors no doubt partly explain the depression in growth rate at low soil temperatures.

The effect of soil temperature on the type and amount of roots produced and on the phosphate uptake patterns of these roots has been studied in detail by Bowen (1970). Increasing soil temperatures from 15-25°C doubled total root length of seedling Pinus radiata, this effect being primarily due to a marked increase in the number and lengths of the lateral roots. A more

interesting result however, was the striking differences in uptake patterns of roots grown below 16°C compared with those grown at 25°C. Phosphorus uptake for roots grown at 25°C was greatest in the apical centimetre, decreasing sharply away from the apex while roots grown at 11, 14 and 16°C showed greatest uptake several centimetres behind the apex, the rate being maintained at approximately this level for 5-10 cms along the root. Bowen was unable to fully explain these results and indicated that further detailed investigation was necessary to interpret the reasons for these temperature-dependent uptake patterns.

II B 2.3 Soil moisture status

As for temperature, the influence of soil moisture on plant phosphorus nutrition results from a combination of the effects of moisture on the availability of soil phosphate to plants and its effects on plant growth and metabolic activity.

II B 2.3(a) Effect on phosphate availability

The reactions of phosphate fertilizers with soil have been reported to depend upon the level of soil moisture. Lehr, Brown and Brown (1959) and Bouldin, Lehr and Sample (1960) all observed that the residues from the dissolution of MCP in soil varied with soil moisture, the proportion of dicalcium phosphate dihydrate relative to the anhydrous form increasing with increases in soil moisture content. Lehr *et al.* (1959) observed, however, that the rate of dissolution and the extent of movement of phosphate from the fertilizer pellet was restricted as soil moisture

increased. They attributed this to a reduction in the movement of water vapour to the pellet thus restricting the formation of solution. These workers, together with Hinman, Beaton and Read (1962), also found that the phosphate content of the fertilizer residue and the amount of water-soluble phosphate present in the reaction zone after initial dissolution was decreased as soil moisture content increased. In contrast to these reports other workers (Beaton, Read and Hinman, 1962; Gough and Beaton, 1963) have reported very little effect of soil moisture on the solubility of added phosphates.

Conflicting results have been obtained concerning the effect of waterlogging on soil phosphates. Williams and Simpson (1965), Smith (1969) and Simpson and Williams (1970) found marked reductions in phosphate availability after temporary waterlogging of soils. Simpson and Williams (loc. cit.) also observed that phosphate extracted by 0.5M$NaHCO_3$ and 0.01M$CaCl_2$ solution decreased as moisture content increased to the saturation point. It was suggested that the decrease in availability was closely associated with the reduction of iron during temporary waterlogging and its subsequent reoxidation upon reduction of the moisture content to 100 cm tension. On the other hand Patrick and Mahapatra (1968) reported that flooding soils generally increased phosphate availability by reducing ferric phosphates to the more soluble ferrous forms and by releasing occluded phosphates from hydrated ferric oxide coatings as these become reduced to ferrous hydroxides.

From these few reports it is obvious that soil moisture can have a marked effect on both the types of fertilizer reaction products formed in soils and the solubility of soil and added phosphates.

II B 2.3(b) Effect on phosphate uptake

The effect of soil moisture on plant growth in general, has been reviewed by Richards and Wadleigh (1952) and more recently by Gates (1964, 1968) and will not be considered here; the main emphasis in this review being placed on the direct effects of moisture on plant phosphate absorption.

Many workers have reported an adverse effect of increasing moisture tension on plant uptake of phosphate. Increasing the moisture content of soil from wilting point to saturation levels was found by Brown, Place and Pettiet (1960) to result in significant increases in the uptake of N, P, K and Ca by cotton and soybean plants. Watanabe, Olsen and Danielson (1960) showed that an increase in soil moisture tension from $\frac{1}{3}$ - 9 bars decreased phosphate uptake by corn plants, the amount absorbed being a linear function of the soil moisture content for a given soil. They attributed this reduction in phosphate absorption to increases in the path length of ion movement through the soil and to decreases in both the rate of ion diffusion to the roots and in the rate of root elongation as the soil moisture tension increased. Likewise, Reichman and Grunes (1966) found that absorption of both soil and fertilizer phosphate by barley was reduced as soil was allowed to dry to high moisture tensions.

Results of short term phosphate uptake experiments at low water potentials in culture solutions support the conclusions presented above. Greenway, Hughes and Klepper (1969), using mannitol to adjust the water potential in solution, report that down to -5.4 atmospheres the amount of P^{32} in the root remained constant but that translocation to the shoot was restricted. Potentials of -10.4 atmospheres, however, reduced both shoot and root P^{32} levels in tomatoes. Even plants treated for one hour at low water potentials and then returned to control solutions at -0.4 atmospheres water potential had much lower rates of phosphate uptake than plants remaining continuously in control solutions. This data supports the idea that a disturbance in plant mineral nutrition is partly responsible for reduced plant growth after moderate water stress.

Somewhat conflicting results were presented by Fawcett and Quirk (1962) who grew wheat in a lateritic podzolic soil and found that absorption of phosphate was not affected by increasing soil moisture stress provided the plants remained undamaged by wilting. They found the plants derived available soil phosphorus mainly from fine soil pores which remained undrained at suctions approaching 15 atmospheres and therefore concluded that water stress per se and not reduced phosphorus absorption restricted plant growth.

II B 2.4 Soil sorption capacity and degree of phosphate saturation

Plant response to phosphate applied to soils is strongly influenced by the phosphate sorption capacity of the soil and the degree of saturation of this capacity.

Hughes and Searle (1964) working with a krasnozem soil showed that the response of tomato plants to current applications of phosphate was dependent on the total phosphate content of the soil, addition of a given amount of phosphate producing larger yields on the high phosphate soils. Kurtz and Quirk (1965) found a reduction in the phosphate adsorption maxima for a brunizem and a lateritic soil due to past phosphate applications but could find no reduction in a red brown earth. They therefore assumed that applied phosphorus did not permanently occupy adsorption sites but was converted to other forms. Obviously the rate of this transformation to other forms may depend on soil mineralogy and pH and would be expected to depend on soil type. Thus Kamprath (1967) has found with high phosphate fixing red soils that yield and phosphate uptake by corn, after banding 22 lbs P/acre, were significantly greater where application of phosphate had been made 8 years previously, the yield and uptake increasing with increases in the initial rates up to 612 lbs P/acre. Similar effects 9-10 years after the last phosphate fertilization have also been reported for a variety of soils by Fox, Plucknett and Whitney (1968) and Fox and Kamprath (1970). The first mentioned workers found the phosphorus requirement (i.e. the amount of phosphorus sorbed by the soil at an equilibrium concentration in solution of 0.2ppmP) varied from 40-1850ppmP depending on the mineralogical

composition of the soils and was in the order amorphous hydrated oxides $\rangle$ gibbsite - goethite $\rangle$ kaolin $\gg$ montmorillonite. Adsorption maxima for two of these soils were obtained using the linear form of the Langmuir isotherm and it was found the measured phosphorus requirements approximated ¼ saturation of these highly fixing Hawaiian soils. This agrees with the earlier work of Woodruff and Kamprath (1965) who found growth of millet was related to the degree of saturation of the phosphorus adsorption maximum; soils with high adsorption maxima required ¼ saturation while soils with lower adsorption maxima required much greater saturation to attain maximum yields.

II B 2.5 Soil pH

Soil pH has a considerable indirect influence on the phosphorus nutrition of plants by its effect on the availability of both inorganic and organic forms of phosphate in the soil.

It is generally recognised (Russell, 1961; Smith, 1965; Black 1968) that the type of phosphate compounds present in soil are at least partly controlled by soil pH and that in acid soils the iron and aluminium phosphates predominate. Hydrated aluminium and iron oxides are very effective in sorbing soluble phosphate from solution in the pH range 3.5 - 7.0 (Drake, 1967) but less than 10% of the phosphate adsorbed at pH 4.0 is solubilized by a rise to pH 6.0. With further increases in hydroxyl ion activity the phosphate is released according to the conventional but oversimplified equation

$$AlPO_4 + 3OH^- \longrightarrow Al(OH)_3 + PO_4^{3-}$$ (Black, 1968).

With calcium phosphates a decrease in pH causes dissolution of these compounds until below pH 5.5 all the phosphate is eventually released (Russell, 1961). Lindsay and Moreno (1960) have presented solubility diagrams for some of the major phosphate compounds and these indicate the stability of soil phosphates at any particular pH value. The importance of pH in determining the stability of a particular phosphate in soil has been stressed by Huffman (1962) and he concluded that soil-fertilizer phosphate reaction products, as a group, had a wide range of availabilities to plants, the extent of availability of a particular source depending upon its capacity to undergo chemical change in the soil environment in which it is placed.

Many workers, including Robertson et al. (1954), Singh and Seatz (1961), Stewart and McConaghy (1963), Munns (1965) and Helyar and Anderson (1970) have reported plant yield increases on application of lime to soils, thus increasing soil pH. In many cases this was attributed to the alleviation of aluminium and heavy metal toxicity associated with low pH values rather than to an effect on phosphorus availability. On the other hand Bouma (1960) has shown that a lowering of pH by application of $(NH4)_2SO4$ may enhance adsorption of phosphorus and reduce availability.

Besides its effect on inorganic phosphorus fractions pH may also effect the behaviour of organic phosphates. Thompson, Black and Zoellner (1954), Halstead, Lapensee and Ivarson (1963) and Kaila (1965) have all reported that part of the beneficial effect of an increased pH on phosphate availability in acid soils is due to mineralization of the organic

phosphate fraction. This may be due to a reduction in the sorption of organic phosphates by hydrous oxides thus increasing the solubility and the susceptibility of these compounds to mineralization. This hypothesis appears to be feasible since Halstead, Lapensee and Ivarson (1963) found the reduction in soil organic phosphorus content to be associated with an increase in both the number of micro-organisms present and in the amount of carbon dioxide produced.

A further effect of pH concerns the ionic form of orthophosphate found in solution. Olsen (1953) has calculated that in the pH range 4-9 only $H_2PO_4^-$ and HPO_4^{2-} ions are important, the latter predominating at pH's above 7.2. Hendrix (1967) reported that plant phosphorus uptake at pH 6.0 closely followed the concentration of $H_2PO_4^-$ in solution causing a marked reduction in uptake as pH increased. He inferred from this that phosphorus was only absorbed by the plant in the $H_2PO_4^-$ ion form. A similar marked reduction in phosphorus uptake with increasing hydroxyl ion concentration was also reported by Hagen and Hopkins (1955) but they concluded that this was the result of a competitive inhibition by hydroxyl ions.

These examples illustrate that, apart from its effect on the solubility of phosphates, pH can also greatly influence the rate of phosphorus uptake by plants from solutions of equal phosphorus concentration.

II B 2.6 Effect of other ions

The effects that the ionic composition of the rooting medium may have on the absorption of phosphate by the plant may be considered in two groups. Those effects producing

changes in the solubility of phosphate by chemical means and those acting by causing alteration in plant growth and metabolic activity.

II B 2.6(a) Direct chemical effects on phosphate solubility

A large part of the literature reporting the influence of other elements on phosphate absorption is concerned with the role of nitrogen. Grunes (1959) reviewed this work and showed that both pH and salt effects are important when considering the effect of nitrogen on phosphate availability.

The beneficial effect of acidic pH values (4.5 to 6.5) on phosphate uptake by plants has been considered earlier in this review and compounds that could maintain or alter the pH to within this range may be expected to increase the relative absorption of phosphate. Thus the enhanced plant uptake of phosphate in the presence of $NH4^+$ relative to $NO3^-$ or no nitrogen, observed by Blair, Miller and Mitchell (1970), Miller, Mamaril and Blair (1970), Blair, Mamaril and Miller (1971) and Riley and Barber (1971), was attributed in each case to differences in the soil pH at the root surface (rhizocylinder soil) induced by differences in the form of nitrogen applied. Both Blair et al. (1971) and Riley and Barber (1971) found that plant phosphate absorption was greatest when $NH4^+$ was applied and least when $NO3^-$ accompanied the phosphate. Reductions in the pH of rhizocylinder soil from soybeans was observed with $NH4^+$ while $NO3^-$ increased pH, the difference between nitrogen treatments being as high as 1.9 pH units in a soil initially

of pH 5.2 (Riley and Barber, loc. cit.). It was concluded that reduction in pH was caused by an exchange of H^+ in the roots for $NH4^+$ in the soil. Kirby and Mengel (1967) in solution culture and Riley and Barber (1969) in soil have similarly shown that the form of nitrogen controls the relative absorption of cations and anions which in turn controls the pH of the medium.

Other fertilizers besides nitrogen may be expected to similarly affect soil pH and ultimately also phosphate absorption. Thus K_2SO4 has been shown (Miller et al., 1970) to act like $(NH4)_2SO4$, but to a lesser degree, causing a reduced rhizocylinder pH relative to phosphate alone and increasing phosphate uptake. The acidifying effect of added elemental sulphur is also well established and a number of workers have reported an appreciable increase in phosphate availability of rock phosphate and other phosphate fertilizers in soils on the addition of elemental sulphur (Mitchell, Dehm and Dion, 1952; Seatz and Stanbery, 1963; Menary and Hughes, 1967).

Soluble salts can also influence the availability of native and applied phosphate in soils and these effects have been reviewed by Olsen (1953) and Grunes (1959). Lehr and van Wesemael (1952) found neutral salts applied to soil decreased phosphate solubility, the reduction in solubility decreasing in the order Ca>Mg>K>Na for salts with a common anion. These differences were reflected in plant phosphate uptake; wheat fertilized with $NaNO3$ having a 46% greater absorption than that found with $Ca(NO3)_2$

(Lehr and van Wesemael, 1956). Bouldin and Sample (1958) found the order of effectiveness of a number of salts in increasing plant phosphate uptake was $KNO_3 > (NH_4)_2SO_4 > NH_4NO_3 > NH_4Cl > KCl$. Bouldin, Lehr and Sample (1960) followed this work by studying the reactions that occurred between MCP – salt mixtures and soil. Salts affected both the amount of residual phosphate remaining at the granule site and the composition of the solution moving away from the granule. With $CaCO_3$, 92% of the original phosphate remained at the site while at the other extreme only 2% remained when $(NH_4)_2SO_4$ was mixed with the phosphate.

Mattson (1966) has explained the salt effect on the basis of the Donnan theory by postulating an electrical double layer at the soil solution - adsorbed phosphate interface. As a result a Donnan distribution of diffusible phosphate ions will occur between inner and outer solutions. A neutral salt tends to depress this distribution producing a new equilibrium in which outside phosphate activity is reduced at the expense of the inner resulting in a reduction in the measured phosphate solubility. Mattson (1967) also considers the root membrane as an ion exchange system similar to the soil colloids so that a neutral salt will depress the Donnan distribution of phosphate ions in the soil solution-root membrane system causing an increase in phosphate concentration at the root membrane and therefore an increase in phosphate uptake by plants relative to no salt-treatments. Addition of $CaCl_2$ to a soil in a pot experiment was found to increase the total phosphate uptake

by barley and pea plants thus supporting Mattson's theory accounting for the salt effect on phosphate uptake.

A further chemical effect that must be mentioned is the effect of additions of anions in counteracting the phosphate retention reactions in soils. Raupach and Piper (1959) in laboratory studies found that phosphate adsorption by soil was reduced in the presence of silicate and that the amount of phosphate released from soil was greater from silicate solutions than from water. In spite of these findings they were unable to show a significant improvement in plant utilization of applied phosphates when silicates were applied in pot trials. Hunter (1965), however, reported that calcium silicate significantly increased plant yields, total phosphate uptake and the proportion of the total derived from the soil phosphate when applied to soil in pot trials using four plant species. Deb and Datta (1967(a); 1967(b)) investigated the effects of both organic and inorganic anions on phosphate retention in soil. Organic anions such as citrate and tartrate were superior to inorganic anions in reducing the phosphate adsorption capacity of soils. Silicate performed the best of the inorganic anions tested and was in some cases as effective as the organic forms. The effects of both types depended considerably on soil properties, esp. pH. These workers concluded that the effectiveness of organic anions in acid conditions was due to their ability to form stable complexes with active iron and aluminium, reducing the formation of insoluble iron and aluminium phosphates. Inorganic anions such as silicate, reduced

phosphate adsorption reactions in a different way, mainly through anion exchange mechanisms and inactivation of exchangeable cations.

II B 2.6(b) Indirect effects on plant growth and metabolic activity

The numerous reports of an increase in phosphate uptake due to the biological effects of nitrogen on the plant have been reviewed by Grunes (1959). These effects include changes in root morphology, increased root growth and changes in the metabolic activity of roots.

Early work by Duncan and Ohlrogge (1958), Grunes, Viets and Shih (1958), Miller and Ohlrogge (1958) and Miller and Viz (1962) concluded that increased root growth in the presence of banded phosphate and nitrogen fertilizers was responsible for the large increases in phosphate uptake. Where the phosphate was finely divided and mixed throughout the entire soil volume, even in the presence of nitrogen, no stimulation of growth or plant phosphate uptake was observed (Grunes et al., 1958; Caldwell, 1960; Engelstad and Allen, 1971). Later work by Cole et al. (1963), Blancher and Caldwell (1966), Humble, El Leboude and Rendig (1969), and Thein and McFee (1970) has been unable to explain the nitrogen effect in terms of increased root proliferation and suggests that nitrogen affects the physiological processes that control phosphate absorption. Cole et al. (1963) and Thein and McFee (1970) agree that phosphate absorption and translocation rates are dependent upon nitrogen preconditioning of the roots and not merely upon simultaneous nitrogen and phosphate absorption. They

therefore conclude that a nitrogen requiring metabolite is involved and together with Humble et al. (1969) suggest that the stimulated phosphate uptake may result from an increased synthesis of nitrogen intermediates with a correspondingly greater turnover of "energy-containing" compounds, such as adenosine triphosphate, coupled to the phosphate absorption reactions. Direct experimental evidence confirming the operation of this sequence is, however, still required.

Besides the effects of nitrogen, phosphate uptake by plants is frequently stimulated by the presence of polyvalent cations. In both short and long term experiments in solution culture this effect has been reported for calcium, magnesium and aluminium by Ragland and Coleman (1962), Randall and Vose (1963), Leggett et al. (1965), Hyde (1966), Edwards (1968), Robson, Edwards and Loneragan (1970), and Franklin (1969, 1970, 1971). Increased phosphate uptake in the presence of calcium was thought by Leggett et al. (1965) to be due to an increase in the rate of turnover of phosphate carriers operating in the root. Most other workers have associated this effect with negative charges on the root. Hyde (1966) and Franklin (1969, 1970, 1971) found the effects of cations on phosphate uptake decreased in the order trivalent cations $>$ divalent cations $>$ monovalent cations and they suggested that pre-treatment or co-treatment with cations screened some negative changes on the root thereby reducing the electrical repulsion experienced by approaching phosphate ions. A similar effect of calcium in increasing the accessibility

of the root absorption sites to phosphate was proposed by Robson et al. (1970). Although Ragland and Coleman (1962) and Randall and Vose (1963) have shown an increased phosphate uptake in the presence of aluminium, the former workers, together with Foy and Brown (1964) and Lance and Pearson (1969), have also shown a decrease in plant phosphate uptake at higher concentrations of aluminium. Metabolic effects of aluminium in root tissue have been reported by Rorison (1965) and Clarkson (1966) who both found a restricted incorporation of absorbed inorganic phosphate into nucleotide and sugar-phosphate compounds in the presence of aluminium. In addition these workers have found that the increased phosphate content of aluminium treated roots was almost entirely due to non-metabolic adsorption of phosphate to aluminium ions held on cell wall material external to the plasmalemma. Clarkson found this phosphate to be almost completely exchangeable and concluded that failure to remove this from the root may give a false impression of enhanced phosphate uptake after treatment with aluminium.

II B 3 SOIL MICRO-ORGANISMS

The soil micro-flora can affect the phosphorus nutrition of plants by either directly influencing soil phosphate availability through solubilization-immobilization reactions or indirectly by affecting plant growth and plant metabolic activity.

II B 3.1 Direct effect on phosphate availability

Gerretsen (1948) appears to be the first to report an increase in plant utilization of insoluble phosphates as a

result of the presence of soil micro-organisms. Furthermore he was able to isolate the organisms responsible and show that they solubilized calcium phosphate suspended in nutrient agar. Subsequently many workers have isolated bacteria from soil and found them to release phosphate from insoluble compounds (Sperber, 1957, 1958a, 1958b; Louw and Webley 1959a, 1959b; Katznelson, Peterson and Rouatt, 1962; Raghu and Macrae, 1966; Taha et al. 1969; Louw, 1970; Bajpai and Sundara Rao, 1971a; Paul and Sundara Rao, 1971). The role of soil fungi in dissolving insoluble phosphates has received much less attention although some results concerning fungi isolated from soil have been reported (Katznelson, Peterson and Rouatt, 1962; Das, 1963; Chhonkar and Subba Rao 1967; and Agnihotri, 1970).

The marked differences in physical and chemical properties between agar or broth substrates and the soil situation make it difficult to extrapolate data obtained under laboratory conditions to the soil environment. Thus, although many workers have demonstrated the ability of various soil isolates to dissolve insoluble phosphates in vitro, few reports have shown dissolution to occur in soil. Also, apart from those of Gerretsen (1948) and the Russian workers (reviewed by Cooper, 1959) few studies have found plant utilization of insoluble phosphates to increase after additions of phosphate-dissolving micro-organisms to the growth substrate. An exception is the study of Bajpai and Sundara Rao (1971c) who found that in sterilized soil, containing apatite, the introduction of Bacillus megaterium, isolated from phospho-bacteria (U.S.S.R.) and Bacillus circulans, isolated from an Indian soil, increased

available soil phosphorus levels above those of uninoculated controls. Furthermore in pot and field experiments these organisms significantly increased crop yields and phosphate absorption above those of control treatments.

The various mechanisms involved in microbial solubilization of insoluble phosphates are now quite well established. Sperber (1958b) found that isolates of two bacteria, two fungi and an actinomycete from rhizosphere soil solubilized apatite by formation of acid end-products, the principal acids produced being lactic, glycollic and citric. She found chemical solutions of these hydroxy-acids were able to dissolve apatite and concluded that chelation of cations by these acids was the mechanism of dissolution. The majority of isolates tested by Louw and Webley (1959b) were found to produce mainly lactic acid, but these workers also found that isolates releasing the greatest amounts of phosphate from gasfa rock phosphate also produced 2-ketogluconic acid. Further work by Duff, Webley and Scott (1963) using a 2-ketogluconic acid producing bacterium isolated from the seed coat of oats showed this bacterium to solubilize 40-50% of the common calcium phosphates and silicates tested. Iron and aluminium were not chelated to the same extent as the divalent metals and naturally occurring iron and aluminium phosphates were usually fairly resistant, less than 5% of the material entering solution. Many other workers have reported organic acid production by rhizosphere isolates of bacteria and fungi (Chandrasekaran, 1969; Taha et al. 1969; Agnihotri, 1970; Louw, 1970; Bajpai and Sundara Rao, 1971b; Paul and Sundara Rao, 1971) and many of these have shown an inverse correlation between phosphate solubilization and the pH produced by the organism in the

culture medium.

Other workers (Bromfield, 1953, 1958; Rose 1957; Sperber, 1958a)have reported considerable release of phosphate from iron phosphates in soil by a reduction process involving hydrogen sulphide produced by soil bacteria and fungi. Bromfield (loc. cit.) even detected hydrogen sulphide production by strains of Bacillus megaterium in well aerated soils and showed that the hydrogen sulphide reduced ferric phosphate to ferrous sulphide liberating phosphate in soluble form.

A third type of microbial solubilization of insoluble phosphates has been documented by Tardieux-Roche (1964) and Tardieux-Roche and Tardieux (1970). They found that bacterial cells were capable of assimilating phosphate from insoluble sources and concentrating it in their cells as polyphosphates. Phosphate from killed bacterial cells previously cultivated on apatite was later found to be significantly more available to plants grown aseptically in sand culture than was apatite alone .

Conversely, soil micro-organisms unable to utilize insoluble sources or under conditions of phosphate stress may be expected to compete with plants for soluble phosphate, immobilizing this in bacterial metabolic products and thereby adversely affecting phosphorus availability to the plant. Akhromeiko and Shestakova (1958) found the addition of rhizosphere micro-organisms to sand cultures containing oak and ash seedlings reduced the uptake of labelled soluble phosphate over a one month growth period. However, the release of phosphate from the death of these organisms over a longer time period reduced the difference in phosphate uptake between inoculated and control pots. This indicates that phosphate immobilized in

microbial cells can be relatively quickly converted to plant available forms. Menzel (1971) obtained a similar result, finding that addition of bacteria to plants grown in sterile agar cultures inhibited phosphate uptake by the plant. These results, as they stand, cannot be unequivocally interpreted as microbial immobilization of phosphate since the micro-organisms may have also affected root physiological activity and metabolism (see Section II B 3.2(a)(ii)) and these different effects have not been considered in the above studies. The work of Barber (1966) and Barber and Loughman (1967) in solution culture suggests that micro-organisms at the root surface compete with the root for phosphate, especially at low concentrations of phosphate. Convincing evidence for microbial trapping of phosphate at the root surface was provided by the micro-autoradiographic studies of Crossett (1967) and Barber, Sanderson and Russell (1968) which revealed a marked accumulation of ^{32}P at the root epidermal cells. By a comparison of sterile and non-sterile root sections the latter workers found that no accumulation of phosphate occured at the root surface in the absence of micro-organisms.

The action of microbial phosphatase enzymes in the release of phosphate from organic combination, thus making it available to the plant, is another possible mechanism by which micro-organisms could directly influence the phosphorus nutrition of the plant. However, although phosphatase enzyme activity has been detected in a variety of fungi and bacteria isolated from soil (Casida, 1959; Szember, 1960; Saric, 1965; Greaves and Webley, 1965, 1969; Shiek and Ware, 1968; Theodorou, 1971) the importance of microbial phosphatase to the plant growing in soil has been, until recently rather illdefined. It now

seems clear that in the soil situation microbial phosphatase activity is much less important than previously thought. Ridge and Rovira (1971) for example found the phosphatase activity of intact seedling wheat roots was not increased by inoculation of roots with pure cultures of bacteria and fungi known to have phosphatase activity. In addition Greaves and Webley (1969) found that microbial hydrolysis of sodium myo-inositol hexaphosphate and release of inorganic ortho-phosphate could only be detected in sand culture. Where soil or sand-soil mixtures were used as substrates no hydrolysis occurred. Hydrolysis of the insoluble salts of myo-inositol hexaphosphate was found to be slight; the iron and aluminium salts being particularly resistant at pH 5.0 - 6.8 even in sand culture. Release of phosphate from sodium inositol hexaphosphate was reduced in the presence of clay minerals, especially montmorillonite and Greaves and Webley (loc. cit.) concluded that the formation of insoluble salts and/or sorption on sesquioxide and clay surfaces, as also shown by Anderson and Arlidge (1962), made inositol hexaphosphates in soil completely resistant to microbial phytases. This conclusion was supported by the later work of Martin and Cartwright (1971). This example illustrates the problems inherent in, and the caution required when extrapolating laboratory tests or sand culture experiments to the situation in the soil.

II B 3.2 Indirect effects of micro-organisms

Micro-organisms can have both detrimental and beneficial effects on plant growth and physiological activity. The overall effect of these microbial reactions and their influence upon phosphate uptake by the plant will depend upon their relative

magnitudes and their interaction with the soil environment. The indirect effects of rhizosphere micro-organisms on plant growth and nutrition have been extensively reviewed by Rovira (1965), Rovira and McDougall (1967), Barber (1968, 1969) and Bowen and Rovira (1969).

For convenience the indirect effects of free living rhizosphere micro-organisms and those of endotrophic mycorrhizaes will be discussed separately in this section.

II B 3.2(a) Free living rhizosphere micro-organisms

II B 3.2(a)(i) Indirect effects on plant growth

Bowen and Rovira (1961) have shown that the addition of soil micro-organisms to agar or sand cultures reduced primary root growth and the total number of laterals of four plant species in comparison to uninoculated controls. In addition the number and length of root hairs of subterranean clover but not of the other species were reduced when grown under non-sterile conditions. The degree of root stunting depended upon the soil type used to prepare the inoculum, the dilution of the inoculum and the environmental conditions during plant growth. The inhibitory effects were not observed in very dilute inoculum (1 to 100,000) and it was concluded that the effects were due to specific soil organisms, probably as a result of a build-up of phytotoxic metabolic products. The substances causing the inhibitory effects have not been identified but both antibiotics (Norman, 1959) and proteins (Ulrich, Luse and McLaren, 1964) are able to depress root elongation and root hair production. Similar depressions of plant growth in the presence of microbes have been observed by

Welte and Trolldenier (1965), Rovira and Bowen (1966) and Darbyshire and Greaves (1970) in solution culture experiments.

On the other hand stimulation of root growth after addition of fungal and bacterial filtrates to various plant species including barley, oats and tomato have been reported by Rempe and Kallagova (1965) and Bakalivanov (1965). They concluded, without attempting to isolate the compound, that a biologically active microbial product was involved. Jackson, Brown and Burlingham (1964) produced correlative evidence that the response of tomato plants to Azotobacter chroococcum inoculation was to the microbial production of gibberellin-like substances. It is known that micro-organisms produce both gibberelins (Katznelson and Cole, 1965) and auxins (Brian, 1957; Katznelson and Sirous, 1961; Domsch, 1965) and these substances influence various growth and developmental processes including induction of root initiation, root elongation and root growth in general (Leopold, 1964).

The importance of root structural properties in the phosphorus nutrition of plants has been stressed by Duncan and Ohlrogge (1958, 1959), Nye (1966) and Lewis and Quirk (1967) and therefore any microbial effects which change the roots' structural properties would be expected to influence phosphorus absorption by plants.

II B 3.2(a)(ii) Indirect effects on plant physiological activity

It is now well established that the presence of micro-organisms on plant roots can greatly influence both

phosphate absorption from the external medium and the subsequent metabolic and translocation processes that phosphorus undergoes within the plant tissues.

Barber (1966, 1967) and Barber and Loughman (1967) have found that in the absence of micro-organisms barley roots absorb up to twice as much phosphate as non-sterile roots and also that the amount transported to the shoot is as much as twenty times greater for sterile roots. These effects were greatest at low phosphate concentrations ($3\text{-}30\times10^{-8}$MP), where competition between microbe and plant would be expected to be greatest, but at higher concentrations of phosphate ($3\text{-}300\times10^{-5}$MP) little difference was observed between sterile and non-sterile roots in either uptake or translocation to the shoot. In addition, Barber and Loughman (loc. cit.) found that under sterile conditions greater than 85% of the total phosphate absorbed during one hour was incorporated into the acid-soluble fraction (i.e. inorganic, nucleotide and sugar phosphates) while under non-sterile conditions up to 70%, depending upon the phosphate solution concentration, was incorporated into nucleic acid, phospholipid and phosphoprotein fractions thus explaining the lower percentage translocation under non-sterile conditions. Experiments in which loss of phosphate from sterile and non-sterile roots was measured suggest that the differences between sterile and non-sterile roots in both uptake and incorporation are due to the direct effect of the micro-organisms rather than to an indirect effect of microbes on the plant's metabolism.

Results conflicting with those presented above have been presented by Bowen and Rovira (1966) who found phosphate uptake by non-sterile plants to be 85% greater for tomato and 45% greater for subclover when compared with sterile plants. Translocation of absorbed phosphate from root to shoot was also found to be greater for the non-sterile than for sterile roots. This indicates that the metabolism of the root itself is changed in the presence of micro-organisms and that this factor, as well as the incorporation pattern of the microbes, contributes to the overall difference between sterile and non-sterile roots. These results were verified in similar studies using wheat plants (Rovira and Bowen 1966 , 1968).

Although the results from the two schools of workers are in many ways in direct conflict both agree that more of the absorbed phosphate is incorporated into the acid-soluble phosphate pool in sterile roots than in their non-sterile counterparts while the reverse is true for the fraction consisting of nucleic acid and residual phosphates. The differences existing between the two schools were believed to be due to differences in plant species, plant age, pretreatment effects and other environmental factors (Rovira and McDougall, 1967; Barber, 1968). This conclusion seems reasonable in view of the fact that both pH (Barber, 1967) and pretreatment procedures (Barber, 1969) have been shown to drastically alter the effects of the plant-microbe interaction. Recently Barber and Frankenburg (1971) have presented results showing a greater rate of phosphate absorption (up to three times

greater) by non-sterile roots than by sterile roots over the concentration range 0.001 - 1.0 mMP. This result is the complete opposite to that obtained in the earlier work, Barber (1966, 1967), but coincides with the findings of Bowen and Rovira (1966) and Rovira and Bowen (1968).

As well as the effect of micro-organisms on the metabolism and translocation of phosphorus by the root, other more general effects of microbes on plant metabolic activity and growth may also be expected to ultimately influence plant phosphorus nutrition. Such effects include the increased activity of plant oxidizing and hydrolytic enzymes and the increased plant amino acid and chlorophyll contents found in non-sterile plants by Rempe and Katagova (1965).

II B 3.2(b) Endotrophic mycorrhizae

According to Gerdemann (1968) the endotrophic mycorrhizae can be grouped into those produced by septate fungi and those produced by non-septate fungi. The latter group called phycomycetous or vesicular-arbuscular mycorrhizae are especially important in the growth and nutrition of agricultural plants because of their occurrence on a wide range of crop species. Only this type of mycorrhizae will be discussed below. Gerdemann (1968) states that the Cruciferae and the Chenopodiaceae are the only plant families containing important species of crop plants in which endotrophic mycorrhizae are absent. Cabbage in particular has been found to be non-mycorrhizal after inoculation with various Endogone spore types (Bowen, private communication).

Plant responses to the presence of endotrophic mycorrhizae have recently been reviewed by Gerdemann (1968) and Bowen and Rovira (1969). Many workers have reported increases in plant growth and/or the amount of phosphate absorbed when plants, grown in sterile or partially sterile substrates of various types, are inoculated with vesicular-arbuscular mycorrhizae (Gerdemann, 1964; Holevas, 1966; Daft and Nicolson, 1966, 1969; Murdock, Jackobs and Gerdemann, 1967; Gray and Gerdemann, 1969; Hayman and Mosse, 1971, 1972). Yield responses to mycorrhizal inoculation have also been recorded for plants grown in non-sterile or untreated soil (Gerdemann, 1964; Hayman and Mosse, 1971; and Jackson, Franklin and Miller, 1972), although these tend to be smaller than those reported under sterile or partially sterile conditions. Daft and Nicolson (1966), Holevas (1966) and Murdock et al. (1967) using different plant species have all found that mycorrhizal and non-mycorrhizal plants grew equally well when the phosphate source was readily available and soluble, such as monocalcium phosphate. Yield differences were found to occur only where the source of phosphate was insoluble and slowly available such as apatite and tri-calcium phosphate. However, this effect may have been due to poor mycorrhizal infection associated with soluble forms of phosphate since Baylis (1967) and Mosse (1971) in soil culture, Daft and Nicolson (1969) in sand culture and Mosse and Phillips (1971) in agar have all found a reduction or even an elimination of mycorrhizal infection under high levels of available phosphate. In addition, Daft and Nicolson (1966) showed that the intensity or

level of root infection was a factor in plant stimulation, highly significant differences between inoculated and uninoculated plants only occurring where root infection was in excess of 50%.

The mechanisms involved in host stimulation by endotrophic mycorrhizae have only been investigated in the last few years. Strong evidence that the increased phosphate uptake of mycorrhizal roots was due to uptake by the fungus itself and not to a fungal stimulation of uptake by root cells was obtained by Bowen and Mosse (cited by Bowen and Theodorou, 1967) who demonstrated a much higher incorporation of ^{32}P labelled phosphate into fungal structures than into neighbouring plant cells. Further evidence of this was obtained by Gray and Gerdemann (1969) who found that a fungitoxicant reduced phosphate uptake by mycorrhizal roots but had no significant effect on non-mycorrhizal roots.

Fungal hyphae of mycorrhizal roots may increase phosphate uptake of the root by either utilizing phosphate sources unavailable to the root or by extending into soil not usually exploited by the root and absorbing soluble phosphate present in these areas. Hayman and Mosse (1972) found no differences in the specific activity of absorbed phosphate between mycorrhizal and non-mycorrhizal onion plants grown in eight soils labelled with ^{32}P thus indicating that both mycorrhizal and non-mycorrhizal roots had access to the same sources of soil phosphate. They concluded that the increased uptake by mycorrhizal roots was due to exploration, by the fungal hyphae, of soil beyond the normal zone of phosphate depletion around the root, thus supporting the earlier conclusions of Baylis (1970) and Sanders and Tinker (1971).

II B 4 PLANT FACTORS

II B 4.1 ROOT EXUDATES

Rovira (1969) has reviewed the relevant literature concerning the nature, the amounts and the factors affecting the production of exudates by plant roots. These compounds exuded into the substrate surrounding the plant roots play an important role in plant phosphorus nutrition. The stimulation of particular nutritional groups of micro-organisms by the presence of root exudates (Clarke, 1949; Starkey, 1958) is particularly important and the term rhizosphere is commonly used to indicate the zone of soil surrounding roots in which microbial activity is affected by the presence of the root. The influence of the plant on the microbial population will not be discussed in this review but the effect of these rhizosphere micro-organisms on soil phosphate availability and plant absorption of phosphate will be discussed in a later section. Apart from this rather indirect effect on phosphorus nutrition, root exudates, provided they remain active in the soil may also act directly by releasing phosphate from both inorganic and organic sources previously either unavailable or only slightly available to the plant.

II B 4.1(a) Effects on inorganic phosphates

Very few studies are available reporting the effects of root exudates on inorganic phosphates, due no doubt to the relatively small quantities of exudates produced and the subsequent difficulties encountered in experimentation. Nevertheless chemical compounds shown by *in vitro* studies to react with insoluble inorganic phosphates have been identified in root exudates. Vancura (1964) and Vancura and Hovadik (1965) examined the root exudates from two cereals and four vegetables, including cabbage, and were able [illegible] of organic acids including hydroxy

di- and tri-carboxylic acids. Some of these had previously been shown by Sperber (1958b) and Johnson (1959) to be very effective in solubilizing synthetic apatite, calcium tri-phosphate and a number of iron and aluminium phosphates. The possibility therefore exists that root exudates may act directly on insoluble phosphates and some workers (see Russell 1961 p.500) have attributed the ability of lupins in extracting soil phosphorus to the exudation of large amounts of organic acids from the roots. However, although qualitative analyses of root exudates have identified compounds capable of dissolving insoluble phosphates, it is the quantity of these components which is important in phosphorus nutrition. Riviere (1960) in one of the few quantitative studies of the organic acid content of root exudates found that a total of twenty milligrams of the four acids, acetic, propionic, butyric and valeric were produced by a single wheat plant grown to the tillering stage in sterile nutrient solution. However, because of the possibility of microbial metabolism or inactivation of these acids by physical or chemical reactions with the soil, the role of root exudates in inorganic phosphorus nutrition remains unclear.

II B 4.1(b) Effect on organic phosphates

A different situation occurs with organic phosphates where the role of plant phosphatase enzymes in releasing phosphate from organic combination has been studied in much greater detail. Szember (1960) growing radishes and Wild and Oke (1966) with clover, both showed that under sterile culture conditions the root phosphatases were able to break down a wide range of organic phosphates thus

providing a source of inorganic phosphate for plant growth. Ikaya, Nisizawa and Muva (1964) studied the specificities of several phosphatase enzymes from plant sources and concluded that the phosphatase from each source was composed of more than one iso-enzyme each of a different specificity range. They found the ability to hydrolyse simple organic compounds such as p-nitrophenol phosphate did not necessarily imply the ability to hydrolyse more complex compounds such as inositol phosphates, a point also stressed by Greaves and Webley (1965) with regard to breakdown by the soil microflora. The presence of enzymes capable of hydrolysing organic phosphates has been reported in extracellular sites in plant roots by Eastermann and McLaren (1961), De Jong (1965), Hall and Butt (1968, 1969) and Hall (1969); in mucigel formed on the root surface by Floyd and Ohlrogge (1970), and in the liquid medium surrounding plant roots by Strauss and Campbell (1963), Chang and Bandurski (1964), Thompson and Black (1970) and Ridge and Rovira (1971). Since some of these experiments were conducted under non-sterile conditions microbial activity may have been involved, although Eastermann and McLaren (1961), Saxena (1964) and Ridge and Rovira (1971) have all shown that under non-sterile conditions plant-produced phosphatase comprises most of the total activity present. The latter workers even reported a significant reduction in activity in the presence of soil micro-organisms. Most information concerning phosphatase activity of plants has been obtained from solution culture experiments and the contribution of root phosphatases to the phosphorus nutrition of soil-grown plants has been neglected due to the difficulties in

experimentation. However, because of sorption reactions with the soil constituents and the insolubility of many organic phosphates in the soil system, (Anderson and Arlidge, (1962), and Greaves and Webley (1969)) together with the possibility of rapid degradation of any root phosphatase by rhizosphere micro-organisms, the role of root phosphatases in phorphorus nutrition of plants in soil is likely to be insignificant. This conclusion would appear to be supported by the work of Thompson and Black (1970) who found the addition of phosphatase preparations to uncropped soil failed to produce a decrease in the content of organic phosphate.

II B 4.2 Root structure

Morphology of roots has for many years been qualitatively implicated as a factor affecting absorption of nutrients. With phosphorus for example, both Smith (1934) and Lyness (1936) have reported that for certain maize varieties, utilization of soil phosphate was directly related to root type, high ratios of secondary to primary roots producing a more efficient utilization than those with lower ratios. In the last decade or so quantitative measurements of phosphate absorption by various root types has clarified the earlier ideas of the effects of root morphology on nutrient uptake.

Russell and Sanderson (1967) have found quantitative differences in phosphate uptake between different types of intact barley roots by feeding ^{32}P to 3.5 mm-long segments of these roots. Absorption per unit length of root was greatest for laterals and least for the nodal axes but the fraction of the absorbed phosphate subsequently translocated to the shoot was greater for nodal roots than for laterals. Uptake per unit

length varied largely with the volume of the root tissue, a highly significant correlation being obtained between phosphate uptake and the volume of the root segment. This suggests that the volume of the free space or the number of cortical cells/ unit length (i.e. the total surface area of the plasmalemma exposed to the external solution) may be an important factor in determining the rate of phosphate absorption. In a soil situation however, where rate of diffusion may limit uptake the total external root surface area (which determines the volume of soil capable of being exploited) may be more important.

Bowen and Rovira (1967) and Rovira and Bowen (1968, 1970) have emphasized the importance of lateral roots in phosphate absorption. Using a gas flow chromatogram scanner to detect ^{32}P activity in single roots they found a major peak of phosphate uptake in the apical 3 cms of root followed by another peak of similar or greater intensity in the basal region, 6-8 cms from the apex, corresponding to the formation of lateral roots. Also, they were able to show that for 14 day-old wheat plants grown at 20°C the lateral roots accounted for 81% of the total phosphate absorbed. Kramer and Wiebe (1952), Canning and Kramer (1958) and Russell and Sanderson (1967) have also found the apical root region to be highly active in phosphate absorption and this implies that an increase in the number of apices per unit of root will increase the phosphate absorption capacity of the root system. Such a scheme may explain the second major peak of phosphate uptake observed by Bowen and Rovira (1967) in the zone of lateral root formation. However, the percentage of the absorbed phosphate that is translocated to the shoot has been found to be much smaller for the apical root region than for more basal segments, (Canning and Kramer, 1958; Russell and

Sanderson, 1967; Clarkson, Sanderson and Russell, 1968; Rovira and Bowen, 1968 and Burley et al. 1970). Using Eosin Y dye to detect movement of solution through xylem vessels of excised roots Burley et al. (1970) have correlated this with root anatomy by concluding that little or no translocation of phosphate occurred from the root apical region because of the absence of functional xylem.

The concept of Bray (1954) that nutrient ions such as phosphate, which are relatively immobile within the soil, can only be extracted by plants from the small volume of soil surrounding the root surface, indicates that factors capable of increasing root surface area would increase nutrient uptake capacity of the plant. One such factor is the presence of root hairs which are known to markedly increase root surface area, (Dittmer, 1937). Although the theoretical treatments of Bouldin (1961), Passioura (1963), Nye (1966) and Olsen and Kemper (1968) have provided detailed analyses of how root hairs could increase the absorption efficiency of roots, conclusive, direct experimental proof of the role of root hairs in phosphate uptake has been hindered by the lack of suitable control roots. Treatments that produce roots "artificially" free of root hairs invariably produce other anatomical or physiological changes, (Cormack, 1962). Studies such as that of Place and Barber, (1964) who measured Rb^{86} uptake by corn root segments, "naturally" with and without root hairs and attributed the increased uptake to root hair activity are inconclusive since the root sections were taken from plants of different age and pre-treatment and no doubt these would have had different absorption capacities per se. Lewis and Quirk (1967) presented indirect evidence that root hairs may be important in plant phosphate uptake from soils of

high phosphate fixing capacity. Using autoradiography with P^{32} labelled soil they found that the zone of depletion around wheat roots coincided with the length of the root hairs. The width of this zone remained relatively unchanged during the period 5-18 days after sowing and was independent of the levels of phosphate added to the soil. From these observations it was concluded that root hair morphology influenced the size of the depletion zones around wheat roots more than the rates of phosphate diffusion, which were very small in this soil. Drew and Nye (1970) have compared experimentally determined phosphate uptake for three plant species with uptake computed from model root systems either with or without root hairs. Experimental uptake by onion and leek roots could be accounted for using the model root without root hairs but phosphate uptake by ryegrass could only be accounted for if the root plus root hair model was used. The presence of root hairs was calculated to increase root phosphate absorption by over 200%. An appreciable increase in phosphate uptake from a clay soil due to the presence of root hairs has been measured by Barley and Rovira (1970) but they found uptake from a stirred solution was unaffected by root hairs. This was to be expected since root surface area is unlikely to be a factor limiting phosphate absorption under these conditions.

Root diameter and root elongation are two other factors likely to be important in the uptake of less mobile nutrients, such as phosphate from soil. For equal volumes of root, halving the root diameter will increase the root length four times and assuming an equal width of depleted soil around each root this will result in more than twice as much "depletion zone soil volume" for the root of smaller radius. Similarly, if nutrient

uptake is limited to a thin zone of soil around the root which is rapidly exploited, the rate of uptake will depend directly upon the rate of root elongation. Although models to assess the importance of root elongation on nutrient uptake have been suggested, (Passioura, 1963; Barley, 1970) no experimental studies concerning root elongation rate and nutrient uptake appear in the literature.

Most of the previous discussion has been concerned with the performance of single isolated roots. If one considers a field situation in which immobile nutrients are present in the soil profile the intensity of rooting (length of root per unit volume of soil) is important, as this determines the volume of soil accessible for exploitation by the plant. Cornforth (1968) has verified this for four species growing in different volumes of soil/sand mix which resulted in different root densities. Absorption of phosphate was directly correlated with root density while nitrogen uptake remained independent of root density. Thus responses to added phosphate were greatest in the larger volumes of soil where rooting density was lowest.

II B 4.3 Root physiology and function

While root structural configuration may be important in determining the rate and pattern of phosphate uptake, especially of soil grown plants, phosphorus nutrition of plants is also dependent upon various plant physiological and biochemical factors which control the absorption, translocation and metabolism of phosphate by plants.

Although entry and accumulation of inorganic ions by plant cells is known to be an active process dependent upon metabolically produced energy (Brouwer, 1965), the active

uptake mechanism is not yet clearly understood. A number of theories to account for active ion uptake into plant roots have been proposed and these have been reviewed recently by Jennings (1963), Brouwer (1965) and Robertson (1968). Active uptake of ions is most commonly described by employing the general concept of acid-base carrier systems. This concept envisages that a functional carrier system present in the impermeable membrane barrier combines with the ion at the outer surface forming a carrier-ion complex. This complex reorientates itself within the membrane and subsequently dissociates, releasing the ion into the compartment bounded by the cell membrane. Return of the ion is impeded by the relative impermeability of the membrane and the configuration of the carrier at the inner surface which favours dissociation rather than ion binding. Epstein and Hagen (1952) suggested that this system was analagous to the reaction mechanism of enzyme catalysis and they were able to apply classical enzyme kinetics (Michaelis - Menten 1913) to the absorption of rubidium by excised barley roots. Following this study a large amount of data on ion uptake has been built up from experiments based on the kinetic model of these workers. This approach is, however, not without criticism. Steward and Sutcliffe (1959) maintain that such mathematical interpretation from an empirical standpoint is of limited value and has little meaning until the carrier systems and their specific mechanisms are identified. Other objections have been reviewed by Brouwer (1965) and Leggett (1968). Despite this criticism the enzyme-kinetic model of carrier mediated absorption has been applied to, and found to describe many diverse systems including long distance ion transport in plants, (Lüttge and Laties, 1966, 1967; Edwards, 1970), ion uptake and transport in leaf tissue,

(Smith and Epstein, 1964; Phillips, Baker and Clagett, 1971), ion accumulation in excised phloem tissue (Bielesk, 1966) as well as ion uptake by micro-organisms (Conway and Duggan, 1958; Leggett, 1961; Leggett, Heald and Hendricks, 1965). Perhaps the most useful achievement of the kinetic model of the carrier theory has been the description of ion interactions associated with membrane selectivity, (Epstein, 1962; Epstein and Hagen, 1952; Epstein, Rains and Elzam, 1963). The overall success of this model in accounting for ion uptake in a wide range of tissues has given credance to the carrier theory and extended our knowledge of the process of active ion absorption.

Characterization of phosphate uptake by roots, in terms of the kinetic model of the carrier theory was first undertaken by Hagen and Hopkins (1955). Rates of phosphate absorption by excised barley roots from phosphate solutions ranging in concentration from 1.0-100uM and in pH from 4.0-7.7 were measured and these workers concluded that the ion species $H_2PO_4^-$ and HPO_4^{2-} were absorbed through separate carrier sites. The uptake of both ions was competitively inhibited by OH^- ions and the extent of absorption by each site was dependent upon substrate concentration and pH. Subsequent studies by Hagen, Leggett and Jackson (1957), Noggle and Fried (1960), Leggett et al. (1965), and Andrew (1966), in the same phosphate concentration range and at pH 4.0, have all been interpreted on the basis of simultaneous absorption of the ion species, $H_2PO_4^-$ and HPO_4^{2-}, through different carrier sites. These have been designated as the "a" and "b" reaction sites. Hagen, Leggett and Jackson (1957) proposed that the "a" reaction, which predominates at high concentrations (0.5-1.0mM), with a low affinity for phosphate, was concerned with uptake of $H_2PO_4^-$

while the "b" reaction, which became saturated at low phosphate concentrations (0-25uM) and had a high affinity for phosphate, was concerned with absorption of $HPO4^{2-}$. The interpretation that two ion species are involved in phosphate absorption has recently been challenged by Carter and Lathwell (1967) and Edwards (1968a, 1970) since at pH 4.0 the $H2PO4^{-}$ ion is considered to be the only ion of any consequence involved in absorption reactions. Consideration of the ionization equilibria of othophosphoric acid by Edwards (1968a) has indicated that at pH 4.0, 98.6% of the total phosphate is present as the $H2PO4^{-}$ ion form and only 0.06% as $HPO4^{2-}$ ion. From temperature studies of orthophosphate uptake by excised maize roots, Carter and Lathwell (1967) also concluded that both "a" and "b" reactions were involved with uptake of the $H2PO4^{-}$ ion and they also showed that in both cases chemical reaction was involved (i.e. active uptake occurred).

Unlike previous experiments on root absorption of phosphate, which had all been performed with solution concentrations in the range 0.001-1mMP, Edwards (1970) studied uptake by intact wheat seedlings over the concentration range 0.0001-50 mMP. Kinetic analysis of his results has led to the characterization of three distinct mechanisms of phosphate absorption; two operating in the range 0.0001-1mMP corresponding to those previously described by Hagen and Hopkins (1955) plus a third mechanism which shows a linear response to phosphate concentration and operates only in the range 1-50mMP. This third mechanism is similar to the absorption isotherms for Cl^{-} and Rb^{+} obtained by Torii and Laties (1966) with non-vacuolate maize root cells (also in the concentration range 1-50mM) and attributed by these workers to a diffusive permeation of ions across the plasmalemma. Edwards (1970) concluded that his

results with phosphate also indicate a passive absorption across the plasmalemma at high phosphate concentrations (lmMP). The operation of two mechanisms of phosphate uptake below lmM is in contrast to the single mechanism obtained for Rb^+, K^+ and Cl^- in the same concentration range (Jackman, 1965; Epstein, Rains and Elzam 1963; Luttge and Laties, 1966; Torii and Laties, 1966; and Welsh and Epstein, 1968). Edwards (1970) has asserted that one of these phosphate absorption mechanisms (Km $2x10^{-4}$ MP) must be located at the plasmalemma while the second (Km $7.4x10^{-6}$ MP) may be either a plant mechanism also located at this membrane or may be a microbial absorption mechanism associated with the root micro-flora, the roots being cultured under non-sterile conditions. In support of the latter proposal he shows that the mechanism with a Km constant of $7.4x10^{-6}$ MP closely corresponds with that of the high affinity mechanism of phosphate absorption found by Leggett (1961) for yeast and Bowen and Theodoru (1967) for excised mycorrhizas of Pinus radiata.

Recently the microbial component of uptake has been studied by Barber and Frankenburg (1971) by comparing phosphate absorption of seedling roots of barley grown, prior to excision, under sterile or non-sterile conditions. The difference in phosphate absorption between sterile and non-sterile roots was attributed to uptake by micro-organisms and over the concentration range 0.001-1.0mMP, phosphate uptake by the microbial component of non-sterile roots was found to exceed that of sterile roots. This method of deriving the microbial component of uptake is, however, open to criticism since it assumes that interactions between root and micro-flora do not occur, the uptake by non-sterile roots being a simple summation of the

individual microbial and root components. This is unlikely since Bowen and Rovira (1966a) have evidence that the metabolism of the root itself is altered in the presence of micro-organisms. Also the work of Brian (1957), Katznelson and Sirous (1961) and MacDonald (1967) indicates that micro-organisms can produce compounds which may influence the growth and metabolism of the root. If microbial products are produced which stimulate nutrient uptake by the root the method of Barber and Frankenburg (1971) will tend to over-estimate the microbial component of the total uptake of non-sterile roots, and vice versa. Disregarding this objection, these workers found the relationship between microbial phosphate absorption and substrate concentration followed the kinetics of a first order reversible reaction. The characteristics of this reaction; a rapid saturation at low phosphate concentrations and a Km constant of 13×10^{-6}M appear to be similar to those of the "b" reaction of phosphate absorption identified in non-sterile roots by Hagen and Hopkins (1955). However, no similar kinetic relationship could be derived by Barber and Frankenburg (1971) to describe the experimental uptake of phosphate by sterile barley roots. Despite the criticism mentioned above, this study strongly suggests that the low concentration, high affinity "b" reaction of phosphate uptake, identified in non-sterile roots, is due to a microbial component associated with these roots. By elimination, the "a" reaction would therefore be associated with the root tissue itself.

In contrast to the results of Barber and Frankenburg (1971), Cartwright (1972) has been able to kinetically analyse his data for phosphate uptake by sterile, excised barley roots and has been able to show that the two first order reactions

of the dual absorption isotherm were present in sterile roots (i.e. both uptake mechanisms were of plant origin and not only one as suggested by Barber and Frankenburg, 1971).

Although the sterile roots used in the studies of Barber and Frankenburg (loc. cit.) and Cartwright (1972) were grown under different cultural conditions, this is unlikely to explain the direct conflict in the interpretation and the results of these studies. Obviously further investigation is required to distinguish and clarify the microbial and plant components of phosphate absorption.

II C REPORTED DIFFERENCES BETWEEN PLANT SPECIES IN RESPONSES TO PHOSPHATE

II C 1 INTRODUCTION

Thomas (1930) was one of the first to review the subject of nutrient variation between species. Even at that time considerable literature on the subject had accumulated and he reviewed the various hypotheses that had been advanced to explain differences in the "feeding power of plants", including ideas that differences in root membrane permeability, root exudates and the extensiveness of the root system may account for the variation. Although none of these hypotheses could be substantiated at that time these factors are now known to influence nutrient uptake.

Many workers have since found that plant species respond differently to additions of phosphate to soil or sand cultures. However, until recently few workers had made follow-up studies to critically evaluate the mechanisms behind these differences. Lilliland, Brown and Conrad (1942), for example, tested the responses of a large number of annual and fruit tree crops to superphosphate additions to a phosphorus deficient soil. Squash, lettuce and cucumbers grew poorly in control plots and gave the greatest yield responses to added phosphate. These were followed in order of decreasing response by cereal crops, legume crops and finally fruit tree crops which gave either very small responses or no response to applied phosphate. In sand culture experiments with vegetables, Woodman (1944) also found large differences between species in the response to soluble phosphate. Lettuce and carrots produced response curves different from, and obtained maximum yields at phosphate levels many times greater than those found for the other

vegetables which included turnips, peas and cabbage. In a similar way, Bradshaw et al. (1960) found the yield response curves of eight grasses to soluble phosphate varied widely depending upon the particular species.

Plant species have also been reported to differ in their abilities to utilize insoluble phosphates, such as rock phosphate. Qualitative experiments have ranked members of four plant families in the order; Leguminosae > Cruciferae > Gramineae > Solanaceae as regards their utilization of sparingly soluble phosphates (Marais, 1922). Recently, Johnston, Warren and Penny (1969) have reported swede and kale to be more efficient than cereals, sugar beet and potatoes in utilizing phosphorus from residues of old phosphate fertilizer applications.

In general terms differences between plant species in their response to phosphorus applications may be due to differences in the absorption of phosphate or due to differences in the utilization of the absorbed phosphate within the plant.

II C 2 DIFFERENTIAL PHOSPHATE ABSORPTION BETWEEN SPECIES

Differences between plant species in absorption of phosphate may be due to a differential utilization of phosphate from sources of different availability or alternatively may be due to differences between species in their rate of uptake from the same phosphate source.

II C 2.1 Differential utilization of soil phosphates

Because of gross differences between plant species in their physiological processes or in their microbial populations the availability of adsorbed phosphate or insoluble phosphate

compounds in the soil system may be expected to vary with the particular plant species involved. The L-value concept of Larsen (1952) or the A-value concept of Fried and Dean (1952) have been widely used to obtain evidence of this occurrence. The A-value and L-value, although conceptually different, are both based on the same mathematical treatment and can be determined using radio-active phosphate and tracer techniques. The L or A-value (the quantity of soil phosphate available to the plant) is calculated from the isotopic dilution equation

$$L \text{ or } A = B\left(\frac{SF}{Sp} - 1\right)$$

where B = amount of fertilizer phosphate added to the soil and SF and Sp = specific activity of phosphate in the fertilizer and plant material respectively.

The advantage of using this technique is that the value does not depend upon the extent or size of the root system nor on the total amount of phosphate absorbed. Thus when L-values determined for a number of plant species on the same soil type are found to be different it can be concluded that phosphate absorption has occurred in these species from different forms of phosphate.

Fried (1953) using monocalcium phosphate as the fertilizer found little difference between species in A-values obtained from untreated soils, however, where the soil had been previously enriched with rock phosphate he found up to two fold differences in the A-values of different plant species. Phosphate from rock phosphate was utilized most efficiently by buckwheat and was more available to legumes than grasses or cereals, a result confirmed by later work of McLean and Hoelsher

(1954) and McLean (1956). Russell, Russell and Marais (1958) found higher L-values for cabbage and rye than for barley when grown on a basaltic soil having large quantities of isotopically exchangeable phosphate. These differences were attributed to the greater ability of cabbage and rye to lower the free energy of phosphate in the soil solution (i.e. labile phosphate at low potential was believed to be inaccessible to barley).

Nye and Foster (1958) conducted a number of experiments on different soil types and measured the L-values of a wide range of plant species from the families, Leguminoseae, Cruciferae, Gramineae and Solonaceae. Although marked differences occurred in the total phosphate absorbed by the different species only small and agriculturally insignificant differences were found in the ratio of $^{31}P:^{32}P$ absorbed by these plants and they concluded that all species obtained their phosphate from the same pool of labile soil phosphate. Similar conclusions were drawn by Jenkins (1962) with perennial ryegrass and clover and by Keay, Biddiscombe and Ozanne (1970) working with a range of annual pasture species.

Results contrary to these have also been reported recently. Ozbek (1967) found A-values of corn and oats to be 300% greater than that for millet while Kalra and Soper (1968) reported differences of up to 200% between A-values for soybeans, rape, oats and flax. Very large differences were found between twelve species examined by Kalra (1971). The A-values decreased in the order soybeans $>$ flax $>$ peas $>$ wheat $>$ rape $>$ mustard with a ten fold difference occurring between soybeans and mustard. It should be noted, however, that A-values reported by Ozbek (1967), Kalra and Soper (1968) and Kalra (1971) were all calculated from analysis of plant tops only, no corrections

being made for the presence of seed-borne phosphate in the shoots. This practice tends to overestimate the A-values for all species, being greatest for the large seeded species such as peas and soybeans. This would partially explain the large differences in A-values between species, especially in the case of Kalra (1971).

Marais et al. (1970) calculated L-values for twelve plant species in eight soils from analysis of both root and shoot tissue with a correction for seed-borne phosphate. In contrast to the results of Kalra (1971), in most soils no difference or differences of less than 10% were recorded for L-values of different plants. An exception occurred in two soils containing rock phosphate where cabbages showed a 39% higher L-value than that found for rye. The relatively small differences reported in this study were concluded to be due to the corrections made for seed phosphate. Marais et al. (1970) did in fact calculate the L-values using the shoot tissue data only and found that the differences observed with rye and cabbage were not apparent in L-values calculated in this way. L-values for maize exceeded those of rye by 50% when calculated on shoot data, but from whole plant data no difference could be shown. Although obvious difficulties in this method have been shown to produce erronous results, it can be concluded that certain species have an ability to utilize forms of phosphate unavailable to others, rock phosphate appears to be especially important in this regard.

Various mechanisms have been put forward to account for differential phosphate availability between species. Drake and Strekel (1955), Asher and Ozanne (1961) and Fox and Kacar (1964) have all correlated the ability of plant species to utilize

sparingly soluble calcium phosphates with their root cation exchange capacity (C.E.C.). Drake and Strekel (1955) attributed the species difference to differences in "the bonding of calcium by plant roots resulting in differential dissolution of rock phosphate crystals", and this theory was supported by the work of Asher and Ozanne (1961) and Fox and Kacar (1964). Additional support has recently come from a study by Crooke and Knight (1971) who found distinctly different cation translocation patterns between monocots (low C.E.C.) and dicots (high C.E.C.). The former favour translocation of monovalent ions thus leaving their roots richer in divalent cations while the opposite was found to occur with the dicot species. Other workers, (Broeshart, 1962; Vose, 1963) have found no correlation between root C.E.C. and cation absorption by roots and Fried and Broeshart (1967) after reviewing the literature were able to conclude that "cation exchange capacity of roots does not appear to be directly related to ion uptake". At the present time it is generally considered that a specific adsorption of ions to carrier sites in the root is the first step in ion uptake but that non-specific adsorption has little effect on ion absorption except in the case of anion uptake where non-specific adsorption of cations may reduce the net negative charge on the root and enhance anion uptake (see Section II B 2.6(b)).

Production of different types or amounts of chemical factors by either the rhizosphere micro-organisms or the roots of different plant species may also explain differential utilization of sparingly soluble phosphates. It is well known that organic acids are very effective in dissolving calcium phosphates (Sperber, 1958(b); Johnson, 1959; Louw and Webley, 1959(b); Duff, Webley and Scott, 1963) and Talibudeen (1957)

has found that increasing concentrations of citrate ion added to soil increases the amount of exchangeable soil phosphate by dissolving the very slowly exchangeable solid phase phosphates. However, although both micro-organisms (Katznelson, Peterson and Rouatt, 1962; Louw, 1970) and sterile plant roots (Riviere, 1960; Vancura, 1964) are known to release organic acids into the external medium no direct attempts have been made to implicate these substances as one cause of differential phosphate solubilization between plant species.

II C 2.2 Differential phosphate absorption from a common phosphate source

Although differences between species in root size have in the past (Thomas, 1930; Smith, 1934) been used to explain the observed interspecific variations in phosphate uptake from a soluble phosphate source the recent studies of Russell and Sanderson (1967) and Rovira and Bowen, (1968) showing variation in the rate and pattern of phosphate absorption along the length of a single root as well as roots of different types, has made direct correlations of root size and total phosphate uptake difficult if not impossible to interpret, especially where whole root systems of different species are involved. The complexity of the root system in terms of its absorption capacity has meant that few recent studies have attributed interspecific differences in phosphate absorption to differences in root size per se. In most cases a combination of plant factors has been required to fully explain these differences in phosphate uptake.

Where available phosphate is restricted within certain areas or profiles of the soil, the configuration and distribution of the root system becomes an important factor in determining the amount of phosphate absorbed. Nye and Foster (1961), for example, found that short term crops such as millet, maize and pigeon pea made less use of subsoil phosphate than perennial grasses and dicots in the natural savanna vegetation which derived more than 30% of their total phosphate from this soil layer. Large differences between species in absorption of fertilizer phosphate from a calcareous soil were concluded by Soper and Kalra (1969) to be due to differences in the extent of rooting in the fertilizer reaction zone. Recently, Baker et al. (1970) suggested that differences in the depth of rooting may explain differences between corn hybrids in the accumulation of phosphate in the ear leaves. Later work by Baker, Wooding and Johnson (1971) showed that genetically controlled accumulation of phosphate by corn was associated with physiological processes within the plant that were independent of the depth of rooting.

Differences in the rate of phosphate absorption, especially from sources of low concentration, have been frequently used to explain or partially explain interspecific differences in phosphate absorption from a common soluble phosphate source. Solution culture experiments of Tidmore (1930) and Sommer (1936) using large volumes of solution at low concentrations of phosphate (0.05-13.00ugP/ml) were some of the first to show that plant species differed in their response to a common source of soluble phosphate. Later studies using ^{32}P

added to soil have indicated that species also differ in their ability to absorb phosphate from low concentrations in the soil solution (Nye and Foster, 1958; Jenkins, 1962). The study of Loneragan and Asher (1967) has emphasized the wide differences that occur between species in their rates of phosphate absorption from solutions of low concentration. For eight annual pasture species the mean rate of phosphate uptake/unit weight of roots was found to vary by as much as 400-500% when the phosphate concentration was maintained at 0.04uM. However, where phosphate concentrations were maintained at 24uM the mean rates of phosphate uptake of all species except lupins were within a narrow range, varying from 9.8-13.9 ug atoms P/g fresh roots/day. Clear differences between species in their specific rates of phosphate absorption at both low and high concentrations of phosphate in the soil solution have been shown by Keay, Biddiscombe and Ozanne (1970). Relative to their maximum rates of phosphate absorption these workers found the grasses had higher rates of phosphate uptake/unit weight of roots than the clovers at low phosphate concentrations in the soil solution. In a series of clever grafting experiments with tomato and datura plants, Otsuka (1968) showed that differences in the rate of phosphate uptake between these species were due to physiological factors residing in the roots and that these remained with the root and were operative even when the roots were grafted to shoots of the other plant species.

Such interspecific differences in the rate of phosphate uptake/unit of roots have been explained by some workers on the basis of differences in the components of the active uptake mechanism determined from kinetic analysis of absorption data

assuming a carrier theory of ion uptake (Epstein and Hagen, 1952; Hagen and Hopkins, 1955). On this assumption a number of factors could act as variables and cause differences between species in the rate of phosphate absorption. These include differences, in the carrier concentrations in the roots, in the affinity of the carriers for phosphate (apparent dissociation constants) and/or in the rate of turnover of the carrier-phosphate complex (rate constant).

Noggle and Fried (1960) reported that the rate of phosphate absorption by excised roots of three species decreased in the order millet > barley > alfalfa. Kinetic analysis of their data according to the methods used by Hagen and Hopkins (1955) showed that although there were small differences between species in their rate constants and apparent dissociation constants the rate of uptake for both reactions (a and b) was primarily controlled by the carrier concentrations, those for millet being almost twenty times those found for alfalfa with barley having intermediate concentrations. Andrew (1966) made a similar study of phosphate uptake by three tropical legumes plus lucerne and barley as reference species. At both low ($1x10^{-6}$MP) and high ($2x10^{-4}$MP) substrate concentrations the rate of absorption/unit weight of roots was three times greater for Townsville lucerne (Stylosanthes humilis) than for the other four species, which had similar rates of uptake. Andrew's results could not be explained on the basis of differences in carrier concentration but were attributed to a much greater relative proportion of the total uptake being contributed by the low concentration, high affinity, "b" reaction mechanism in the case of S. humilis. In line with this the rate constant of the "b" reaction of S. humilis was five times greater than that of the other species.

Differences between species in the number of excised roots/g weight or in the type and length of root sampled for experimentation may explain some of the observed differences between species since the pattern and rate of phosphate uptake is known to vary along the length of a single root (Russell and Sanderson, 1967; Rovira and Bowen, 1968), being highly active at the apex and in the zone of lateral root initiation. This approach does not explain the results of Noggle and Fried (1960), however, since the length of root sampled and the number of roots/g sample were approximately the same for both millet and alfalfa although these species differed in carrier concentration. Large differences between species were also apparent in the root properties of the samples used by Andrew (1966). Townsville lucerne had the greatest number of roots/g fresh weight (443) and the shortest length (3.4 cm) while at the other extreme barley had the smallest number of roots/g fresh weight (44) and the longest length (15.5 cm). Thus the high proportion of very actively absorbing root apices in the case of S. humilis root samples may have been responsible for its higher rate of short term phosphate uptake in comparison with the other species.

Alternatively since these experiments were all carried out under non-sterile conditions microbial differences between plant species may be involved in the differential rates of phosphate absorption observed by these workers. Barber and Frankenburg (1971) have presented evidence associating the phosphate absorbed by the "b" reaction mechanisms with uptake of phosphate by the micro-organisms present on the root surface. If this is so then it suggests that the disproportionately high rate of phosphate uptake observed for the "b" reaction mechanism of S. humilis (Andrew 1966) was the result of greater

numbers and/or metabolically more active micro-organisms being associated with these roots in comparison with those of the other species. Although more research is required on the role micro-organisms play in the total phosphate absorption of non-sterile roots the concept that differences between species in rates of phosphorus uptake may be due to microbial differences appears quite feasible especially as Elkan (1962) has shown that differences between plants of a single gene are sufficient to produce differences in the microbial populations around the plant roots.

From the above appraisal of the literature it is evident that many different factors can contribute to the absorption of phosphate by non-sterile plant roots. Where comparisons between species are to be made the conditions during plant growth and the selection of root tissue for experimentation must be rigidly controlled so that the effect of various factors can be examined individually and meaningful conclusions can be obtained.

II C 3 DIFFERENTIAL UTILIZATION OF ABSORBED PHOSPHATE WITHIN THE PLANT

In general terms a difference in total phosphate absorption within a particular plant species may be expected to produce different yields, provided the difference is not due to luxury consumption of phosphate. This, however, does not usually apply when different plant species are involved since responses to phosphate in terms of yield also depend upon the species relative efficiency in utilizing the absorbed phosphate for growth. Thus, for example, a yield difference between species may occur even though the total amount of absorbed

phosphate is identical if either, the species differ in their distribution of phosphate between roots and shoots or differ in their phosphate utilization quotients (i.e. units of dry plant material produced per unit of absorbed phosphate).

Differences between species in their relative yield responses to applied phosphate have been shown to be associated with differences in the distribution of absorbed phosphate between roots and shoots. Keay, Biddiscombe and Ozanne (1970) found the species having the greatest yield response at low levels of applied phosphate also tended to translocate a higher percentage of the absorbed phosphate to the shoots. Clovers were relatively inefficient in translocating absorbed phosphate to the shoots (less than 50% was translocated to the shoots by three clover species) while grasses and other species translocated an average of 79% to the shoots when grown at the lowest level of applied phosphate for a period of four weeks. Tropical grasses grown under low levels of nitrogen and phosphate were shown by Wilson and Haydock (1971) to produce proportionately higher yields of shoots than did the temperate grasses. They associated this with the relatively larger root systems of the temperate grasses which immobilized a greater proportion of the absorbed nutrients in the root system allowing less for translocation to the shoot in comparison with the tropical species. This conclusion highlights the inaccuracies that are inherent in experiments where total nutrient absorption of different species is inferred and comparisons between species are made on the basis of shoot analysis alone.

White (1972) has also found significant differences between species in the retention of phosphate by the roots of three tropical legumes. Although these were associated with the root weight ratios (root weight as % of total plant

weight) of each species (as suggested by Wilson and Haydock, 1971), the correlation was not exact and following the suggestion of Williams (1948), White (1972) found that the nitrogen status of the plant influenced phosphate retention in the roots. In each case,both within and between species, comparatively less of the total phosphate was immobilized in the roots as the nitrogen concentration in the plants declined. This relationship was attributed to the fact that under nitrogen stress, breakdown of protoplasm in older root tissues and translocation of nitrogen to the deficient shoot also produced an accompanied mobilization and transport of phosphate from the roots. Indirect support for this theory comes from the studies of Baker et al. (1970, 1971) who attribute high accumulation of phosphate in the ear leaves of some maize hybrids to high levels of nitrogen and sulphur (leaf protein) which has a high bonding energy for othophosphate. This effect of nitrogen on phosphorus nutrition of plants is a further factor that must be considered when comparing the response of plant species to applied phosphate.

Differences between species in their phosphate utilization quotients have also been reported by a number of workers. Substantial differences were found to occur (Loneragan and Asher, 1967) when phosphate supply restricted plant growth but where the phosphate level allowed maximum yield to be attained all eight species utilized the absorbed phosphate with similar efficiencies. A similar conclusion was reached by Keay, Biddiscombe and Ozanne (1970). In both cases the results showed that clovers were approximately twice as efficient as annual grasses and pasture herbs in the amount of dry matter produced per unit of absorbed phosphate during the first four weeks of growth. Wuenscher and Gerloff (1971) noted differences

between two grasses in their utilization of phosphate when grown in sand culture but when these species were grown in soil the phosphate utilization quotients of both species were identical. They concluded that the considerably greater phosphorus stress imposed on the plants in sand culture than those in the soil was responsible for the results they obtained.

The correspondence in the results of these three studies using a range of pasture plants suggests that in general inter-specific differences in phosphate utilization quotients only occur where plants are grown under conditions of phosphorus stress.

Nassery (1970) has suggested that plants with slow relative growth rates are the most efficient in the utilization of accumulated phosphate because of the greater time period available for remobilization and retranslocation of phosphate from senescing tissues to the young actively growing meristematic tissues. White (1972) has obtained results supporting this hypothesis through his finding that the relative growth rates and phosphate utilization quotients of three tropical legumes were inversely correlated, the species with the highest growth rate having the lowest utilization quotient for phosphate.

III GENERAL MATERIALS AND METHODS

TABLE OF CONTENTS

In this section the techniques and experimental materials common to experiments in more than one of the following sections will be discussed.

III A SOIL AND SOIL PREPARATION

Unless otherwise stated the soil used in all experiments was the surface 15 cm (A horizon) of a "red loam" or krasnozem soil developed on basalt on the North West Coast of Tasmania. Soils of this area have been surveyed and described by Stephens (1937) and Loveday and Farquhar (1958) and chemical and morphological data for some of these have been presented by Graley and Loveday (1961). The soil was sampled from an uncultivated area set aside on the Tasmanian Department of Agriculture's Forthside Vegetable Research Farm at Forth. Although not in its virgin state this soil had remained unfertilized and uncultivated since the establishment of the research farm (at least seven years) and had a plant cover of mixed pasture species and weeds at the time of sampling. The soil was trucked to Hobart in moist condition where it was passed through a 5 mm mesh sieve to remove large stones and plant remains before being air-dried and stored. Several physical and chemical properties of this air-dried soil were determined and the results together with the method of analyses are given in Table III A. This particular soil was selected because of its low level of "available phosphate".

III B PLANT SPECIES AND SEED

The four plant species grown throughout this study were cabbage, Brassica oleracea v. capitata L. cultivar Ballhead Hybrid; lettuce, Lactuca sativa L. cultivar Pennlake; perennial ryegrass, Lolium perenne L. cultivar Tasmanian No. 1; and phalaris, Phalaris tuberosa L. cultivar Australian.

TABLE III A

CHEMICAL AND PHYSICAL PROPERTIES OF FORTHSIDE KRASNOZEM SOIL

Determination	Mean Value	Replication	Method
Total P	2044ug/g	5	Tandon, Cescas and Tyner (1968)
"Available" P	7 ug/g	3	Colwell (1963)
Organic P	647 ug/g	3	NaOH extraction method of Saunders and Williams (1955)
Sorbed P	950 ug/g (to give 0.3 ug/ml in supernatant)	2	Fox and Kamprath (1970)
Total N	2733 ug/g	3	Kjeldahl-Markham (1942)
NO_3 N	43 ug/g	3	Mack and Sanderson (1971)
Moisture Content			
pF 2	451 mg/g	4	
15 atmos.	240 mg/g	4	
pH	4.7	2	0.01M$CaCl_2$ White (1969)
pH	5.3	2	H_2O

TABLE III C

COMPOSITION OF NUTRIENT SOLUTION (FULL STRENGTH)

Salt	Concentration
KH_2PO_4	1mM
KNO_3	5mM
$CaNO_3$ $4H_2O$	5mM
$MgSO_4$ $7H_2O$	2mM
H_3BO_3	46.2uM
$MnCl_2$ $4H_2O$	9.1uM
$ZnSO_4$ $7H_2O$	0.76uM
$CuSO_4$ $5H_2O$	0.32uM
Na_2MoO_4 $2H_2O$	0.11uM
Sequestrene NaFe	8.9uM

All plants in all experiments were grown from one batch of seed/species, the vegetable seed being obtained from Arthur Yates & Co. Pty. Ltd., Sydney, and the grass seed from Roberts, Stewart and Co. Ltd., Hobart.

III C NUTRIENT SOLUTIONS

A nutrient solution based on that given by Hoagland and Arnon (1950) was used at full strength or at fractional dilutions for all solution and sand culture experiments. The salts used and their concentration in full strength solution are given in Table III C. Apart from Sequestrene Na Fe all the salts used were reagent grade chemicals. Solutions were made up with deionized water (less than 10umhos conductance) produced in a Twin Bed Deionizer Unit (Commando Products Aust., St. Mary's, South Australia) and subsequently stored in a 450 litre black polythene reservoir. Cation and anion beds were recharged whenever water conductance rose above 10umhos.

III D ENVIRONMENTAL CONDITIONS DURING PLANT GROWTH

III D 1 GLASSHOUSE CONDITIONS

Plants grown under glasshouse conditions were all grown in an air conditioned glasshouse at the University of Tasmania, Hobart. The flow of air within the glasshouse and the rate of change were controlled to provide a minimum of 20 changes/hr. The air stream was heated by an oil fired furnace or cooled by refrigeration as required, the control being automatic so that temperatures were maintained above 10^{o}C at night and below 27^{o}C during the day. Relative humidity was automatically controlled above 50% by injection of water sprays into the air stream. No artificial lighting was provided and the day length varied from a maximum of 15 hours in summer to a minimum of 9 hours in winter.

III D 2 GROWTH CABINET CONDITIONS

Where environmental conditions during plant growth were required to be more rigorously controlled, plants were placed in growth cabinets ("Controlled Environments", Model No. EF7H - Winnipeg, Canada). Plants were grown in these cabinets at a constant temperature of 20°C, a relative humidity of 65% and a light intensity of 16000 lux during the daily 12 hour photo-period. Nutrient solutions in these cabinets were continuously aerated using small pumps, ("Hy-Flo", Model C, Medcalf Brothers Ltd., Herts., England).

III E TISSUE PREPARATION AND CHEMICAL ANALYSIS

III E 1 PREPARATION OF PLANT TISSUE FOR ANALYSIS

Harvested plant material was dried in a forced draft oven at 65°C for 48 hours. After cooling to room temperature in a desiccator the oven dry weight of each sample was determined. Large samples were then ground in a "Culatti" hammer-mill but small samples were ground by hand in a glass mortar to avoid loss of tissue. Each finely ground sample was thoroughly mixed and stored in closed glass vials. Immediately before chemical analysis the ground samples were redried at 65°C and cooled in a desiccator.

III E 2 DIGESTION OF TISSUE

Approximately 0.2 g of ground, oven-dry plant tissue was accurately weighed out and transferred to "Pyrex" test tubes (1.5 cm diameter) marked to hold a volume of 20 ml. These tissue samples were wet digested in 5 ml of 1:5 perchloric acid (70%) - nitric acid (70%) mixture and the final clear digestate was diluted to a total volume of 20 ml. Where less than 0.2 g of tissue was available for analysis the volume of acid mixture used for digestion was reduced proportionately and

the aliquot of diluted digestate required for phosphorus determination was increased.

III E 3 DETERMINATION OF PHOSPHORUS

Plant phosphorus was determined on an aliquot of the diluted digestate after reaction with ammonium molybdate - ammonium metavanadate reagent (Chapman and Pratt, 1961). A 2.5 ml aliquot of test solution was usually taken and after further dilution, 5 ml of vanado-molybdate reagent were added. The mixture was diluted to a final volume of 25 ml with distilled water and after a thorough mixing was allowed to develop colour for 30 min., before the optical density of the solution was measured at 400 nm against a phosphorus free digest on a Hitachi 101 Spectrophotometer fitted with a flow-through cell. Phosphorus in solution was determined from a standard curve constructed in the range 0-10ppmP.

III E 4 DETERMINATION OF NITROGEN

Plant tissue samples were analysed for total nitrogen (excluding nitrates) using the micro-Kjeldahl method of Markham (1942), a mixture of K_2SO_4 and $CuSO_4.5H_2O$ being used as a catalyst.

IV PLANT GROWTH AND PHOSPHATE ABSORPTION FROM SOIL

TABLE OF CONTENTS

IV A RESPONSE TO PHOSPHATE WHEN ALL PLANTS ARE HARVESTED AT THE SAME CHRONOLOGICAL AGE

IV A 1 INTRODUCTION

The aim of this experiment was to compare the utilization of soil phosphorus by the four species, cabbage, lettuce, rye-grass and phalaris, and to compare their response, in terms of growth and phosphate uptake, to levels of fertilizer phosphate applied to the krasnozem soil. From the results of this experiment it was anticipated that a measure of the plant tissue phosphorus levels and soil phosphate levels required for maximum growth of each species could be obtained.

IV A 2 MATERIALS AND METHODS

IV A 2.1 Design and treatments

A 5x4 factorial design in a randomized block layout of three replicates was used in this experiment. Treatments consisted of the four plant species and five levels of phosphate applied to the krasnozem soil; 0, 100, 200, 400 and 800 ug P/g soil. The phosphate was added as calcium phosphate monobasic, $Ca(H_2PO_4)_2H_2O$. In addition, basal nutrients consisting of 200 ug N and 558 ug K/g soil as KNO_3, 25 ug S/g soil as $MgSO_4.7H_2O$ and 4.24 mg $CaCO_3$/g soil were added to each treatment, all chemicals used being of A.R. grade. Pots were randomized within replicates according to tables of random numbers (Fisher and Yates, 1948) and re-randomization was carried out every two weeks.

IV A 2.2 Potting and seeding technique

Plants were grown in No. 10 tin cans (17.8 cm high by 15.2 cm diameter) lined with polythene bags and containing the equivalent of 2700 g oven dry (106°C) krasnozem soil/pot.

Calcium carbonate was mixed with the air dry soil one month before seed sowing and after incubation at field capacity for three weeks in the glasshouse the soil was allowed to air dry. To facilitate this process the soil was removed from each pot and placed on polythene sheeting. The pH of this soil was found to be 5.7, measured in 0.01 M $CaCl_2$ solution at a soil: solution ratio of 1:2 (White 1969). The basal nutrients and the five levels of monobasic calcium phosphate were added to the limed, air dry soil as fine powders (less than 100 mesh) using the following procedure. The air dry soil from each pot was spread thinly on a 1 metre square of polythene sheeting and the powdered chemicals were individually sprinkled as evenly as possible over the entire soil surface. Between applications of each chemical, the soil was thoroughly mixed in a uniform manner by consecutively lifting each side of the sheet and rolling the soil to the opposite side. After addition of all nutrients the treated, air dry soil was packed uniformly into the polythene lined, tin cans.

A weighed amount of air dry soil equivalent to the top 1 cm of soil was removed from each pot and the seeds were sown on the top of the remaining soil. After addition of the 1 cm depth of air dry soil previously removed, the soil in each pot was carefully watered to field capacity (pF 2). Black polythene sheeting was used to cover the pots to prevent drying of the surface soil thus ensuring an even germination. Most seed of all four species germinated during the period 24-72 hours after sowing and seedlings appearing after this period were removed. One week after sowing the seedlings were thinned to 6 plants/pot for lettuce and cabbage and 12 plants/pot for the grasses. All plants were also sprayed with 0.1%

(w/v) ammonium molybdate solution to prevent molybdenum deficiency and this was followed up with two further sprays at weekly intervals. Two weeks after sowing the number of plants/pot was further reduced to four for the vegetables and eight for the grasses. Throughout the period of growth the soil was maintained at field capacity by watering each pot to weight. For the first three weeks of growth, pots were watered every two days but later, daily and in some cases twice daily watering was required.

IV A 2.3 Harvest technique

Both shoots and roots of all species were harvested at the same chronological age (i.e. a set time period after sowing), a complete replicate being completed each day. Replicate 1 was harvested sixty days after sowing and the other two replicates were harvested on the following two days. At the time of harvest the plants had attained the growth stages shown in Plates IV A 2.3. Shoots were removed close to soil level and after determination of fresh weight were quickly rinsed in deionized water to remove dust contamination and placed in brown paper bags in a forced draught oven to dry. With the grasses, the tillers were lifted slightly before separation from the roots so that the bulbous tiller bases in the soil could be included in the shoot sample. The roots were separated from the soil by sieving on a 0.3 cm mesh screen. Small roots and root fragments passing through the screen were recovered from the soil using fine forceps. Once separated the roots were placed in an empty bucket and washed under a stream of cold tap water. The clean washed roots were collected on a fine mesh screen (0.1 cm aperture) and given a final rinse in deionized water before oven drying. In most

PLATE IV A 2.3

Top to bottom:- Cabbage, Lettuce, Ryegrass and Phalaris grown in krasnozem soil with no applied phosphate (P1) and 100, 200, 400 and 800 ug applied P/g soil (P2, 3, 4 and P5 respectively). Photographed at time of harvest, 60 days after sowing.

cases 1-2 minutes washing was sufficient but for the grasses, especially the nodal roots of phalaris, a further more vigorous washing period was required as rhizosphere soil particles were strongly adhered to the root surface.

IV A 3 RESULTS

IV A 3.1 Dry matter yields

The average dry weight yields of shoots and roots of the four plant species are shown in Table IV A 3.1(i). Because of inherent differences between species in seed size and rate of growth the total dry matter produced during the sixty day growth period varied widely (Table IV A 3.1(i)) and comparisons between species were therefore made on the basis of relative dry weight yields (Figure IV A 3.1(i)). The basic form of the response curve for each species was similar although the curve for lettuce tended towards a more sigmoid type. At the three lowest levels of applied phosphate the relative dry weight yields of cabbage and lettuce were significantly different ($P = 0.001$) with cabbage having the greater response per increment of phosphate. No significant differences were found between the relative yields of ryegrass and phalaris at each level of phosphate. In general, the relative yield responses to applied phosphate were in the order, cabbage $>$ grasses $>$ lettuce. Cabbage achieved 90% of maximum dry weight with an application of 200 ug P/g soil but this did not occur for the other species until almost double this level, 350-400 ug P/g soil. Cabbage also achieved the highest relative yield when comparisons were made between species grown without added phosphate.

TABLE IV A 3.1(i)

Average Dry Weight Yields of Four Plant Species

(g/plant - mean of three replicates)

Species	Plant Part	Applied Phosphate (ug P/g soil) 0	100	200	400	800
Cabbage	Shoot	2.22	6.06	6.84	7.26	7.76
	Root	0.33	0.67	0.86	0.82	0.92
Lettuce	Shoot	0.10	0.74	1.52	3.26	3.72
	Root	0.03	0.13	0.30	0.55	0.41
Ryegrass	Shoot	0.36	1.04	1.38	1.79	1.80
	Root	0.14	0.35	0.37	0.43	0.38
Phalaris	Shoot	0.17	0.61	0.86	1.22	1.17
	Root	0.09	0.30	0.32	0.39	0.27

TABLE IV A 3.1(ii)

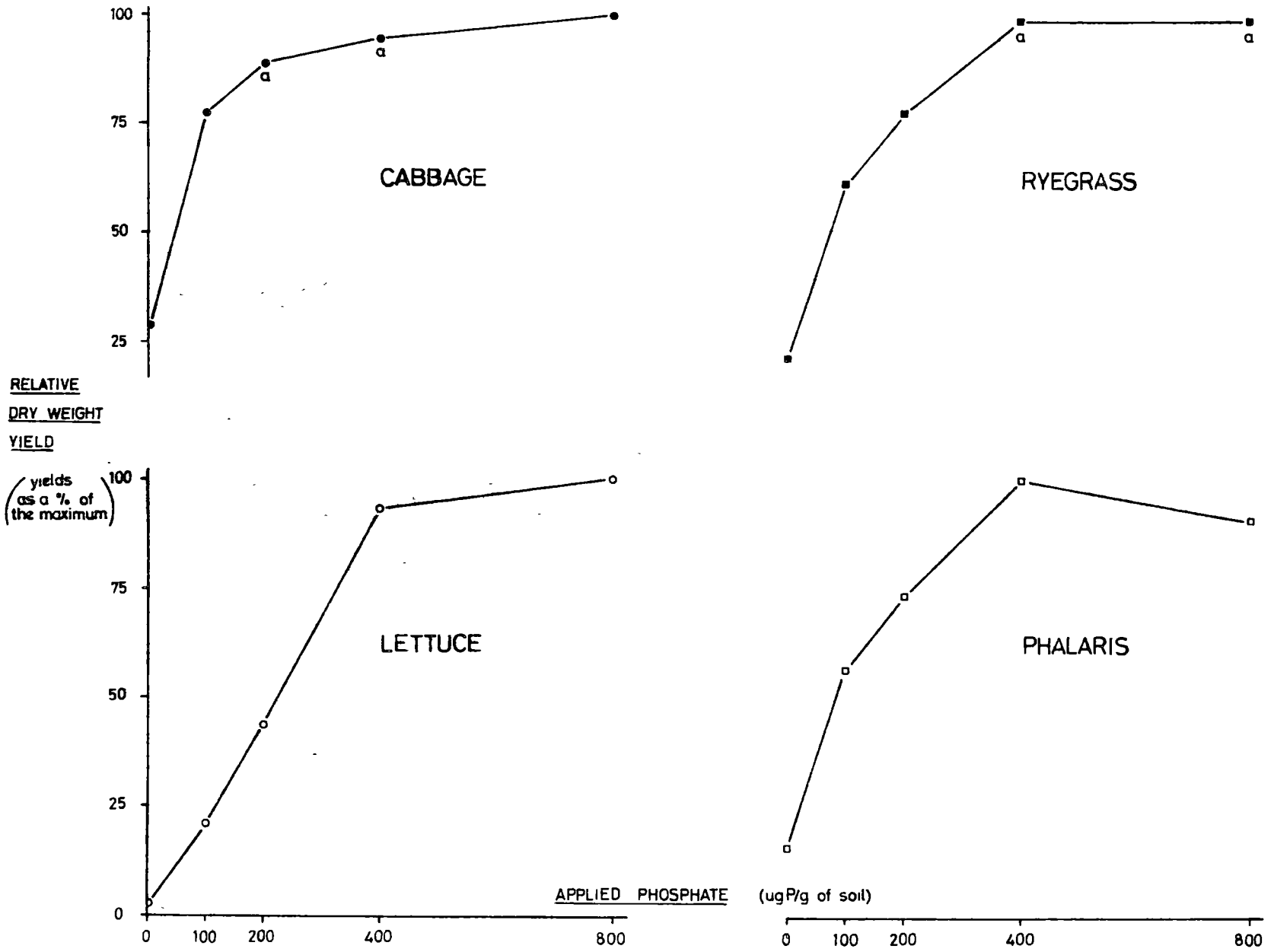
Average Root Weight Ratios of Four Plant Species

(Root dry weight as a % of total dry weight - mean of three replicates)

Species	Applied Phosphate (ug P/g soil) 0	100	200	400	800
Cabbage	12.9	11.1	11.2	10.2	10.6
Lettuce	23.1	14.9	16.5	14.4	11.0
Ryegrass	28.0	25.2	21.1	19.4	17.4
Phalaris	34.6	33.0	27.1	24.2	18.8

FIGURE IV A 3.1(1)

Relative dry weight yield responses of cabbage, lettuce, ryegrass and phalaris to phosphate applied to a krasnozem soil. (All treatments harvested at the same chronological age.) (Values with the same letter are not significantly different (P = 0.05) by Duncan's multiple range test.)


CABBAGE
RYEGRASS
LETTUCE
PHALARIS
RELATIVE DRY WEIGHT YIELD (yields as a % of the maximum)
100
75
50
25
0
a
a
a
a
APPLIED PHOSPHATE (ugP/g of soil)
0
100
200
400
800

The distribution of the total dry weight between roots and shoots is presented in Table IV A 3.1(ii) as root weight ratios (the root dry weight expressed as a percentage of total dry weight). For all species the root weight ratio decreased with increasing applications of phosphate, the values for the grasses being higher than those for lettuce and cabbage at each phosphate level. Cabbage, especially at the lower levels of applied phosphate had much lower root weight ratio than those of the other plant species.

IV A 3.2 Relative growth rates

The large differences between species in absolute yields (Table IV A 3.1(i)) indicated that differences in relative growth rates may also occur. Using seed weight as a measure of initial yield the mean relative growth rates over the total growth period were calculated using the formula:-

$$R.G.R. = \frac{\log_e W2 - \log_e W1}{t2 - t1} \times 100 \quad \text{(Williams 1948)}$$

where W2 = plant dry weight yield at harvest, W1 = seed weight and t2 - t1 = growth period, sowing to harvest (days).

Although use of this formula may introduce errors because of changes in relative growth rates over long time periods the mean values obtained (Table IV A 3.2) indicate that, with the exception of the zero phosphate treatment for lettuce, the relative growth rates of both grasses were lower than those of lettuce and cabbage at all levels of applied phosphate. Ryegrass had a slightly higher value than phalaris at the zero phosphate level but at all other levels the values for both grasses were identical. At the zero treatment level the relative growth rate of cabbage was 25% higher than that of lettuce. This trend also

TABLE IV A 3.2

Average Relative Growth Rates of Four Plant Species

(g dry matter/100g dry matter/day)

Species	Applied phosphate (ug P/g soil) 0	100	200	400	800
Cabbage	10.9	12.5	12.7	12.8	12.9
Lettuce	8.1	11.3	12.5	13.7	13.9
Ryegrass	9.2	10.9	11.3	11.7	11.7
Phalaris	8.8	10.9	11.3	11.8	11.6

TABLE IV A 3.3(i)

Average Tissue Phosphorus Concentrations of Four Plant Species

(ug P/g dry weight - mean of three replicates)

Species	Plant Part	Applied Phosphate (ug P/g soil) 0	100	200	400	800
Cabbage	Shoot	911 a*	1717 b	2512 c	3292 d	4099 e
	Root	2217 a	2525 b	3154 c	4154 d	5125 e
Lettuce	Shoot	3062 a	3205 a	3208 a	3674 b	5006 c
	Root	3397 a	3391 ab	2950 b	3167 b	4175 c
Ryegrass	Shoot	3420 a	3200 a	3427 a	4299 b	5614 c
	Root	2138 a	2054 a	2196 a	2759 b	3988 c
Phalaris	Shoot	3003 a	2493 ab	2753 b	3578 c	4761 e
	Root	1828 a	1604 a	1663 a	1863 a	3200 b

*Values, within a row, followed by the same letter are not significantly different (p = 0.05) by Duncan's Multiple Range Test.

continued, but to a lesser extent, at the 100 and 200 ug P/g soil treatments while at the highest levels of applied phosphate the position was reversed, the value for lettuce being slightly greater than that of cabbage.

IV A 3.3 Phosphorus concentration and total phosphorus absorbed

For lettuce, ryegrass and phalaris the phosphorus concentrations of both roots and shoots remained relatively constant as the level of applied phosphate was increased from 0-200 ug P/g soil (Table IV A 3.3(i)). Thereafter these concentrations increased (significant at P = 0.05) as further increments of phosphate were added to the soil. With cabbage, however, the tissue phosphorus concentrations were significantly different at each level of applied phosphate, the increase resulting from the first and second increments of phosphate being large compared with those of the other species. Cabbage also differed from the other species in having higher concentrations of phosphate in the root than in the shoot tissue. The slight increase in root and shoot phosphorus concentrations of grasses and root concentration of lettuce at the zero phosphate treatment is probably the result of high levels of tissue nitrogen. This was not observed in cabbage because the greater dry matter production diluted plant nitrogen to a lower level than found in the other species. Total nitrogen analyses of the remaining bulked shoot tissue showed that the highest nitrogen levels were associated with plants grown without added phosphate (Table IV A 3.3(ii)).

Root tissue of lettuce and cabbage plants producing 90% of the maximum yield had similar phosphorus concentrations (3650 ug/g) but the shoot phosphorus concentrations of these plants were significantly different, cabbage having the lower value

TABLE IV A 3.3(ii)

Average Total Shoot Nitrogen Content

(excluding nitrates)

(% dry weight - each value mean of duplicate determinations on a bulked sample of the three replicates)

Species	Applied Phosphate (ug P/g soil)				
	0	100	200	400	800
Cabbage	3.97	2.50	2.42	2.22	2.09
Lettuce	4.91	4.24	4.20	4.21	4.10
Ryegrass	5.03	4.76	4.58	4.41	3.96
Phalaris	5.58	4.91	5.24	5.42	5.33

TABLE IV A 3.3(iii)

Average, Phosphorus Uptake (mg/plant) (a) and Relative Phosphorus Uptake (as a % of the maximum) (b) of Four Plant Species

(Each value is the mean of three replicates)

Species		Applied Phosphate (ug P/g soil)				
		0	100	200	400	800
Cabbage	(a)	2.76	12.08	19.92	27.25	36.43
	(b)	7.6	33.2	54.7	74.8	100
Lettuce	(a)	0.38	2.80	5.76	13.75	20.37
	(b)	1.9	13.8	28.3	67.5	100
Ryegrass	(a)	1.51	4.03	5.53	8.82	11.63
	(b)	13.0	34.7	47.6	75.8	100
Phalaris	(a)	0.67	2.01	2.90	5.08	6.40
	(b)	10.5	31.4	45.3	79.4	100

(Figure IV A 3.3). For the grasses the shoot and root tissue phosphorus concentrations of phalaris were significantly lower than those of ryegrass at each phosphate level.

As expected from the yield data the total amount of phosphate absorbed during the growth period was greatest for cabbage and, with the exception of the zero phosphate level of lettuce, was least for phalaris at each level of applied phosphate (Table IV A 3.3 (iii)). Because of the great variability in the morphology of the root systems, both between treatments within a species and between species at one phosphate treatment, the phosphate uptake data were not expressed in terms of root parameters. Instead, phosphate absorption at each level was expressed as a percentage of the maximum absorbed by the species concerned. On this basis the values for the lettuce plants at the three lowest levels of applied phosphate were disproportionately low in comparison with those for cabbage and the grasses. (Table IV A 3.3(iii))

The proportion of the total plant phosphorus that was present in the roots, termed the root phosphorus ratio is shown for each species at each phosphate level (Table IV A 3.3 (iv)). With few exceptions these values parallel the respective root weight ratios, being greatest under phosphate deficiency and decreasing as each increment of phosphate is applied to the soil. Differences between species are small at each phosphate level and comparisons made with plants of each species attaining 90% of the maximum yield show that the root phosphorus ratios vary in the narrow range of 12.7-14.4%.

FIGURE IV A 3.3

Relationship between shoot phosphate concentrations and shoot dry weight of cabbage, lettuce, ryegrass and phalaris grown in krasnozem soil.
(All treatments harvested at the same chronological age.)

Note the greater shoot yields and lower shoot phosphate concentrations of cabbage, in comparison with the other species.

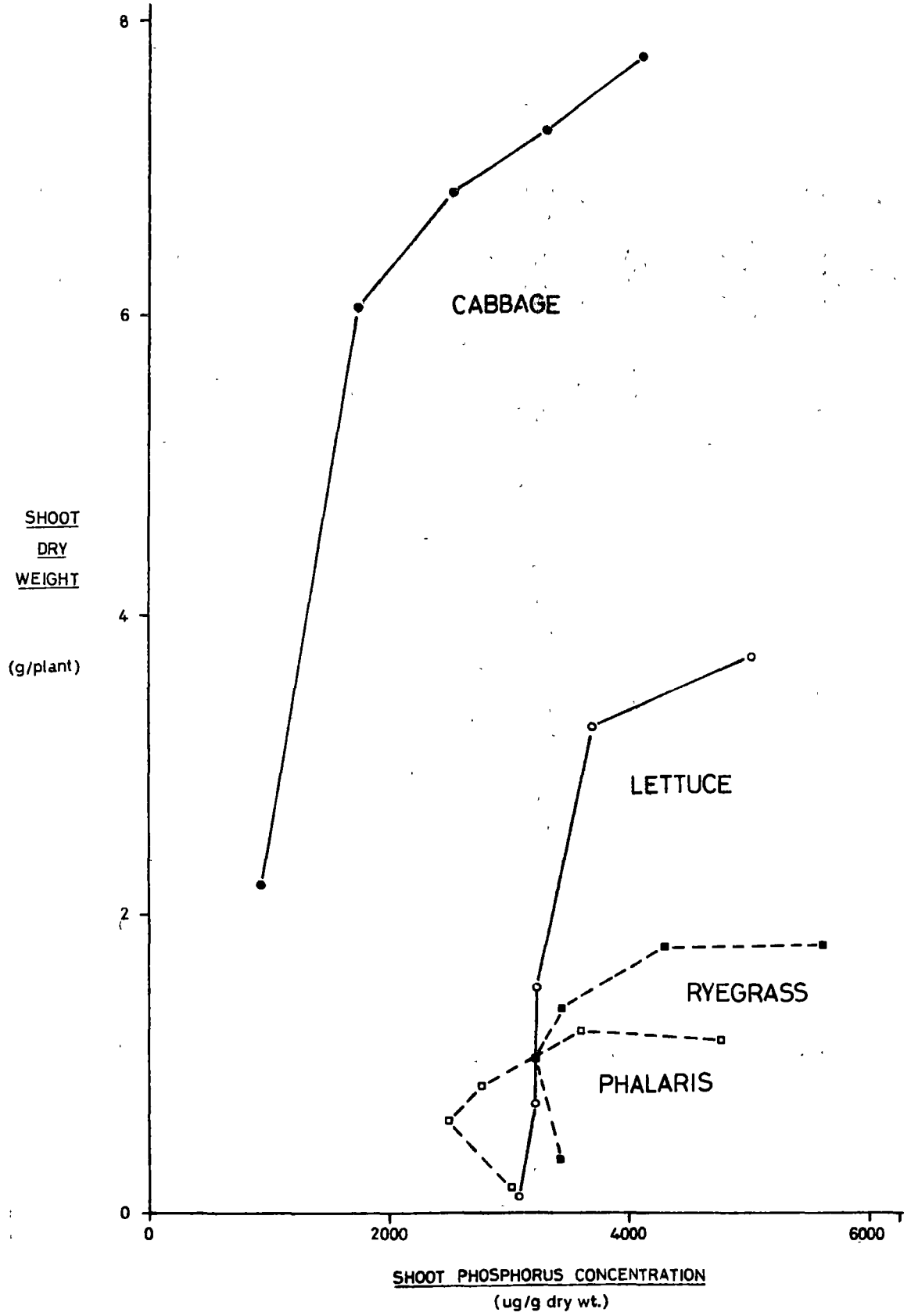

8
6
4
2
0
0
2000
4000
6000
CABBAGE
LETTUCE
RYEGRASS
PHALARIS
SHOOT DRY WEIGHT
(g/plant)
SHOOT PHOSPHORUS CONCENTRATION
(ug/g dry wt.)

TABLE IV A 3.3(iv)

Average Root Phosphorus Ratios of Four Plant Species

(Root P as a % of total plant P - each value mean of three replicates)

Species	Applied Phosphate (ug P/g soil)				
	0	100	200	400	800
Cabbage	26.5	14.0	13.6	12.5	12.9
Lettuce	23.7	15.7	15.5	12.7	8.4
Ryegrass	19.2	17.6	15.0	13.3	13.0
Phalaris	23.9	24.4	18.3	14.4	13.6

TABLE IV A 3.4

Average Phosphorus Utilization Quotients

(g total plant dry matter/mg atom P)

Species	Applied Phosphate (ug P/g soil)				
	0	100	200	400	800
Cabbage	28.6	17.3	12.0	9.2	7.4
Lettuce	10.6	9.6	9.8	8.6	6.3
Ryegrass	10.3	10.7	9.8	7.8	5.8
Phalaris	12.0	14.0	12.6	9.8	7.0

IV A 3.4 Utilization quotients

The efficiency with which absorbed phosphate is utilized in plant growth is given by the reciprocal of the tissue phosphorus concentration and this value, called the phosphorus utilization quotient, provides a clearer and more useful index of utilization efficiency than do tissue concentrations. The average phosphorus utilization quotients for each treatment were calculated from the mean dry weight yields and the mean tissue concentrations and these values appear in Table IV A 3.4. With few exceptions the greatest dry matter production per unit of phosphorus occurred in plants grown without added phosphate. Each increment of applied phosphate reduced the value of the utilization quotients and the lowest values were obtained at the highest level of applied phosphate. The utilization quotients of lettuce, ryegrass and phalaris were within a similar range of values at each level of applied phosphate but for cabbage at the zero and 100 ug P/g soil levels the efficiency of utilization was two to three times that of any other species.

IV B RESPONSE TO PHOSPHATE WHEN ALL PLANTS ARE HARVESTED AT THE SAME PHYSIOLOGICAL AGE

IV B 1 INTRODUCTION

All plants in the previous experiment were harvested at the same chronological age (i.e. a specified number of days after sowing) with the result that plants of any one species grown at different phosphate levels or different species at the same phosphate level were harvested at different physiological ages (i.e. different stages of growth). This was especially marked with lettuce due to its very slow growth rate at the low phosphate levels and comparisons between lettuce and cabbage over the range of phosphate levels were, therefore, made with plants varying widely in physiological age. Since the physiological age of tissue is one of the most important factors affecting the mineral composition of plant species (Smith, 1962), in the following experiment the response of lettuce and cabbage to applied phosphate was compared for plants harvested at the same physiological age, determined on the basis of leaf number. Because ryegrass and phalaris responded similarly to applications of phosphate in experiment IV A and because of the greater difficulty in determining the physiological age of the grasses compared with the vegetables, the grass species were not included in this experiment.

IV B 2 MATERIALS AND METHODS

IV B 2.1 Design and treatments

Rather than have a limited number of replicated treatments it was thought more desirable in this experiment to increase the number of treatments at the expense of replication and compare the response surfaces obtained on the basis of fitted

curves. With this in mind plants of lettuce and cabbage were grown at twenty levels of phosphate applied as $Ca(H_2PO_4)_2.H_2O$. These were 0, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 350, 400, 450, 500, 600, 800, 1000 ug P/g soil. All treatments were unreplicated except the zero level (i.e. the treatment indicating utilization of "natural" soil phosphate) which was set up in triplicate. To avoid the problem of high soil nitrogen levels at low phosphate levels as occurred in experiment IV A, nitrate nitrogen was added in strict proportion to the amount of phosphorus applied. Each pot received a basal dressing of KNO_3 equivalent to 50 ug N/g soil to prevent nitrogen deficiency at low levels of added phosphate, plus a further dressing of nitrogen, at the same time, equivalent numerically to one quarter of the phosphorus applied/pot (e.g. if P treatment = 250 ug P/g soil the corresponding nitrogen level was 50 (basal) + $\frac{250}{4}$ = 112.5 ug N/g soil). At high levels of phosphate the nitrogen applied by this formula was equivalent to that supplied in experiment IV A where tissue levels of nitrogen were found to be adequate. Other basal nutrients were applied to each pot at the same rates used in the previous experiment.

The pots were set out on glasshouse benches in two closely adjacent rows parallel to the long axis of the glasshouse (i.e. N-S orientation) and were completely randomized, re-randomization being carried out at weekly intervals. In addition to these "experimental pots", twelve additional "observation pots" were set up for each species, for determination of leaf numbers during the experiment and before harvesting of the "experimental pots". One pot of each species was set up at each of the following phosphate levels, 0, 50, 100, 150, 200,

250, 300, 400, 500, 600, 800 and 1000 ug P/g soil. These pots were treated in the same manner as the "experimental pots" throughout the experiment.

IV B 2.2 Potting and seeding technique

Soil preparation, addition of fertilizers and the method of seed sowing were the same as previously described (Section IV A 2.1). One week after germination each pot was thinned to 12 plants/pot and all pots were sprayed to incipient run-off with 0.1% ammonium molybdate solution. One week later the seedlings were sprayed again and thinned to a final number of eight lettuce or cabbage plants/pot. Water was applied throughout the experiment as described previously.

IV B 2.3 Harvest technique

Both lettuce and cabbage plants at all phosphate levels were harvested when fifteen true leaves had been produced. This included all the young leaves at the shoot apex visible under a stereoscopic microscope. The correct harvest time for each species at each phosphate level was determined by destructive dissection of plants from the "observation pots". When these plants had produced the required number of leaves the corresponding treatment among the "experimental pots" was harvested. Times of harvest for each treatment are given in Appendix II . Shoots and roots were harvested from each pot using the same technique as in experiment IV A but before washing and oven drying, the shoots from each pot were dissected under the microscope and the leaf number was counted.

IV B.3 RESULTS

Plants of both lettuce and cabbage grown at the higher levels of applied phosphate reached the growth stage required for harvest 4-5 weeks after sowing. However, plants grown without added phosphate, which had the slowest growth rates, were the last to reach the 15 leaf stage and were not ready for harvesting until nine weeks after sowing (see Appendix II). In general lettuce plants reached the required growth stage a few days before cabbage plants grown at the same level of applied phosphate. In most cases all plants in a single treatment were at the same growth stage and were harvested at the correct leaf number but in a few cases slight variations in specified leaf number did occur (see Appendix II). In these cases the treatments were analysed and the results were collected but they were not included in the analyses and interpretation of the overall results.

IV B 3.1 Dry matter yields

Root and shoot dry weight yields of cabbage were from 3-6 times those of lettuce at each phosphate level in the range 0-1000 ug P/g soil, (Table IV B 3.1). Total plant dry weight yields ranged from 1.8 - 4.4 g/plant for cabbage but from only 0.38 - 1.0 g/plant for lettuce. However, the relative total dry weight yield response to phosphate was similar for both lettuce and cabbage (Figure IV B 3.1), the maximum plant dry weight occurring in each case when about 250 ug P/g soil had been applied. Attempts were made to fit the total dry weight yield data of each species to theoretical curves so that a more reliable comparison of the maximum yield points could be obtained. However, the great variability between adjacent

TABLE IV B 3.1

Tissue Dry Weights and Phosphorus Concentrations of Cabbage and Lettuce

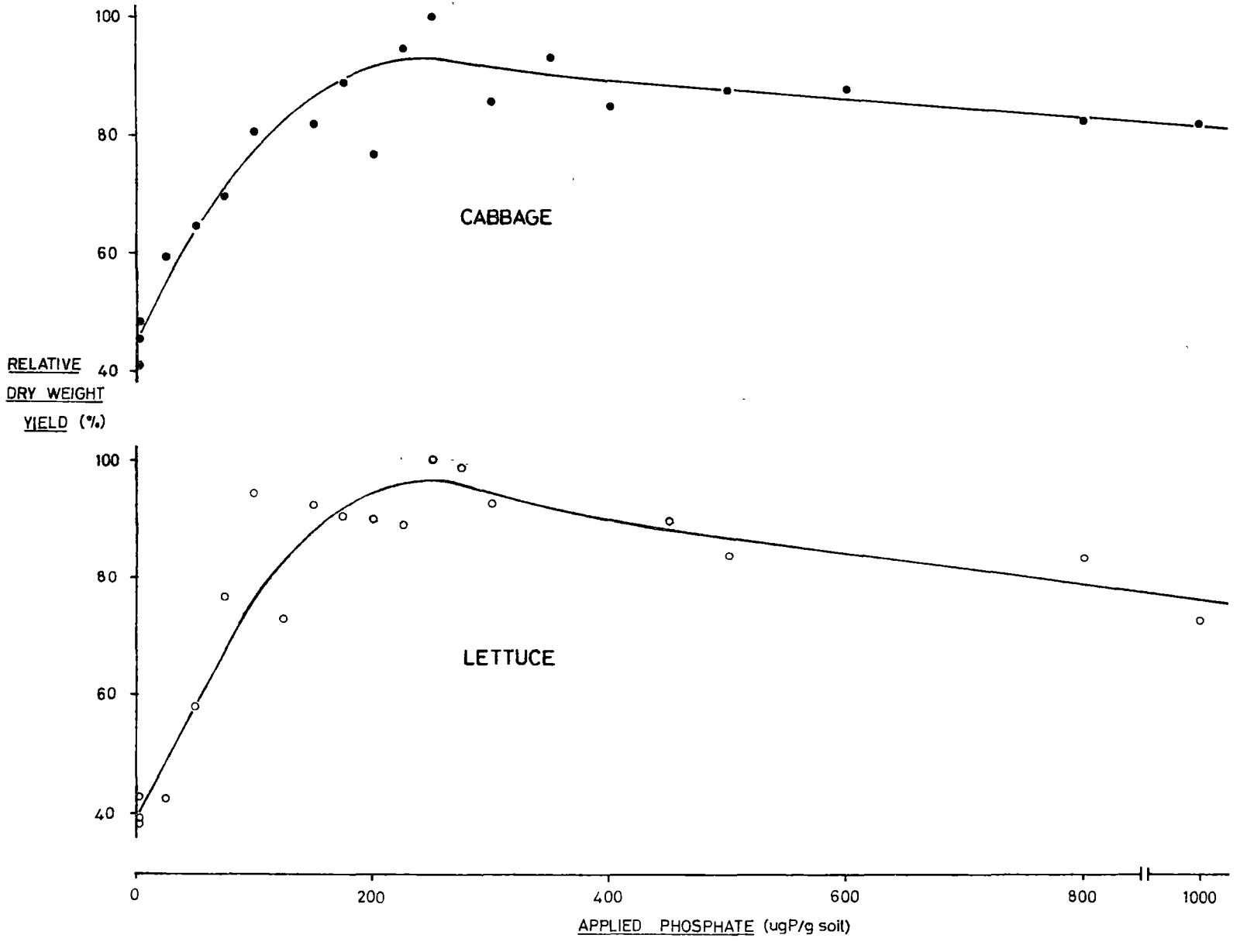
Applied Phosphate (ug P/g soil)	Tissue Dry Weight (g/plant)				Tissue Phosphorus Concentration (ug P/g plant dry weight)			
	Shoot		Root		Shoot		Root	
	C*	L*	C	L	C	L	C	L
O_1	1.470	0.331	0.331	0.095	588	2175	1738	3450
O_2	1.645	0.289	0.360	0.098	600	2225	1588	3488
O_3	1.746	0.294	0.375	0.086	619	2013	1438	3363
25	2.254	0.323	0.383	0.100	762	2288	1850	3338
50	2.464	0.461	0.389	0.114	800	2363	1988	3513
75	2.693	0.625	0.373	0.136	738	2513	2288	3300
100	3.123	0.739	0.433	0.201	857	2150	2225	2825
125	2.380	0.614	0.275	0.109	675	2363	2138	3425
150	3.189	0.760	0.410	0.158	1025	1700	2013	2288
175	3.423	0.756	0.478	0.138	1188	2075	2738	2750
200	3.035	0.764	0.324	0.128	1051	2213	3013	2475
225	3.613	0.738	0.528	0.144	1431	2013	2800	2325
250	3.865	0.863	0.534	0.128	1382	2375	2975	2550
275	2.978	0.861	0.148	0.119	807	2863	3313	3275
300	3.346	0.800	0.419	0.113	1619	2825	3050	3250
350	3.666	1.070	0.403	0.159	1563	2800	3700	3000
400	3.299	1.168	0.416	0.140	2125	3188	4300	3075
450	3.229	0.798	0.358	0.093	1988	3888	4550	3888
500	3.484	0.824	0.376	0.096	2238	4100	4550	4038
600	3.460	0.598	0.388	0.068	2306	4725	4400	4575
800	3.194	0.743	0.429	0.081	2688	5188	4550	5300
1000	3.215	0.649	0.388	0.066	3331	5863	5338	6338

* C = cabbage, L = lettuce

FIGURE IV B 3.1

Relative dry weight yield response curves of cabbage and lettuce grown at a range of phosphate levels applied to krasnozem soil.

(All treatments harvested at the same physiological age).


100
80
60
40
RELATIVE DRY WEIGHT YIELD (%)
CABBAGE
100
80
60
40
LETTUCE
0
200
400
600
800
1000
APPLIED PHOSPHATE (ugP/g soil)

points was a major problem, making the fitting process and therefore the point of maximum yield rather arbitrary. Under these conditions it was considered unreasonable to use and draw conclusions from such fitted curves. For the relative yield response curves the line of best fit was drawn through the points by eye and as there was no marked difference between the set of points for each species this approach was considered satisfactory.

The distribution of total dry weight between root and shoot as given by the root weight ratios (Table IV B 3.2) shows a variation with applied phosphate of from 9.2 - 25.3% for lettuce and from 9.6 - 18.4% for cabbage. These follow the same trend and were similar in value to those found in experiment IV A. In both cases root weight ratios increased as applied phosphate decreased and the phosphorus stress on the plant increased.

IV B 3.2 Phosphorus concentrations

The shoot and root phosphorus concentrations of lettuce plants at all levels of applied phosphate were approximately double those of cabbage and for both species the root phosphorus concentrations were generally greater than those found in the shoot (see Table IV B 3.1). Cabbage was found to have low to extremely low levels of phosphorus in the shoot tissue and this was so even at the point of maximum plant yield (Figure IV B 3.2). These low tissue phosphorus concentrations were found to be associated with low concentrations of nitrogen in the shoots (Table IV B 3.2). Whereas the levels of nitrogen in lettuce shoots ranged from 3.2% at the point of maximum yield

TABLE IV B 3.2

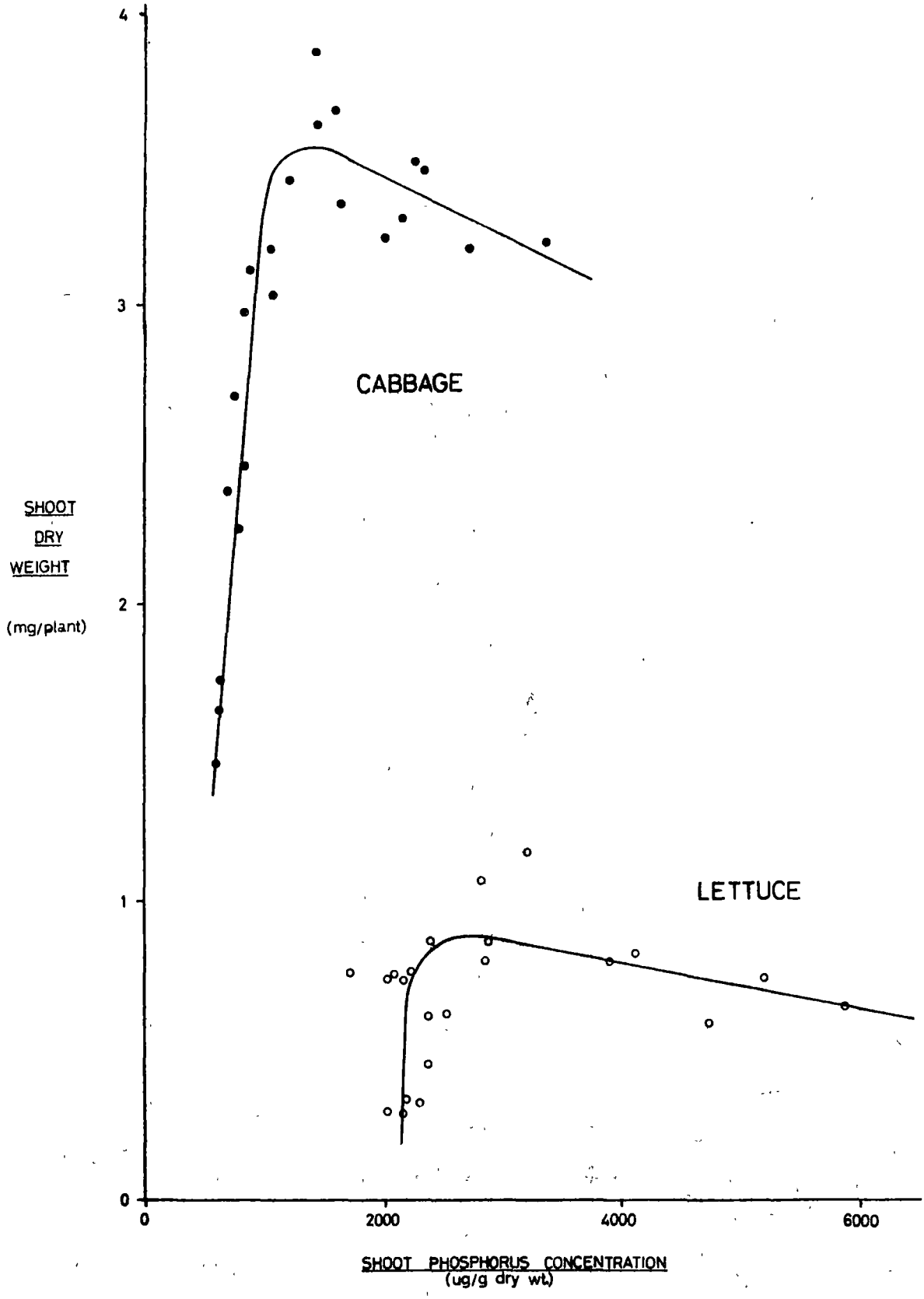
Root Weight and Root Phosphorus Ratios, Phosphorus Utilization Quotients and Shoot Nitrogen Content of Cabbage and Lettuce

Applied Phosphate (ug P/g soil)	Root Weight Ratio (% Dry Weight)		Root Phosphorus Ratio (% Plant Phosphorus)		Phosphorus Utilization Quotients (g dry matter/mg atom P)		Total Nitrogen Content of Shoot (excluding nitrates) (% N, Dry Weight)	
	C*	L*	C	L	C	L	C	L
O_1	18.4	22.3	40.0	31.3	38.8	12.6	2.30	3.66
O_2	18.0	25.3	36.7	34.7	39.8	12.2	2.03	3.78
O_3	17.7	22.6	33.3	32.8	40.6	13.4	1.92	3.65
25	14.5	23.6	29.2	31.1	33.7	12.2	1.35	3.82
50	13.6	19.8	28.2	26.9	32.2	12.0	1.31	3.95
75	12.2	17.9	30.0	22.2	33.4	11.7	1.05	3.72
100	12.2	21.4	26.5	26.3	30.3	13.5	1.01	3.32
125	10.4	15.1	26.8	20.4	37.5	12.3	1.24	3.70
150	11.4	17.2	20.2	21.9	27.2	17.3	1.15	3.20
175	12.2	15.4	24.3	19.5	22.5	14.2	1.10	3.61
200	9.6	14.3	23.4	15.8	25.0	13.8	1.22	3.72
225	12.7	16.3	22.2	18.4	19.3	15.0	1.14	3.63
250	12.1	12.9	22.9	13.7	19.7	12.9	1.07	3.74
275	4.7	12.1	16.9	13.7	33.5	10.6	1.50	4.08
300	11.1	12.4	19.1	14.0	17.4	10.8	1.38	4.34
350	9.9	12.9	20.6	13.7	17.5	11.0	1.55	4.04
400	11.2	10.7	20.3	10.4	13.1	9.8	1.69	4.19
450	10.0	10.4	20.2	10.4	13.8	8.0	1.83	4.80
500	9.8	11.6	18.0	10.3	12.6	7.6	1.82	4.89
600	10.1	10.2	17.6	9.9	12.3	6.6	2.08	5.08
800	11.8	9.8	18.5	10.0	10.7	6.0	2.62	5.09
1000	10.8	9.2	16.2	9.9	8.7	5.2	2.98	5.19

* C = cabbage, L = lettuce

FIGURE IV B 3.2

Relationship between shoot phosphate concentration and shoot dry weight of cabbage and lettuce plants grown in krasnozem soil and harvested at the same physiological age.


4
3
2
1
0
SHOOT
DRY
WEIGHT
(mg/plant)
CABBAGE
LETTUCE
0
2000
4000
6000
SHOOT PHOSPHORUS CONCENTRATION
(ug/g dry wt.)

to 5.2% at the lowest levels of applied phosphate the corresponding values for cabbage were only 1.1 - 3.0% N. The nitrogen:phosphorus ratios for cabbage shoots at maximum yield were also low, being only half those of the corresponding lettuce shoots despite the much lower tissue phosphorus levels in cabbage.

The proportion of the total plant phosphorus present in the roots was in most cases and especially at the low levels of applied phosphate, greater than the corresponding values found in experiment IV A. However, in contrast to the previous experiment the root phosphorus ratios of lettuce were lower than those of cabbage at each phosphorus level (Table IV B 3.2).

IV B 3.3 Utilization quotients

Phosphorus utilization quotients calculated from the average total plant dry weight and average total phosphorus content are present in Table IV B 3.2. These values reveal a major difference between lettuce and cabbage in the efficiency of phosphorus utilization, the quotients for cabbage being from 1.7 - 3.0 times those for lettuce, depending upon the level of applied phosphate added to the soil.

IV C PHOSPHORUS FRACTIONS IN PLANT SHOOT TISSUE

IV C 1 INTRODUCTION

The ability of cabbage to produce high shoot yields at low levels of phosphorus in the tissue is in direct contrast to the performance of lettuce (see experiments IV A and IV B) and it appears that the internal utilization of the absorbed phosphate differs between these two species. The following experiment was, therefore, performed to study the concentration of each form of phosphate within the shoot.

IV C 2 MATERIALS AND METHODS

Lettuce and cabbage plants were grown at two levels of added phosphate, 0 and 100 ug P/g soil, applied as $Ca(H_2PO_4)_2 H_2O$. There were six replicates of each combination. The phosphate levels selected were both in the deficiency range where differences in the efficiency of utilization were expected to be maximal. Three replicates of each species were required for "whole shoot" sampling while the remaining three replicates were used for sampling young and old leaf tissue. For comparison with the vegetables, three replicates of ryegrass and phalaris were grown at the zero level of added phosphate and whole shoots were sampled from these pots.

The addition of fertilizers and the potting and seeding technique were identical to those used in experiment IV A. Seedlings were thinned to 8 plants/pot for the vegetables and 16 plants/pot for the grasses. All plants were harvested after seven weeks growth. Half of the shoots in each pot of three replicates of vegetable and grass treatments were harvested, fresh weights were recorded and the shoots were oven dried and prepared for total phosphorus analysis. The other

half of the shoots in each pot were harvested and after fresh weight determinations the sample was placed in a polythene bag and immediately frozen at -10°C for later analysis of their orthophosphate and total trichloroacetic acid (T.C.A.) - soluble phosphate contents. From the remaining three replicates of each cabbage and lettuce treatment, samples of old and young leaf tissue were obtained. Laminar tissue cut from each side of the mid-vein was removed, in all cases half the leaf was placed in one sample for oven drying, after fresh weights were recorded, while the other half comprised part of the sample which was frozen (-10°C). Young leaf samples were composed of leaf tissue from the youngest fully expanded leaf of eight plants while the old leaf samples were from the second and third oldest leaves of each of eight plants.

Inorganic orthophosphate and T.C.A. - soluble phosphorus was extracted from the frozen plant tissue by homogenising the tissue in an appropriate volume of cold (2°C) 2% T.C.A. The volume used depended on the tissue weight and varied from 75-200 ml. The extraction procedure was carried out in an "Omni-mixer" (I. Sorvall Inc., Connecticut, U.S.A.), the temperature being maintained at 2°C by immersion of the mixing vessel in an ice bath. After a 2 min. extraction period the slurry was filtered using a buchner funnel maintained at 2°C in a cool room and the residue was washed with two 10 ml. portions of cold 2% T.C.A. The filtrate was made to volume (100-250 ml) and stored at 2°C until the inorganic ortho-phosphate content was determined. This was always performed within 24 hours of extraction. Ten millilitre aliquots of each filtrate were taken for determination of inorganic ortho-phosphate and total T.C.A. - soluble phosphate using the

method of Allen (1940). Some of the T.C.A. filtrates were pink in colour due to the presence of anthocyanin pigments, however, recovery studies with added KH_2PO_4 showed that these did not interfere with the analysis of phosphate by this method. Absorbance readings of the coloured solutions were made on a Unicam SP800 Ultraviolet Spectrophotometer at a wavelength of 730 nm using either 10 or 40 mm pathlength curvettes depending upon the concentration of phosphorus in solution. Total phosphorus content of oven dried tissue was also determined using the method of Allen (1940) and the amount of residual phosphorus was determined by difference.

The phosphorus concentrations of each tissue fraction were expressed on a dry weight basis, by conversion from a fresh weight basis, using the percentage dry matter values obtained from the fresh and dry weight determinations of the tissue used for total phosphorus analysis. Inorganic orthophosphate was expressed as a percentage of the total phosphorus content of each treatment and these values were subjected to an analysis of variance. Both the arcsine $\sqrt{\ }$ transformed values and untransformed values were analysed but as the overall results were the same for each analysis only the results using the untransformed values are presented.

IV C 3 RESULTS

The concentrations of inorganic orthophosphate, total T.C.A. - soluble phosphate and residual phosphate in whole shoot tissue and in young and old leaf tissue of plants grown in krasnozem soil with and without added phosphate are given in Table IV C 3.1. Without applied phosphate whole shoot tissue of ryegrass and phalaris had higher concentrations of total

TABLE IV C 3.1

Phosphorus Concentrations in Various Phosphate Fractions of Four Plant Species (ug P/g dry matter)

(Each value is the mean of three replicates)

Species	Applied Phosphate (ug P/g soil)	Plant Part	Phosphorus Forms			
			Inorganic Ortho-Phosphate	T.C.A. - Soluble Phosphate	Residual Phosphate	Total Phosphate
Cabbage	0	Whole Shoot	242	280	618	898
		Young Leaf	405	462	972	1434
		Old Leaf	178	178	515	693
	100	Whole Shoot	558	617	796	1413
		Young Leaf	861	927	1245	2172
		Old Leaf	222	262	468	730
Lettuce	0	Whole Shoot	835	924	1009	1933
		Young Leaf	1148	1212	1569	2781
		Old Leaf	713	782	389	1171
	100	Whole Shoot	1265	1349	1200	2549
		Young Leaf	1862	2071	1349	3420
		Old Leaf	692	774	606	1380
Ryegrass	0	Whole Shoot	710	765	1568	2333
Phalaris	0	Whole Shoot	863	893	1492	2385

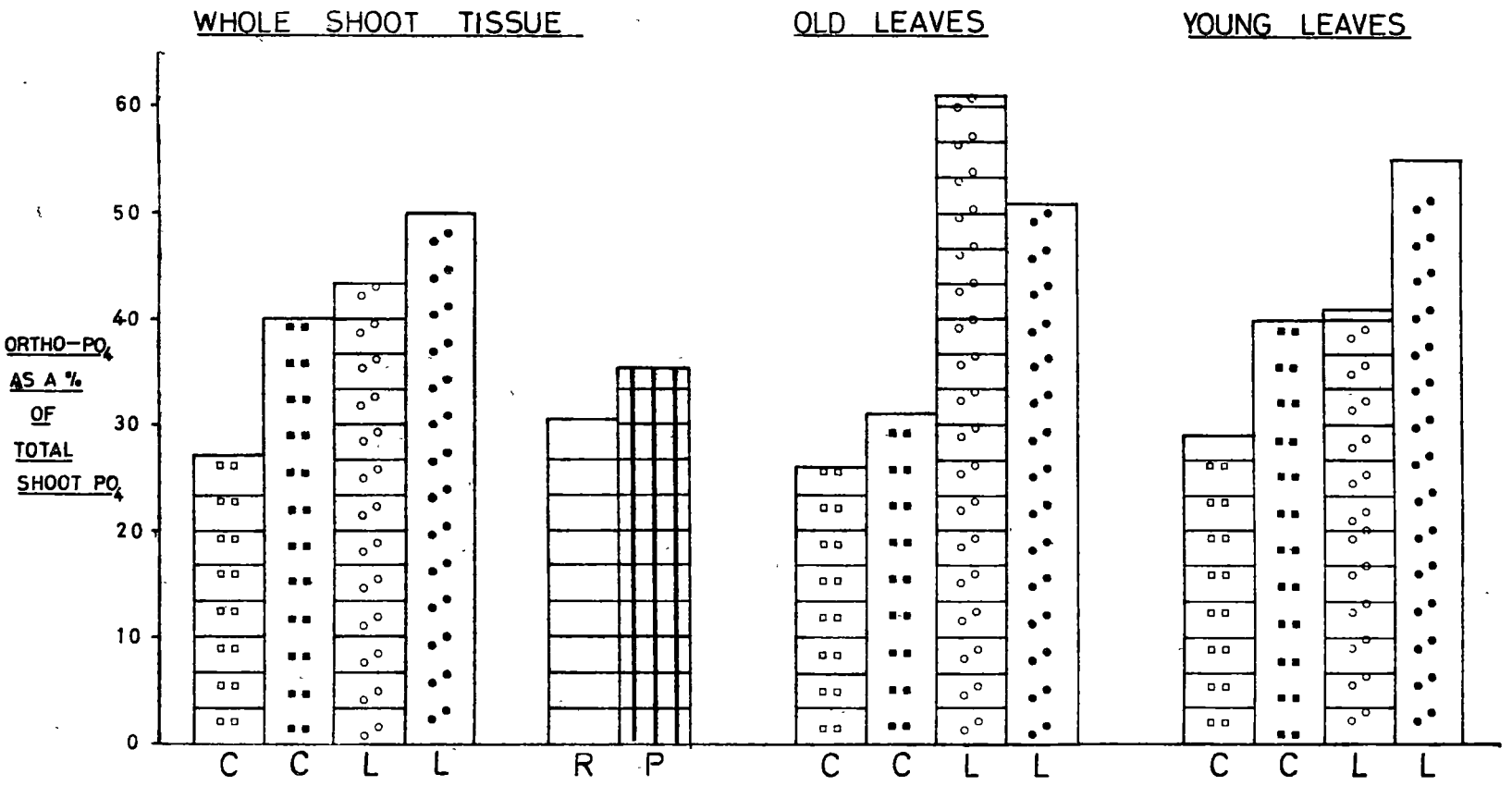
and residual phosphate than that found for lettuce and cabbage. Cabbage had much lower tissue phosphorus levels than lettuce which is in agreement with the results of previous experiments (IV A and IV B). As expected, the concentration of phosphorus in each fraction, for both lettuce and cabbage tissue, was highest for young leaf tissue and lowest for old leaves, the concentration in whole shoot tissue being intermediate between these values. In general the concentrations of phosphorus in young leaves were 2 to 4 times those found in old leaves (Table IV C 3.1).

Inorganic orthophosphate expressed as a proportion of the total tissue phosphate varied from 27-50% when whole shoots were analysed and from 26-61% when old and young leaves were analysed separately (Figure IV C 3.1). The percentage of inorganic orthophosphate in shoot tissue also varied markedly between species and between soil treatments. When comparisons between species were made at each level of added phosphate, inorganic orthophosphate expressed as a percentage of the total phosphate in both whole shoots and in young and old leaf tissue was found to be significantly different ($P = 0.05$) for lettuce and cabbage. In all cases cabbage tissue had lower percentages of inorganic orthophosphate and consequently higher percentages of residual phosphate than the corresponding values found for lettuce. T.C.A. - soluble organic phosphate (i.e. total T.C.A. - soluble phosphate minus inorganic orthophosphate) made up less than 10% of the total phosphate content of each tissue sample and was, therefore, a relatively minor component of the phosphate in shoot tissue grown under these conditions of phosphorus deficiency.

FIGURE IV C 3.1

Orthophosphate content (expressed as a percentage of the total phosphorus) of whole shoots and leaf tissues of vegetables and grasses grown under phosphate deficient conditions in krasnozem soil.

C = Cabbage, L = Lettuce, R = Ryegrass and P = Phalaris. Horizontal bars indicate plants grown without applied phosphate, the other column of each pair denoting plants grown with 100 ug applied P/g soil.


WHOLE SHOOT TISSUE
OLD LEAVES
YOUNG LEAVES
ORTHO-PO4 AS A % OF TOTAL SHOOT PO4
0
10
20
30
40
50
60
C C L L
R P
C C L L
C C L L

IV D PHOSPHATE DEPLETION ZONE STUDY

IV D.1 INTRODUCTION

The work of Lewis and Quirk (1967) has shown that in a soil of high phosphate fixing capacity wheat roots remove phosphate from only a limited volume of soil around each root; the size of this zone of depletion being largely controlled, at least in these types of soil, by the length of the root hairs. This result implies that phosphate uptake from such soils, per unit length of root, would be expected to be greatest for plants having the longest root hairs and therefore a larger volume of soil available for phosphorus exploitation by the plant. Since the Forthside krasnozem soil has a high capacity to adsorb phosphate from solution (Table III A) differences between plant species in phosphate absorption from this soil may be due to differences in the zone of phosphate depletion around the roots. Lewis and Quirk (1967) found that for one soil and one plant species the size of the depletion zone was independent of soil phosphate level but they concluded that between soils the depletion zone size would be expected to vary due to differences in the rate of phosphate diffusion in the soil.

In this study the phosphorus depletion zones around roots of the four species, lettuce, cabbage, ryegrass and phalaris were measured in two soils of different sorption capacity but at only one level of applied phosphate.

IV D 2 MATERIALS AND METHODS

A modification of the technique of Lewis and Quirk (1967) was used since in an initial experiment, using narrow boxes similar to those of Lewis and Quirk's, difficulties were encountered in maintaining soil moisture at the required level,

even when water was applied daily. In addition frequent watering should be avoided since it tends to cause movement of radio-active soil particles. To avoid this problem wider boxes, 17.8 x 12.7 cm x 4.4 cm wide, with removeable front sections were constructed from 0.5 cm thick perspex. Soil blocks, 17.0 x 12.4 cm x 3.8-4.0 cm thick, at a bulk density of 1.5 and at field capacity were placed in these perspex boxes to provide a moisture reservoir for the thin layer of ^{32}P - labelled soil later added to the front of the box. The high bulk density of these blocks restricted root growth to the less dense, thin layer of labelled soil. Soil blocks were made by hydraulically compressing into a specially constructed steel mould (17.0 x 12.4 x 7.6 cm) a weighed quantity of soil, held at field capacity, so that a bulk density of 1.5 was achieved when the block was compressed to a thickness of 3.8 cm.

The ^{32}P - labelled soil was prepared by adding, to a weighed quantity of air-dry soil passing a 1 mm sieve, the required amounts of KNO_3, $Ca(H_2PO_4)_2H_2O$ and ^{32}P (as orthophosphate in dilute HCl, Australian Atomic Energy Commission, Lucas Heights, N.S.W.) in a sufficient volume of water to form an immediate soil slurry. This was thoroughly mixed, equilibrated for 24 hours, dried at 45°C and finally reground, in a closed container, to pass a 1 mm mesh sieve. This soil, containing 2.5 uCi ^{32}P, 200 ug N and 150 ug P/g soil, was packed uniformly over the unlabelled soil block contained in the perspex box. A thin film of polythene was secured across the front of the box using plastic cement and the front section was replaced. Gentle tapping of the entire perspex box, in a vertical position, consolidated the soil particles as the remaining ^{32}P labelled soil was added, producing a uniformly packed soil layer of bulk

density about 1.0. Two series of these boxes were set up, one using the Forthside krasnozem soil already described and a second series using the A horizon of a grey-brown podzolic soil developed on dolerite on an unimproved site on Mount Nelson, near Hobart. It was anticipated that the latter soil, having a lower phosphate sorption capacity than the krasnozem and consequently a greater degree of phosphate mobility within the soil, would allow plant roots to develop larger zones of phosphate depletion than those occurring in krasnozem soil.

After watering each box to field capacity 2 or 3 germinated seeds of either cabbage, lettuce, ryegrass or phalaris were planted in the labelled soil close to the polythene film. The boxes were inclined at a slight angle encouraging root growth to occur along the soil-polythene film interface. The plants were grown in the glasshouse and watered to weight twice a week initially and more frequently at later stages of growth.

Autoradiographs of the soil surface and plant roots were taken in a photographic dark room by placing a sheet of 17.8 x 12.5 cm (7" x 5") "Kodirex" x-ray film, plus some foam rubber backing material, between the polythene film and the perspex front of the box. Good contact between the x-ray film and the polythene was obtained by applying light pressure in the form of weights to the front of the box. Initially an exposure period of one hour was sufficient, but this was continually increased with time from sowing to allow for the loss in activity as decay of ^{32}P occurred. Exposed x-ray film was processed in Kodak Type II x-ray Developer and Kodak x-ray Fixer and Replenisher according to the manufacturer's recommendations.

All plant species were grown for thirty-two days and autoradiographs of each box were taken every three days for the first eighteen days and finally at thirty-two days after planting. At the end of this period the box fronts and polythene film were removed, with the box in a horizontal position and the soil surface was allowed to air dry before root diameter and root hair lengths were determined under a stereoscopic microscope. Air drying allowed the soil particles to be gently brushed from the roots and root hairs facilitating microscopic examination and root measurement. "Mean" root hair length was obtained by visually estimating the line where the majority of root hairs terminated and then measuring the distance between this line and the root surface.

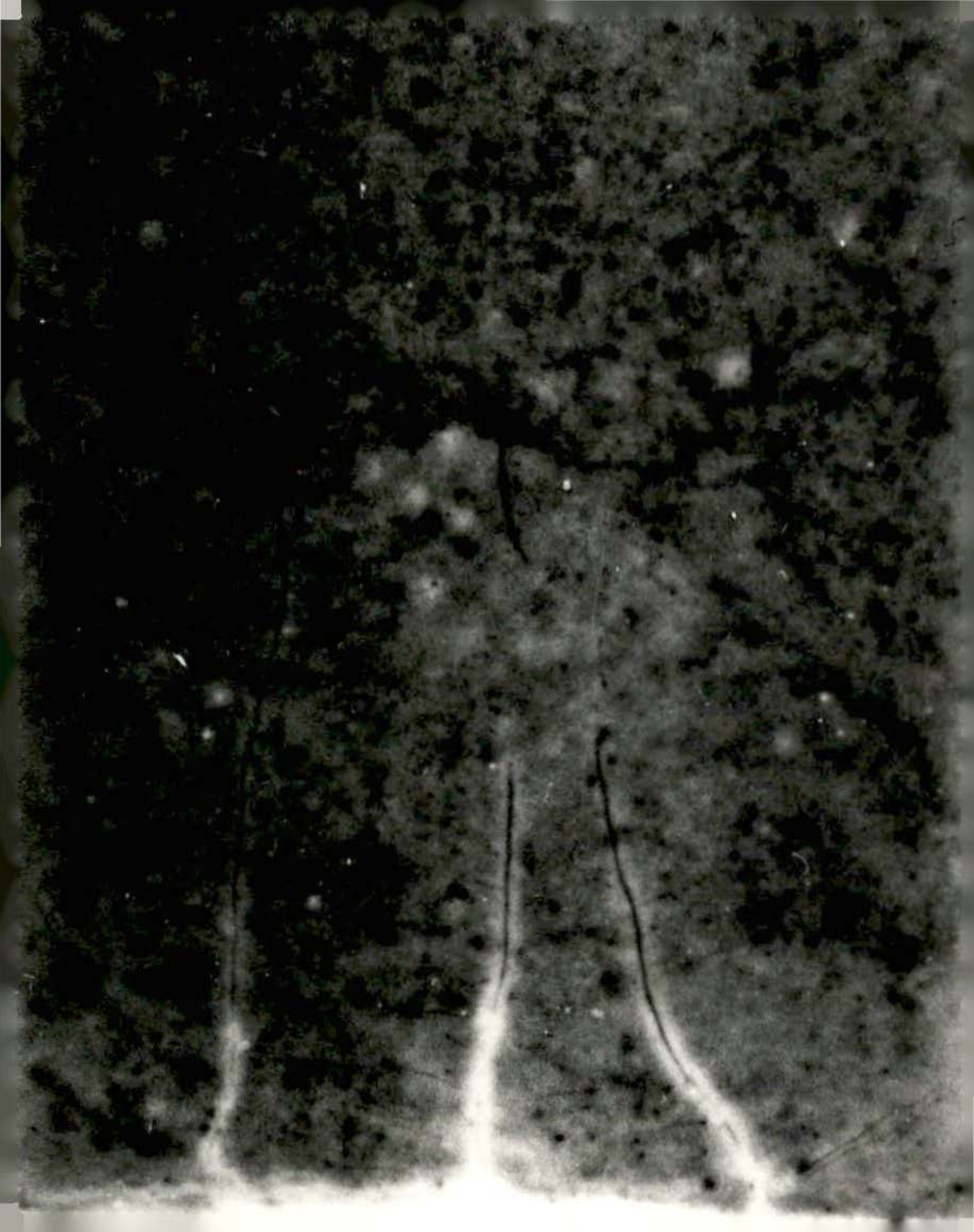
IV D 3 RESULTS

Zones of phosphorus depletion around primary roots of both lettuce and cabbage were evident on autoradiographs from both podzolic and krasnozem soil three days after planting the pregerminated seeds. The depletion zones at this stage were rather diffuse because of the small quantities of phosphorus absorbed, but even at this time a difference was detected between the depletion zones in podzolic and krasnozem soils. In the krasnozem soil the depletion zone diameter was approximately 0.4 - 0.6 mm which corresponds closely with the measured root diameter, while in the podzolic soil the diameter of this zone was about three times greater ranging from 1.5 - 1.9 mm. There was no obvious difference between species in the size of the depletion zone when comparisons were made within the one soil type. Growth of the grasses was much slower than that of lettuce and cabbage and although very diffuse depletion

zones were observed after six days, well defined depletion zones around the grass roots were not apparent until nine days after planting. At this time the depleted areas around grass roots in krasnozem soil were approximately 0.5 mm, and in the podzolic soil 2.0 - 2.5 mm, with little if any difference between ryegrass and phalaris.

As further growth and phosphorus absorption of all four species occurred the zone of phosphorus depletion and the boundary of this zone became better defined, particularly in the podzolic soil where these zones extended further from the root axes than in the krasnozem. Increases in the diameter of the depletion zones occurred in both soils until 8-12 days after planting, depending on the species, but subsequent autoradiographs showed little if any further increase with time. The maximum diameter of the phosphorus depletion zones was approximately 3.0 - 3.5 mm in the podzolic soil and approximately 0.7 - 0.8 mm in the krasnozem soil for each species. Plate IV D 3.1 shows the depletion zones of cabbage and lettuce in both podzolic and krasnozem soils after nine days growth. In both soils cabbage reached its maximum depletion zone diameter three days earlier than did lettuce.

After six days growth of the vegetables and nine days growth of the grasses the root apices of plants growing in the podzolic soil were sufficiently radio-active to appear as dots on the x-ray film. This did not occur with plants growing in the krasnozem soil treatments. Eight to twelve days after planting, the entire root systems of cabbage, ryegrass and phalaris in the podzolic soil had accumulated radio-active phosphate and these root systems appeared on the x-ray film as black lines (Plate IV D 3.1 and 3.2).



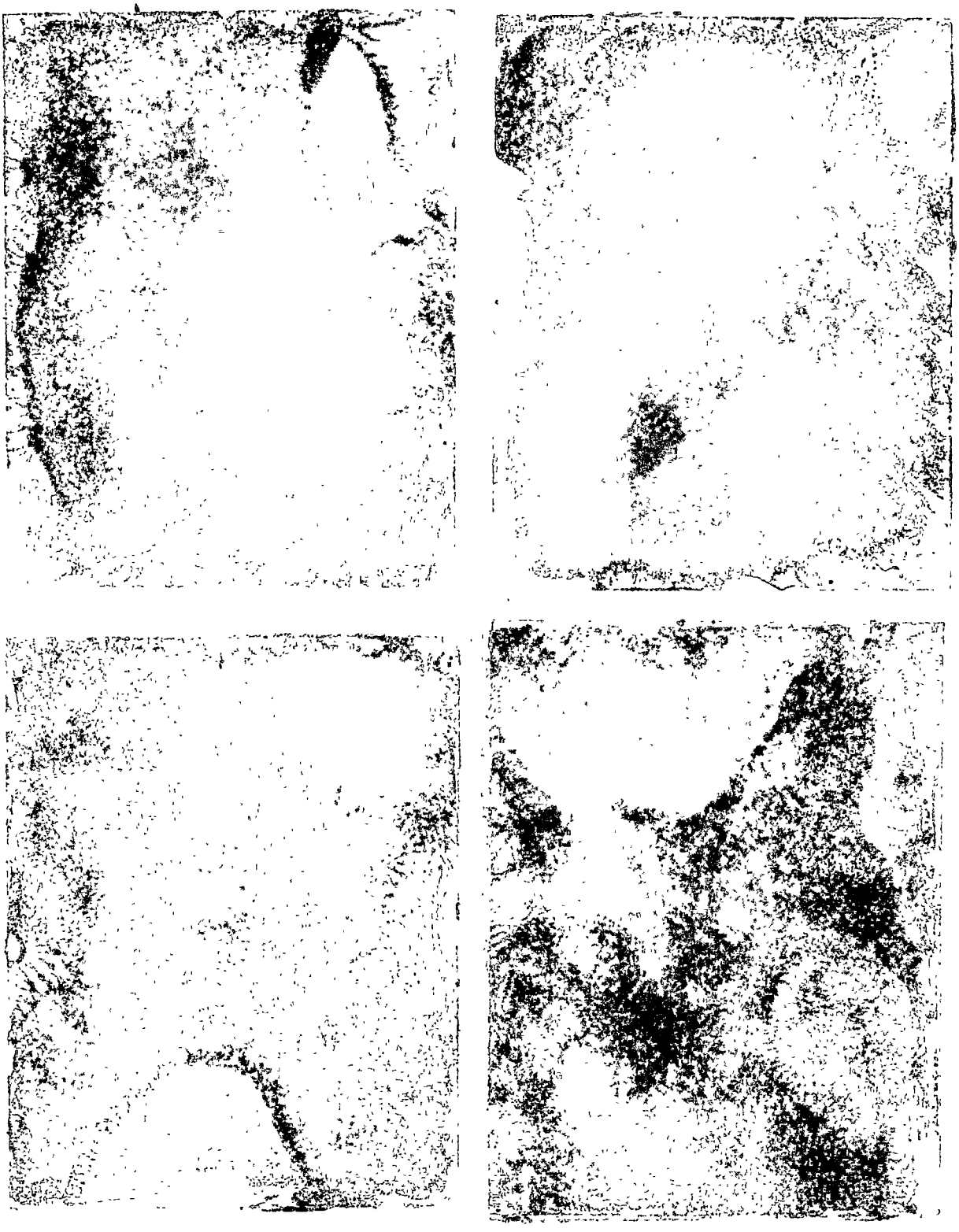


PLATE IV D 3.1

Autoradiographs of cabbage and lettuce roots grown 9 days in krasnozem and podzolic soils (X½). Top:- podzolic soil. Bottom:- krasnozem soil. Left:- lettuce. Right:- cabbage. Similar autoradiographs were obtained for the grasses.

Root systems of lettuce, however, were not detected in this manner until 32 days after sowing. With krasnozem soil some root apices of all species eventually became sufficiently labelled to appear on auto-radiographs, but at no stage did the entire root system or large lengths of roots appear on the x-ray film. Where roots did appear as black areas on the x-ray film the diameter of these lines was in all cases equivalent to the measured root diameter. This was also checked using auto-radiographs of boxes in which only the bottom half of the soil layer was radio-actively labelled. In these cases the roots eventually became radio-active along their length and were detected on the auto-radiographs as black lines against a clear unexposed background produced by the non-radio-active soil (Plate IV D 3.2).

In the podzolic soil where plant growth was more vigorous and phosphorus more mobile than in the krasnozem, the production of numerous roots and the frequent applications of water in the later stages of growth resulted in the obliteration of the phosphorus depletion zones. In the krasnozem soil, however, plants of all species did not develop so rapidly and clear depletion zones were still apparent thirty-two days after planting.

Root diameter and root hair lengths measured at the end of the experiment were found to be within the same range for each plant species. In addition, these measurements were found to be the same for plants grown in both podzolic and krasnozem soils. These values are presented in Table IV D 3. Root diameter varied slightly within any one species depending upon the root type examined and the stage of development. In general

PLATE IV D 3.2

Autoradiograph (X½) of phalaris roots grown for 32 days in podzolic soil. Note that only the bottom half of the soil in the box was radio-actively labelled with ^{32}P. The roots have become radio-actively labelled along their entire length, the intensity of ^{32}P accumulation being greatest at the root tips, including lateral apices.

TABLE IV D 3

Mean Root Diameter and Root Hair Length of Plants Grown in ^{32}P-labelled Krasnozem and Podzolic Soils.

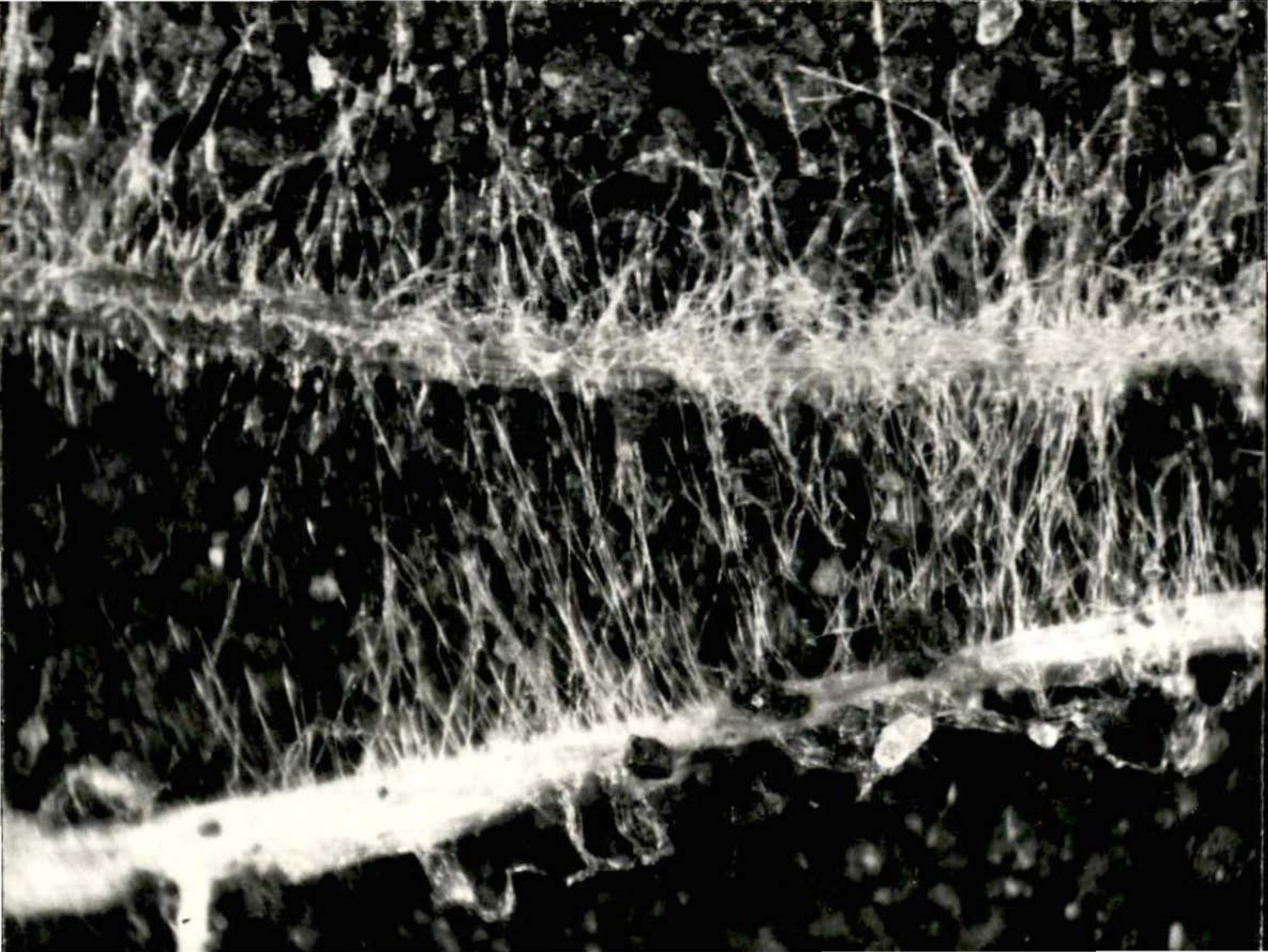
(Each measurement the mean of five observations)

Species	Primary Roots			Lateral Roots		
	Root Diameter (mm)	Root Hair Length (mm)		Root Diameter (mm)	Root Hair Length (mm)	
		"Mean"	Max.		"Mean"	Max.
Cabbage	0.3-0.6	1.3	2.3	0.1-0.3	1.0	2.1
	1.0-1.5*	No functional root hairs				
Lettuce	0.3-0.6	1.2	2.0	0.2-0.4	1.0	2.0
	0.8-1.0*	No functional root hairs				
Ryegrass	0.3-0.5	1.3	2.1	0.1-0.3	0.8	1.3
Phalaris	0.5-0.8	1.0	2.0	0.2-0.4	0.9	1.5

* Oldest basal root sections

the diameter of ryegrass roots were less than those of phalaris but the root hair lengths were similar for both species. The root diameters and root hair lengths of cabbage and lettuce were also similar and these varied within the same range of values. The extent of root hair development and the relative sizes of the roots and root hairs are shown in Plate IV D 3.3.

Qualitative observations of root growth in this experiment indicated that the rate of root extension varied widely between species. Initial rates of root extension decreased in the order cabbage > lettuce > grasses, so that initially, the total root length of cabbage seedlings exceeded those of lettuce and the grasses. At later growth stages (Plate IV D 3.4) the total root length of cabbage plants remained greater than those of lettuce partly because of greater production of long, branched laterals. However, total root length of the grasses at this time probably exceeded that of cabbage because of the extensive and highly ramified nature of the grass root system.



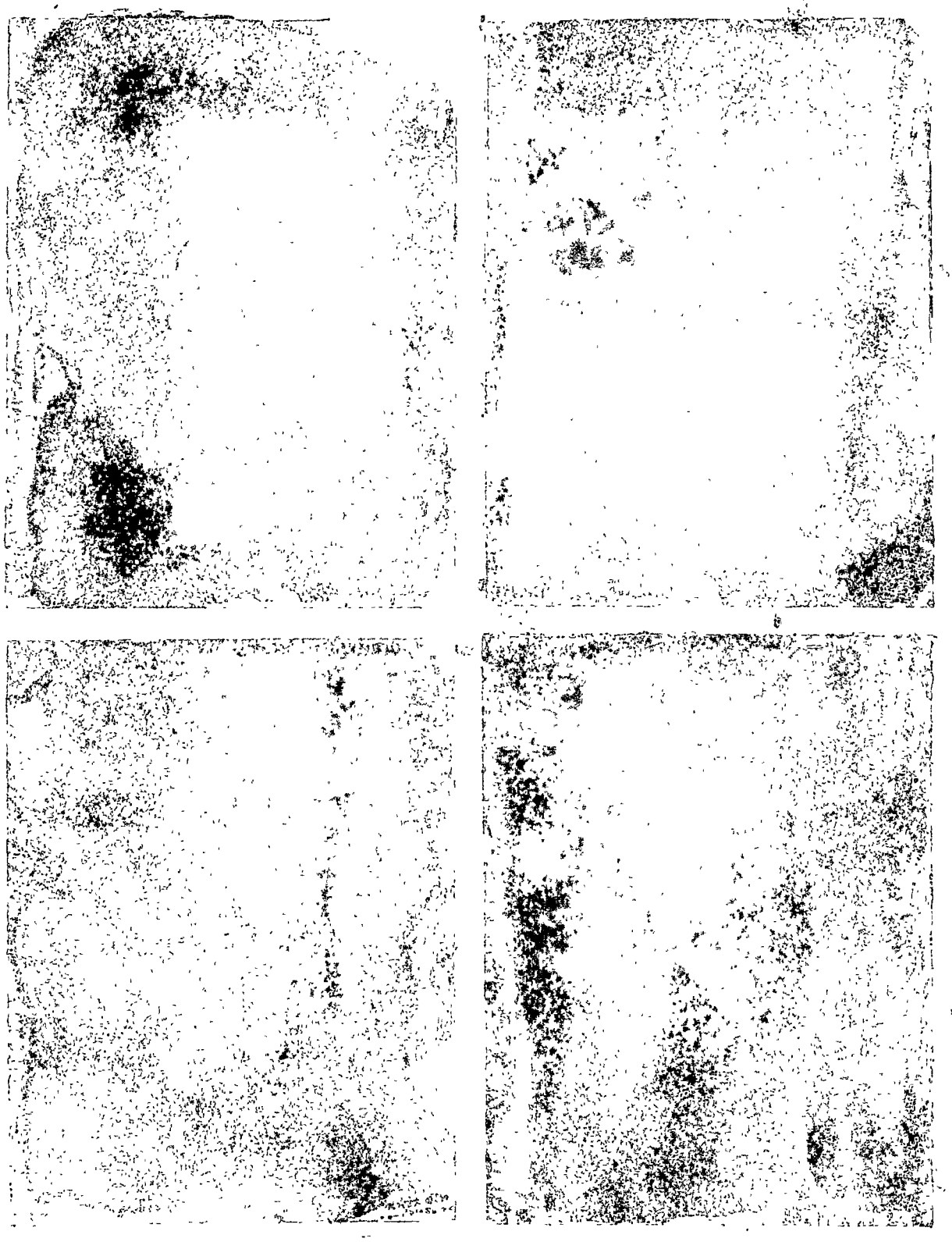


PLATE IV D 3.3

Photomicrographs (X480) of vegetable and grass roots and root hairs grown in ^{32}P labelled krasnozem soil for the depletion zone study. Top: left, cabbage; right, lettuce. Bottom: left, phalaris; right, ryegrass.

PLATE IV D 3.4

Rooting patterns of vegetables and grasses observed during the depletion zone study. Top: left, lettuce; right, cabbage. Bottom: left, phalaris; right, ryegrass.
Note the more extensive root branching of cabbage and ryegrass in comparison with lettuce and phalaris respectively.

IV E DISCUSSION

The relative responses of a wide range of plant species to applications of phosphate are known to depend broadly upon two factors; internal or plant factors governing the absorption and subsequent utilization of phosphate and external factors controlling the availability of phosphate to the plant root. The indirect effects of phosphate treatment on growth and hence on the demand for phosphate have been considered by Williams (1948) to be more important than the direct effects of external phosphate concentration on the rate of phosphate absorption, although more recently Loneragan and Asher (1967) have shown that the concentration of phosphate can in some cases become the dominant factor.

In the present study the four species exhibited marked differences in the yield responses to applied phosphate and also in phosphate uptake. These differences can be attributed to variation between species in the plant factors which influence rate of growth and total phosphate absorption. Compared with the other species cabbage achieved higher relative growth rates and relative yields at each of the three lowest levels of applied phosphate. This was largely the result of the much lower levels of phosphorus in shoot tissue and to a lesser extent root tissue of cabbage, in comparison with the other species (i.e. higher phosphorus utilization quotients than the other species) and also to a higher proportion of shoot to root (i.e. lower root weight ratios) in cabbage than in the other species. In contrast, lettuce, with the lowest relative dry weight yields at the low levels of applied phosphate, had the lowest root phosphorus utilization quotients and had higher root weight ratios and

lower shoot phosphorus utilization quotients than cabbage. All of these would tend to reduce the dry matter production of lettuce per unit of absorbed phosphate in comparison with that for cabbage and the grasses.

Differences between species and also between cultivars in phosphorus utilization quotients have been reported in the past by Lipsett (1964), Loneragan and Asher (1967), Keay, Biddiscombe and Ozanne (1970) and Wuenscher and Gerloff (1971). These workers found up to three fold differences in phosphorus utilization quotients between species, when grown under phosphorus deficient conditions and harvested at the same chronological age. However, with adequate phosphorus supply all species were found to utilize phosphorus for dry matter production with similar efficiencies. A similar pattern occurred in this study where the phosphorus utilization quotients of the four species harvested at the same chronological age ranged from 28.6 - 10.3 g dry matter per mg-atom P at the zero level of applied phosphate but were grouped in a much narrower range (7.4 - 5.8 g dry matter per mg-atom P) at the highest level of applied phosphate.

Apart from the zero phosphate treatment the relative growth rates of ryegrass and phalaris were identical at each phosphate level and this is reflected in the similarity of their relative dry weight response curves. At the three lowest levels of applied phosphate the relative dry weight yields of the four species decreased in the order cabbage $>$ grasses $>$ lettuce which corresponds with the order of decreasing seed weights of these species. This suggests that large seed reserves may confer an advantage to a species during early seedling growth that is maintained during later growth stages. Black (1957) and Scaife and Jones (1970) have shown, for a single species, that

differences in plant size arising from seeds of different sizes are maintained throughout ontogeny of the plants and a similar situation could occur between species. The differences between cabbage and lettuce in early phosphate absorption by seedling roots, noted in the depletion zone study (Section IV D), lend support to the conclusion that the size of the seed may at least partly control the rate of seedling growth and ultimately the final yield. However, when more than one species is compared, especially under phosphorus deficient conditions, such factors as seed phosphorus content, root weight ratios and phosphorus utilization quotients must also play a part in determining a species absolute and relative yield response. In the present study it is clear that differences between species in both root weight ratios and phosphorus utilization quotients have had a major role in determining the relative yields obtained, especially at the low levels of applied phosphate. The low root weight ratios and high phosphorus utilization quotients of cabbage, coupled with its high seed phosphorus content, have enabled this species to produce higher absolute and relative yields than lettuce and the grasses at the low phosphate treatment levels.

In terms of total phosphorus absorption per plant the species were ranked in the order cabbage > lettuce > ryegrass > phalaris at the three highest levels of applied phosphorus, but at the zero level the species were ranked cabbage > ryegrass > phalaris > lettuce. Because of gross differences in root morphology between species at harvest, and since different root types have different rates of phosphorus absorption (see Section II B 4.2), the total plant phosphorus content was not expressed

as a function of root weight. Instead, phosphorus absorption was expressed relative to the maximum absorbed by each species. These results (Table IV A 3.3(ii)) indicate that the four species, ryegrass, phalaris, cabbage and lettuce may differ either in their ability to absorb phosphorus from low concentrations in the soil solution or that under conditions of limited, "available phosphorus" supply some species are able to utilize sources of soil phosphorus unavailable to other species. Differences between species in their rates of phosphorus absorption from solutions of low phosphate concentration have been reported previously, (Sommner, 1936; Nye and Foster, 1958; Jenkins, 1962; Loneragan and Asher, 1967; Otsuka, 1968; and Keay, Biddiscombe and Ozanne, 1970). However, both Loneragan and Asher (1967) and Keay, et al. (1970) have stressed that this factor alone could not fully explain the different species response to phosphorus and they implicated other physiological and anatomical factors as well. For example, Keay et al. (1970) found the species responding most at the low levels of applied phosphate were also those tending to translocate a higher proportion of the absorbed phosphorus to the shoots. A similar trend was observed in this study where cabbage and ryegrass plants harvested from treatments at low levels of applied phosphate had in most cases a greater proportion of their total phosphorus in the shoots than was found for the corresponding phalaris and lettuce plants. With lettuce plants in particular, the low mean relative growth rate at the zero level of applied phosphate, associated with high root weight ratio and low phosphorus utilization efficiency, coupled with the possibility of an inefficient absorption mechanism at low phosphate concentrations, has resulted in a reduced plant

demand for phosphate and much lower relative phosphorus yields of lettuce than was observed for the other species.

Results from experiments IV A and IV B indicate that the shoot phosphorus concentration required for maximum growth of lettuce was greater than that of cabbage. The lower value for cabbage, however, may be misleading because of the use of whole shoots for analysis. It is possible that a greater remobilization of plant phosphorus has occurred in cabbage than in lettuce, depleting the older leaves of phosphorus in favour of the younger tissues and, therefore, reducing the overall shoot phosphorus concentration. Williams (1948) suggested that translocation of phosphorus from old to young tissues depends in part on the nitrogen supply, plants inadequately supplied with nitrogen undergoing a senescent break-down of protoplasm in old tissue with subsequent translocation of the remobilized nitrogen and associated phosphorus to younger tissues. This process would tend to lower the total phosphorus content of the plant in comparison with plants adequately supplied with nitrogen because the demand for phosphorus is met from within the plant rather than from phosphate absorbed from the external medium. White (1972) found this hypothesis held for three legumes and he was able to correlate low retention of phosphate in the roots with low nitrogen concentrations in the shoot. In the present study the nitrogen levels in the shoots of cabbage were low at the point of maximum yield (2.2% and 1.1% for experiments IV A and IV B respectively) compared with those of lettuce (4.2% and 3.6% respectively) but in spite of this the root phosphorus ratios of cabbage were always equal to or higher than those of lettuce. Nevertheless it does appear that the low levels of

tissue nitrogen in cabbage have reduced phosphorus tissue levels (i.e. increased the phosphorus utilization quotients) and may have affected final yields.

Fractionation of the total shoot phosphorus in plants grown under phosphorus deficient conditions has shown that cabbage leaf tissue, with a higher dry matter production per mg-atom P than lettuce tissue, had a lower percentage of its total phosphorus in the form of inorganic orthophosphate and a correspondingly higher percentage as organic phosphate. This implies that, for plants grown under phosphate deficient conditions, the efficiency of incorporation of phosphate into organic compounds required for growth is greater for cabbage than for lettuce. This may explain the higher phosphorus utilization quotients and partly explain the higher relative growth rate of cabbage under phosphate deficient conditions in comparison with those of lettuce. In agreement with this, Nassery (1970, 1971) has found that species containing higher proportions of organically combined phosphorus had higher relative growth rates and also higher rates of phosphate absorption than plants containing relatively more of their phosphate in the form of inorganic orthophosphate.

Although the efficiency of incorporation of phosphorus into organic compounds undoubtably depends upon the bio-chemical make-up of the particular plant species, because inorganic orthophosphate in plant tissue would be almost entirely associated with the solution phase present in the cell cytoplasm and vacuole there may be an association between plant water content and the proportion of phosphorus present as inorganic orthophosphate. This may occur if the bio-chemical reactions within the cell can reduce the concentration of inorganic orthophosphate in the root pool to the same level for each

species. Under these conditions it would follow that plants of greater water content would have more phosphorus in the inorganic form. The dry matter content of lettuce and cabbage leaves are different (about 6-7% and 12-14% for lettuce and cabbage respectively) and although it is not possible to implicate this as a factor controlling "incorporation efficiency", this difference between the species must be considered in the presentation and interpretation of the results.

Throughout this study the results have been expressed on a dry weight basis, this being considered a better criterion for species comparisons than fresh weight. In the latter case, the compartment in mature cells containing the majority of the plant's water, the vacuole, would not be expected to contain an equivalent proportion of the total phosphorus content, except perhaps under conditions of luxury consumption. This statement appears to be supported by the work of Läuchli and Schwander (1966) and Läuchli (1967) using the electron probe. They showed the highest concentrations of phosphorus were associated with, or close to the cell walls, much lower concentrations occurring in the vacuoles. From this work it appears there is little correlation between phosphorus concentration and cell water thus justifying the use of dry weight as a basis for inter-specific comparisons. Despite the presentation of data on a dry weight basis it must it must be emphasized that because of the large difference in percentage dry matter content between lettuce and the other species, comparisons between species of data expressed on a fresh weight basis can in some cases be almost the complete reverse of those made on a dry weight basis. A similar situation was also found by Asher and Loneragan (1967)

in connection with their data involving the response of eight annual pasture species to phosphorus.

The critical tissue concentrations and the yields produced in experiments involving a wide range of levels of a particular nutrient depend to a large extent on the physiological age of the plants at harvest. Since the phosphorus concentrations of plant tissue, determined from analyses of whole shoots are known to decrease with increasing physiological age (Smith, 1962; Bates, 1971) it is possible that the differences between species in phosphorus concentration at the point of maximum yield may have resulted from interspecific differences in physiological age at harvest. However, in comparison with plants harvested after a certain period of time (i.e. harvested at different physiological ages - experiment IV A) plants harvested at the same physiological age had lower tissue phosphorus concentrations at the point of maximum yields although the difference in tissue phosphorus concentration between lettuce and cabbage was still present and in the same direction. In addition, the maximum yields of lettuce and cabbage appear to occur at the same level of applied phosphorus. Unfortunately, these results were obtained from plants deficient in nitrogen, especially cabbage, which produced more dry weight than lettuce, and this would have the effect of reducing maximum plant yields and the total phosphorus content of the plant tissue. Thus the results of this experiment (IV B) cannot be interpreted solely as a plant response to increasing applications of phosphate. Nevertheless the experiment does indicate once again the large difference between lettuce and cabbage in their phosphorus utilization quotients. At the zero level of added phosphate where the total tissue nitrogen levels were comparatively close (2.1%

and 3.7% for cabbage and lettuce respectively) the utilization quotient of cabbage was more than three times greater than that of lettuce. The similarity of the phosphorus utilization quotients in experiments IV A and IV B for any one species suggests that this value is not dependant on the physiological age of the plant but varies markedly with changes in the phosphate status of the growth medium.

In the depletion zone experiment (IV D), areas of low ^{32}P activity around plant roots grown in the podzolic soil were observed on auto-radiographs 3-6 days after planting, but accumulation in the roots, so that the activity could be detected on the x-ray film, did not occur until some days later. Lewis and Quirk (1967) have observed a similar occurrence with wheat roots and it appears that in both cases the rate of translocation from root to shoot in the early period of growth was sufficient to accumulate the majority of the absorbed phosphate in the shoot. In the krasnozem soil the whole roots never became sufficiently radio-active to allow detection on auto-radiographs. This reflects the much lower total phosphorus content of these plants in comparison with those grown in the podzolic soil and indicates that the "availability" and mobility of added phosphorus is restricted more in the krasnozem soil. The delayed appearance of activity in the roots of lettuce grown in the podzolic soil, in comparison with the other species, indicates that either the rate of phosphorus absorption from the soil solution or the percentage of the absorbed phosphorus accumulated in the root is lower for lettuce than for cabbage, ryegrass and phalaris.

Lewis and Quirk (1967) found the volume of soil depleted of phosphorus was only slightly influenced by time; however, in the present study the zone of depletion around roots of all four species was found to increase with time, in some cases a 2-fold increase occurring in 6-9 days. Such an increase in the size of the depletion zone with time may be expected since it may take some days for the root to elongate and to initiate and develop root hairs to their full length. In the podzolic soil the size of the depletion zone around plant roots of each species corresponded with their root hair length (i.e. the diameter of the depletion zone approximated the root diameter plus twice the root hair length). However, in the krasnozem soil the phosphate depleted zone was severely restricted and in all cases was little more than the root diameter although root hair development was similar to that in the podzolic soil. This suggests that the root hairs were unable to utilize adsorbed phosphate in the krasnozem soil and that only very close to the root surface, where the effects of root exudates and microbial activity would be greatest, could phosphate adsorbed to soil constituents be effectively utilized. In the podzolic soil the soil solution phosphorus concentration would be higher and it is this source of phosphorus that root hairs appear to absorb. Even so, it appears from the radio-autographs that the labile phosphate pool in the podzolic soil has been almost completely depleted.

Although the auto-radiographic method used in this study was able to detect marked differences in the size of the phosphate depletion zones between roots in the podzolic and krasnozem soils, no differences between plant species were observed when comparisons were made in either one of the soil types. This may

be due to the failure of the method to detect small changes in radio-activity (phosphorus concentration) around roots. However, the primary cause appears to be due to the lack of any significant difference between species in their root dimensions, the root diameters and root hair lengths of all species being within a similar range (Table IV D 3).

The depletion zone study has shown that the differential species response to phosphorus applied to a krasnozem soil are not due to differences between species in the volume of soil around individual roots that can be exploited of phosphorus. However, there were indications that differences in either the rate of phosphorus absorption or in the utilization of phosphorus in the roots did exist between lettuce plants and those of the other three species, thus supporting the conclusions made from the results of the previous experiments.

V PLANT UTILIZATION OF ORGANIC AND INORGANIC PHOSPHATE SOURCES

TABLE OF CONTENTS

V A PHOSPHATASE ENZYME ACTIVITY OF PLANT ROOTS

V A 1 INTRODUCTION

Chemical analysis of the krasnozem soil (Table III A) has revealed that more than 30% of the total soil phosphorus is present in the organic form. From this data it seemed reasonable to speculate that differences between species in phosphorus uptake from the unfertilized soil may have been due to interspecific differences in the utilization of organic phosphorus compounds. The following experiments were designed to measure the extra-cellular phosphatase activity of roots of each species (i.e. their capacity to hydrolyse organic phosphates) and also to determine the contribution of the rhizosphere micro-organisms to the total root phosphatase activity.

V A 2 MATERIALS AND METHODS

V A 2.1 Production of root material

V A 2.1(a) Soil-grown roots (non-sterile)

Root material for this experiment was obtained from plants grown in the "50 ug P/g soil treatment" of the "observation pots" from experiment IV B. Plants of each species were harvested twice, four and six weeks after sowing and in each case three replicate root samples of either four or six 2 cm tip sections/sample were obtained from each species. The apices were excised from the seminal roots of the grasses and the primary roots of the vegetables after the entire root systems had been washed carefully under running tap water and aerated in 0.5 mM $CaSO_4$ solution at room temperature (20°C) for two to three hours after harvesting.

V A 2.1(b) Solution-grown roots (non-sterile and sterile)

Seeds of the four species were surface sterilized as follows; a two minute treatment in ethanol followed by one hour in 7% calcium hypochlorite filtrate at room temperature. One hundred ml of filtrate was used for each gram of seed and the flasks were initially partially evacuated and then hand shaken intermittently during sterilization. After washing for 15 min. in four changes of sterile distilled water the seeds were plated onto nutrient agar (10g glucose, 1g yeast extract (Oxoid), 1g neutralized bacteriological peptone (Oxoid) and 15g agar in 1 l water). Forty-eight hours later two sterile germinated seeds (radicles 0.3-1.0 cm long) were aseptically transferred to each of 24 sterile, cotton-wool plugged tubes (6 tubes of each plant species). The seedlings were placed on a disc of 2 mm aperture, stainless steel mesh supported 8 cm from the base of 3 cm x 20 cm glass boiling tubes and immediately above 40 ml of half strength Hoagland's solution (Hoagland and Arnon, 1950). Tubes plus nutrient solution were sterilized for 20 min at 121^{o}C prior to the transplantation of the sterile, germinated seeds. Non-sterile treatments were produced in three tubes of each plant species by adding 0.1 ml of a 1% aqueous suspension of krasnozem soil to the appropriate tubes. All tubes were placed in a specially designed wooden box to shield the root systems from the light and the plants were grown for 21 days in the growth cabinet. Prior to harvest 0.5 ml of solution from each tube was aseptically removed and plated onto sterile nutrient agar. After three days incubation at 28^{o}C the plates were inspected for microbial growth and those without micro-

organisms were concluded to be solution aliquots from sterile tubes. At harvest the plants were removed from the tubes, rinsed in sterile distilled water and the two 2 cm apical root sections per tube were excised and placed in sterile 0.5 mM $CaSO_4$ solution prior to phosphatase analysis.

V A 2.2 Assay of root phosphatase activity

The assay procedure was adapted from that described by Woolhouse (1969). Two excised root tips/sample were placed into a small glass vial containing 1 ml of 1 mM sodium citrate buffer, pH 4.5. A 1 ml aliquot of 3 mM p-nitrophenyl phosphate was added and the tubes were incubated for 1 hour at 25°C in polystyrene racks which were hand shaken at about 5 minute intervals. The enzyme substrate, 4-nitrophenyl di-sodium orthophosphate (B.D.H.) was dissolved in distilled water to form a 3 mM solution and this was stored at 2°C until required. After the incubation period, 1.5 ml of each reaction solution was removed from the vials containing the roots and pipetted into another series of vials each of which contained 4.5 ml 0.1 M NaOH. Under alkaline conditions the nitrophenol released from the substrate forms the yellow coloured phenolate ion which has an absorption peak at 410 nm. Absorbance readings of the solutions were made at that wavelength using 1 cm pathlength cuvettes fitted to a Unicam SP 800 Ultraviolet spectrophotometer. For the solution-grown roots a Scale Expansion Accessory (x10) was used to measure the small amounts of nitrophenol released. Standard solutions containing 4.5 ml 0.1 M NaOH, 0.75 ml 1 mM sodium citrate buffer and known quantities of p-nitrophenol were prepared to give a calibration curve in the concentration range 0-50 ugs p-nitro-

phenol/6 ml aliquot. Readings obtained from solutions containing root samples were converted to ugs p-nitrophenol released, by reference to the standard curve.

The mean diameter of each root tip section was measured under a microscope using an eyepiece micrometer and from these total surface area/root sample was calculated assuming the root to be a perfect cylinder of length 2 cm. The results were expressed as ugs p-nitrophenol released per unit surface area per hour and these were statistically analysed by an analysis of variance and by Duncan's multiple range test.

V A 3 RESULTS

V A 3.1 Soil grown roots

Root tip sections of all species were found to have root hairs present at their basal ends, but the difficulties in accurately measuring the root hair surface area of each sample prevented its inclusion in the total root surface area values which were determined ignoring the contribution of the root hairs. Since the root hairs of the grasses appeared to be more numerous than those of the vegetables the error associated with such values would be greater in the case of the grasses.

The mean phosphatase activities of apical root sections of the four plant species are presented in Table V A 3.1. Significant differences ($P = 0.01$) were found to occur between species, lettuce having the lowest phosphatase activity/unit surface area of root while those of cabbage and phalaris had the highest values. The phosphatase activity was similar for both harvest times in the case of lettuce and cabbage but there was a significant increase in phosphatase

TABLE V A 3.1

Mean Phosphatase Activities for 2 cm Root Tip Sections of Soil-grown Roots (Non-sterile) of Four Plant Species

(ug p-nitrophenol released/cm^2 root surface area/hr at 25^oC)

Harvest	Species			
	Lettuce	Cabbage	Ryegrass	Phalaris
1	11.2* a	21.1 b	14.2 a	19.7 b
2	11.7 a	22.2 bc	19.5 b	25.5 c

*Each value is the mean of three replicates; values followed by the same letter are not significantly different (P = 0.05) by Duncan's Multiple Range Test.

TABLE V A 3.2

Mean Phosphatase Activities for 2 cm Root Tip Sections of Sterile and Non-sterile Solution-grown Roots of Four Plant Species

Microbial Treatment	Species			
	Lettuce	Cabbage	Ryegrass	Phalaris
Sterile	9.4* b	19.0 c	3.3 a	12.3 b
Non-sterile	11.7 a	20.9 b	9.6 a	18.4 b

*same meaning as above.

activity of both grasses between the first and second harvests. The greater variability in phosphatase activity of the roots of grass than of those of the vegetables is probably the result of soil contamination of the grass roots. Soil particles were quickly and easily removed from root tips of lettuce and cabbage by washing gently under running tap water but with the grasses, especially phalaris, this procedure was not completely effective and some soil particles still remained firmly adhered to the roots after washing. Rather than damage the roots by a more severe washing technique these remnants of soil were allowed to remain on the roots. To get some idea of the phosphatase activity of the soil itself the activity of air dried krasnozem soil samples were determined, in duplicate, using the method of Tabatabai and Bremner (1969), with and without added toluene to suppress microbial activity. With no toluene a mean phosphatase activity of 274 ug nitrophenol/g air dry soil/hour was obtained but this dropped to 181 ug nitrophenol when toluene was added.

V A 3.2 Solution-grown roots

Sterility checks made on the nutrient solutions prior to harvest showed that all tubes of the sterile series were sterile and that a wide range of micro-organisms were present in those of the non-sterile series. At harvest there was little visible difference between sterile and non-sterile plants apart from a tendency for grasses grown under non-sterile conditions to have a greater number of lateral roots than those grown aseptically. As with the soil-grown roots, root hairs were present on the basal ends of the root tips of all species, although these were shorter and fewer in number than those found on soil-grown roots. Phosphatase activity/

unit of root surface area (Table V A 3.2) was again calculated ignoring the contribution made by the root hairs.

Comparisons of root phosphatase activities between sterile and non-sterile series within any one species were found to be significantly different (P = 0.05) for both ryegrass and phalaris but there was no significant difference between microbial treatments for lettuce and cabbage. In both sterile and non-sterile series the mean phosphatase activity of cabbage roots was significantly greater than those of other species and was approximately double that of lettuce in both cases. The phosphatase activity of phalaris was also double or more than double that of ryegrass when grown under both sterile and non-sterile conditions. Except in the case of ryegrass, comparisons of phosphatase activities for non-sterile, solution-grown roots of each species with those of the soil-grown roots determined previously (see Section V A 3.1), show that the mean values agree closely. This is especially so with the two vegetables where virtually no difference was recorded between the differently cultured roots.

V B UTILIZATION OF INOSITOL HEXAPHOSPHATES

V B 1 INTRODUCTION

As a result of the consistent differences between plant species in p-nitrophenyl phosphatase activity of the roots it became necessary to determine if more complex organic phosphates were also utilised by these species in a similar pattern. Inositol hexaphosphate was chosen as the more complex organic phosphate since it is widely accepted as the most important single organic phosphate occurring in soils. Because it was of interest to perform these experiments under sterile and non-sterile conditions, sand culture techniques were used in preference to plant growth in soil.

V B 2 MATERIALS AND METHODS

V B 2.1 Utilization of sodium inositol hexaphosphate

This experiment was a 4 x 3 factorial in a completely randomized design with three phosphorus treatments and the usual four plant species. Two of the phosphorus treatments, no added phosphate and 250 ug P/tube as sodium inositol hexaphosphate (Sigma Type V from corn) were replicated six times while the third treatment, 250 ug P/tube as disodium orthophosphate was replicated three times only. This latter treatment was included to confirm that plants grown under the experimental conditions imposed, were able to utilize inorganic orthophosphate present in the sand culture system. Sodium sulphate was added to the zero phosphate treatment so that the sodium concentrations in the sand culture of all three phosphorus treatments were identical.

Basal nutrients and phosphate treatments were added in solution to 80 g air dry acid washed sand (see Section V C 2.2) in 3 x 20 cm boiling tubes. Each tube received 15 ml of solution containing the phosphate treatment and a basal nutrient level equivalent to half strength Hoagland's solution minus phosphorus. The cotton wool plugged tubes containing sand plus nutrients in solution was sterilized by autoclaving (1 hour at 121^{o}C) and two seedlings of each species, sterilized and germinated as previously described (see Section V A 2.1(b)), were aseptically transferred to appropriate tubes. The tubes were placed in the specially constructed rack and the plants were grown in a growth cabinet for six weeks (see Plate V B 2.1). Prior to harvest the sterility of each tube was tested by plating some of the sand medium onto nutrient agar. Shoots and roots were harvested together, washed in distilled water, oven dried at 65^{o}C and weighed. After dry weight determination, the plant sample from each tube was wet digested and an aliquot of the diluted digest was taken for phosphorus analysis using the method of Watanabe and Olsen (1965). This was used in preference to the vanado-molybdate method (Chapman and Pratt, 1961) because of its greater sensitivity.

To account for any "natural" hydrolysis of the organic phosphate during autoclaving or during the growth period a number of sterile culture tubes were set up for analysis at the end of the experiment. At the time of plant harvest the sand in each tube was shaken for 30 minutes with 50 ml of distilled water, the solution was centrifuged to remove suspended material and an aliquot of the supernatant was taken for phosphorus analysis by the method of Watanabe and Olsen (1965).

PLATE V B 2.1

Cabbage, lettuce and grass seedlings growing in sterile sand culture tubes in the growth cabinet. Note the specially constructed, enclosed rack to allow root development to occur in the dark.

Duplicate batches of 100 or 50 seeds of each species were also wet digested and their phosphorus contents were determined by the vanado-molybdate method (see Section III E 3). Subtraction of seed phosphorus and inorganic phosphorus in the unplanted tubes at the end of the experiment from the total plant phosphorus content at harvest allowed the amount of organic phosphorus hydrolysed and subsequently absorbed by the plant to be calculated.

V B 2.2 Utilization of calcium inositol hexaphosphate

A 4 x 2 x 2 factorial combination in a randomized complete block design was used for this experiment each treatment being replicated four times. Four plant species were grown in sand culture at two phosphorus treatments; no added phosphate and 5000 ug P/tube as calcium inositol hexaphosphate. This was produced from the sodium form (Sigma Type V from corn) using the method of Greaves and Webley (1969). Two microbial treatments were also established by addition of 0.1 ml of a 1% aqueous suspension of krasnozem soil to half the sterile culture tubes of each species and each phosphorus treatment. Four replicated tubes without plants were also set up for each phosphorus treatment so that orthophosphate contents of these tubes could be determined at the end of the growth period.

Calcium inositol hexaphosphate (31 mg/tube) in the form of a fine powder passing a 60 mesh sieve was thoroughly mixed with 80 g of air dry acid washed sand and the mixture was placed in a boiling tube with 16 ml of Hoagland's solution minus phosphorus. Apart from the seed sterilization technique all subsequent operations were the same as those described in Section V B 2.1. Vegetable seeds were sterilized using a 7% filtrate

of calcium hypochlorite as before but because of a fungal contamination problem the grass seeds were surface sterilized using mercuric chloride and hydroxylamine hydrochloride as described by Kylin (1950).

Plants were harvested after six weeks growth. Sterility checks and orthophosphate analyses of the plant tissue and the unplanted culture tubes were performed as described previously (see Section V B 2.1).

V B 3 RESULTS

V B 3.1 Utilization of sodium inositol hexaphosphate

The mean dry weights and phosphorus contents of seedlings of each species grown in sand culture with three sources of phosphorus are shown in Table V B 3.1. Sterility tests of the sand medium prior to harvest showed that all lettuce, cabbage and phalaris seedlings were growing aseptically but that the tubes containing ryegrass were in most cases non-sterile, fungi being the major contaminants. As a result of this and also because of an apparent lack of utilization of organic phosphorus the results of this experiment were not statistically analysed. Except for the non-sterile ryegrass seedlings there was little if any difference in the phosphorus content and dry weight of plants grown with inositol hexaphosphate as the phosphorus source and those grown without added phosphate. However, with sodium orthophosphate as the phosphorus source there was a large increase in the phosphorus content of all plant species indicating that orthophosphate, when present in the sand culture, was readily absorbed by all plant species. In contrast there was not a corresponding increase in plant dry weight.

TABLE V B 3.1

Mean Plant Dry Weights and Total Phosphorus Content of Four Plant Species Grown on Three Phosphate Sources

Species#	Phosphate Source		
	Control (NOP)*	Sodium Inositol* Hexaphosphate	Di-sodium + Orthophosphate
	Mean Plant Dry Weight (mg/tube)		
Cabbage	33	31	30
Lettuce	33	26	22
Ryegrass	15	18	19
Phalaris	18	21	26
	Mean Plant Phosphorus Content (ugs/tube)		
Cabbage	79	79	126
Lettuce	27	28	93
Ryegrass	7	14	112
Phalaris	12	12	83

*Each value in these columns is the mean of six replicates

+Each value in this column is the mean of three replicates

#All species except ryegrass were grown under sterile conditions

V B 3.2 Utilization of calcium inositol hexaphosphate

Full experimental results, together with statistical analysis of the data of the total phosphorus contents of each species, are given in Appendix IVB2. Average values of the plant dry weight and total plant phosphorus content for each treatment at harvest are presented in Table V B 3.2

Within species comparisons of plants in both the sterile and non-sterile series showed that there was significantly less phosphorus ($P = 0.01$) in plants grown without added phosphorus than in those grown with added calcium inositol hexaphosphate. Even when allowances were made for the seed phosphorus contents and the inorganic phosphorus released from the organic form during the experiment (i.e. inorganic phosphorus content of sand cultures without plants) it is obvious that all species were able to hydrolyse calcium inositol hexaphosphate and utilize the inorganic phosphorus released as a source of phosphorus for plant growth. This occurred whether the plants were grown under sterile or non-sterile conditions and within any one species and any one phosphorus treatment there was no significant difference ($P = 0.05$) between the total phosphorus content of sterile and non-sterile plants although there was a tendency for plants from the non-sterile series to have slightly lower phosphorus contents than those from the sterile series.

Phosphorus in seedlings from the "no added phosphorus" treatment was approximately equivalent to the phosphorus present in the seed. In these treatments the phosphorus content of cabbage seedlings was significantly greater ($P = 0.01$) than those of the other three species reflecting the much greater reserve of phosphorus in the seed of cabbage. In the calcium inositol hexaphosphate treatments the phosphorus content of

TABLE V B 3.2

Mean Plant Dry Weights and Total Phosphorus Content of Four Plant Species Grown Under Sterile and Non-sterile Conditions on Two Phosphate Sources

(Each value is the mean of four replicates)

Species	Phosphate Source and Microbial Treatment: Control Sterile	Control Non-sterile	Calcium Inositol Hexaphosphate Sterile	Calcium Inositol Hexaphosphate Non-sterile	Seed Phosphorus Content (ugs P/ tube)
	Mean Plant Dry Weight (mg/tube)				
Cabbage	53	56	67	63	
Lettuce	28	24	27	29	
Ryegrass	20	14	35	27	
Phalaris	24	16	30	23	
	Mean Plant Phosphorus Content (ugs/tube)				
Cabbage	57	42	167	154	57
Lettuce	25	21	75	75	19
Ryegrass	10	7	45	39	8
Phalaris	13	11	34	30	11
Blanks - No Plants	1	2	13	9	

the seedlings also fell in the order cabbage > lettuce > grasses which is the order of decreasing seed phosphorus contents.

V C KRASNOZEM-PHOSPHORUS REACTION PRODUCTS AND THEIR UTILIZATION BY PLANTS

V C 1 INTRODUCTION

Although there are many reports in the literature comparing the abilities of a wide range of soil-phosphorus reaction products to supply phosphorus to the plant most of these have been concerned with only a single plant species. Few workers have considered the possibility that interspecific differences in the availability of phosphorus in these products may occur. The following experiment was conducted in an attempt to detect differences between species in their ability to utilize phosphorus from a range of artificially produced reaction products. Initially an attempt was made to isolate and identify the reaction products produced from mono-calcium phosphate in the krasnozem soil so that artificially prepared compounds identical to those actually found in soil could be used for the later pot-study.

V C 2 MATERIALS AND METHODS

V C 2.1 Reaction products from krasnozem soil

The technique of Lindsay and Stephenson (1959b) which simulates the movement of fertilizer phosphate solution from a dissolving fertilizer granule into successive layers of surrounding soil was used to produce fertilizer-soil reaction products by reaction of saturated mono-calcium phosphate solution with krasnozem soil.

A saturated solution of mono-calcium phosphate was prepared by shaking 1600 g $Ca(H_2PO_4)_2H_2O$ (Lab. grade) in 2 l of distilled water at 25°C. After 10 days the suspension was removed from

the shaker, centrifuged and the clear supernatant was decanted and stored in plastic bottles. This solution had a pH of 1.43 and a phosphorus concentration of 4.16M. One kg of air dry krasnozem soil was shaken for 2 hours at 25°C with 2 l of the saturated mono-calcium phosphate solution. After centrifugation, the clear filtrate obtained was filtered (Whatman No. 41) to remove floating organic debris and was shaken with a fresh sample of krasnozem soil for a further 2 hours at 25°C, the soil:solution ratio being maintained at 1:2. Centrifugation and filtration were carried out as before and the supernatant was retained for further reaction. This process was repeated a total of six times at the end of which the volume of clear filtrate was approximately 200 mls. This was stored in a well stoppered plastic bottle and left for a period of twelve weeks at room temperature for precipitation reactions to occur. At the end of this period the insoluble precipitate was washed into a centrifuge tube with distilled water and was then washed four times with distilled water by centrifugation and decantation before being finally washed in acetone. After drying in a desiccator the product was stored in a stoppered bottle for chemical and x-ray diffraction analyses. The x-ray diffraction analyses were made on a Phillips Diffractometer fitted with a Li F monochromator, a curved focussing specimen holder and a 4° divergent slit. It was operated at a scan rate of ½° 2Θ, a ratemeter setting of 16 and a time constant of two. The phosphorus content of the reaction product was determined in duplicate using the method of Tandon, Cescas and Tyner (1968).

V C 2.2 Utilization of artificially produced reaction products

From the results obtained using the methods outlined above (Section V C 2.1) together with a study of published data a number of phosphate reaction products were selected as those most likely to occur in the krasnozem soil. These reaction products with their method of preparation were, colloidal ferric phosphate (Cate, Huffman and Deming, 1959), colloidal aluminium phosphate, potassium iron phosphate and potassium taranakite (Taylor, Gurney and Lindsay, 1960) and strengite (Juo and Ellis, 1968). Analytical grade reagents (Ajax) were used as sources of mono-calcium phosphate and dicalcium phosphate dihydrate. Calcium phytate was also included as a source of phosphate and this was prepared as described previously (V B 2.2). "No phosphorus" treatments were also included as control pots. All the prepared reaction products were characterized by x-ray diffraction analysis and their phosphorus contents were determined in duplicate using the methods described in Section V C 2.1.

Because of the differences between species in yield and phosphorus uptake known to occur when grown on unfertilized krasnozem soil and to prevent the confounding of this effect with those of the species responses to the reaction products, it was decided to conduct the experiment in a sand culture system where phosphorus-free controls could be established. Phosphorus-free sand was obtained by washing a local grade of river sand three times with tap water, both before and after treatment with 5% HCl for two weeks in 45 l plastic bins. Washing was carried out in a concrete mixer lined internally with fibre-glass. After washing in deionized water the sand was leached with Hoagland's solution minus phosphorus (pH 5.5) until the pH no longer

decreased upon standing. The sand was finally leached with deionized water and air dried. Processed sand had a water holding capacity of 19% and had the following particle size analysis, expressed as a percentage of the total sample; 1 mm, 6.6; 1.0-0.5 mm, 32.1; 0.5-0.25 mm, 45.7; and $<$ 0.25 mm, 16.6. Sand of similar grading has been found satisfactory for the vigorous growth of a wide range of crop plants (Hewitt, 1966).

Plastic pots (10 cm, surface diameter) lined with polythene bags were filled with 750 g air dry sand/pot. The phosphate sources were added as finely divided powders passing a 100 mesh sieve and were mixed throughout the sand at the rate of 150 mg P/pot. The phosphate compounds were lightly ground with a small amount of fine sand prior to application to prevent aggregate formation of the material during the adding and mixing operations which were carried out on a polythene sheet. All pots were watered to 80% of water holding capacity after first adding 100 mls of Hoagland's solution minus phosphate (pH 5.5) to each pot. To provide a wide spectrum of soil micro-organisms in the sand culture each pot received a 1 ml aliquot of a 1% aqueous suspension of krasnozem soil. Seeds of each species were germinated on cheese cloth supported on stainless steel screens over 6 l 0.5 m M $CaSO_4$ and four pregerminated seeds of either ryegrass or phalaris or three of cabbage or lettuce were planted per pot. The pots were randomized within replicate blocks and re-randomized each fortnight. Pots were watered to weight each day with deionized water and during the first three weeks of growth 50 ml of nutrient solution was added per pot each week. Thereafter 100 mls of nutrient solution was added per pot each week except where growth was limited because of acute phosphorus deficiency where the volume was reduced to 25 ml per week.

Plant shoots were harvested after seven weeks growth by cutting the stems at ground level. Tissue weights were recorded after oven drying and the total phosphorus content of the shoots from each treatment were determined using the methods described in Section III E.

V C 3 RESULTS

V C 3.1 Reaction products from krasnozem soil

X-ray diffraction analysis of the crystalline material obtained from the reaction filtrate after a three month storage period gave reflections at 7.60, 4.23, 3.04, 2.93, 2.62, 2.42 and 2.17 ^{o}A etc, this being the diagnostic pattern of brushite, $CaHPO_4 2H_2O$. This was the only compound identified as the result of x-ray diffraction analyses. Chemical analyses of the reaction product showed a phosphorus content of 16.82% which is lower than the theoretical figure for brushite of 18.21%. This suggests that other compounds of lower phosphorus content may also have been present in the reaction precipitate. Compounds such as colloidal iron and aluminium phosphates were suspected as being involved since these compounds give no x-ray diffraction patterns and in addition have lower phosphorus contents than brushite.

V C 3.2 Utilization of artificially produced reaction products

V C 3.2(a) Characterization of the compounds

All of the laboratory prepared reaction products gave the predicted diagnostic patterns when analysed by x-ray diffraction methods. As expected no peaks were detected for the colloidal ferric and aluminium phosphates. The patterns determined for the remaining products are given in Table V C 3.2(a) together with the phosphorus analyses of all compounds. In general the

TABLE V C 3.2(a)

X-ray Diffraction Patterns of Laboratory Prepared Reaction Products

Strengite		Potassium taranakite		Potassium iron phosphate	
Intensity*	d(A)	Intensity	d(A)	Intensity	d(A)
s	5.50	vs	15.84	s	7.17
w	4.95	s	7.92	s	5.77
vs	4.38	m	7.45	s	4.57
m	3.99	m	5.94	w	4.01
w	3.28	w	5.02	m	3.58
s	3.11	w	4.66	s	3.23
m	3.00	m	4.31	vs	3.09
m	2.95	s	3.81	w	2.98
w	2.54	m	3.74	w	2.94
m	2.53			m	2.88
w	2.44			m	2.81

*Intensity of peaks:- vs = very strong, s = strong, m = moderate, w = weak.

Phosphate Content of Laboratory Prepared Reaction Products

Compound	Phosphate Contents Determined (%P)			Theoretical Value (%P)
	Rep. 1	Rep. 2	Mean	
Colloidal aluminium phosphate	15.13	15.03	15.08	15.6
Colloidal ferric phosphate	17.21	17.31	17.26	14.8
Potassium taranakite	18.56	18.47	18.52	18.6
Potassium iron phosphate	20.87	20.32	20.59	21.0
Strengite	14.15	14.69	14.42	16.6

measured phosphorus content of these compounds closely approach the theoretical values although the value for colloidal aluminium phosphate is higher than the figure published by Taylor, Gurney and Lindsay (1960) for a compound with a P:Al molar ratio of 1.19.

V C 3.2(b) Plant response to the reaction products

Mean shoot dry weight yields (Table V C 3.2(b)(i)) and mean shoot phosphorus contents (Figure V C 3.2(b)) of all plant species were severely depressed when plants were grown with no added phosphate or with strengite as the phosphorus source. Maximum dry weight yields of lettuce and cabbage were obtained using dicalcium phosphate dihydrate (brushite) and calcium phytate as the phosphate sources but for the grasses the colloidal aluminium and iron phosphates produced the greatest dry weight yields. In most cases the shoot phosphorus concentrations of each species (Table V C 3.2(b)(ii)) followed the trend in dry weight yields for each phosphate source with the exception that for all four species the highest shoot phosphorus concentrations occurred in plants of depressed dry weight yield grown with mono-calcium phosphate as the source of phosphorus. Because of this abnormal response, plants from pots fertilized with mono-calcium phosphate could not be used as a reference for estimating the response of the plants to the reaction products and the data from plants grown with mono-calcium phosphate were not included in the subsequent analyses of the results.

TABLE V C 3.2 b(i)

Mean Shoot Dry Weight Yields (g/pot) of Four Plant Species Grown on Nine Different Phosphate Sources

(Each value the mean of two replicates)

Phosphate Source	Cabbage	Lettuce	Ryegrass	Phalaris
Control (no P)	0.130	0.021	0.014	0.016
Strengite	0.295	0.152	0.226	0.133
Pot. iron phosphate	3.824	1.366	2.042	1.087
Pot. taranakite	4.960	4.814	3.783	1.836
Col. ferric phosphate	6.414	3.505	4.095	2.975
Col. aluminium phosphate	4.871	4.669	4.206	2.772
Calcium phytate	8.310	8.899	3.756	2.227
D.C.P.D.	8.554	8.105	3.809	2.055
M.C.P.	5.464	5.525	2.911	1.357

TABLE V C 3.2 b(ii)

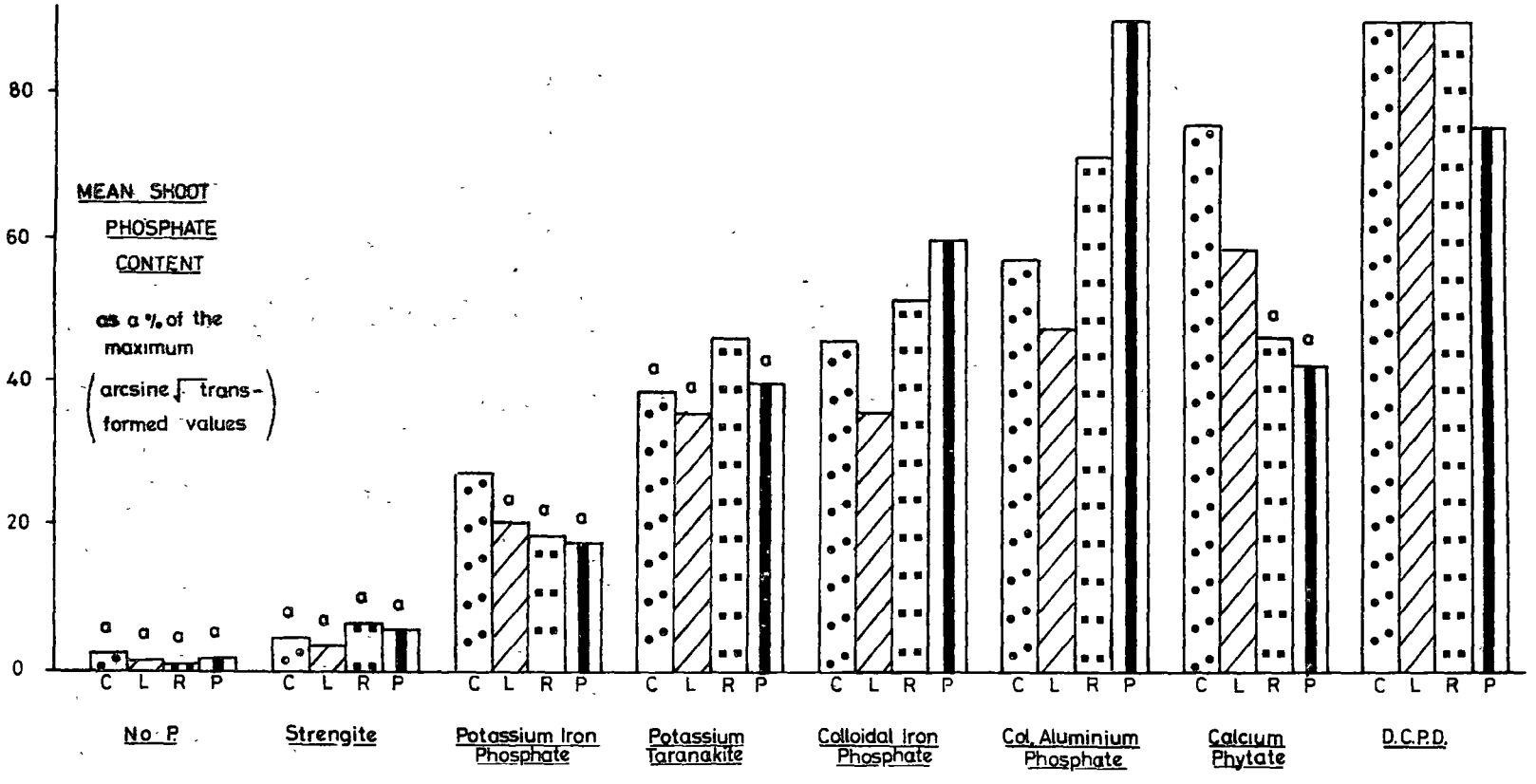
Mean Shoot Phosphate Content (ug P/g dry tissue) of Four Plant Species Grown on Nine Different Phosphate Sources. (Each value the mean of two replicates)

Phosphate Source	Cabbage	Lettuce	Ryegrass	Phalaris
Control (no P)	412	822	579	611
Strengite	563	718	1211	1097
Pot. iron phosphate	1759	3000	1093	1176
Pot. taranakite	2459	2389	3037	3304
Col. ferric phosphate	2562	3329	3349	3736
Col. aluminium phosphate	4627	3987	4786	5367
Calcium phytate	3608	2824	3114	3099
D.C.P.D.	3734	4219	5857	6769
M.C.P.	9578	9527	5967	7022

FIGURE V C 3.2(b)

Shoot phosphate content (expressed as a percentage of the maximum shoot phosphate content) of cabbage (C), lettuce (L), ryegrass (R) and phalaris (P) grown in sand culture with eight different phosphate sources.

(For each source, species with the same letter are not significantly different (P = 0.05), in shoot phosphate content, when determined using Duncan's multiple range test.)


MEAN SHOOT PHOSPHATE CONTENT
as a % of the maximum
(arcsine √ trans-formed values)
80
60
40
20
0
a
C L R P
No P
Strengite
Potassium Iron Phosphate
Potassium Taranakite
Colloidal Iron Phosphate
Col. Aluminium Phosphate
Calcium Phytate
D.C.P.D.

From shoot dry weight yields and shoot phosphorus concentrations the total phosphorus content of the shoots was calculated. These values for each phosphorus source were expressed as a percentage of the maximum for each species and the arcsine $\sqrt{\ }$ transformations of these were subjected to statistical analyses, (Appendix IV C 2). Figure V C 3.2(b) shows the mean of these values plotted for all species and each phosphorus source. For the vegetables the shoot phosphorus content increased in the order no P < strengite < potassium iron phosphate < potassium taranakite < colloidal iron phosphate < colloidal aluminium phosphate < calcium phytate < dicalcium phosphate dihydrate. The ranking for the grasses was similar except that calcium phytate produced shoots of lower phosphate content than did both the colloidal phosphate sources. Comparison of the shoot phosphorus content of plants grown with no added phosphate and with strengite showed that these were not significantly different ($P = 0.05$) for both lettuce and cabbage. However, for both ryegrass and phalaris the shoot phosphorus contents of plants grown with strengite were significantly greater than those grown without added phosphate. Between species comparisons made for each phosphate source showed there was no significant difference between species when plants were grown without phosphate or with strengite at the phosphorus source. However, large differences between species were found for plants grown with colloidal aluminium and iron phosphates and calcium phytate as the phosphate sources. In all cases the values for cabbage were significantly greater ($P = 0.05$) than those for lettuce, and for the vegetables as a group the values were significantly different from those of the grasses being greater with calcium phytate and less with the colloidal phosphates as the sources of plant phosphorus.

V D DISCUSSION

Although this section of the study suffers somewhat from a lack of direct positive results the experiments have helped to eliminate a number of factors concerned with plant utilization of phosphates that conceivably could have been important in determining the relative responses of these species to phosphate applied to a krasnozem soil.

Determination of the phosphatase activity of excised root tips showed up to two-fold differences between species in their ability to hydrolyse p-nitrophenol phosphate. The use of excised root tips in these experiments may be criticized because of the possibility of phosphatase enzyme leakage from the cut root surfaces. However, during a half hour incubation period the amount of phosphatase released into solution was found to be negligible in comparison with the activity associated with the root surface. The close correlation between the amount of p-nitrophenol released and the root surface area of the sample for any one species and for roots of different sizes is further evidence of the surface character of this enzyme. The relatively close agreement between the phosphatase activities of non-sterile soil-grown roots and roots grown in nutrient solution receiving soil inoculum was probably due to the analytical technique employed (i.e. phosphatase activities of both root types were measured in solution) rather than to a similarity in microbial root populations and root physiological condition. As pointed out by Ridge and Rovira (1971) the phosphatase activity of soil-grown roots measured *in situ*, if that were possible, is likely to be totally different from that measured in solution because of the presence of factors such as root-soil contact, aeration

and the rhizosphere which are peculiar to the soil situation.

Comparison of the phosphatase activity of sterile and non-sterile roots reveals that the rhizosphere populations of cabbage and lettuce roots have little influence on the total phosphatase activity of the root whereas for both grasses the activity of non-sterile roots was greater than their sterile counterparts. This suggests that the rhizosphere populations of the grasses have different properties or have different effects on the roots than do those of the vegetables, at least under these culture conditions. The increase in phosphatase activity after microbial inoculation of grass roots is contrary to the reports of Gilliam (1970) and Ridge and Rovira (1971). The increase is probably due to physiological changes in the roots produced by the presence of micro-organisms rather than to a direct effect, due to the phosphatase activity of the rhizosphere micro flora. Ridge and Rovira (1971) have found that a suspension of 10^9 viable micro-organisms of high phosphatase activity made a negligible contribution to the total phosphatase activity of a root sample.

Although up to two-fold differences between species in p-nitrophenol activity of excised root tips was observed in these experiments it does not necessarily follow that similar results would apply to the hydrolysis of more complex organic phosphates that occur in the soil situation. Ikaya, Nisizawa and Miwa (1964) found the phosphatase activity of three different plant tissues was due to the presence of more than one isozyme, each with a different specificity range. Some were capable of attacking a wide range of esterphosphates including aryl phosphates, phosphomonoesters and phytin while others were

restricted to only a few substrates. In general where the specificity of the isozyme was broad, the rate of hydrolysis of a substrate decreased as the structure of the organic phosphate became more complex. Greaves and Webley (1965) and Ridge and Rovira (1971) have all cautioned the practice of extrapolating the results from hydrolysis of simple organic compounds to those situations involving hydrolysis of more complex organic phosphates such as occur in soil. Under these circumstances more detailed work is required in order to establish the significance of phosphatases in a natural situation.

The results of experiment V B 2.1 involving utilization of sodium inositol hexaphosphate were disappointing. The low dry weight yields and the apparent inability of all plant species to utilize this form of phosphate may be attributed to the presence of sodium and to the low levels of phosphorus applied. In the second experiment (V B 2.2) these deficiencies were rectified by increasing the amount of phosphorus and basal nutrients applied and by adding the organic phosphate in the form of a calcium salt. Under these conditions sterile and non-sterile plants of all species were able to hydrolyse inositol hexaphosphate and utilize the phosphate released for plant growth. This agrees with the results of Wild and Oke (1966) for clover plants grown in solution culture and also those of Szember (1960) and Martin and Cartwright (1971) working with radish and ryegrass plants respectively in sand culture. As observed in experiment V A the presence of micro-organisms appeared to have little effect on the hydrolysis and utilization of inositol hexaphosphate by each plant species although there was a tendency for sterile plants in both the calcium inositol hexaphosphate and no phosphate treatments to have a slightly greater

total phosphorus content than their non-sterile counterparts. Immobilization of some of the available inorganic phosphate within cells of the microflora as shown by Menzel (1971) may explain this phenomenon although other changes such as subtle biochemical or physiological alterations of the plant, induced by the presence of micro-organisms, may also be involved.

Despite the known ability of these and other plant species to hydrolyse inositol hexaphosphate in sand and solution culture the recent reports of Greaves and Webley (1969) and Martin and Cartwright (1971) have shown that in soil or sand-soil mixtures no hydrolysis of this organic phosphate could be detected even though phosphatases of microbial and plant origin were known to be present. Apparently in the soil situation the formation of insoluble salts of inositol hexaphosphate and its adsorption to soil colloids together with adsorption and inactivation of the phosphatase enzyme effectively prevents the hydrolysis of this compound. For these reasons it would appear that inorganic phosphate reaction products are more important in the phosphate nutrition of soil-grown plants, than are organic phosphates, at least in the short-term.

In the present study only one reaction product, brushite ($CaHPO_4.2H_2O$), was positively identified in the precipitate resulting from reaction of krasnozem soil with saturated mono-calcium phosphate solution. However, a number of other products were probably present in trace amounts and remained undetected. Longer reaction times may have been an advantage in this regard by increasing the amounts of these products so that identification was possible. Das and Datta (1968) found similar results; brushite being one of the major component of the precipitates

obtained after reaction of mono-calcium phosphate with a red sandy-clay loam for half, 5 and 72 hours. The amount of brushite tended to decrease with time whilst that of other products increased.

The availability of a selected range of phosphate reaction products to the four species, lettuce, cabbage, ryegrass and phalaris was determined on the basis of the plant's total shoot phosphorus content at harvest. Root phosphorus was not determined because of the problem of root surface contamination with particulate and adsorbed phosphate present in high concentration in the sand culture. Phosphorus adsorbed to root cell walls may be expected to vary with the particular source of phosphate present and would be of major importance with aluminium phosphate sources (see Section II B 2.2(b)). Nevertheless, the use of shoot phosphorus as the criteria of availability may be criticized because of the differences in root phosphorus ratio that occur between plants of different phosphorus status (see Tables IV A 3.3(iii) and IV B 3.2). Thus the use of shoot phosphorus only, tends to underestimate the availability of the poor phosphate sources, such as strengite and acid potassium iron phosphate, relative to the more available sources such as brushite.

Relative to the maximum shoot phosphorus contents the availability of the various phosphate sources, averaged for the four plant species decreased in the order brushite $>$ colloidal aluminium phosphate $>$ colloidal iron phosphate $>$ potassium taranakite $>$ potassium iron phosphate $>$ strengite. This is similar to the ranking given these phosphate compounds by Lindsay and Taylor (1960) for corn grown on neutral soils and is also similar to the relative initial rates of solution

of these compounds in water, as given by Huffman (1962). The correlation of availability ranking with the relative initial rates of solution of these compounds suggests that their effectiveness as sources of phosphorus to plants depends directly upon their relative reactivities in the medium in which they are placed. Since changes in pH of the medium or changes in surface area of the reaction product will alter the rate of phosphorus dissolution from such compounds these factors will also effect the availability of the reaction products to plants.

Comparisons between species of the relative shoot phosphorus content of plants grown on each phosphate source (Figure V C 3.2(b)) show that the relative availability of some sources depends upon the plant species involved. Thus with colloidal aluminium and iron phosphates as phosphorus sources, the relative shoot phosphorus content of the grasses were greater than those of the vegetables, an approximate two-fold difference occurring between the extreme values of lettuce and phalaris. With the calcium phosphate sources, however, the position was reversed, the vegetables having the higher phosphorus contents. Of the vegetable species, cabbage in all cases had higher relative phosphorus contents than lettuce. This was most marked with potassium iron phosphate, colloidal iron and aluminium phosphates and calcium phytate as the phosphorus sources.

The differences between species groups also occurred with the yield data; grasses achieving maximum yields when grown on colloidal aluminium and iron phosphates while the vegetables produced their maximum yields where calcium phosphates were used as the phosphorus sources. Since an increase in pH would

make the iron and aluminium phosphates more available (i.e. increase the rate of phosphate dissolution) and calcium phosphates less available the different responses of the grasses and vegetables may be the result of differential rhizosphere pH's between the two plant groups. Plant species have been found by Barber (see Riley and Barber, 1971) to differ in the pH change induced in the rhizocylinder by different nitrogen treatments and it is possible that in the present study the rhizocylinder pH of the grasses is higher than that of the vegetables. The experimental results, however, (Tables V C 3.2 (b)(i) and (ii)), indicate that other factors are involved since the reductions in plant yield are not always accompanied by reduced shoot phosphorus concentrations suggesting that nutrient toxicity problems or an imbalance of nutrients may have developed in some treatments.

VI PLANT GROWTH AND PHOSPHATE ABSORPTION FROM NUTRIENT SOLUTION

TABLE OF CONTENTS

VI A CONTINUOUS-FLOW NUTRIENT SOLUTION EXPERIMENTS

VI A 1 INTRODUCTION

It was suggested (Section IV E) that the differential response of cabbage, lettuce, ryegrass and phalaris to low levels of phosphate applied to a krasnozem soil may be at least partly explained by species differences in the ability to absorb phosphate from the soil solution. However, in soil experiments, other factors such as the amount of root extension and soil exploitation and the effects of rhizosphere micro-organisms and root exudates on the solubility and plant utilization of insoluble organic and inorganic soil phosphates also operate simultaneously, so that the importance of each factor becomes impossible to determine. In addition the fluctuation in phosphate concentration around the root with time, as shown in Section IV D, means that all species grown at the same treatment level were not subjected to equal and constant phosphate concentrations around their roots. Because of this difficulty it was not possible to make accurate measurement and species comparisons of the rate of phosphate absorption.

In the following experiments the mean rate of phosphate absorption and the growth characteristics of the four plant species have been measured under controlled solution culture conditions. Unlike the soil situation, the phosphate concentration of each treatment was maintained relatively constant throughout the growth period so that all roots of a single treatment whether developing or senescing absorbed phosphate from a medium of constant phosphate concentration. In addition

results from solution culture experiments can be interpreted without reference to the factors, mentioned above, which complicate the interpretation of results in soil experiments.

VI A 2 MATERIALS AND METHODS

VI A 2.1 Apparatus

The continuous-flow nutrient system constructed for these experiments was an adaption of the design described by Asher, Ozanne and Loneragan (1965). Eight individual units were built, each unit consisting of a storage tank (a 450 l galvanized iron water tank painted internally with three coats of Epoxy-4 High Gloss Enamel - British Paints Ltd.), a poly-vinyl chloride (P.V.C.) or polythene head tank, a small capacity centrifugal water pump with a plastic impellor (Eilbeck and Co. Pty. Ltd., Sydney), six 3 l plastic pots and various P.V.C. connecting pipes.

Nutrient solution, pumped from storage tanks below the glasshouse benches to head tanks above these benches, was allowed to flow under gravity through the six pots of each unit and back into the storage tanks. Solution was pumped into the head tank at a rate slightly exceeding the total flow rate through the six pots, the excess solution being returned to the storage tank via a rigid P.V.C. overflow pipe (2.25 cm, diameter). This system provided a constant head of nutrient solution above the pots. From the head tank solution flowed into a rigid P.V.C. manifold (2.25 cm diameter pipe) and then via a 10 cm length of 0.4 cm diameter flexible P.V.C. tubing and a narrow bore, rigid P.V.C. connecting tube into each of the six pots per unit, which were arranged in parallel with respect to solution flow. Solution entered each pot near the

base and left via a 0.6 cm diameter, flexible P.V.C. tube situated near the top of each pot on the opposite side. To prevent plant roots blocking the outlet tubes fine stainless steel mesh (0.1 cm aperture and 3.5 cm square) was hung from the lip of each pot to cover the outlet.

The flow rate through each pot was controlled by the head of water above the pots and the length and internal diameter of the rigid P.V.C. connecting tubes. Using a 60 cm head of water and P.V.C. connecting tubes, 5.2 cm long and 0.25 cm internal diameter, a mean flow rate of 704 $\pm$ 18 ml/min (approximately 1000 l/pot/day) was recorded by measurement of flow rates in 32 pots (4 pots/unit). The top of each pot was covered with a 20 cm square of "Darvic" P.V.C. sheeting (0.4 cm thick) and plants were supported, in 1 cm diameter holes drilled in the sheet, by means of a plug of crimped "Terylene" fibre (Fibremakers Ltd., Bayswater, Victoria). Nutrient solution in each pot was aerated via a glass tube passing through the centre of each "Darvic" square. Air was supplied from a compressor which automatically provided aeration for five minutes in every twenty, the air being reticulated throughout the glasshouse in overhead polythene pipes. The eight units constructed were set up on two metal and lathe glasshouse benches (2 m square) in the air-conditioned glasshouse (see Plate VI A 2.1). All external surfaces of the units were painted with aluminium paint to reflect radiant heat and to prevent light entering the system through the P.V.C. and plastic components. The top surface of the "Darvic" pot covers were painted with a white plastic paint to avoid the possibility of plant aluminium toxicity. Although no provision was made for controlling the temperature of the nutrient solution, the

PLATE VI A 2.1

Eight continuous-flow, nutrient solution culture units, each of six pots, set up on two metal and lathe benches in the glasshouse. Note the 450 l storage tanks below the benches, the head tanks to the right and the centrifugal pumps beneath these tanks. Nutrient solution flows under gravity from the head tank to the six pots via the P.V.C. manifold seen in the foreground and air is supplied via the yellow P.V.C. tubing above the pots.

circulation of the solution, the reflective external surfaces, the air-flow in the glasshouse and the fact that the storage tanks were shaded by the benches meant that the nutrient solution temperature rarely exceeded the air temperature.

VI A 2.2 Experiment 1

VI A 2.2(a) Design and treatments

A split plot design with phosphate concentration in solution as the main effect (unreplicated) and species as sub-plots within each mainplot was used in this experiment. As comparisons within vegetable species and within grass species were considered more important than comparisons between vegetables and grasses the two groups were grown in separate units. Four levels of phosphate in solution were established, the nominal values of these being 0.1, 0.3, 0.9 and 2.7u MP. One bench of four units was used for the vegetables (3 pots of lettuce and 3 pots of cabbage/unit), each unit being maintained at a different phosphate concentration. This gave three replicates of each species within each mainplot. A second bench of four units was set up in a similar manner for the grasses. Phosphate concentrations were randomly assigned to the four units of each bench and the six pots within each unit were also randomly assigned to the two plant species concerned. To reduce variations in growth and phosphate absorption, resulting from slight differences in solution flow rates between pots of any one phosphate level, the "Darvic" pot covers with their plants were systematically moved to the adjacent pot every three days throughout the growth period.

VI A 2.2(b) Plant culture and harvest

Seedlings were raised in conventional nutrient solution cultures in growth cabinets. Seeds were germinated on cheese-cloth squares supported on stainless steel mesh screens (0.2 cm aperture) over six litres of aerated Hoagland's solution minus phosphorus. During germination no phosphorus was added to the vegetable cultures but 2 mg P/culture vessel was added to the nutrient solutions of the grass cultures as the grasses had lower seed reserves of phosphorus than the vegetables. All solutions were replaced after one week. After two weeks the young seedlings were transferred to the continuous-flow nutrient solution units. Four seedlings of the vegetable species or eight grass seedlings were transplanted into the appropriate pots, one species being used for each individual pot. At the same time a subsample of 20 lettuce or cabbage plants and 40 ryegrass or phalaris seedlings were harvested, oven dried and kept for chemical analysis.

Each continuous-flow nutrient unit held a total volume of 450 l of solution consisting of a basal nutrient solution of $^1/_5$ Hoagland's solution minus phosphorus to which the required concentration of phosphate was added as a buffer mixture of KH_2PO_4 and K_2HPO_4 having a pH of 5.6. The solution in each unit was drained and replaced at weekly intervals to ensure an adequate supply of basal nutrients. The solution in each unit was sampled each day and analysed for phosphate using the ascorbic acid reduction method of Watanabe and Olsen (1965). The optical density of the reduced phosphomolybdate complex was determined on a Unicam SP800 Ultraviolet Spectrophotometer fitted with a scale expansion accessory. The solutions were read at a wavelength of 710 n m against a distilled water blank in 4 cm pathlength cuvettes. Using the scale expansion

accessory at x10 or x20 even the lowest phosphate concentration could be determined directly and alcohol extraction to concentrate the phosphomolybdate complex was not required. Where necessary, as indicated by the daily analyses, the solution in each unit was readjusted to the correst phosphate concentration by additions of phosphate buffer at pH 5.6. The pH of each solution was measured daily using a Radiometer (Type PHM26) pH Meter and was readjusted to pH 5.6 with either dilute KOH or H_2SO_4. To help maintain the phosphate concentration and pH constant during the period between the daily analyses, 24 hr drip-feed systems (300 ml Mariotte's resevoirs, Hewitt (1966)) were constructed for each unit. These continually supplied phosphate buffer and dilute acid to the nutrient solution of each unit at rates approximately equivalent to their rates of removal from solution by the growing plants. Drip-feed systems were refilled each day, the amount of phosphate or H_2SO_4 added being determined by extrapolation from requirements of the preceeding two or three days.

All plants were harvested after 25 days growth in the continuous-flow nutrient units. Shoots were removed and after fresh weight determination were rinsed in deionized water and oven dried. Roots were rinsed in two changes of deionized water, hand shaken in fine terylene mesh "teabags" to remove free water and oven dried after recording fresh weights.

VI A 2.3 Experiment 2

VI A 2.3(a) Design and treatments

This was a split plot design of two species, cabbage and lettuce, set up as subplots within mainplots which consisted of eight phosphate concentrations, 0.06, 0.12, 0.24, 0.48, 0.96,

(see Appendix VI D)
3.84, 7.68 and 30.72 uMP. Mainplots were not replicated but each species was replicated three times at each phosphate concentration, four plants of either lettuce or cabbage per pot comprising one replicate. Eight continuous-flow nutrient units were used, each unit being maintained at a different phosphate concentration. The randomization procedures employed in experiment 1 (Section VI A 2.2) were used to determine the arrangement of the various treatments and to minimize variations in growth and nutrient uptake between plants in different pots of the one unit.

VI A 2.3(b) Plant culture and harvest

Lettuce and cabbage seeds were germinated as previously described (Section VI A 2.2(b)) but after one week the seedlings were placed on modified Hoagland's solution in which phosphorus was 1/10 of full strength (100µMP) and the other macro- and micro-nutrients were at full strength. Twelve days after germination the seedlings were transferred, four per pot, to the continuous-flow nutrient units. Three subsamples of each species (20 and 25 plants/subsample for cabbage and lettuce respectively) were also harvested at the same time and kept for chemical analysis.

From the experience gained in experiment 1 the basal nutrient solution was modified so that macro-nutrients apart from phosphorus were at a concentration equivalent to 1/5 Hoagland's solution while micro-nutrients and iron were adjusted to 2/5 Hoagland's solution. Apart from this change the units were operated in an identical manner to that described earlier (Section VI A 2.2(b)).

After 35 days growth in the continuous-flow units all plants were harvested. Shoots were removed from the roots, fresh weights were recorded and after rinsing in deionized water the shoot samples were oven dried. Roots were rinsed in two changes of deionized water, centrifuged at approximately 300 g in an M.S.E. Super Minor bench centrifuge to remove free water and fresh weights were recorded prior to oven drying.

VI A 3 RESULTS

VI A 3.1 Experiment 1

(a) General plant growth and conditions during growth

This experiment was conducted in mid-summer when the mean daylength during the growth period was 14.7 hours and the mean maximum and mean minimum daily temperatures in the glasshouse were 28°C and 16°C respectively. Under these conditions plant growth was rapid, where phosphate levels were adequate, and visual differences in plant size and colour between treatments were apparent after seven days' growth. After ten days' growth in the nutrient system an interveinal chlorosis appeared on young leaves of cabbage and to a lesser extent on young lettuce leaves. Grass plants at the highest level of phosphate were also slightly chlorotic. The symptom appeared on vegetable leaves at all phosphate concentrations except the lowest and was most marked at the highest phosphate concentration. The chlorotic symptom was diagnosed as iron deficiency and all plants were immediately sprayed with a solution of "sequestrene NaFe". In addition the iron concentration of the nutrient solution of all treatments was adjusted to 2/5th of normal Hoagland's concentration. These measures corrected the chlorosis

and the higher iron concentration was, therefore, maintained for the remainder of the experiment.

The mean phosphate concentration and the mean pH of the nutrient solution of each treatment were calculated from the daily determinations made over the growth period and these are given in Table VI A 3.1(a). The mean phosphate concentrations were in all cases slightly lower than the nominal values. The greater phosphate absorption by vegetables in comparison with grasses resulted in slightly lower mean values and greater variation of phosphate concentration for treatments containing vegetables than for the grasses.

TABLE VI A 3.1(a)

Mean Phosphate Concentration and pH ($x \pm t_{0.05}\, s\bar{x}$) of Nutrient Solutions Containing Vegetables and Grasses

(Each value the Mean of 22 readings)

Solution Number	Nominal Value	Measured Value Vegetables	Grasses
		Solution Phosphate Concentration (uMP)	
1	0.1	0.08 ± 0.02	0.09 ± 0.02
2	0.3	0.25 ± 0.06	0.28 ± 0.01
3	0.9	0.79 ± 0.12	0.89 ± 0.07
4	2.7	2.56 ± 0.09	0.27 ± 0.09
		Solution pH Value	
1	5.6	5.66 ± 0.22	5.64 ± 0.21
2	5.6	5.70 ± 0.23	5.67 ± 0.24
3	5.6	5.71 ± 0.27	5.68 ± 0.24
4	5.6	5.58 ± 0.14	5.62 ± 0.20

VI A 3.1(b) Dry weight yields and tissue phosphorus concentrations

The mean plant dry weights and the mean phosphorus concentration in root and shoot tissues are presented in Table VI A 3.1(b). Both dry weight yields and tissue phosphorus concentrations of all species increased with increasing phosphate concentration in solution but at the highest level, 2.7uMP, there was in most cases a depression of dry weight yield while the level of phosphorus in the tissue increased in comparison with lower treatment concentrations. The data for dry weight and tissue phosphorus concentrations were statistically analysed using analysis of variance (Appendix V A). No valid comparisons could be made between the grasses and the vegetables because these two groups of plants were grown in different continuous-flow units. However, it was possible to make orthogonal comparisons between lettuce and cabbage at each phosphate concentration and a separate comparison between ryegrass and phalaris at each phosphate concentration using the least significant difference (lsd) test, (Steel and Torrie, 1960). Due to the large differences between species in dry weight yields, the total dry weight yield (shoot plus root) of each species, at each treatment, was expressed as a percentage of the maximum yield of that species and the arcsine $\sqrt{\quad}$ transformed values of these were analysed. On this basis the dry weight yields of cabbage were significantly greater ($p = 0.001$) than those of lettuce at the two lowest levels of phosphate. In addition the root and shoot phosphorus concentrations of cabbage at the lowest level of phosphate (0.1uMP) were significantly greater ($p = 0.001$) than those of lettuce. For the grasses there was no significant difference between the two

TABLE VI A 3.1(b)

Mean Dry Weight Yields and Tissue Phosphorus Concentrations of Vegetables and Grasses.

(Each value the mean of three replicates)

Phosphate Concentration (uM)	Shoot Dry Weight (g/plant)	Root Dry Weight	Shoot P Concentration (ug/g dry tissue)	Root P Concentration
		Cabbage		
0.1	1.364	0.306	1956	3040
0.3	2.343	0.461	2349	3273
0.9	3.869	0.434	4032	4451
2.7	2.265	0.280	4931	5560
		Lettuce		
0.1	0.035	0.021	1452	1992
0.3	0.203	0.094	2617	3372
0.9	1.010	0.204	4826	5032
2.7	0.721	0.186	5284	5335
		Ryegrass		
0.1	0.136	0.084	3582	3297
0.3	0.262	0.138	3841	3679
0.9	0.257	0.125	5161	4693
2.7	0.266	0.138	6958	6301
		Phalaris		
0.1	0.077	0.047	3501	2758
0.3	0.117	0.61	3513	3082
0.9	0.125	0.61	4805	4240
2.7	0.103	0.54	8136	6898

species when comparisons of total yields, as a percentage of the maximum, were made at each phosphate level. This occurred despite the fact that at the three lowest levels of phosphate the root tissue phosphorus concentrations of ryegrass were higher ($p = 0.05$) than those of phalaris. There was no significant difference between the shoot phosphorus concentrations of the grasses at the three lowest levels of phosphate.

VI A 3.1(c) Mean relative growth rates and rates of phosphate absorption

These figures (Table VI A 3.1(c)) were calculated from the mean values of dry weight and phosphorus concentration given in Table VI A 3.1(b) and were not statistically analysed. At all phosphate concentrations in solution the rate of phosphate absorption by cabbage was from two to three times that of lettuce and the relative growth rate of cabbage was always greater than that of lettuce, this being especially marked at the lowest level of phosphate where the species differed by a factor of 2.7. On the other hand, the rates of phosphate absorption and the relative growth rates of ryegrass and phalaris were similar when comparisons were made at each treatment level. In comparison to the vegetables the rate of phosphate absorption by the grasses was greater at the lowest concentration of phosphate in solution while at the higher phosphate concentrations the rate of uptake decreased in the order cabbage $>$ grasses $>$ lettuce.

VI A 3.1(d) Plant utilization of phosphorus

The distribution of both the absorbed phosphate and the plant dry weight between root and shoot components is given in Table VI A 3.1(c) as the mean values for root phosphorus ratio and

TABLE VI A 3.1(c)

Mean Values of the Following Parameters of Cabbage, Lettuce, Ryegrass and Phalaris Grown at Four Phosphate Levels

Solution Phosphate Concentration (uM)	Root Weight Ratio (% D.W.)		Root Phosphorus Ratio (% Plant P)		Phosphorus Utilization Quotient (g D.M./mg atom P)		Relative Growth Rate* (g D.M./100 g D.M./day)		Rate of Phosphorus Absorption + (ug atoms P/g F.W. root/day)	
					VEGETABLES					
	C	L	C	L	C	L	C	L	C	L
0.1	18.4	38.2	25.9	44.9	14.3	18.7	18.8	7.0	2.2	0.7
0.3	16.4	31.6	21.5	37.3	12.4	10.8	20.9	13.7	7.2	2.2
0.9	10.1	16.8	11.0	17.3	7.6	6.4	22.6	19.3	18.4	7.5
2.7	11.0	20.5	12.2	20.7	6.2	5.9	20.5	18.2	18.2	6.7
					GRASSES					
	R	P	R	P	R	P	R	P	R	P
0.1	38.0	37.9	36.2	33.7	9.0	9.9	16.0	15.4	5.1	4.2
0.3	34.5	34.3	33.6	31.2	8.2	9.2	18.4	16.9	6.6	5.2
0.9	32.7	32.6	30.7	30.2	6.4	6.7	18.2	17.1	9.6	7.3
2.7	34.2	34.4	32.0	30.6	4.8	4.0	18.5	16.4	11.3	10.8

* $R.G.R. = \frac{\log e\ D.W._2 - \log e\ D.W._1}{t2 - t1} \times 100$ where $D.W._2$ and $D.W._1$ = Plant dry weight at harvest and transplanting respectively.

t2 - t1 = period of growth (25 days)

+Williams (1948)

root weight ratio (i.e. the mean root value expressed as a percentage of the mean total plant value). These values for ryegrass and phalaris are remarkably similar at each phosphate concentration but large differences in both root phosphorus and root weight ratios were found between lettuce and cabbage. In both cases and at each phosphate concentration cabbage had the lower value, the differences between the two species being greatest at the lowest solution phosphate level.

The mean phosphorus utilization quotients (g dry plant material/unit of phosphorus) of ryegrass and phalaris were similar when comparisons were made at each treatment level and compared with the values for the vegetables, the grasses produced less plant dry weight/unit of absorbed phosphate at all four concentrations of phosphate. In contrast to the earlier soil experiments (Sections IV A and B), cabbage had a smaller utilization quotient than lettuce at the lowest level of phosphate but had slightly higher values than lettuce at the other levels of phosphate.

VI A 3.2 Experiment 2

(a) General plant growth and conditions during growth

Unlike the previous experiment carried out in mid-summer, this experiment was conducted in late autumn and the shorter day length (9.6 hours) and lower temperatures (mean daily maximum and minimum being 19°C and 12°C respectively) together with lower light intensities necessitated a longer growing period (35 days vs 25 days) so that sufficient plant tissue could be obtained for analysis. Even so, the total plant dry weights were less than those obtained for cabbage and lettuce in the previous experiment. Despite the higher concentrations of micro-nutrients and iron in the nutrient solutions (see Section VI A 2.3(b)) slight chlorosis of cabbage and lettuce leaves occurred at the higher concentrations of phosphate. To alleviate the problem iron and micro-nutrients were added to these treatments midway between the weekly change of solutions to give an increase in concentration equivalent to that in 1/5th Hoagland's solution. Phosphorus deficiency at the lower phosphate concentrations produced a marked stunting of both lettuce and cabbage plants and resulted in pink pigmentation of cabbage leaves and stems and the death of cotyledons on both species (Plate VI A 3.2(a)). At the two highest phosphate concentrations (7,7 and 30.7 uMP) plant size appeared to be depressed, plants in these treatments having a general chlorosis of the older leaves.

The mean of the daily determinations of phosphate concentration and pH of each nutrient solution are presented in Table VI A 3.2(a). These values are close to the nominal values, the greatest departure occurring at the highest phosphate concen-

PLATE VI A 3.2(a)

Cabbage and lettuce plants grown at eight levels of phosphate in the continuous-flow nutrient units. Photographed at harvest after 35 days growth in the units. Numbers 1-8 are plants grown in solutions of 0.06, 0.12, 0.24, 0.48, 0.96, 3.84, 7.68 and 30.72 uMP respectively. Note the relatively better growth of cabbage than of lettuce at the lower solution phosphate concentrations.

TABLE VI A 3.2(a)

Mean Phosphate Concentration and pH ($x \pm t_{0.05}\, s\bar{x}$)

of Nutrient Solutions Containing Lettuce and Cabbage

(Each value the mean of 30 readings)

Nominal Phosphate Concentration (uMP)	Measured Phosphate Concentration (uMP)	Measured pH (Nominal pH = 5.6)
0.06	0.07 ± 0.01	5.55 ± 0.09
0.12	0.12 ± 0.01	5.52 ± 0.13
0.24	0.23 ± 0.02	5.55 ± 0.13
0.48	0.45 ± 0.02	5.57 ± 0.11
0.96	0.96 ± 0.03	5.48 ± 0.13
3.84	3.95 ± 0.08	5.47 ± 0.11
7.68	8.08 ± 0.09	5.54 ± 0.11
30.72	28.33 ± 0.37	5.49 ± 0.09

tration. However, the standard error of the means were less than those of the corresponding phosphate concentrations in experiment 1 (Table VI A 3.1(a)).

VI A 3.2(b) Dry weight yields and relative plant growth rates

As in experiment 1 (Section VI A 3.1(b)) the total plant dry weight yields at each phosphate concentration were expressed as a percentage of the maximum dry weight of each species and the arcsine $\sqrt{\ }$ transformations of these values were subjected to an analysis of variance. The means of these values are presented in Figure VI A 3.2(b). Because the phosphorus treatments were not replicated the main effect of phosphorus could not be tested, however, for species and species x phosphorus interaction components a significant result was obtained ($p = 0.001$). At four of the five lowest phosphate treatments the relative dry weight yields of cabbage were significantly greater than those of lettuce. In addition the maximum plant dry weight for cabbage was achieved at a solution concentration of 0.45 uMP whereas for lettuce a concentration almost nine times that (3.95 uMP) was required to achieve maximum yield. At all phosphate concentrations lettuce had a higher percentage of its total dry weight present in the root component, 29.7 - 18.4% for lettuce compared with 20.5 - 12.1% for cabbage (Table VI A 3.2(b)).

The mean relative growth rates for cabbage were also higher than those for lettuce at each phosphate level (Table VI A 3.2 (b)). However, when comparisons were made at the point of maximum yield the relative growth rates of lettuce and cabbage were much closer being 14.0 and 14.4 g dry matter/100 g dry matter/day, respectively.

FIGURE VI A 3.2(b)

Relative dry weight yields of lettuce and cabbage expressed as a percentage of the maximum yields (arcsine $\sqrt{}$ transformed values), when grown at eight phosphate concentrations in solution culture. Note difference between cabbage and lettuce in the solution phosphate concentration required for maximum yield.

FIGURE VI A 3.2(c)

Relative total plant phosphate content of cabbage and lettuce grown at eight levels of phosphate in solution culture (values expressed as a percentage of the maximum phosphate content).

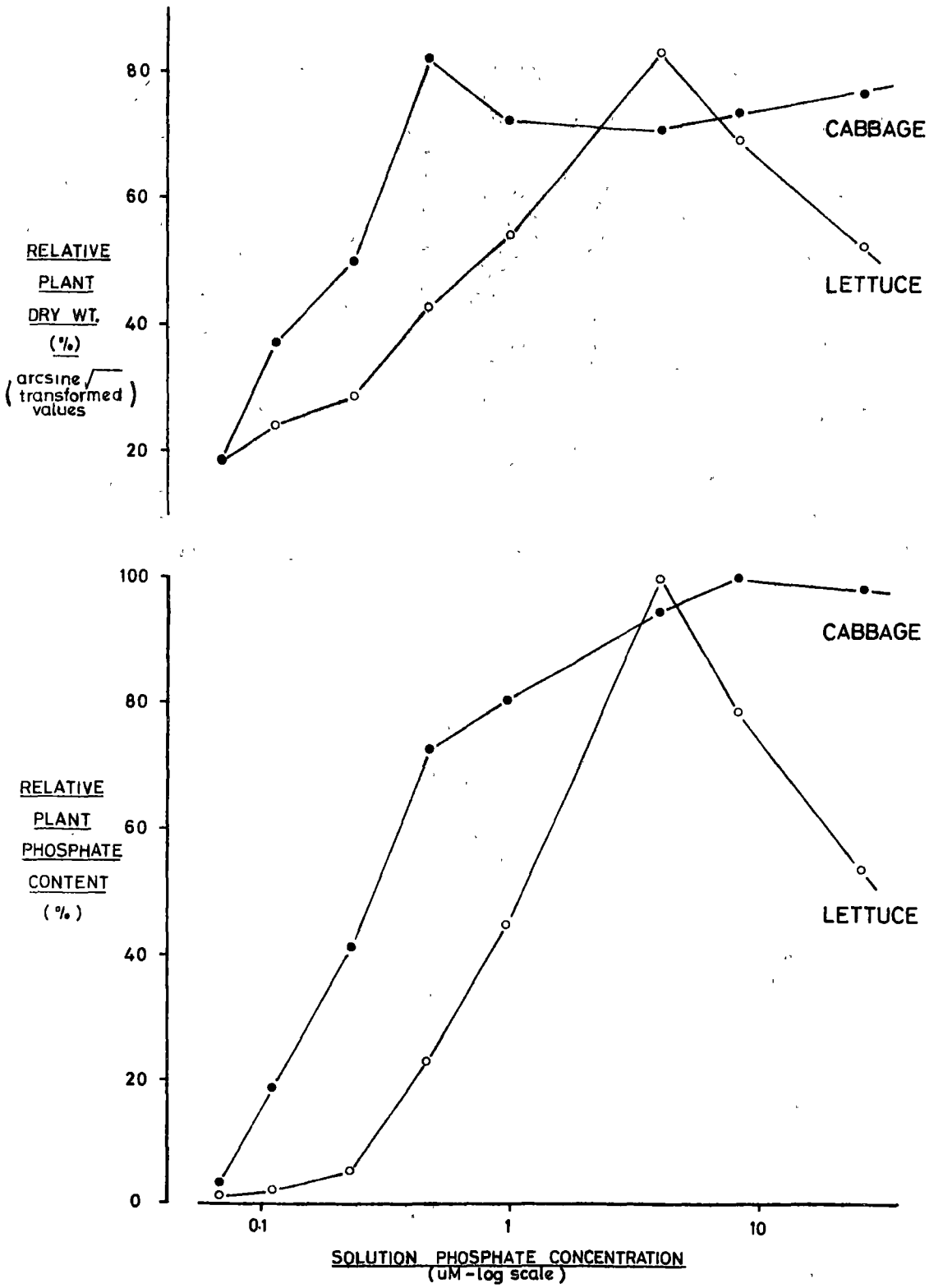

80
60
40
20
RELATIVE PLANT DRY WT. (%) (arcsine √ transformed values)
CABBAGE
LETTUCE
100
80
60
40
20
0
RELATIVE PLANT PHOSPHATE CONTENT (%)
CABBAGE
LETTUCE
0.1
1
10
SOLUTION PHOSPHATE CONCENTRATION (uM - log scale)

TABLE VI A 3.2(b)

Mean Values of the Following Parameters of Cabbage and Lettuce Grown at Eight Phosphate Levels

Solution Phosphate Concentration (uM)	Root Weight Ratio (% D.W.)		Root Phosphorus Ratio (% Plant P)		Phosphorus Utilization Quotient (g D.M./mg atom P)		Relative Growth Rate (g D.M./100 g D.M./day)		Rate of Phosphorus Absorption (ug atoms P/g F.W. root/day)	
	C	L	C	L	C	L	C	L	C	L
0.06	20.0	25.7	34.3	41.5	21.2	38.4	8.3	7.9	1.3	0.6
0.12	20.5	24.5	27.5	45.4	11.9	34.5	11.6	9.0	3.4	0.8
0.24	15.9	28.7	19.1	44.3	8.7	19.6	12.9	10.0	5.8	1.3
0.48	14.0	29.7	16.3	33.6	8.1	8.8	14.4	11.8	7.4	2.7
0.96	13.7	24.7	14.4	24.2	6.6	6.4	14.0	12.9	9.4	4.1
3.84	12.5	18.4	15.0	17.5	5.9	4.3	14.1	14.0	11.3	8.4
7.68	13.0	21.0	16.2	21.8	5.7	4.6	14.2	13.6	11.2	6.8
30.72	12.1	22.7	14.5	26.6	5.9	5.2	14.3	12.8	13.7	6.9

VI A 3.2(c) Tissue phosphorus concentrations, total phosphate absorbed and rates of absorption

The average shoot and root phosphorus concentrations of both species are presented in Table VI A 3.2(c). In both cases there was a significant phosphate x species interaction (see Appendix VB2), the root and shoot phosphorus concentrations of cabbage being significantly greater ($p = 0.001$ for roots and $p = 0.05$ for shoots) than those of lettuce at the four lowest levels of solution phosphate while at the three highest levels, the values for lettuce were significantly greater ($p = 0.05$) than those of cabbage. However, at the point of maximum yield the root and shoot tissue concentrations of cabbage (4484 ppmP and 3741 ppmP for roots and shoots) were much lower than those of lettuce, (6886 ppmP and 7373 ppmP for roots and shoots).

The total plant phosphorus contents of both species were determined from the dry weight and phosphorus concentration data for roots and shoots and the mean values, expressed as a proportion of the maximum total phosphorus content of each species, are presented in Figure VI A 3.2(c). These curves show a similar trend to the corresponding dry weight curves although the difference between lettuce and cabbage is more marked because of the proportionately greater depression of the lettuce tissue phosphorus concentrations at the lower levels of phosphate in solution.

Due to large differences in root dry weight between lettuce and cabbage (Appendix V B.1) the relative or total phosphorus content/plant is not a true indication of the amount of phosphate absorbed/unit of root weight. The mean rates of phosphate absorption/unit root weight were, therefore,

TABLE VI A 3.2(c)

Mean Shoot and Root Tissue Phosphate Concentrations of Lettuce and Cabbage Grown at Eight Phosphate Levels

(Each value the mean of three replicates)

Phosphate Concentration (uM)	Shoot Phosphate (ug P/g shoot D.W.)		Root Phosphate (ug P/g root D.W.)	
	Cabbage	Lettuce	Cabbage	Lettuce
0.06	1197	639	2748	1316
0.12	2374	647	3498	1672
0.24	3413	1233	4274	2441
0.48	3741(a)*	3324(a)	4484	3972
0.96	4664(b)	4871(b)	4941(c)	4728(c)
3.84	5124	7373	6336	6886
7.68	5222	6737	6771(d)	7003(d)
30.72	5151	5675	6286	6926

*Orthogonal comparisons between species at each phosphate concentration - values followed by same letter not significantly different (P = 0.05) by the least significant difference test.

calculated using the formula of Williams (1948). These values were based on root fresh weights rather than dry weights since fresh weight was considered by Loneragan and Asher (1967) to be the better measure of the actual absorbing surface of the root. Mean rates of phosphate absorption per unit of root fresh weight were greater for cabbage than for lettuce at each phosphate concentration (Table VI A 3.2(b)). From solutions containing 0.06 to 0.96 uMP the rate of phosphate absorption by cabbage was from two to four times greater than that of lettuce but at higher concentrations the difference was less. Maximum plant yield occurred at a mean rate of phosphate uptake of 7.4 and 8.4 ug atomsP/g root fresh weight/day for cabbage and lettuce respectively.

VI A 3.2(d) Plant utilization of phosphorus

Phosphorus utilization quotients, calculated from the data for mean total plant dry weight and mean total plant phosphorus content (Table VI 3.2(b)); show a rapid decrease for each increase in solution phosphate concentration until a steady value of about 5-6 g dry matter/mg atom P was attained for both species at the higher levels of phosphate. The inefficiency of lettuce in absorption of phosphate at the three lowest concentrations of phosphate is illustrated by its much higher utilization quotients in comparison with those of cabbage. At the point of maximum yield of each species, however, cabbage was able to produce about twice as much dry matter/unit of phosphate as did lettuce plants.

The distribution of total plant phosphorus between roots and shoots is shown in Table VI A 3.2(b). These root phosphorus ratio values follow a similar trend to the root weight ratios, i.e. decreasing with each increase in solution phosphate concentration, with a tendency for lettuce to show an increase at the two highest levels of phosphate. Throughout the range of phosphate levels used in this study cabbage had a substantially lower percentage of its total phosphorus in the root component than that found for lettuce.

VI B SHORT TERM PHOSPHATE ABSORPTION BY EXCISED ROOTS

VI B 1 INTRODUCTION

As a result of the marked differences between species in their mean rates of phosphate absorption calculated for growth periods of four to five weeks and reported in the previous section (Tables VI A 3.1(c) and VI A 3.2(b)), the following short term uptake experiments were carried out to study the process of plant phosphate absorption. Kinetic analysis of the uptake data was performed to evaluate the biological constants controlling the uptake of phosphate by the roots of the four plant species. Such constants may at least partly explain the different response of each species to increasing increments of phosphate applied to either soil or solution cultures. Experiments were performed using both sterile and non-sterile root tissue in order to evaluate the role of micro-organisms in phosphate absorption.

VI B 2 MATERIALS AND METHODS

VI B 2.1 Phosphate absorption by non-sterile roots

VI B 2.1(a) Production of root material

A weighed quantity of seed of each species (equivalent to about 2000 seeds) was surface sterilized in 100 ml 7% calcium hypochlorite filtrate for one hour and then washed in four changes of sterile distilled water. Sterilized seeds were placed in cheesecloth "teabags" and immersed in aerated 0.5 mM $CaSO_4$ solution at 20°C for six hours before they were placed in the growth cabinet on stainless steel screens (30 x 25 cm)

suspended over 10 l of continuously aerated 0.5 mM $CaSO_4$ solution. After 48-72 hours in the growth cabinet at 20°C germination had occurred and the $CaSO_4$ was replaced by complete 1/10 Hoagland's solution at pH 5.6. These solutions were replaced at three day intervals and root material required for experimentation was excised after 10-12 days growth of lettuce and cabbage seedlings (Plate VI B 2.1) or after 16-18 days for the slower growing grasses.

VI B 2.1(b) Short-term uptake technique

Basically the method followed was that outlined by Epstein, Schmid and Rains (1963). Briefly this consists of incubating a known weight of freshly excised root tissue in solutions of ^{32}P labelled phosphate, precisely controlled with respect to concentration, temperature and pH. After a short period of absorption the exchangeable ^{32}P is desorbed from the root tissue and the amount of actively absorbed ^{32}P in the root tissue is measured. Knowing the specific activity of $(PO_4)^{2}P$ in the original absorption solutions and assuming an identical specific activity in the root tissue the amount of phosphorus absorbed during the time period can be calculated.

$$\text{Since } \frac{^{32}P \text{ solution}}{^{31}P \text{ solution}} = \frac{^{32}P \text{ root}}{^{31}P \text{ root}},$$

$$^{31}P \text{ root} = \frac{^{31}P \text{ solution} \times {}^{32}P \text{ root}}{^{32}P \text{ solution}}$$

PLATE VI B 2.1

Non-sterile cabbage (right front) and lettuce (left front) seedlings photographed after 12 days growth and immediately prior to excision of root tissue for use in short-term phosphate uptake experiments. Note stainless steel stand and screen, covered with a single layer of cheesecloth, for supporting seedlings (at rear).

Roots were excised below the stainless steel screens, rinsed in deionized water and placed in 0.5 mM $CaSO_4$ solution. Root samples were removed, blotted gently with paper towels to remove free water and 0.3 g samples were quickly weighed and placed on a square (20 x 20 cms) of nylon mesh (1 mm apertures) which was formed into a "teabag" and closed with a plastic clip as described by Epstein, Schmid and Rains (1963). Each root sample was labelled with an aluminium strip tied to the plastic clip by a length of cotton thread. The "teabags" with their root samples were each immediately placed in an equilibration solution of 10 l of aerated 0.5 mM $CaSO_4$ solution maintained at 25°C by an immersion thermostat unit, ("Thermomix II" - B. Braum; Western Germany). After a 45 min. equilibration period the root samples were removed individually, at two minute intervals, shaken to remove excess solution and at zero time each sample was immersed in the appropriate, aerated experimental solution (see Plate VI B 2.2). All experimental solutions contained 0.5 mM $CaSO_4$ and ^{32}P labelled KH_2PO_4 (as potassium dihydrogen orthophosphate P32 aqueous solution (Radiochemical Centre, Amersham, Bucks., England) or as high specific activity orthophosphate - P32 in dilute HCl (Australian Atomic Energy Commission, Lucus Heights, N.S.W.). In the latter case the chloride was removed using the method of Leggett et al. (1965)). The solutions were adjusted to pH 5.5 using dilute KOH and H_2SO_4. Just prior to the absorption period an aliquot of solution was removed from each treatment so that the specific activity of the phosphate in solution could be determined. The experimental solutions were held in plastic pots and were maintained at 25°C by immersion in water baths heated by "Thermomix II" units.

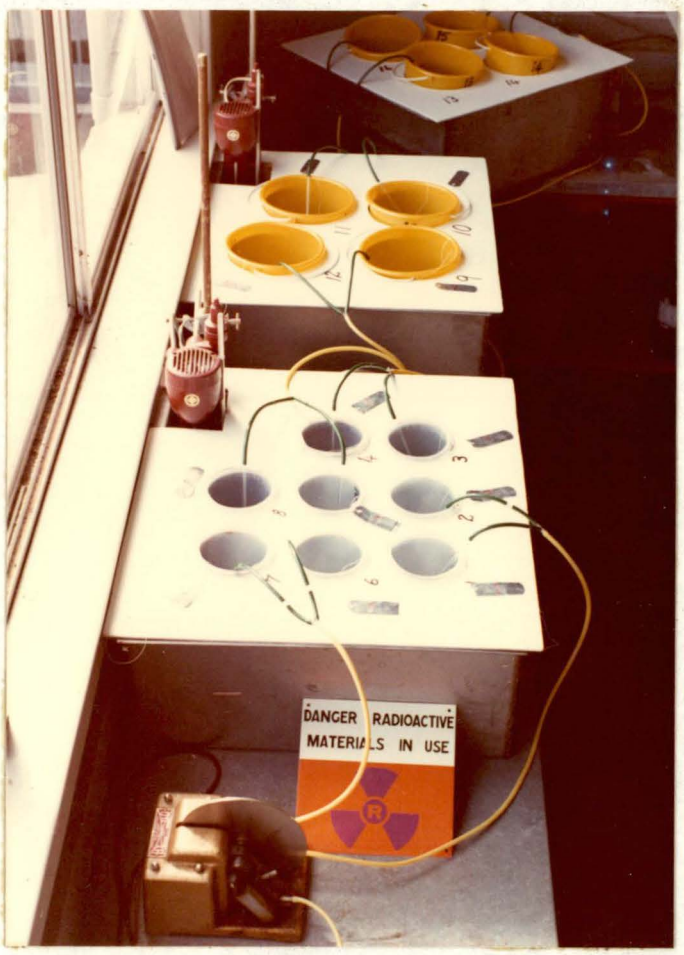


PLATE VI B 2.2

Apparatus used for conducting short-term uptake experiments with excised roots and radio-active phosphate. Radio-active solutions, held in plastic vessels which were immersed in heated water baths, were continuously aerated using the small pump in the foreground. Root samples in nylon mesh "teabags" attached to a cotton thread and a labelled aluminium strip can be seen immersed in each solution.

To prevent depletion of phosphate in solution during the absorption period the volume of solution used varied with phosphate concentration from 2 l at 0.1 uMP to 250 ml at 1000 uMP. The specific activity of phosphate in these solutions also varied with concentration but at no time exceeded 20 uCi/uMP. The highest specific activities were used at the lowest phosphate concentration to ensure that suitable final count rates were obtained in all root samples. At the end of the uptake period of 20 min., each root sample in its "teabag" was individually removed from the solution, washed for 1 min. under cold running tap water and placed in 400 ml of desorbing solution (10 uM KH_2PO_4 (unlabelled) and 0.5 mM $CaSO_4$) maintained at 3-4^{o}C in a refrigerated water bath. After a 45 min. desorption period the "teabags" were rinsed under cold running tap water and the root samples were removed and arranged loosely in a glass counting vial. These samples were oven dried overnight at 65^{o}C.

VI B 2.1(c) ^{32}P Counting procedure

Cerenkov radiation (Jelley, 1958), produced by the radioactive samples' emission of β particles was counted using a Unilux I Liquid Scintillation Counter (Model 6850, Nuclear-Chicago Corp., America) with the amplifier gain control adjusted for maximum counting efficiency for ^{32}P. Distilled water and a wavelength shifter (2-naphthyamine 6, 8-disulphonic acid, NADA), to increase counting efficiency (Läuchli, 1969), were added to each sample to give a total volume of 15 ml solution/counting vial. A final concentration of 2.5 mM NADA was found to produce the optimum count rate and this concentration was established in all vials, including the solutions used for determining back-

ground count rate and absolute counting efficiency. The efficiency of counting ^{32}P in root and solution aliquot samples was determined using a standardised ^{32}P solution (Type S, accuracy 3% - Australian Atomic Energy Commission, Lucas Heights, N.S.W.) and the method of Läuchli (1969). The counting efficiency of ^{32}P in solution was 30.7% while for the root samples it varied with the plant species and ranged from 15.2% for ryegrass to 24.3% for cabbage. These differences in counting efficiency were corrected for in the final calculations.

VI B 2.1(d) Experimental design

The design of these experiments was controlled by the total number of experimental units (i.e. ^{32}P labelled absorption solutions) that could be set up and by the time required to weigh and prepare the corresponding number of excised root samples for experimentation. A maximum number of 20 units was decided upon and with this number of root samples and experimental solutions the entire experimental procedure for each root sample was completed within 3 hours from root excision.

Two types of studies were conducted; "concentration" experiments where phosphate uptake by excised roots in solutions of different phosphate concentrations were measured over a fixed period of absorption, and "time" experiments where absorption was measured for a number of different uptake periods. In each "concentration" experiment excised root samples of two species, either lettuce and cabbage or ryegrass and phalaris (ten samples/species) were incubated at 25°C for absorption periods of twenty minutes in solutions of ten different phosphate concentration; 0.1, 0.5, 1, 5, 10, 25, 50,

100, 500 and 1000 uMP. A separate experimental solution was used for each root sample and solutions at the same phosphate concentration but containing root samples of different species were always incubated in a single water bath. Three different "concentration" experiments were performed, each requiring a separate batch of plant seedlings. In the first experiment phosphate absorption of excised lettuce and cabbage roots were compared, each root sample consisting of the entire seedling root (primary plus laterals). Because of differences in rate of phosphorus uptake between primary and lateral roots of a single species (Russell and Sanderson, 1967; Rovira and Bowen, 1968, 1970) in the second and third experiments, using grass and vegetable species respectively, the roots were excised more carefully so that as far as possible only that part of the primary root without laterals (apical 2-4 cm) was included in the samples.

For each experiment, additional root samples were excised from the same plant batch and "time" experiments at high and low phosphate concentrations were conducted to check that a steady state of root phosphate absorption was maintained over the twenty minute uptake period. In the first and second experiments "time" studies were conducted with the solutions remaining after the "concentration" study had been completed, absorption being measured for 7, 13 and 20 min. uptake periods. In the third experiment with lettuce and cabbage the "time" study was made prior to the "concentration" study and separate experimental solutions were used for each. In the "time" study, phosphate absorption by lettuce and cabbage roots was measured for five time periods, 4, 8, 12, 16 and 20 mins. and two phosphate

concentrations, 0.5 uMP (1500 ml solution) and 500 uMP (250 ml solution), a total of ten solutions/species.

VI B 2.2 Comparison of phosphate absorption by sterile and non-sterile roots

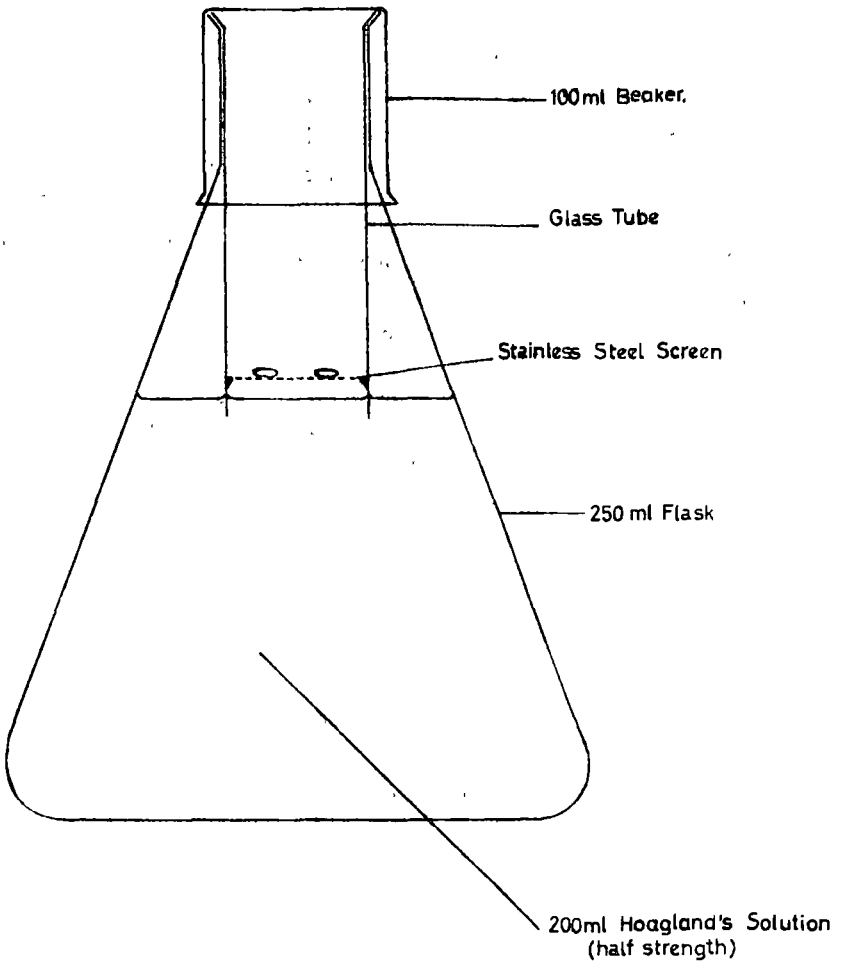
VI B 2.2(a) Production of root material

Seed of lettuce and cabbage was separately surface sterilized by treatment in 0.02% Thiram solution for 48 hours to kill fungi, followed by treatment for 1 hour in 7% calcium hypochlorite filtrate. After washing the seed in four changes of sterile distilled water the seeds were aseptically transferred to sterile plates containing nutrient agar, (Barber, 1967). Where aseptic conditions were required to be maintained all operations were performed on the bench of a laminar air-flow unit (Gelman Clemco Pty. Ltd., Artarmon, N.S.W.). The plates containing the sterilized seeds were placed in the growth cabinet at 20°C and after 72 hours most seed had germinated. Those germinated seeds found to be sterile (free of visible microbial growth) and normally developed were aseptically transferred to sterile 250 ml Erylenmeher flasks capped with inverted 100 ml beakers. Each flask contained 200 ml of sterile ½ strength Hoagland's solution and a stainless steel screen was suspended in a glass tube just above this solution (Figure VI B 2.2). Six sterile, germinated seeds of either lettuce or cabbage were placed on the screen in each flask. Twelve flasks of each plant species were rendered non-sterile by addition of a 1 ml aliquot of a 1% aqueous suspension of krasnozem soil to each flask and the remaining flasks (15) were maintained aseptic. All flasks were replaced in the growth cabinet and three days before harvest a 1 ml aliquot of solution was removed from each flask and plated onto sterile nutrient agar. After 18 days growth the whole

FIGURE VI B 2.2

Diagramatic representation of the apparatus used to grow sterile lettuce and cabbage plants.

The complete apparatus was heat-sterilized prior to the addition of surface-sterilized seeds.


100ml Beaker.
Glass Tube
Stainless Steel Screen
250 ml Flask
200ml Hoagland's Solution
(half strength)

root system of each seedling was excised; the six root systems in each tube comprising one sample for the absorption study.

VI B 2.2(b) Experimental

The short term uptake and counting procedures used in this experiment were the same as those given in Section VI B 2.1(b) and (c) but with the following exceptions:-

(i) Root samples were weighed after the uptake procedure to obtain root fresh weights

(ii) All washing, equilibrating and experimental solutions were sterilized by autoclaving at 121°C for 1 hour, the distilled water, potassium dihydrogen phosphate and calcium sulphate solutions being autoclaved separately

(iii) Sterile and non-sterile root samples were equilibrated in different containers, each holding 2 l of sterile 0.5 mM $CaSO_4$ solution at 25°C.

(iv) Sterile and non-sterile roots of both lettuce and cabbage were incubated in the same sterile experimental solutions during the 30 min. uptake period.

(v) The specific activity of phosphate in the experimental solutions was increased to a maximum of 50 uCi/uMP to obtain sufficient final count rates in the small root samples.

(vi) Desorption of sterile and non-sterile roots was carried out in separate solutions.

Rates of phosphate uptake by sterile and non-sterile lettuce and cabbage excised roots were determined for ten phosphate concentrations in the range 1-1000 uMP. The total

volume of the experimental solutions varied from 500-200 ml and was greatest at the lowest phosphate concentration. Twenty root samples of each species, ten sterile and ten non-sterile were used for this study, one root sample of each treatment group being incubated in each of the ten experimental solutions of different phosphate concentration.

VI B 3 RESULTS

VI B 3.1 Phosphate absorption by non-sterile roots

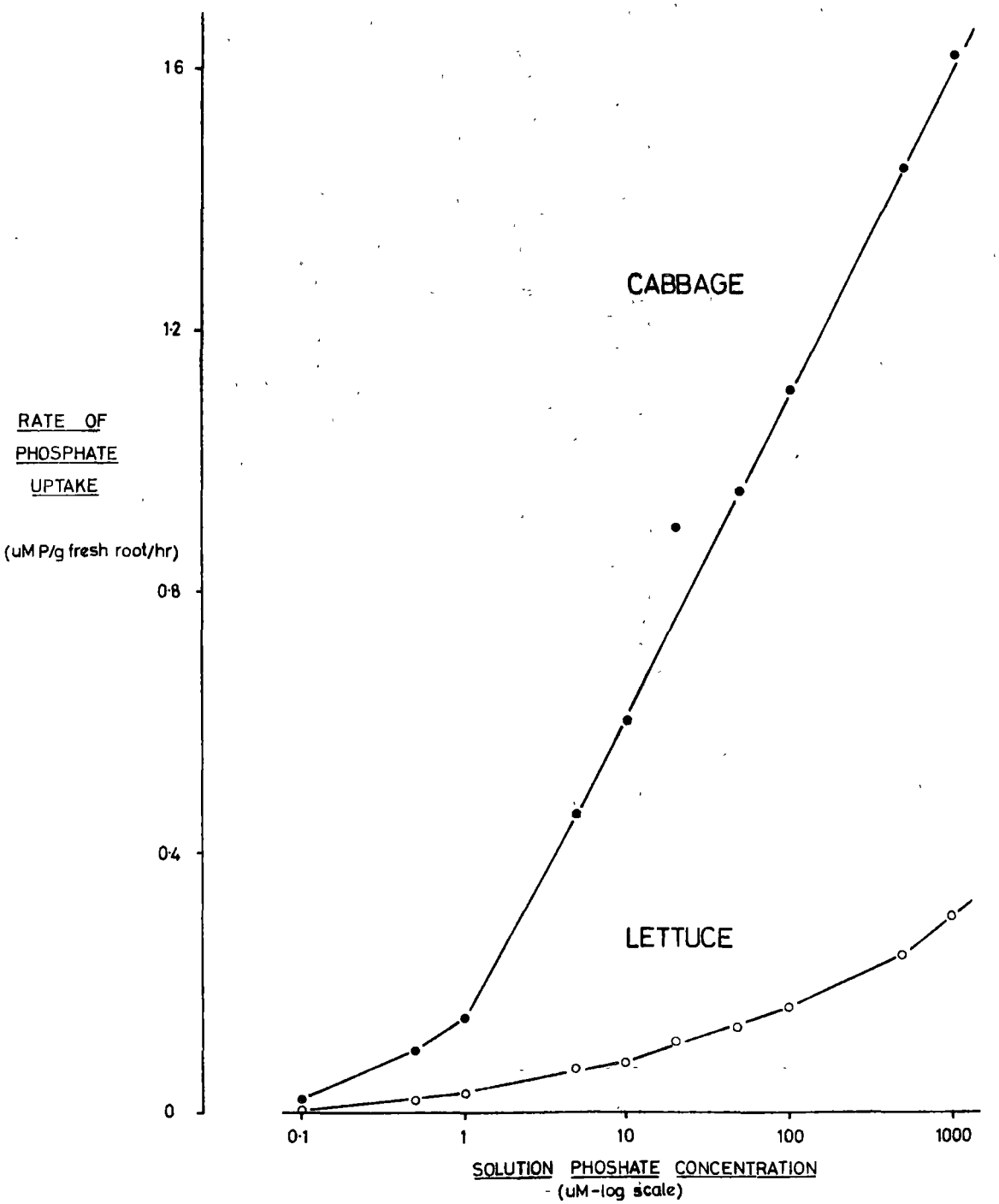
The rates of phosphate absorption by excised roots of the four plant species over the concentration range 0.1 - 1000 uMP are presented in Figures VI B 3.1, 3.2 and 3.3. In both experiments using the vegetable species the rate of phosphate uptake by cabbage roots was found to be two to six-fold greater than that of lettuce roots at all phosphate levels studied. The difference between these species was greater in experiment 1 where all the root (primary plus laterals) was sampled. The absorption plots for lettuce were similar, although not identical, for the two experiments, but for cabbage two to three-fold differences in uptake rate were observed when comparisons were made between experiments. The absorption plots for both grasses were similar to each other but ryegrass had a slightly higher rate of absorption at each phosphate concentration in the range 0.1 - 1000 uMP. The rate of uptake by the grasses was greater than that for both lettuce and cabbage roots when similar samples were used (i.e. primary roots, no laterals).

Kinetic analysis of the phosphate absorption data was carried out in an attempt to isolate the reaction mechanisms involved and explain in detail the components of phosphate absorption in each species. The use of Michaelis-Menten kinetics for the analysis of ion absorption data as described

FIGURE VI B 3.1

Rate of phosphate absorption from solution culture for whole, excised roots of cabbage and lettuce grown under non-sterile conditions.

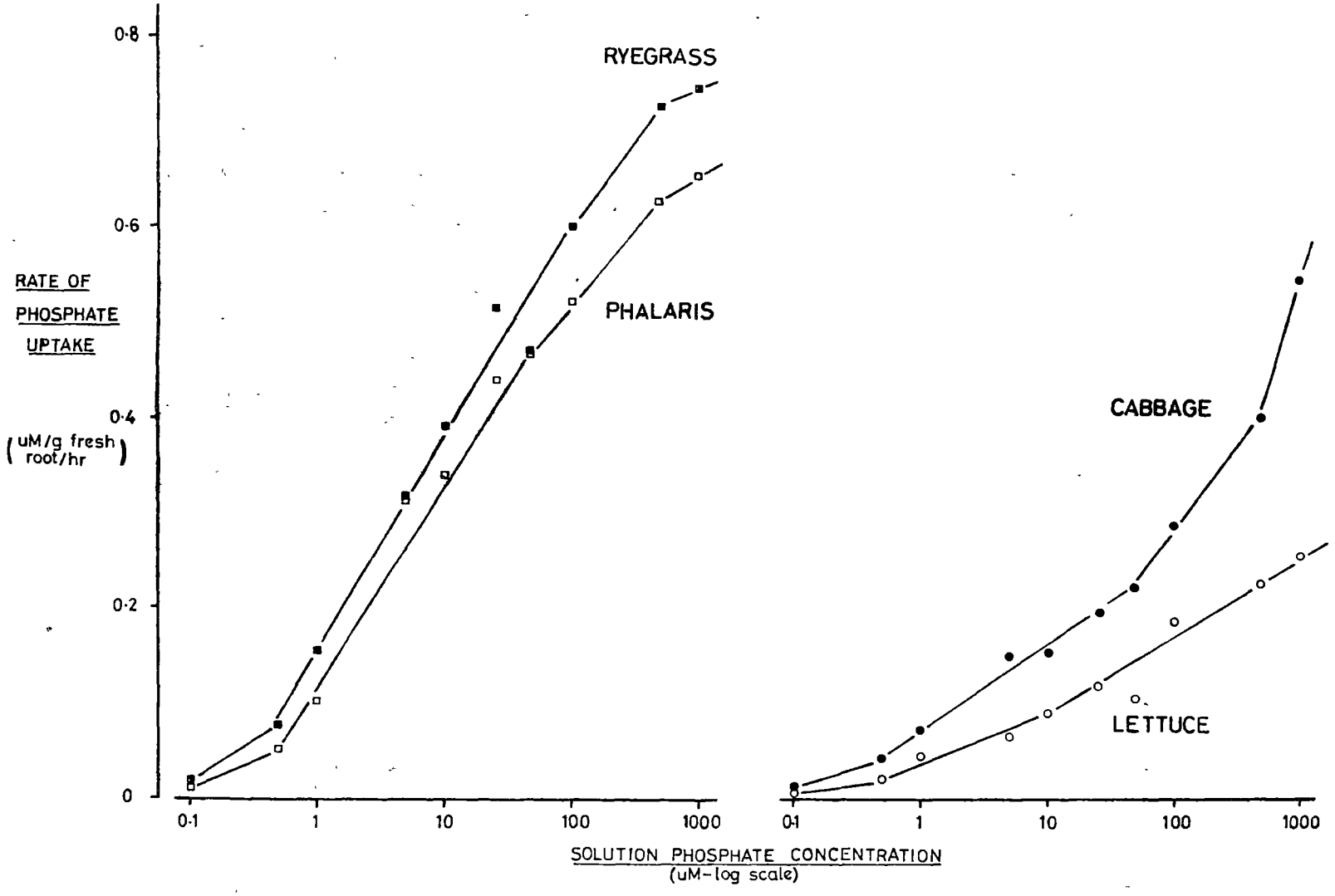
Note the greater rate of phosphate uptake by cabbage over the entire range of phosphate concentrations (0.1 - 1000 uM).


1·6
1·2
0·8
0·4
0
RATE OF PHOSPHATE UPTAKE
(uM P/g fresh root/hr)
CABBAGE
LETTUCE
0·1
1
10
100
1000
SOLUTION PHOSHATE CONCENTRATION
(uM-log scale)

FIGURES VI B 3.2 and 3.3

Rate of phosphate absorption by excised roots (free of laterals) of cabbage, lettuce, ryegrass and phalaris grown under non-sterile conditions in solution culture.

Note the greater rate of phosphate uptake by the grasses in comparison with that of the vegetables, and the greater rate of phosphate uptake by cabbage when comparisons are made between vegetable species.


0·8
0·6
0·4
0·2
0
RATE OF PHOSPHATE UPTAKE
(uM/g fresh root/hr)
RYEGRASS
PHALARIS
CABBAGE
LETTUCE
0·1
1
10
100
1000
SOLUTION PHOSPHATE CONCENTRATION
(uM-log scale)

by Epstein and Hagen (1952) and Hagen and Hopkins (1955) is conditional upon steady state uptake during the period of absorption. This requirement was fulfilled by the "time" studies in which the rate of phosphate uptake by excised roots of all species remained essentially linear at both high and low phosphate concentrations over the 20 min. uptake period, (Figures VI B 3.4 and 3.5). In most cases these "time" lines intersected the axis at the origin and there was no positive intercept on the y-axis.

When rate of phosphate absorption/unit weight of root was plotted against $\frac{\text{rate of absorption/unit weight root}}{\text{phosphate concentration in solution}}$ (Eadie, 1942) a curvilinear relationship was obtained for each species over the concentration range 0.1 - 1000 uMP. These are shown in Figures VI B 3.6 - 3.7 and indicate that for all species two or more first order reactions are involved in the absorption of phosphate by the roots. These curvilinear plots were resolved into two linear components, the "a" and "b" reaction mechanisms, using the graphical method of Hofstee (1952) together with a mathematical solution based on simultaneous equations (see Appendix VIB) to solve for Vmax (the maximum absorption rate) and Km (the apparent dissociation constant of the carrier-phosphate complex) of both "a" and "b" reactions for all four plant species. The values of Vmax and Km are given in Table VI B 3.1 and the contribution of the "a" and "b" reaction mechanisms to the total phosphate absorption capacity of each species was calculated in the concentration range 0.1 - 1000 uMP using the equation

FIGURES VI B 3.4 and 3.5

"Time curves" (phosphate uptake vs absorption time) at high and low solution phosphate concentrations for excised, lateral-free roots of grasses and vegetables.

(R, P, C and L = ryegrass, phalaris, cabbage and lettuce respectively)

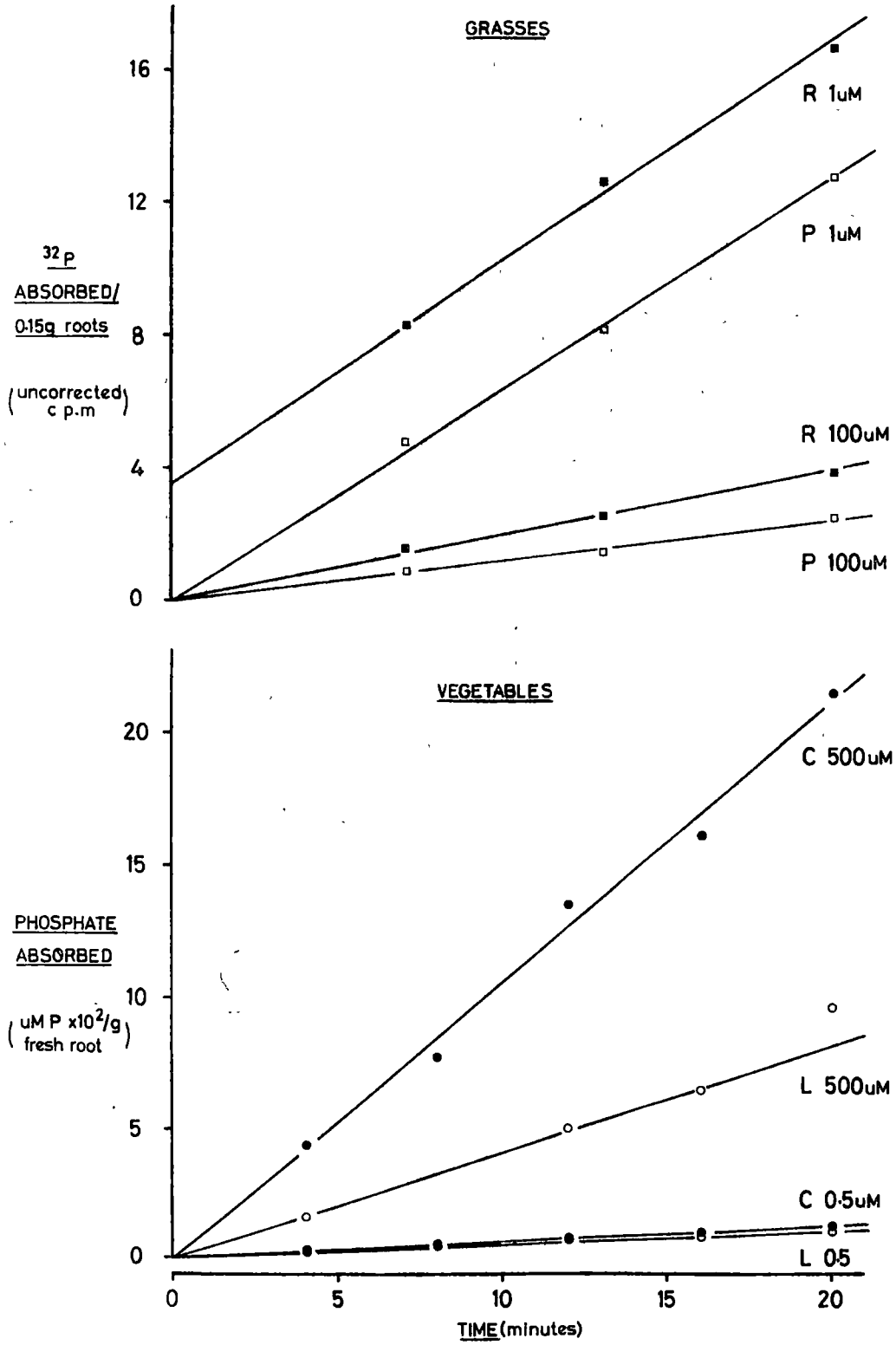

GRASSES
16
12
8
4
0
32P ABSORBED/ 0·15g roots (uncorrected c p.m)
R 1uM
P 1uM
R 100uM
P 100uM
VEGETABLES
20
15
10
5
0
PHOSPHATE ABSORBED (uM P x10²/g fresh root)
C 500uM
L 500uM
C 0·5uM
L 0·5
0
5
10
15
20
TIME (minutes)

FIGURE VI B 3.6

Eadie plots of the phosphate absorption data for whole, excised roots of cabbage and lettuce grown under non-sterile conditions. These have been resolved into the two linear components, the "a" and "b" reaction mechanisms.

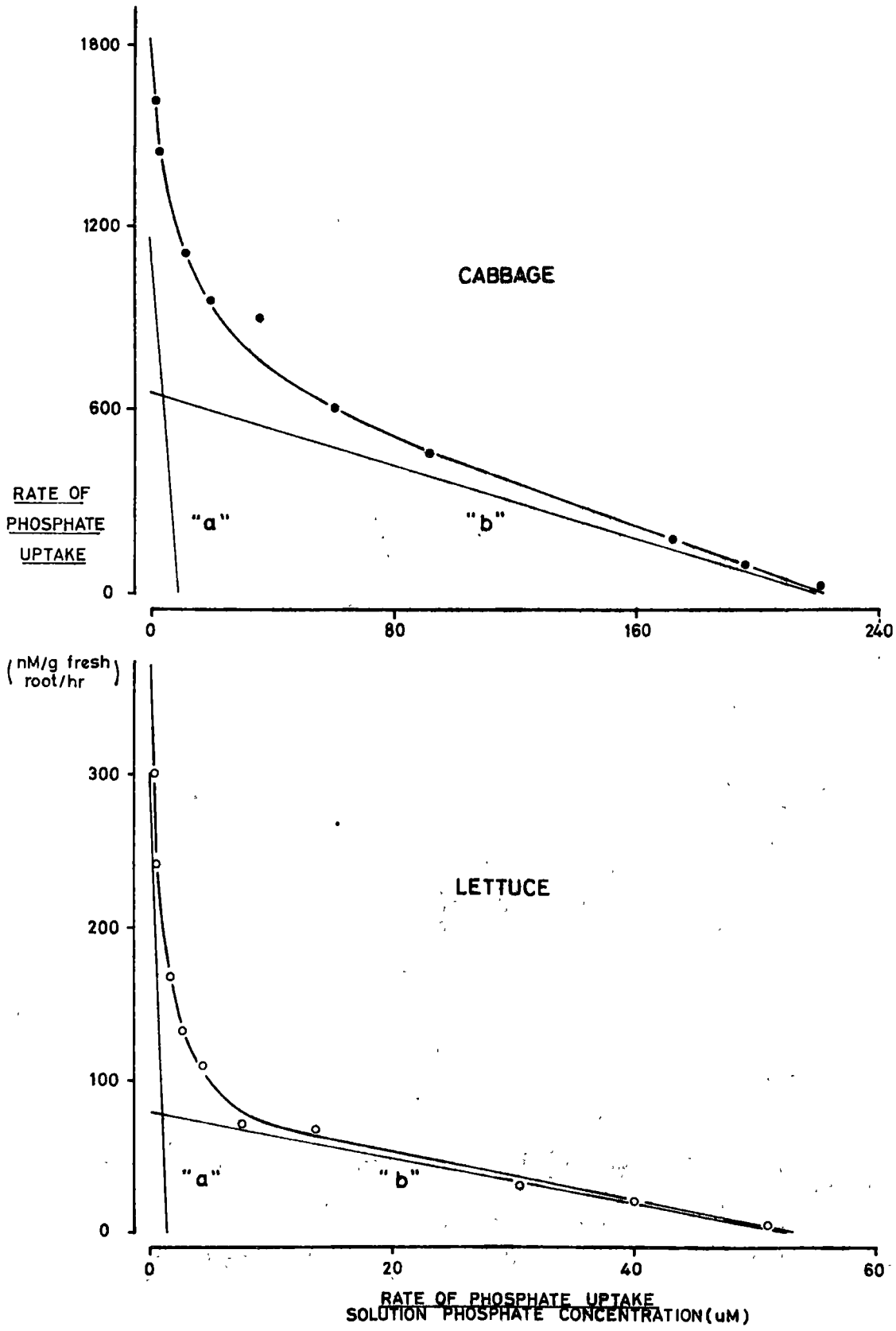

1800
1200
600
0
CABBAGE
RATE OF PHOSPHATE UPTAKE
(nM/g fresh root/hr)
"a"
"b"
0
80
160
240
300
200
100
0
LETTUCE
"a"
"b"
0
20
40
60
RATE OF PHOSPHATE UPTAKE
SOLUTION PHOSPHATE CONCENTRATION (uM)

FIGURE VI B 3.7

Eadie plots and their resolution into two linear components for the phosphate absorption data for non-sterile, lateral-free, excised roots of grasses and vegetables.

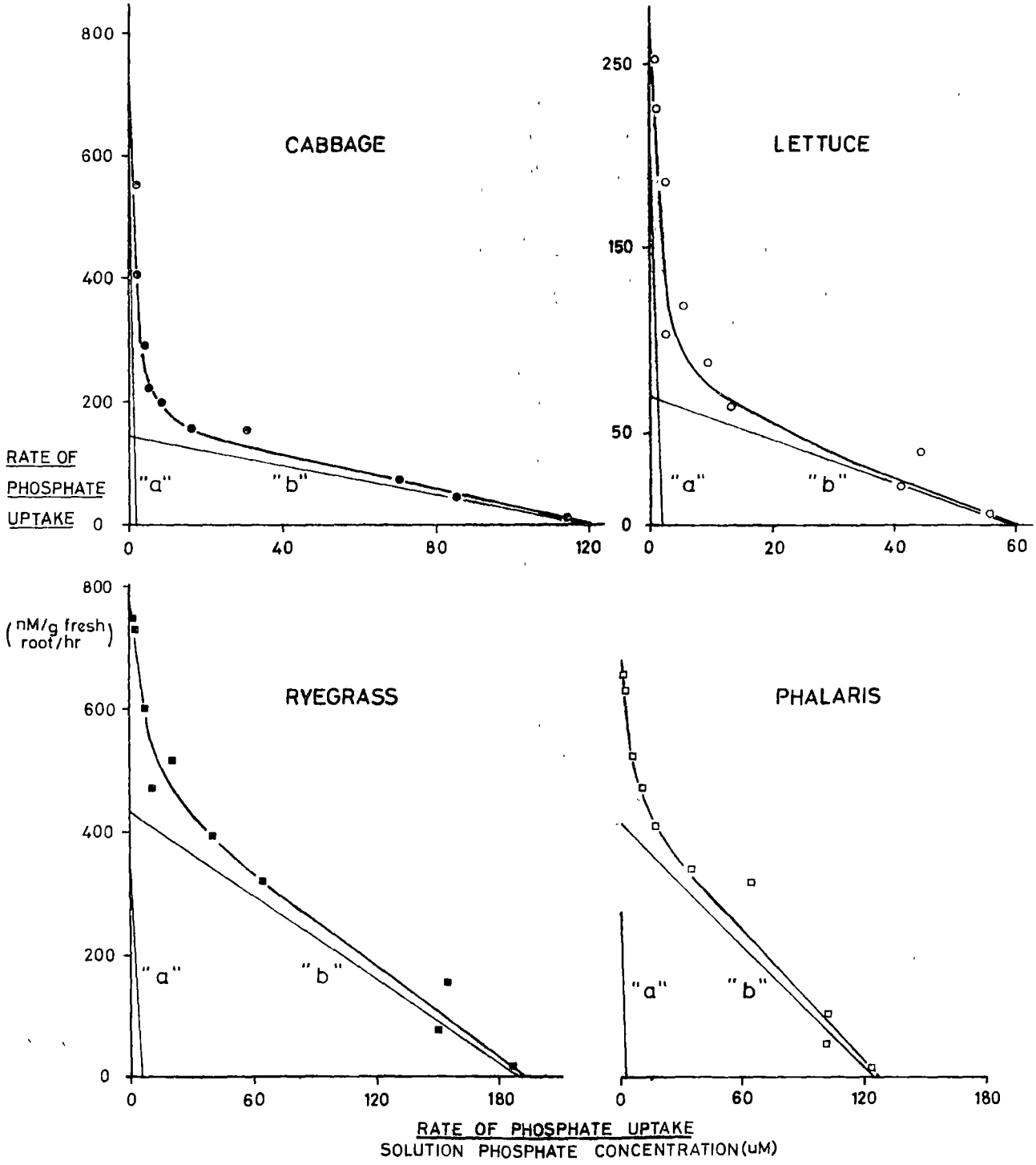

CABBAGE
LETTUCE
RYEGRASS
PHALARIS
"a"
"b"
RATE OF PHOSPHATE UPTAKE
(nM/g fresh root/hr)
RATE OF PHOSPHATE UPTAKE
SOLUTION PHOSPHATE CONCENTRATION (uM)

TABLE VI B 3.1

Maximum Rates of Phosphate Uptake (Vmax, nMP/g fresh root/hr) and Apparent Dissociation Constants (Km, MP) for the "a" and "b" Reactions of Phosphate Absorption of Non-sterile Cabbage, Lettuce, Ryegrass and Phalaris Roots.

Treatment	$Vmax_a$	$Vmax_b$	$Km_a x10^4$	$Km_b x10^6$
Cabbage 1*	1160	652	1.4	3.0
Lettuce 1*	301	74	2.2	1.5
Ryegrass 2	337	431	0.7	2.3
Phalaris 2	266	413	1.1	3.3
Cabbage 3	570	148	2.6	1.3
Lettuce 3	199	70	1.1	1.2

*1 = Whole excised root (primary + laterals)

2 and 3 = Excised root tip, free of laterals

$$v = \frac{Vmax\ (P)}{(P) + Km}$$

where v = rate of phosphate absorption at any phosphate concentration (P).
These values are plotted in Figures VI B 3.8.

For the vegetables Vmax a was always greater than Vmax b (Table VI B 3.1) and as the "b" reaction became saturated at a concentration of 50-100 uMP, at the higher concentrations of phosphate, the phosphate absorbed by the "a" reaction comprised the major component of the total phosphate absorbed. However, with ryegrass and phalaris the reverse was true, Vmax b being greater than Vmax a, and at all concentrations in the range 0.1 - 1000 uMP the "b" reaction remained the principle absorption mechanism. The affinity of the two reaction mechanisms for phosphate, given by Kma and Kmb (Table VI B 3.1) show that a difference of approximately two orders of magnitude occurs between the values of these constants for each plant species, the values for Kma being the greatest (i.e. lower affinity for phosphate). Differences in Km between species also occurred and in general the Kma values for the grasses were less and the Kmb values were greater than those of the vegetables. Values of Kma and Kmb for cabbage and lettuce varied between experiments 1 and 3, where different types of roots were used to measure rate of phosphate absorption.

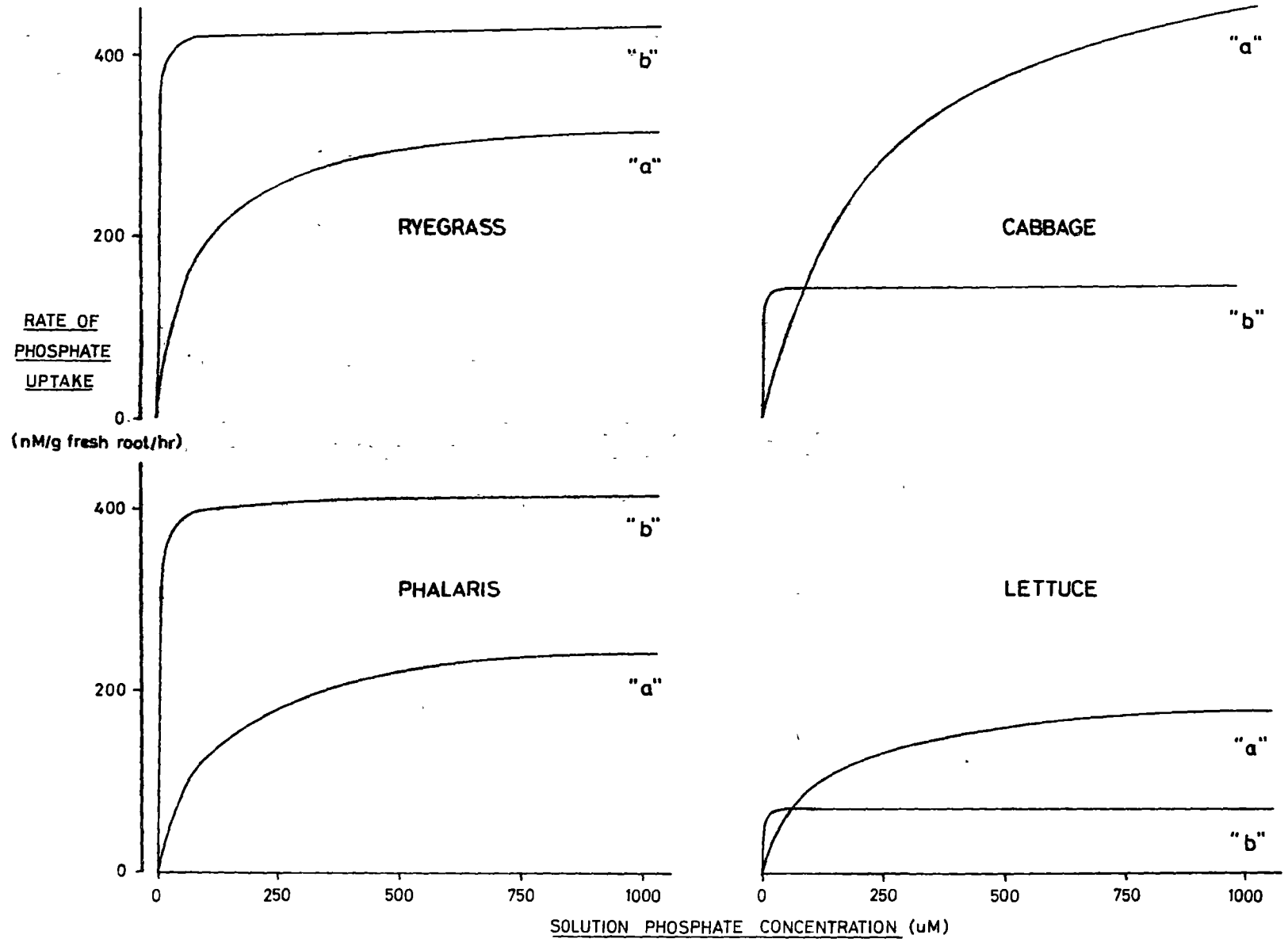
VI B 3.2 Comparison of phosphate absorption by sterile and non-sterile roots

Of the fifteen flasks of each species set up as sterile cultures only two flasks, both containing lettuce plants, were

FIGURE VI B 3.8

Rate of phosphate absorption by the "a" and "b" reaction mechanisms in relation to solution phosphate concentration, for non-sterile, excised roots of vegetables and grasses.
(Data derived from Figure VI B 3.7)

Note the difference between the grasses and the vegetables in the relative magnitudes of their "a" and "b" reaction mechanisms.


RYEGRASS
CABBAGE
PHALARIS
LETTUCE
"a"
"b"
400
200
0
RATE OF PHOSPHATE UPTAKE
(nM/g fresh root/hr)
0
250
500
750
1000
SOLUTION PHOSPHATE CONCENTRATION (uM)

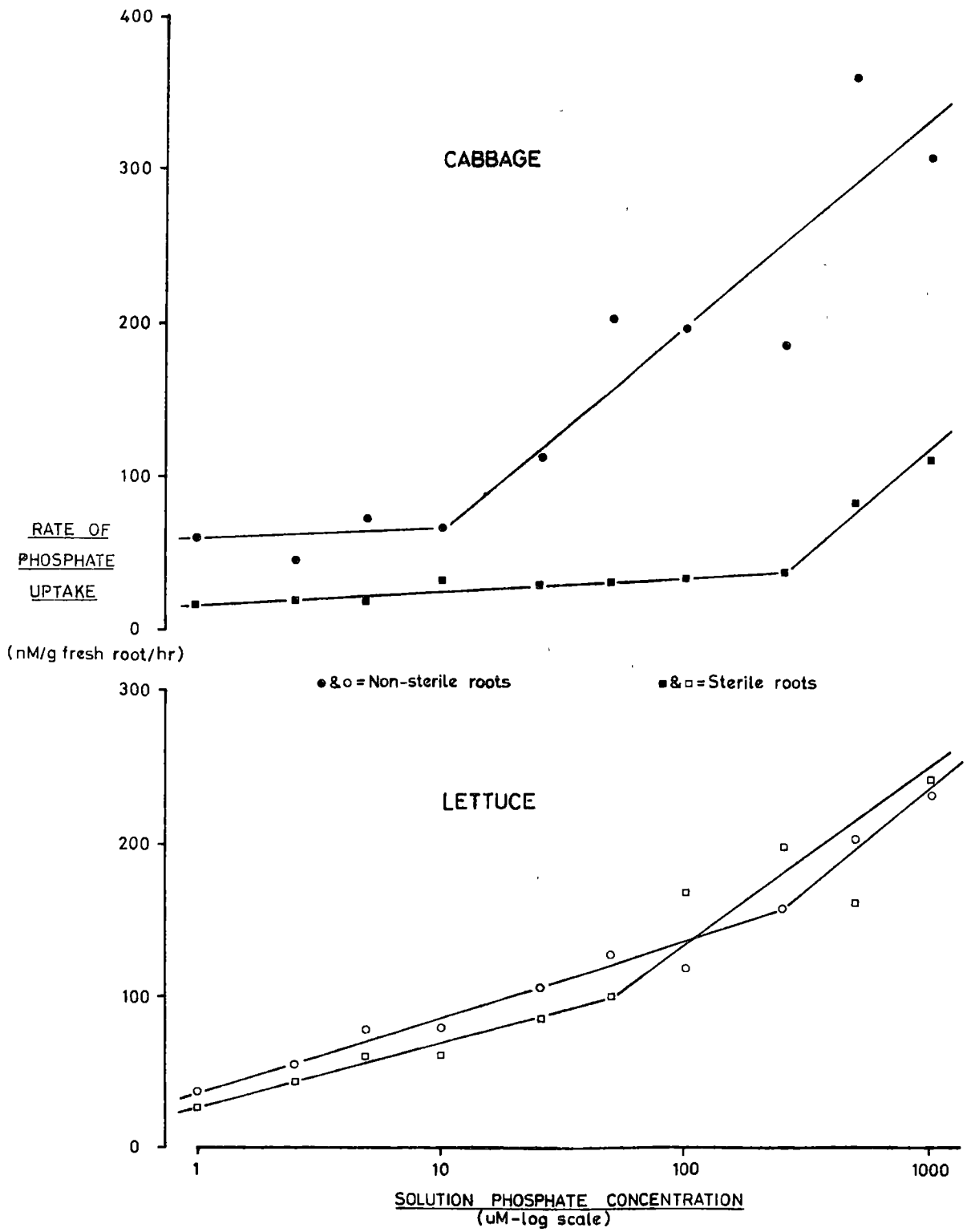
found to be contaminated with micro-organisms at harvest time. Plants from these flasks were not used in the subsequent absorption experiment. In the non-sterile cultures, solution aliquots taken from each flask were all found to contain a wide variety of bacterial species when incubated on sterile agar plates. Despite this difference between sterile and non-sterile cultures there appeared to be no difference in either total plant growth or in root structure or length between plants grown in the different culture treatments.

The rates of phosphate absorption determined for sterile and non-sterile roots of lettuce and cabbage at each phosphate concentration are presented in Figures VI B 3.9 and 3.10. For cabbage the rate of phosphate uptake by non-sterile excised roots was from two to four times that of the corresponding sterile roots at all concentrations in the range 1 - 1000 uMP. For lettuce, however, there was little difference in the rate of phosphate uptake between sterile and non-sterile excised roots although below 100 uMP there was a trend showing the sterile roots to have slightly lower rates of phosphate absorption than their non-sterile counterparts.

Kinetic analysis of the absorption data was carried out as described previously (Section VI B 3.1). Eadie plots (Eadie, 1942) of the uptake data (Figures VI B 3.11 - 3.14) show that a curvilinear relationship was obtained for both sterile and non-sterile roots of each species and these were resolved into two first order reactions, "a" and "b" in each case. The values Vmax, Km determined from these plots are given in Table VI B 3.2. In all treatments V_{max_a} was greater than V_{max_b} and the values of Kma were approximately two orders of magnitude greater than those of Kmb. Comparison of absorption

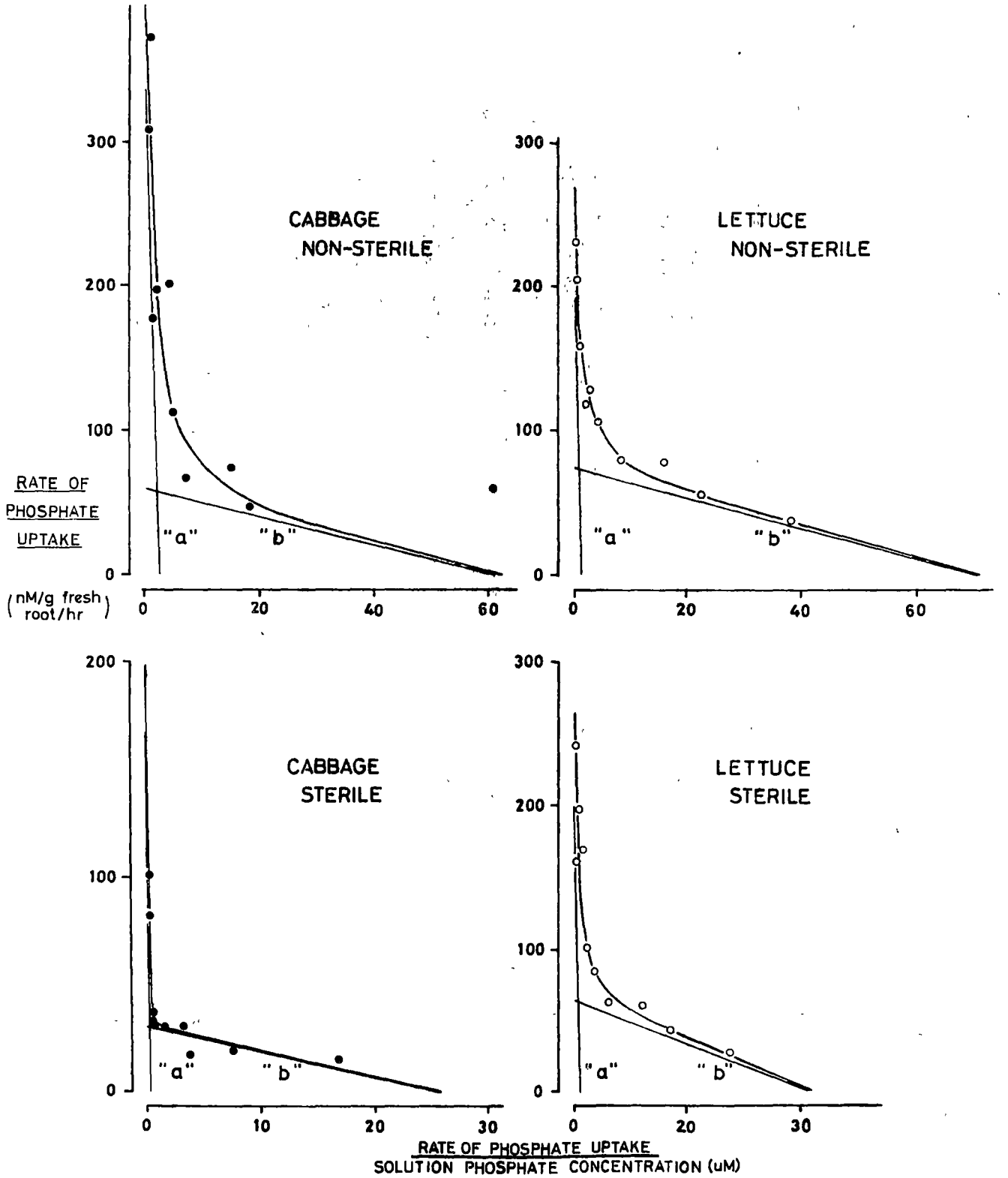
FIGURES VI B 3.9 and 3.10

Rates of phosphate absorption by sterile and non-sterile excised roots of cabbage and lettuce from solutions ranging in concentration from 1 - 1000 uMP.


CABBAGE
400
300
200
100
0
RATE OF PHOSPHATE UPTAKE
(nM/g fresh root/hr)
●&o = Non-sterile roots
■&□ = Sterile roots
LETTUCE
300
200
100
0
1
10
100
1000
SOLUTION PHOSPHATE CONCENTRATION
(uM-log scale)

FIGURES VI B 3.11 - 3.14

Eadie plots of the phosphate absorption data (Figures VI B 3.9 and 3.10) and their resolution into "a" and "b" reaction mechanisms.


CABBAGE
NON-STERILE
LETTUCE
NON-STERILE
CABBAGE
STERILE
LETTUCE
STERILE
"a"
"b"
RATE OF PHOSPHATE UPTAKE
(nM/g fresh root/hr)
RATE OF PHOSPHATE UPTAKE
SOLUTION PHOSPHATE CONCENTRATION (uM)

rates of sterile and non-sterile, excised roots of lettuce show that the values of $Vmax_a$ and $Vmax_b$ are similar. Similarly there was little difference between the Km values obtained for excised lettuce roots grown under sterile and non-sterile conditions. For cabbage, however, the values of $Vmax_a$ and $Vmax_b$ obtained with non-sterile roots were approximately double those of the sterile roots. In addition there was also a large difference in the value of the constant Km_a between sterile and non-sterile cabbage roots although the values of Km_b were similar for both treatments.

In these experiments the major difference between lettuce and cabbage in phosphate uptake was due to differences between species in the value of Vmax, since with one exception (Kma, cabbage) the Km values of both lettuce and cabbage were found to be within a narrow range of values (Table VI B 3.2). Because of the different response of the two species to sterile conditions, relative to their response under non-sterile conditions, the difference in rate of phosphate uptake between lettuce and cabbage depended very largely on the microbial treatment considered. Under non-sterile conditions the value of $Vmax_a$ for lettuce was less than 60% of that for cabbage whilst the $Vmax_b$ value of lettuce was about 125% that of cabbage. However, under sterile conditions the values of $Vmax_a$ and $Vmax_b$ were both lower than those of lettuce being about 80% and 50% of those for lettuce respectively.

TABLE VI B 3.2

Maximum Rates of Phosphate Uptake (Vmax, nMP/g fresh root/hr) and Apparent Dissociation Constants (Km, MP) for the "a" and "b" Reactions of Phosphate Absorption of Lettuce and Cabbage Roots.

Treatment	$Vmax_a$	$Vmax_b$	$Km_a x10^4$	$Km_b x10^6$
Cabbage Non-sterile	338	61	1.4	1.0
Cabbage Sterile	168	31	10.3	1.1
Lettuce Non-sterile	192	77	1.5	1.1
Lettuce Sterile	199	65	1.7	1.6
Ratio, Cabbage Non-sterile:Sterile	2.0	2.0	0.1	0.9
Ratio, Lettuce Non-sterile:Sterile	1.0	1.2	0.9	0.7

VI C DISCUSSION

Studies in which plants were grown in a range of phosphate concentrations using a continuous-flow nutrient solution system have indicated that the four species, cabbage, lettuce, ryegrass and phalaris differ in their response to low concentrations of phosphate applied in solution. Although responses in terms of both dry weight yield and phosphate absorbed were obtained for all species as the phosphate concentration increased, both experiments were marred by depressions in yield at the highest level of phosphate, especially in the case of the vegetable species. These depressed yields were apparently not the result of phosphorus toxicity since the shoot phosphorus concentrations at these levels were not excessively high (4931-8136 ppmP) and no leaf necrosis was observed. Plants were found by Asher and Loneragan (1967) to develop phosphorus toxicity and leaf necrosis only when shoot phosphorus concentrations approached 10,000 ppm or more. Apparently in the present study an imbalance of nutrients or a marginal deficiency of iron or micro-nutrients at the highest level of phosphate has resulted in the depressed yields, probably because of precipitation reactions with phosphate, either within the plant tissue or in the nutrient solution. As a result, the maximum dry weight yields of lettuce and cabbage in experiment 1, achieved at a concentration of 0.9 uMP, were probably below the absolute maximum that these species could produce had the plants at the highest levels of phosphate grown normally. Nevertheless the high rate of phosphate absorption and high relative growth rate of cabbage at the 0.9 uMP level suggests that this concentration of phosphate was near optimal for growth under these environmental

conditions. In contrast the much lower rate of phosphate absorption by lettuce at the same phosphate level (Table VI A 3.1(c)) suggests that further increases in phosphate concentration may have produced further growth responses. Loneragan and Asher (1967) found that at rates of phosphorus absorption of 10 ug atoms P/g fresh roots/day all plant species in their study achieved 78% or more of their maximum yields with three species achieving maximum yields. In addition all species except one achieved relative growth rates of 11.5g dry matter/100 g dry matter/day or more. At the point of maximum yield in experiment 1 of the present study the rates of phosphate absorption were 18.4, 7.5, 9.6 and 7.3 ug atoms P/g fresh roots/day while the relative growth rates were 22.6, 19.3, 18.5 and 17.1 g dry matter/100 g dry matter/day for cabbage, lettuce, ryegrass and phalaris respectively. Thus although the relative growth rates of these species were higher than those reported by Asher and Loneragan (1967), for a different range of plant species, the rates of phosphate absorption were in general lower than those reported by Loneragan and Asher (1967). This apparent contradiction occurs because the mean phosphorus utilization quotients of cabbage, lettuce, ryegrass and phalaris are greater, at the point of maximum yield, than the corresponding values reported by Loneragan and Asher (loc. cit.) for their plant species. The generally lower rates of phosphate absorption recorded in this study may also result from the high proportion of root: shoot found with these species. At maximum yield, ryegrass and phalaris had root weight ratios of about 33% compared with only 16-22% for the grasses used by Loneragan and Asher (loc. cit.). The relatively greater root system of ryegrass and

phalaris may have the effect of reducing overall plant demand for phosphate and also of reducing the rate of phosphate absorption, when expressed per unit of root. Loneragan and Asher (1967) have suggested that root weight ratios may be important in determining species response to phosphate since in their study the species achieving maximum rates of growth at low rates of phosphate uptake also had substantially higher root weight ratios than did the remaining species.

The similarity of the phosphorus utilization quotients of lettuce and cabbage (Table VI A 3.1(c)) suggests that the much higher total dry weight and relative dry weight yields of cabbage, compared with those of lettuce, cannot be attributed to a difference between species in the efficiency with which absorbed phosphate is used in dry matter production. Instead, the difference between lettuce and cabbage in dry weight yields and also in total phosphate uptake appears to be due to the much lower rate of phosphate absorption by lettuce roots. This reduces the relative growth rate and ultimately the overall plant demand for phosphate below the corresponding values for cabbage. The situation is further accentuated by the higher root weight and root phosphorus ratios of lettuce compared with those of cabbage.

In comparison with the results of the first experiment, the lower temperatures and light intensities and the shorter photoperiods operating during the second experiment caused a reduction in both the rate of phosphate absorption and the relative growth rates of lettuce and cabbage. These conditions reduced the yield and total phosphorus content of both species at harvest. Nevertheless this experiment once again demonstrated that the rate of phosphate absorption per unit of root weight was greater

for cabbage than for lettuce, especially at low concentrations of phosphate in solution. The differences observed between these species in the other plant factors, such as root weight and root phosphorus ratios (Table VI A 3.2(b)), appear to be the result rather than the cause of the interspecific difference in rate of phosphate absorption.

Further studies on the rate of phosphate uptake by the four plant species from solution culture were made using excised roots and short-term uptake experiments. Phosphate absorption by lettuce, cabbage, ryegrass and phalaris was characterized by the simultaneous operation of two separate absorption mechanisms which act in the concentration range 0.1 - 1000 uMP. This occurred for both sterile and non-sterile roots. The same dual mechanisms of phosphate absorption, designated the "a" and "b" reactions have been described previously for phosphate uptake by a number of plant species (see Section II B 4.4). In the present study it was not practical to make comparisons between all species at the same time. However, the use of excised roots of similar physiological development and the growth and testing of these under rigidly controlled nutritional and environmental conditions should allow valid comparisons to be made between the absorption mechanisms of the grasses and the vegetables.

The "time curves" (Figures VI B 3.4 and 5) obtained for phosphate uptake by the four species, at both high and low concentrations, were found to pass through the origin in all cases except one and because of the lack of an intercept on the ordinate it was not possible to calculate either the carrier concentrations (Ra and Rb) or the rate constants of the limiting

step in phosphate absorption $RP \xrightarrow{k3} R^1 + P$ inside. Both Carter and Lathwell (1967) and Edwards (1970) have found similar "time lines" with the intercept at the origin, but previous workers; Hagen and Hopkins (1955), Hagen, Leggett and Jackson (1957), Noggle and Fried (1960), and Andrew (1966) found these lines to intercept the ordinate and they considered this value to be the amount of phosphate in combination with the carriers (i.e. Ra P and RbP). Using these values the above workers calculated the rate constants (k3) and the carrier concentrations for both reaction mechanisms. Bange and van Germerden (1963) have studied the uptake of caesium in short term experiments and they questioned the view that the ordinate intercepts represent the amount of ion initially bound to the carrier, because when a divalent ion was added to the experimental solutions the intercept on the ordinate vanished and the time lines passed through the origin. With phosphate experiments where ordinate intercepts have been found (Hagen et al., 1957; Noggle and Fried, 1960; Andrew, 1966), no divalent cations or other anions were present in the experimental solutions. However, in the present study and in those of Carter and Lathwell (1967) and Edwards (1970) calcium sulphate was added to all solutions and the "time lines" passed through the origin. Leggett et al. (1965) on the other hand found that although addition of $CaCl_2$ increased the rate of phosphate uptake, it had no effect in changing the intercept of the "time line". These differences in the "time line" intercepts and their correct interpretation have not yet been satisfactorily resolved. In the present studies where differences in Vmax were apparent between species it was not possible, because of the zero intercept of the "time lines", to identify which

component of Vmax, k3 or Σ R (since Vmax = ΣR k3) was responsible for the differential rates of phosphate absorption.

For non-sterile roots the values of $Vmax_a$ + $Vmax_b$ for the four species used in this study varied from 250 - 1600 n MP/g fresh root/hr. The values obtained by Hagen and Hopkins (1955), Noggle and Fried (1960), Andrew (1966) and Cartwright (1972) for excised roots of barley, by Noggle and Fried (1960) and Andrew (1966) for lucerne excised roots, and by Carter and Lathwell (1967) for excised corn roots all fall within the range of values found in the present study. The apparent dissociation constants (Km's) found for the four species are also similar to those previously reported by Fried and Broeshart (1967). Km values determine the proportion of the total carrier concentration that is associated with phosphate at any particular phosphate concentration. Thus at 1 uMP, a concentration commonly present in soil solutions, the carrier associated with mechanism "b" is 45% saturated with phosphate for both cabbage and lettuce but only 20-30% saturated for the grasses (Figure VI B 3.8). Since the total rate of phosphate uptake (Va + Vb) at this concentration is greater for the grasses than the vegetables and as absorption by mechanism "b" predominates at 1 uMP, it follows that either the carrier concentration (Rb) or the rate constant (k3b) must be much greater for grasses than for vegetables. For carrier mechanism "a" at 1 uMP, the sites are only 1.5, 1.0, 0.5 and 1.1% saturated while at 100 uMP they are 62.5, 53.5, 35.1 and 53.7% saturated for ryegrass, phalaris, lettuce and cabbage respectively. These differences between species in the affinity of their carrier sites for phosphate cannot fully explain the variations in rate of phosphate absorption. This also suggests

that the other biological constants governing the rates of phosphate uptake (i.e. rate constants and carrier concentrations) must vary widely between different plant species. In previous studies species differences in both carrier concentration (Noggle and Fried, 1960) and rate constants (Andrew, 1966) have been cited as the primary cause of variation between species in the rate of phosphate absorption.

In some cases (Andrew loc. cit.) these species differences have been associated with differences in the size and number of roots per sample and since Bowen and Rovira (1967) and Rovira and Bowen (1970) have shown that the rate of phosphate uptake is not uniform along a length of root it becomes important, when comparing plant species, to use roots selected for uniformity in size, type and physiological age. In the present study primary root sections of similar length and development were excised from each species and the small differences in physical root characteristics are unlikely to account for the observed differences in absorption rates between species. The type of root used for the uptake studies does, however, appear to be important. When rates of phosphate absorption were compared for roots of different type (i.e. whole root systems, experiment 1, with primary root tip sections free of laterals, experiment 3) there were large differences between experiments in the values of Vmax and Km obtained with both lettuce and cabbage roots, (Table VI B 3.1). This was most marked for cabbage roots where a three-fold difference in total Vmax occurred between experiments, the higher rate occurring where lateral roots were included in the sample. The relatively smaller increase in total Vmax obtained with whole root samples of lettuce in

comparison to primary root tips can be explained as a lack of extensive lateral root development in lettuce seedlings when compared with cabbage seedlings of the same chronological age. These results imply that lateral roots, per unit of root weight, have a higher rate of phosphate absorption than primary roots and this conclusion is supported by the studies of Russell and Sanderson (1967) and Rovira and Bowen (1970).

Comparisons of the rate of phosphate uptake by excised primary root sections free of laterals (experiment 2 and 3) show that the four species were ranked in order of decreasing rates of absorption as ryegrass $>$ phalaris $>$ cabbage $>$ lettuce for all concentrations in the range 0.1 - 1000 uMP, (Figures VI B 3.2 and 3.3). When the total rate of absorption was resolved into that due to each reaction mechanism obvious differences were apparent between the grasses and the vegetables. While the vegetables had a $Vmax_a$:$Vmax_b$ ratio of more than 2.8 the ratio for both grasses is less than 0.8, (Table VI B 3.1). That is, at all concentrations in the range 0.1 - 1000 uMP absorption by the "b" reaction of the grasses exceeds that of the "a" while for the vegetables the "a" reaction contributes the majority of the total phosphate absorbed by the root when the concentration exceeds 50 - 100 uMP, (Figures VI B 3.10 to 3.13). Similar results have been reported by Andrew (1966) who found the $Vmax_a$:$Vmax_b$ ratio varied from approximately 1.0 - 5.0 for five plant species, the species most efficient in absorbing phosphate from low concentrations having the lowest ratio, as found with the grass species in the present study.

As the experiments discussed above have all been conducted under non-sterile conditions and since micro-organisms present on roots are known to influence the rate of phosphate absorption (Rovira and Bowen, 1968; Barber and Frankenburg, 1971), the differences between species in the rate and pattern of phosphate uptake may be due to interspecific differences in either the quantity or activity of the root micro-flora or to a differential effect of the rhizosphere micro-flora on root physiological and bio-chemical functions.

Absorption of phosphate by sterile excised roots of lettuce and cabbage has been shown (Figures VI B 3.16 and 3.18) to be characterized by the operation of two different hyperbolic isotherms representing sites of different affinities for phosphate. The presence of this dual absorption isotherm under sterile conditions supports the recent work of Cartwright (1972) using sterile, excised barley roots and puts in doubt the conclusion of Barber and Frankenburg (1971) that the low concentration, high affinity mechanism is due to micro-organisms associated with non-sterile roots. In the present study the reaction mechanisms operating under sterile conditions appeared to be of a similar type to those operating under non-sterile conditions although differences in magnitude were apparent between treatments. The absence of micro-organisms affected the rate of phosphate absorption but the magnitude of this effect was dependant on the plant species involved. When comparisons were made between sterile and non-sterile excised roots of lettuce the values of Vmax and Km for both "a" and "b" reaction mechanisms were similar. The lack of any significant difference in either the rate of uptake or the proportion of

the total phosphate absorbed by each mechanism, between sterile and non-sterile lettuce roots, may be explained as the result of either an insignificantly small or inactive rhizosphere microbial population or due to a negligible effect of rhizosphere micro-organisms on the bio-chemical functioning of the root. The former alternative was not the result of a lack of micro-organisms in the non-sterile culture flasks since plating tests using aliquots of solution from each flask had shown a wide spectrum of bacterial types to be present in each case. For cabbage roots there were much larger differences in phosphate absorption rates between microbial treatments. Although the values of Km_b were similar for both sterile and non-sterile cabbage roots, $Vmax_b$ for non-sterile roots was approximately double the value found for sterile cabbage roots. In a similar way the $Vmax_a$ value for non-sterile cabbage roots was twice that of sterile roots but in this case the change was accompanied by a ten-fold decrease in the value of Km_a. How the presence of micro-organisms may affect the affinity of the "a" carrier site for phosphate is not yet clear, however, it appears that rhizosphere micro-organisms are essential for the "normal" functioning of cabbage roots with respect to absorption of phosphate. In view of the recent and convincing arguments put forward by Epstein (1972), to substantiate his claim that ion uptake by non-sterile plant tissue at low external ion concentrations ($<$1000 uM) is not due to ion absorption by rhizosphere bacterial cells (except for phosphate absorption at very low concentrations), the large difference in the rates of phosphate absorption between sterile and non-sterile cabbage roots at all concentrations in the range

1 - 1000 uMP must be attributed to a microbe induced change in the physiological and/or bio-chemical function of the plant root.

VII GENERAL DISCUSSION

By experimentation along a number of different, but related lines of approach, this study has attempted to define the factor or group of factors which are of primary importance in controlling the phosphate nutrition and growth of a number of plant species. The use of four plant species enabled comparisons to be made between species and the interspecific differences in phosphate nutrition observed under a variety of experimental conditions allowed the important factors in plant phosphate nutrition to be identified.

In both the soil and the continuous-flow, nutrient culture experiments interspecific differences in plant phosphate nutrition resulted in different relative yield responses of the four species to applied phosphate. These differences could not be attributed to the effects of a single factor operating differentially between species, but were found to be due to the simultaneous operation of a number of different factors. For the purposes of discussion the factors affecting phosphate nutrition can be divided into those that operate only in the soil situation, such as the efficiency of plant utilization of insoluble phosphates, the rate of root extension and the total root surface area, and secondly those that operate in both the soil and in experimental, solution culture situations (i.e. the rate of phosphate absorption per unit of root and the efficiency with which absorbed phosphate is used in plant growth).

The relative yield responses of each species to applied phosphate were found to be similar when comparisons were made between the soil and the solution culture experiments (i.e. all

four species were ranked in the same order of relative yields in both experiments). This suggests that the primary factors controlling the responses of these species to applied phosphate are common to both the soil and the solution culture situation. Thus it appears unlikely that factors operating only in the soil situation are involved in the interspecific differences in growth and phosphate absorption observed in this study. This does not imply that all factors which operate exclusively in the soil (i.e. do not operate in solution cultures) are of no importance in plant phosphate absorption. In this study, root phosphatase activity varied considerably between species when p-nitrophenol phosphate was used as a substrate. However, there was little apparent difference between species in the utilization of inositol hexaphosphate and it was concluded that the observed differences in p-nitrophenol phosphatase activity would have little significance in the soil situation because of the nature of the soil organic phosphate. Despite the fact that there was little apparent difference between species in the utilization of inositol hexaphosphate (Section V B) or in the volume of soil surrounding the root which was depleted of phosphate (Section IV D), there were differences in the ability of these species to utilize inorganic phosphates. With aluminium and iron phosphate reaction products, the species were ranked in order of increasing efficiency of utilization as lettuce < cabbage < grasses. Since these forms are of special importance in krasnozem soils this factor may partly explain the differences in total and relative phosphate uptake, between lettuce and the other three species, observed in the soil experiment (Table IV A 3.3(iii)). However, in the overall study other factors were considered to be of greater importance in determining species

responses to phosphate.

The total phosphate absorbed per plant also depends upon the total root length and the rate of root extension since these determine the total volume of soil available for phosphate exploitation. Although no specific study was made of these factors the observations made during the depletion zone study indicated that the rate of root extension and the total root length per plant were greater for cabbage than for lettuce at all stages of seedling growth. This was probably the result of the much greater weight and phosphate content of cabbage seeds which produced an initial growth advantage for this species in comparison with lettuce. In line with this conclusion, the rate of root extension in the grasses (smaller seeds than the vegetables) was initially less than that of the vegetables. However, at later growth stages the total root length of the grasses appeared to be greater than that of the vegetables because of their finer more highly ramified root systems. Study of the importance of these root characteristics in the phosphate nutrition of soil-grown plants was not undertaken in detail and further work on these aspects may have been warranted.

The observation of interspecific differences in plant growth and phosphate absorption in solution culture experiments, where the ability of a species to utilize insoluble phosphates or produce large root surface areas may be neglected, indicates that other factors, applicable to both soil and solution culture situations, are responsible for determining the yield and phosphate absorption responses of these species to applied phosphate. These factors are obviously plant characteristics associated with uptake and metabolism of phosphate. In the

present study the most important factors were found to be the rate of phosphate absorption per unit of root and the efficiency with which absorbed phosphate was utilized for plant growth. Both of these varied with the particular plant species and phosphate substrate concentration considered and together were largely responsible for the interspecific differences in yield and phosphate uptake observed in this study.

The amount of plant growth produced per unit of absorbed phosphate depended upon the tissue phosphate concentrations required for maximum growth rate and also upon the plant's capacity for remobilization of the previously absorbed and utilized phosphate. Although reports in the literature (Beeson, 1941; Bear, Toth and Prince, 1949) had shown that the species used in this study required similar tissue phosphate levels for maximum growth, the response curves obtained in the soil experiments (Sections IV A and IV B) indicated that the critical level of phosphate in cabbage shoot tissue was much lower than that of the other species. As explained in Section IV E this was attributed to a greater remobilization of phosphate in cabbage plants due to a nitrogen deficiency resulting from the high dry matter production of cabbage plants in comparison with the other species. However, in the solution culture experiments (Section VI A), where nitrate was provided at adequate levels throughout the growth period, the critical level of phosphate in cabbage shoots corresponded more closely to that found for the other species. The value for cabbage, however, was still lower than those for lettuce and the grasses. This difference between the soil and solution culture experiments was also reflected in the mean phosphate utilization quotients, particularly

of lettuce and cabbage. In the soil experiments the utilization quotients of cabbage were greater than those of lettuce at each phosphate concentration (at least double those of lettuce at the lowest concentration) while in the solution cultures the values for cabbage were less than those of lettuce at the low phosphate levels and equal to, or slightly greater than those of lettuce at the higher levels. The difference in the relative phosphate utilization quotients of lettuce and cabbage between soil and solution culture experiments was due possibly to an interaction between phosphate and nitrogen within the plant. Where high utilization quotients were found, they may be attributed to a deficiency of nitrogen within the plant which increased the remobilization of absorbed phosphate and therefore the utilization quotient. This highlights the need for adequate and balanced levels of all other nutrients when yield response curves to a particular nutrient are used for determination of critical nutrient concentrations.

The efficiency with which absorbed phosphate was used in plant growth also depended upon the proportion of the total absorbed phosphate that was translocated to the shoot. Thus in the solution culture experiments and to a lesser extent in the soil experiments the greater relative yields of cabbage at low phosphate concentrations, in comparison with those of lettuce, were in part due to species differences in the proportion of the plant's total phosphate and dry matter accumulated in the roots. For example, at the three lowest levels of phosphate in the second continuous-flow nutrient experiment (Section VI A 2.3) the root phosphorus ratio varied from 45.1 - 41.5% for lettuce but from only 34.3 - 19.1% for cabbage while the corresponding values for the root weight ratios were 28.7 - 24.5% and 20.5 - 15.9% for lettuce and cabbage respectively.

These differences were also accompanied by differences between species in the proportion of the total phosphate present in organic combination at low levels of applied phosphate (Section IV C). The lower percentage of organically combined phosphate and the corresponding higher percentage of inorganic orthophosphate in lettuce shoot tissue grown under phosphate deficient conditions may be the result of a lack of carbohydrates or other organic building blocks. Kakie (1969) has shown a severe inhibition of ^{14}C incorporation into carbohydrates under phosphate deficiency and Hartt (1972) found translocation of ^{14}C within the plant to be impaired when phosphate deficiency was severe enough to decrease growth. Carbohydrate production and subsequent translocation within the plant may therefore be expected to be inhibited by phosphate deficiency, the severity of this inhibition possibly depending on the plant species involved. Interspecific comparisons concerning this aspect of phosphate nutrition do not appear to be available and studies of this type may be valuable in partly explaining the different yield response curves of lettuce and cabbage when grown under phosphate deficiency.

Since the presence of micro-organisms is known to increase the percentage of plant phosphate present in nucleic acid, phospholipid and phosphoprotein forms, in comparison with sterile plants (Bowen and Rovira, 1969), the difference between lettuce and cabbage in the percentage of their phosphate found in T.C.A. insoluble forms may be used to indicate that these species have different rhizosphere populations or have a different interaction with their micro-flora. Such an observ-

ation would support the conclusion made in Section VI B 2 concerning the rhizosphere populations or rhizosphere effect produced with these species.

The other major factor which played an important role in defining the growth and phosphate responses of the four species to applied phosphate was the rate of phosphate absorption per unit weight of roots. Interspecific differences in uptake rates were greatest at the lowest external phosphate concentrations and in general the rates of uptake from these concentrations were greater for the grasses than for the vegetables, the reverse being true at the higher concentrations. This situation may not be unreasonable considering the conditions against which these two groups of plants, the grasses and the vegetables have been selected and also considering the fact that absorption capacity is a bio-chemical process under normal genetic control. Whereas vegetables have been selected and bred under conditions of high soil nutrient status with levels of phosphate of up to 200 lbs P/acre/annum or more being applied to vegetable crops (Webster, 1969), the grasses have been selected under lower nutrient levels and in general, pasture grasses rarely receive more than 20-30 lbs P/acre/annum. These differences in soil nutrient status may have selected for genetic variation between these species which is displayed as differences between species in the bio-chemical or physiological components of the phosphate absorption process. Pigott (1971), for example, has indicated that many species characteristic of stable vegetation, such as woodland or grassland, show little or no significant responses to concentrations of phosphate above those normally occurring in the soils they occupy (i.e. the

absorption mechanisms have become adapted to operate most efficiently at certain nutrient concentrations which are the norm in the environment in which the species grows). A similar type of response pattern may also apply to the grasses and vegetables in this study, the differential absorption responses of these species being ultimately dependent upon the range of phosphate concentrations to which each species has become adapted. At concentrations above and below this range the mechanisms of phosphate uptake may be expected to be less efficiently adapted.

Genetic variation in the mechanism of ion uptake should be revealed in short term uptake studies where phosphate absorption of each species was measured over a wide range of phosphate concentrations. Fundamental differences in the relative magnitude of the two absorption reactions "a" and "b" were observed in these experiments, the grasses absorbing a much greater proportion of their total phosphate than the vegetables, via the low concentration, high affinity "b" carrier mechanism. Under non-sterile conditions the rate of phosphate uptake by the "b" mechanism of the grasses was from 3-5 times greater than that for the "b" mechanisms of cabbage and lettuce. This explains why the grasses had higher rates of phosphate absorption than the vegetables, especially at concentrations in the range 0-10 uMP, and supports the conclusion that the relative yield responses of these species to applied phosphate are due in part to differences in their rates of phosphate absorption. The observation that at low phosphate concentrations the rate of phosphate uptake by cabbage was greater than that of lettuce (i.e. uptake via the "b" reaction mechanism) also

contributes evidence to explain why the amounts of phosphate absorbed and the relative yield responses at low phosphate concentrations were different for the two vegetable species in both the soil and the solution culture experiments. The much lower rates of phosphate uptake by lettuce, in comparison with the other species (see Section VI B 3.1), also explains why, in the depletion zone study, the formation of a clear zone of phosphate depletion in the soil around lettuce roots lagged behind that for cabbage and the grasses.

It must be emphasized that in both the soil pot trials and the solution culture studies the concentration of phosphate in the external solution was such that for all species well over 70% of the total phosphate was absorbed via the "b" reaction mechanism. This situation would apply to most agricultural soils with the exception of some micro-environments in close proximity to phosphate fertilizer granules, thus highlighting the fact that the "b" reaction mechanism is the most important in determining the rate of phosphate absorption from soils.

In the short-term uptake studies the use of excised roots of adequate phosphate status may raise some doubt as to the validity of applying the results to interpret the behaviour of species grown at a range of phosphate concentrations, where plants of widely different phosphate status are present. For example, phosphate deficient roots may be expected to behave differently to roots of adequate phosphate status. However, the work of Cartwright (1972) with excised barley roots of different phosphate status has shown that the phosphate status of the root had no effect on the value of $Vmax_b$ and had little effect on either the rate constant k_{3b} or in the "b" carrier concentration. The only change was a large reduction in

k_{mb} as the roots became phosphate deficient so that the same maximum rate of uptake was reached at a lower external phosphate level. Cartwright's results suggest that values of $Vmax_b$ obtained under conditions of adequate phosphate status may be validly applied to roots grown under phosphate deficient conditions. His observation that values of K_{mb} change with variations in phosphate status may also explain the slight differences in K_{mb} values of a single species obtained in different short-term uptake experiments of this study. The extrapolation of results obtained with excised roots to studies using whole plants is more difficult to justify especially in view of the differences in phosphate uptake between excised and whole roots observed by both Bowen and Rovira (1967) and Nassery (1970). However, the short-term nature of these experiments and the greater convenience of experimentation with excised roots, rather than whole plants, may perhaps justify their use. Even so, where possible, short-term absorption studies with whole plants are to be recommended as being more closely correlated to the natural field situation.

Barber and Frankenburg (1971) have concluded from kinetic analysis of phosphate uptake data of excised roots grown under sterile and non-sterile conditions that the low concentration, high affinity "b" carrier site was associated with the rhizosphere micro-organisms present on the root rather than with the plant root cells. Their conclusions applied to the present study imply that the differences between grasses and vegetables in the rate of phosphate uptake by the "b" reaction mechanism are the result of species differences in the total numbers or activities of micro-organisms associated with the

roots, the grasses having a greater microbial absorption of phosphate than the vegetables. Such an assumption means that in the concentration range 0.1 - 1000 uMP, phosphate uptake by the rhizosphere micro-organisms of the grasses (i.e. the "b" reaction) exceeds that of the grass root tissue despite the fact that the root cell volume would be at least 100 times that of the associated microbial cell volume. The conclusions of Barber and Frankenburg (loc. cit.) were based on an assumption that the microbial component of phosphate uptake could be determined as the difference in phosphate absorbed between non-sterile and sterile roots. Their assumption appears to be incorrect since it fails to allow for the effect the presence of micro-organisms may have on the root's physiology and bio-chemistry, both of which influence the rate of phosphate absorption. Thus in the present study and in the study of Cartwright (1972) both "a" and "b" reaction mechanisms were apparent in plant roots grown in the absence of micro-organisms. These findings contradict the conclusions made by Barber and Frankenburg (1971).

The possible role of endotrophic mycorrhizae in increasing the rate of phosphate absorption by these species, when grown in soil of low phosphate status, has not been investigated in this study. Since grasses have been widely recorded as being mycorrhizal (Nicolson 1960, 1967), the difference in phosphate uptake between vegetables and grasses at low phosphate concentrations may be attributed to the differential activity of mycorrhizal fungi. However, the different response of lettuce and cabbage to low concentrations of phosphate could not be attributed to a differential mycorrhizal stimulation of phosphate uptake since it is known (Bowen, private communication)

that cabbage, the more efficient species, is one of the few crop plants in which mycorrhizal fungi appear to be absent.

Comparisons between sterile and non-sterile roots of cabbage and lettuce have indicated that micro-organisms may influence phosphate absorption from solutions by affecting the rate of phosphate uptake of plant roots (Section VI B 3.2). Considering the complexity of root exudates and microbial species and their interactions in the root rhizosphere it is not surprising that differences in the relative importance of micro-organisms in phosphate uptake were found between lettuce and cabbage plants. These results indicated that under non-sterile conditions the rate of phosphate absorption by plant roots depends to a large extent on the interaction between the microbial population and the plant root, the particular plant species involved in this interaction being an important factor. Loutit (1970) showed that different soil types produce different rhizosphere populations on the same plant species, so that for phosphate, the effect of micro-organisms on the plant's rate of phosphate uptake may be different for each particular soil type as well as for each plant species.

The object of this study was to examine the growth and phosphate nutrition of four plant species, cabbage, lettuce, ryegrass and phalaris and to determine and compare the relative importance of various factors involved in absorption and utilization of phosphate by these species. The factors selected for study were those considered most relevant to plant growth and phosphate nutrition in the krasnozem soil but in most cases the results would also apply to plant growth in other soils and culture systems.

Species differences in growth and in phosphate absorption and utilization were recorded under a variety of culture conditions including both soil and solution culture situations. In general the differences between species in response to phosphate were most marked between lettuce and cabbage. The behaviour of the grasses was found to more closely approximate that of cabbage than of lettuce, but cabbage and the grasses responded differently to some treatments. The marked difference between lettuce and cabbage in response to phosphate made it difficult to compare the behaviour of the vegetables (dicotyledons) and the grasses (monocotyledons) directly as groups. However, kinetic data from the short term uptake study did show a distinct difference between these groups in the relative magnitude of the two phosphate absorption mechanisms. Whether or not this is a general phenomenon associated with these two plant groups is not clear, but in this study the relatively greater magnitude of the "b" reaction mechanism of the grasses was at least partly responsible for their higher rates of phosphate uptake at low phosphate concentrations in comparison with those of the vegetables. In contrast to the vegetables, the relative responses of ryegrass and phalaris to phosphate were remarkably similar and as a result, the emphasis in some sections of this study was directed to defining the differences between the vegetable species, as these were known to respond differently to phosphate. This approach appears to have been justified since it enabled more detailed experimentation with cabbage and lettuce and produced results which emphasized the importance, in plant growth and phosphate nutrition, of both the ability of roots to absorb phosphate from dilute solutions and the interaction of roots and microflora.

Another factor found to be of major importance to the growth and phosphate nutrition of these species was the efficiency with which absorbed phosphate was utilized in plant growth. For example, the relatively poor utilization efficiency of lettuce, in comparison with other species, was partly responsible for its depressed growth under phosphate deficient conditions. This study also emphasised the importance of seed reserves in determining the rate of root extension in seedlings and the rate of seedling growth in general. The rate of seedling growth was important in phosphorus nutrition, especially for soil-grown plants, since the effect of an early growth advantage was maintained at later stages of growth.

BIBLIOGRAPHY

Acquaye, D.K. (1963). Some significance of soil organic phosphorus mineralization in the phosphorus nutrition of cocoa in Ghana. Pl. Soil 19: 65-80.

Adams, F. (1971). Ionic concentrations and activities in soil solutions. Proc. Soil Sci. Soc. Am. 35: 420-426.

Agnihotri, V.P. (1970). Solubilization of insoluble phosphates by some soil fungi isolated from nursery seed beds. Can. J. Microbiol. 16: 877-880.

Akhromeiko, A.I. and Shestakova, V.A. (1958). Role of rhizosphere micro-organisms in the nutrition of woody plants. Mikrobiologiya 27: 67-74.

Allen, R.J.L. (1940). The estimation of phosphorus. Biochem. J. 34: 858-865.

Anderson, G. (1956). The identification and estimation of soil inositol phosphates. J. Sci. Fd. Agric. 7: 437-444.

Anderson, G. (1957). Nucleic acid derivatives in soil. Nature, Lond. 180: 287-288.

Anderson, G. (1961). A partial fractionation of alkali-soluble soil organic phosphate. J. Soil Sci. 12: 276-285.

Anderson, G. (1967). Nucleic acids, derivatives and organic phosphates. In "Soil Biochemistry", (McLaren, A.D. and Peterson, G.H., Eds.) Marcel Dekker Inc., New York. pp 67-90.

Anderson, G. and Arlidge, E.Z. (1962). The adsorption of inositol phosphates and glycerophosphate by soil clays, clay minerals and hydrated sesquioxides in acid media. J. Soil Sci. 13: 216-224.

Andrew, C.S. (1966). A kinetic study of phosphate absorption by excised roots of Stylosanthes humilis, Phaseolus lathyroides, Desmodium uncinatum, Medicago sativa and Hordeum vulgare. Aust. J. agric. Res. 17: 611-624.

Apple, S.B. and Butts, J.S. (1953). Soil temperature studies. I The effect of soil temperature and phosphorus on growth and phosphorus uptake by pole beans. Proc. Am. Soc. Hort. Sci. 61: 325-332.

Asher, C.J. and Loneragon, J.F. (1967). Response of plants to phosphate concentration in solution culture. I Growth and phosphorus content. Soil Sci. 103: 225-233.

Asher, C.J. and Ozanne, P.G. (1961). The cation exchange capacity of plant roots and its relationship to the uptake of insoluble nutrients. Aust. J. agric. Res. 12: 755-766.

Asher, C.J., Ozanne, P.G. and Loneragan, J.F. (1965). A method for controlling the ionic environment of plant roots. Soil Sci. 100: 149-154.

Bache, B.W. (1963). Aluminium and iron phosphate studies relating to soils. I Solution and hydrolysis of varascite and strengite. J. Soil Sci. 14: 113-123.

Bajpai, P.D. and Sundara Rao, W.V.B. (1971a). Phosphate solubilising bacteria. I Solubilisation of phosphate in liquid culture by selected bacteria as affected by different pH values. Soil Sci. Pl. Nutr. 17: 41-43.

Bajpai, P.D. and Sundara Rao, W.V.B. (1971b). Phosphate solubilising bacteria. II Extracellular production of organic acids by selected bacteria solubilising insoluble phosphate. Soil Sci. Pl. Nutr. 17: 44-45.

Bajpai, P.D. and Sundara Rao, W.V.B. (1971c). Phosphate solubilising bacteria. III Soil inoculation with phosphorus solubilising bacteria. Soil Sci. Pl. Nutr. 17: 46-53.

Bakalivanov, D. (1965). The influence of soil fungi on plants. In "Plant Microbes Relationship" (Macura, J., and Vancura, V., Eds.) Publ. Czek. Acad. Sci., Prague, 1965. pp 236-240.

Baker, D.E., Wooding, F.J. and Johnson, M.W. (1971). Chemical element accumulation by populations of Corn (Zea mays L.) selected for high and low accumulation of P. Agron. J. 63: 404-406.

Baker, D.E., Jarrell, A.E., Marshall, L.E. and Thomas, W.I. (1970). Phosphorus uptake from soils by corn hybrids selected for high and low phosphorus accumulation. Agron. J. 62: 103-106.

Bange, C.G.J. and Van Gemerden, H. (1963). The initial phase of ion uptake by plant roots. Pl. Soil. 18: 85-98.

Barber, D.A. (1966). The effect of micro-organisms on nutrient absorption by plants. (Hordeum vulgare). Nature, Lond. 212: 638-640.

Barber, D.A. (1967). Influence of pH on phosphorus uptake by sterile and non-sterile roots. Nature, Lond. 215: 779-780.

Barber, D.A. (1967). Effect of micro-organisms on the absorption of inorganic nutrients by intact plants. I. - Apparatus and culture technique. J. Exp. Bot. 18: 163-169.

Barber, D.A. (1968). Micro-organisms and the inorganic nutrition of higher plants. Ann. Rev. Pl. Physiol. 19: 71-88.

Barber, D.A. (1969). The influence of the microflora on the accumulation of ions by plants. In "Ecological Aspects of the Mineral Nutrition of Plants". (Rorison, I.H., Ed.) Blackwell Scientific Publ., Oxford. pp 191-200.

Barber, D.A. and Frankenburg, U.C. (1971). The contribution of micro-organisms to the apparent absorption of ions by roots grown under non-sterile conditions. New Phytol. 70: 1027-1034.

Barber, D.A. and Loughman, B.C. (1967). The effects of micro-organisms on the absorption of inorganic nutrients by intact plants. II Uptake and utilization of P by barley plants grown under sterile and non-sterile conditions. J. exp. Bot. 18: 170-176.

Barber, D.A., Sanderson, J. and Russell, R.S. (1968). Influence of micro-organisms on the distribution in roots of phosphate labelled with phosphorus - 32. Nature, Lond. 217: 644.

Barley, K.P. (1963). Influence of soil strength on the growth of roots. Soil Sci. 96: 175-180.

Barley, K.P. (1970). The configuration of the root system in relation to nutrient uptake. Adv. Agron. 22: 159-201.

Barley, K.P. and Greacen, E.L. (1967). Mechanical resistance as a soil factor influencing the growth of roots and underground shoots. Adv. Agron. 19: 1-43.

Barley, K.P. and Rovira, A.D. (1970). The influence of root hairs on the uptake of phosphate. Communs. Soil Sci. Pl. Anal. 1: 287-292.

Barley, K.P., Farrell, D.A. and Greacen, E.L. (1965). The influence of soil strength on the penetration of a loam by plant roots. Aust. J. Soil Res. 3: 69-79.

Bates, T.E. (1971). Factors affecting critical nutrient concentrations in plants and their evaluation : A review. Soil Sci. 112: 116-130.

Baylis, G.T.S. (1967). Experiments on the ecological significance of phycomycetous mycorrhizas. New Phytol. 66: 231-243.

Baylis, G.T.S. (1970). Root hairs and phycomycetous mycorrhizas in phosphorus deficient soil. Pl. Soil 33: 713-716.

Bear, F.E., Toth, S.J. and Prince, A.L. (1949). Variation in mineral composition of vegetables. Proc. Soil Sci. Soc. Am. 13: 380-384.

Beaton, J.D. and Read, D.W.L. (1963). Effects of temperature and moisture on phosphorus uptake from a calcareous Saskatchewan soil treated with several pelleted sources of phosphorus. Proc. Soil Sci. Soc. Am. 27: 61-65.

Beaton, J.D., Read, D.W.L. and Hinman, W.C. (1962). Phosphorus uptake by alfalfa as influenced by phosphate source and moisture. Can. J. Soil Sci. 42: 254-265.

Beaton, J.D., Speer, R.C. and Brown, G. (1965). The effect of soil temperature and length of reaction period on water solubility of phosphorus in soil fertilizer reaction zones. Proc. Soil Sci. Soc. Am. 29: 194-198.

Beauchamp, E.G. (1967). Root-zone temperature effects on the early development of maize. Pl. Soil 26: 224-234.

Beeson, K.C. (1941). The mineral composition of crops with particular reference to the soils in which they were grown. A review and compilation. U.S.D.A. Misc. Publ., No. 369.

Bell, L.C. and Black, C.A. (1970). Crystalline phosphates produced by interaction of orthophosphate fertilizers with slightly acid and alkaline soils. Proc. Soil Sci. Soc. Am. 34: 735-740.

Bieleski, R.L. (1966). Accumulation of phosphate, sulfate and sucrose by excised phloem tissues. Pl. Physiol., Lancaster. 41: 447-454.

Bjerrum, N. (1949). "Selected papers" pp 245-248. Munksgaard, Copenhagen.

Black, C.A. (1968). Phosphorus. In "Soil-Plant Relationships". 2nd Edn. Wiley and Sons Inc., New York. pp 558-653.

Black, J.N. (1957). Seed size as a factor in the growth of subterranean clover (Trifolium subterraneum L) Under spaced and sward conditions. Aust. J. agric. Res. 8: 335-351.

Blair, G.J., Mamaril, C.P. and Miller, M.H. (1971). Influence of nitrogen source on phosphorus uptake by corn from soils differing in pH. Agron. J. 63: 235-238.

Blair, G.J., Miller, M.H. and Mitchell, W.A. (1970). Nitrate and ammonium as sources of nitrogen for corn and their influence on the uptake of other ions. Agron. J. 62: 530-532.

Blanchar, R.W. and Caldwell, A.C. (1966). Phosphate-ammonium-moisture relationships in soils. II Ion concentrations in leached fertilizer zones and effects of plants. Proc. Soil Sci. Soc. Am. 30: 43-48.

Bouldin, D.R. (1961). Mathematical description of diffusion processes in the soil-plant system. Proc. Soil Sci. Soc. Am. 25: 476-480.

Bouldin, D.R. and Sample, E.C. (1958). The effect of associated salts on the availability of concentrated superphosphate. Proc. Soil Sci. Soc. Am. 22: 124-129.

Bouldin, D.R. and Sample, E.C. (1959). Calcium phosphate fertilizers. III The effect of surface area on the availability coefficients of the dicalcium phosphates. Proc. Soil Sci. Soc. Am. 23: 276-281.

Bouldin, D.R., Lehr, J.R. and Sample, E.C. (1960). The effect of associated salts on transformations of monocalcium phosphate monohydrate at the site of application. Proc. Soil Sci. Soc. Am. 24: 464-468.

Bouma, D. (1960). The effect of ammonium sulphate usage on the availability of soil phosphorus to citrus. Aust. J. agric. Res. 11: 292-303.

Bowen, G.D. (1970). Effects of soil temperature on root growth and on phosphate uptake along Pinus radiata roots. Aust. J. Soil Res. 8: 31-42.

Bowen, G.D. and Rovira, A.D. (1961). Effects of micro-organisms on plant growth. I Development of roots and root hairs in sand and agar. Pl. Soil 15: 166-168.

Bowen, G.D. and Rovira, A.D. (1966). The microbial factor in short-term PO_4 uptake studies with plant roots. Nature, Lond. 211: 665-666.

Bowen, G.D. and Rovira, A.D. (1967). Phosphate uptake along attached and excised wheat roots measured by an automatic scanning method. Aust. J. biol. Sci. 20: 369-78.

Bowen, G.D. and Rovira, A.D. (1969). The influence of micro-organisms on root growth and metabolism. In "Root Growth" (Whittington, W.J. Ed.), Butterworth, London. pp 170-201.

Bowen, G.D. and Theodorou, C. (1967). Studies on phosphate uptake by mycorrhizas. Proc. 14th Congr. Int. Union Forest Res. Organ., Munich, 5, Sect. 24: 116-138.

Bradshaw, A.D., Chadwick, M.J., Jowett, D., Lodge, R.W. and Snaydon, R.W. (1960). Experimental investigations into the mineral nutrition of several grass species. III Phosphate level. J. Ecol. 48: 631-637.

Bray, R.H. (1954). A nutrient mobility concept of soil-plant relationships. Soil Sci. 78: 9-22.

Brian, P.W. (1957). The effect of some microbial metabolic products on plant growth. In "The Biological Action of Growth Substances". Symp. Soc. exp. Biol. 11: 166-182.

Broeshart, H. (1962). Cation adsorption and absorption by plants. In "Radioisotopes in Soil Plant Nutrition Studies", Symp. Soil Pl. Nutr. I.A.E.A. Vienna. pp 303-313.

Bromfield, S.M. (1953). Sulphate reduction in partially sterilized soil exposed to air. J. gen. Microbiol. 8: 378-383.

Bromfield, S.M. (1958). The properties of a biologically formed manganese oxide, its availability to oats and its solution by root washings. Pl. Soil 9: 325-332.

Bromfield, S.M. (1965). Studies on the relative importance of iron and aluminium in the sorption of phosphate by some Australian soils. Aust. J. Soil Res. 3: 31-44.

Brouwer, R. (1965). Ion absorption and transport in plants. Ann. Rev. Pl. Physiol. 16: 241-266.

Brown, D.A., Place, G.A. and Pettiet, J.V. (1960). The effect of soil moisture upon cation exchange in soils and nutrient uptake by plants. Trans 7th Intern. Congr. Soil Sci., Madison, Wisc., 1960. III: 443-449.

Burley, J.W.A., Nwoke, F.I.O., Leister, G.L. and Popham, R.A. (1970). The relationship of xylem maturation to the absorption and translocation of P^{32}. Am. J. Bot. 57: 504-511.

Caldwell, A.C. (1960). The influence of various nitrogen carriers on the availability of fertilizer phosphorus to plants. Trans. 7th Intern. Congr. Soil Sci., Madison, Wisc., 1960. III: 517-525.

Canning, R.E. and Kramer, P.J. (1958). Salt absorption and accumulation in various regions of roots. Am. J. Bot. 45: 378-382.

Carter, O.G. and Lathwell, D.J. (1967). Effects of temperature on orthophosphate absorption by excised corn roots. Pl. Physiol., Lancaster. 42: 1407-1412.

Cartwright, B. (1972). Effect of phosphate deficiency on kinetics of phosphate absorption by sterile excised barley roots, and some factors affecting ion uptake efficiency of roots. Communs. Soil Sci. Pl. Anal. 3: 313-322.

Casida, L.E. Jr. (1959). Phosphatase activity of some common soil fungi. Soil Sci. 87: 305-310.

Cate, W.E., Huffman, E.O. and Deming, M.E. (1959). Preparation of crystalline ferric phosphates. Soil Sci. 88: 130-132.

Chakravarti, S.N. and Talibudeen, O. (1962). Phosphate equilibria in acid soils. J. Soil Sci. 13: 231-240.

Chandrasekaran, S. (1969). Production of organic acids by soil micro-organisms. Pl. Soil 30: 299-304.

Chang, C.W. and Bandurski, R.S. (1964). Exocellular enzymes of corn roots. Pl. Physiol., Lancaster 39: 60-64.

Chapman, H.D. and Pratt, P.F. (1961). "Methods of Analysis for Soils, Plants and Waters". (Uni. Calif., Div. Agric. Sci.).

Chhonkar, P.K. and Subba-Rao, N.S. (1967). Phosphate solubilization by fungi associated with legume root nodules. Can. J. Microbiol. 13: 749-753.

Clarke, F.E. (1949). Soil micro-organisms and plant roots. Adv. Agron. 1: 241-288.

Clarkson, D.T. (1966). Effect of aluminium on the uptake and metabolism of phosphorus by barley seedlings. Pl. Physiol., Lancaster 41: 165-172.

Clarkson, D.T., Sanderson, J. and Russell, R.S. (1968). Ion uptake and root age. Nature, Lond. 220: 805-806.

Cole, C.V. and Olsen, S.R. (1959). Phosphorus solubility in calcareous soils II Effects of exchangeable phosphorus and soil texture on phosphorus solubility. Proc. Soil Sci. Soc. Am. 23: 119-121.

Cole, C.V., Grunes, D.L., Porter, L.K. and Olsen, S.R. (1963). The effects of nitrogen on short-term phosphorus absorption and translocation in corn (Zea mays). Proc. Soil Sci. Soc. Am. 27: 671-674.

Coleman, R. (1944). Phosphorus fixation by the coarse and fine clay fractions of kaolinitic and montmorillonitic clays. Soil Sci. 58: 71-77.

Coleman, N.T., Thorup, J.T. and Jackson, W.A. (1960). Phosphate sorption reactions that involve exchangeable aluminium. Soil Sci. 90: 1-7.

Colwell, J.D. (1963). The estimation of the phosphorus fertilizer requirements of wheat in southern New South Wales by soil analysis. Aust. J. exp. agric. Anim. Husb. 3: 190-197.

Conway, E.J. and Duggan, F. (1958). A cation carrier in the yeast cell wall. Biochem. J. 69: 265-274.

Cooper, R. (1959). Bacterial fertilizers in the Soviet Union. Soils Fertil. 22: 327-333.

Cormack, R.G.H. (1962). Development of root hairs in angiosperms. Bot. Rev. 28: 446-464.

Cornforth, I.S. (1968). Relationships between soil volume used by roots and nutrient accessibility. J. Soil Sci. 19: 291-301.

Cosgrove, D.J. (1967). Metabolism of organic phosphates in soil. In "Soil Biochemistry" (McLaren, A.D. and Peterson, G.H., Eds.), Marcel Dekker Inc., New York. pp 216-228.

Crooke. W.M. and Knight, A.H. (1971). Crop composition in relation to soil pH and root cation-exchange capacity. J. Sci. Fd. Agric. 22: 235-241.

Crossett, R.N. (1967). Autoradiography of P^{32} in maize roots. Nature, Lond. 213: 312-313.

Cunningham, R.K. (1963). The effect of clearing a tropical forest. J. Soil Sci. 14: 334-345.

Daft, M.J. and Nicolson, T.H. (1966). Effect of Endogone mycorrhiza on plant growth. New Phytol. 65: 343-350.

Daft, M.J. and Nicolson, T.H. (1969). Effect of Endogone mycorrhiza on plant growth. II Influence of soluble phosphate on endophyte and host in maize. New Phytol. 68: 945-952.

Darbyshire, J.F. and Greaves, M.P. (1970). An improved method for the study of the inter-relationships of soil micro-organisms and plant roots. Soil Biol. Biochem. 2: 63-71.

Das, A.C. (1963). Utilization of insoluble phosphates by soil fungi. J. Indian Soc. Soil Sci. 11: 203-207.

Das, D.K. and Datta, N.P. (1966). Nature of phosphate reaction products in black and brown soils. Sci. Cult. 32: 459-461.

Das, D.K. and Datta, N.P. (1967). Nature of reaction products from phosphate fertilizers in acid and calcareous soils of India. Indian J. Agric. Sci. 37: 526-536.

Das, D.K. and Datta, N.P. (1968). Reaction products from phosphate fertilizers in red and laterite soils of India. Indian J. Agric. Sci. 38: 382-390.

Deb, D.L. and Datta, N.P. (1967a). Effect of associating anions on phosphorus retention in soil. I Under variable phosphorus concentration. Pl. Soil 26: 303-316.

Deb, D.L. and Datta, N.P. (1967b). Effect of associating anions on phosphorus retention in soil. II Under variable anion concentration. Pl. Soil 26: 432-444.

De Jong, D.W. (1965). Histochemical demonstration of extracellular distribution of acid phosphatase in onion roots. Phyton. (Argentina). 22: 141-146.

Dittmer, H.J. (1937). A quantitative study of the roots and root hairs of a winter rye plant (Secale cereale). Am. J. Bot. 24: 417-420.

Domsch, K.M. (1965). The action of physiologically active substances in the root region. In "Plant Microbe Relationships", (Macura, J. and Vancura, V., Eds.) Publ. Czek. Acad. Sci., Prague., pp 186-192.

Dormaar, J.F. (1967). Distribution of inositol phosphates in some chernozemic soils of southern Alberta. Soil Sci. 104: 17-24.

Drake, M. (1967). Soil chemistry and plant nutrition. In "Chemistry of the Soil", (Bear, F.E., Ed.) Reinhold, New York. pp 395-444.

Drake, M. and Streckel, J.E. (1955). Solubilization of soil and rock phosphate as related to root cation exchange capacity. Proc. Soil Sci. Soc. Am. 19: 449-450.

Drew, M.C. and Nye, P.H. (1970). The supply of nutrient ions by diffusion to plant roots in soil. III Uptake of phosphate by roots of onion, leek and ryegrass. Pl. Soil 33: 545-563.

Duff, R.B., Webley, D.M. and Scott, R.O. (1963). Solubilization of minerals and related materials by Z-ketogluconic acid-producing bacteria. Soil Sci. 95: 105-114.

Duncan, W.G. and Ohlrogge, A.J. (1958). Principles of nutrient uptake from fertilizer bands. II Root development in the band. Agron. J. 50: 605-608.

Duncan, W.G. and Ohlrogge, A.J. (1959). Principles of nutrient uptake from fertilizer bands. III Band volume, concentration and nutrient composition. Agron. J. 51: 103-108.

Eadie, G.S. (1942). The inhibition of cholinesterase by physostigmine and prostigmine. J. biol. Chem. 147: 85-93.

Edwards, D.G. (1968). Cation effects on phosphate absorption from solution by Trifolium subterraneum. Aust. J. biol Sci. 21: 1-11.

Edwards, D.G. (1968a). The mechanism of phosphate absorption by plant roots. Trans 9th Intern. Congr. Soil Sci., Adelaide, Aust., 1968. II: 183-190.

Edwards, D.G. (1970). Phosphate absorption and long-distance transport in wheat seedlings. Aust. J. biol. Sci. 23: 255-264.

Elkan, G.H. (1962). Comparison of rhizosphere micro-organisms of genetically related nodulating and non-nodulating soybean lines. Can. J. Microbiol. 8: 79-87.

Engelstad, O.P. and Allen, S.E. (1971). Effect of form and proximity of added N on crop uptake of P. Soil Sci. 112: 330-337.

Engelstad, O.P. and Moreno, E.C. (1965). Effect of P concentration and distribution on P uptake and root growth of corn. Soil Sci. 99: 227-233.

Epstein, E. (1962). Mutual effects of ions in their absorption by plants. Agrochimica. 6: 293-322.

Epstein, E. (1972). Ion absorption by roots: the role of micro-organisms. New Phytol. 71: 873-874.

Epstein, E. and Hagen, C.E. (1952). A kinetic study of the absorption of alkali cations by barley roots. Pl. Physiol., Lancaster. 27: 457-474.

Epstein, E., Rains, D.W. and Elzam, O.E. (1963). Resolution of dual mechanisms of potassium absorption by barley roots. Proc. natn. Acad. Sci. 49: 684-692.

Epstein, E., Schmid, W.E., and Rains, D.W. (1963). Significance and technique of short term experiments on solute absorption by plant tissue. Pl. Cell Physiol., Tokyo. 4: 79-84.

Ericsson, Yngve. (1949). Enamel-apatite solubility, investigations on the calcium phosphate equilibrium between enamel and saliva and its relation to dental caries. Acta Odontol. Scand. Suppl. 8: 1-137.

Estermann, Eva. F. and McLaren, A.D. (1961). Contribution of rhizosphere organisms to the total capacity of plants to utilize organic nutrients. Pl. Soil 15: 243-260.

Fardeau, J.C., Delille, D. et Abramovici, C. (1968). Utilisation de la phytine par les plantes. In "Isotopes and Radiation in Soil Organic-matter Studies" (International Atomic Energy Agency, Vienna). pp 555-565.

Fawcett, R.G. and Quirk, J.P. (1962). The effect of soil-water stress on the absorption of soil phosphorus by wheat plants. Aust. J. agric. Res. 13: 193-205.

Fisher and Yates (1948). "Statistical Tables for Biological, Agricultural and Medical Research". (Oliver and Boyd, London.)

Flocker, W.J. and Nielsen, D.R. (1962). The absorption of nutrient elements by tomatoes associated with levels of bulk density. Proc. Soil Sci. Soc. Am. 26: 183-186.

Flocker, W.J., Vomocil, J.A. and Howard, F.D. (1959). Some growth responses of tomatoes to soil compaction. Proc. Soil Sci. Soc. Am. 23: 188-191.

Floyd, R.A. and Ohlrogge, A.J. (1970). Gel formation on nodal root surfaces of Zea mays. I Investigation of the gel's composition. Pl. Soil 33: 331-343.

Fox, R.L. and Kacar, B. (1964). Phosphorus mobilization in a calcareous soil in relation to surface properties of roots and cation uptake. Pl. Soil. 20: 319-330.

Fox, R.L. and Kamprath, E.J. (1970). Phosphate sorption isotherms for evaluating the phosphate requirements of soils. Proc. Soil Sci. Soc. Am. 34: 902-907.

Fox, R.L., Plucknett, D.L. and Whitney, A.S. (1968). Phosphate requirements of Hawaiian latosols and residual effects of fertilizer phosphorus. Trans. 9th Intern. Congr. Soil Sci., Adelaide, Aust., 1968. II: 301-310.

Foy, C.D. and Brown, J.C. (1964). Toxic factor in acid soils. II Differential aluminium tolerance in plant species. Proc. Soil Sci. Soc. Am. 28: 27-32.

Franklin, R.E. (1969). Effect of adsorbed cations on phosphorus uptake by excised roots. Pl. Physiol., Lancaster, 44: 697-700.

Franklin, R.E. (1970). Effect of adsorbed cations on phosphorus absorption by various plant species. Agron. J. 62: 214-216.

Franklin, R.E. (1971). Cation effects on chloride, sulphate and phosphate uptake by excised roots. Soil Sci. 112: 343-347.

Franklin, W.T. and Reisenauer, H.M. (1960). Chemical characteristics of soils related to phosphorus fixation and availability. Soil Sci. 90: 192-200.

Fried, M. (1953). The feeding power of plants for phosphate. Proc. Soil Sci. Soc. Am. 17: 357-59.

Fried, M. and Broeshart, H. (1967). "The Soil-plant System in Relation to Inorganic Nutrition". (Academic Press, New York and London).

Fried, M. and Dean, L.A. (1952). A concept concerning the measurement of available soil nutrients. Soil Sci. 73: 263-272.

Gates, C.T. (1964). The effect of water stress on plant growth. J. Aust. Inst. agric. Sci. 30: 3-22.

Gates, C.T. (1968). Water deficits and growth of herbaceous plants. In "Water Deficits and Plant Growth" (T.T. Kozlowski, Ed.), Academic Press, New York and London, Vol. II, pp 135-190.

Gerdemann, J.W. (1964). The effect of mycorrhizas on the growth of maize. Mycologia 56: 342-349.

Gerdemann, J.W. (1968). Vesicular-arbuscular mycorrhiza and plant growth. Ann. Rev. Phytopath. 6: 397-418.

Gerretsen, F.C. (1948). The influence of micro-organisms on phosphate uptake by the plant. Pl. Soil 1: 51-81.

Gilliam, J.W. (1970). Hydrolysis and uptake of pyrophosphate by plant roots. Proc. Soil Sci. Soc. Am. 34: 83-86.

Gough, N.A. and Beaton, J.D. (1963). Influence of phosphorus source and soil moisture on the solubility of phosphorus. J. Sci. Fd. Agric. 14: 224-228.

Graley, A.M. and Loveday, J. (1961). Chemical and morphological data for soils of the Burnie-Table Cape districts, Tasmania. Div. Rep. 13/60, C.S.I.R.O., Div. of Soils, Adelaide.

Gray, L.E. and Gerdemann, J.W. (1969). Uptake of phosphorus-32 by vesicular-arbuscular mycorrhizae. Pl. Soil 30: 415-421.

Greaves, M.P. and Webley, D.M. (1965). A study of the breakdown of organic phosphates by micro-organisms from the root region of certain pasture grasses. J. appl. Bact. 28: 454-465.

Greaves, M.P. and Webley, D.M. (1969). The hydrolysis of myo-inositol hexaphosphate by soil micro-organisms. Soil Biol. Biochem. 1: 37-43.

Greenway, H., Hughes, P.G. and Klepper, Betty. (1969). Effects of water deficit on phosphorus nutrition of tomato plants. Physiologia Pl. 22: 199-207.

Grunes, D.L. (1959). Effect of nitrogen on the availability of soil and fertilizer phosphorus to plants. Adv. Agron. 11: 369-396.

Grunes, D.L., Viets, F.G., Jr. and Shih, S.H. (1958). Proportionate uptake of soil and fertilizer phosphorus by plants as affected by nitrogen fertilizer. I Growth chamber experiments. Proc. Soil Sci. Soc. Am. 22: 43-48.

Gunary, D. and Sutton, C.D. (1967). Soil factors affecting plant uptake of phosphate. J. Soil Sci. 18: 167-173.

Hagen, C.E. and Hopkins, H.T. (1955). Ionic species in ortho-phosphate absorption by barley roots. Pl. Physiol., Lancaster, 30: 193-199.

Hagen, C.E., Leggett, J.E. and Jackson, P.C. (1957). The sites of orthophosphate uptake by barley roots. Proc. Natn. Acad. Sci. 43: 496-506.

Hagin, J. (1958). Availability of dicalcium phosphate to plants when applied in various forms. Pl. Soil 10: 101-113.

Hagin, J. and Berkovitis, J. (1961). Efficiency of phosphatic fertilizers of varying water-solubility. Can. J. Soil Sci. 41: 68-80.

Hall, J.L. (1969). Histochemical localization of B-glycero-phosphatase activity in young root tips. Ann. Bot. 33: 399-406.

Hall, J.L. and Butt, V.S. (1968). Localization and kinetic properties of B-glycerophosphatase in barley roots. J. exp. Bot. 19: 276-287.

Hall, J.L. and Butt, V.S. (1969). Adenosine triphosphatase activity in cell-wall preparations and excised roots of barley. J. exp. Bot. 20: 751-762.

Halstead, R.L., Lapensee, J.M. and Ivarson, K.C. (1963). Mineralization of soil organic phosphorus with particular reference to the effect of liming. Can. J. Soil Sci. 43: 97-106.

Hance, R.J. and Anderson, G. (1963). Extraction and estimation of soil phospholipids. Soil Sci. 96: 94-98.

Hannapel, R.J., Fuller, W.H. and Fox, R.H. (1964). Phosphorus movement in a calcareous soil. II Soil microbial activity and organic phosphorus movement. Soil Sci. 97: 421-427.

Hannapel, R.J., Fuller, W.H., Bosma, Shirley and Bullock, J.S. (1964). Phosphorus movement in a calcareous soil. I Predominance of organic forms of phosphorus in phosphorus movement. Soil Sci. 97: 350-357.

Hartt, Constance, E. (1972). Translocation of carbon-14 in sugarcane plants supplied with or deprived of phosphorus. Pl. Physiol, Lancaster. 49: 569-571.

Haseman, J.F., Brown, E.H. and Whitt, C.D. (1950). Some reaction of phosphate with clays and hydrous oxides of iron and aluminium. Soil Sci. 70: 257-271.

Haseman, J.F., Lehr, J.R. and Smith, J.P. (1951). Mineralogical character of some iron and aluminium phosphates containing potassium and ammonium. Proc. Soil Sci. Soc. Am. 15: 76-84.

Hayman, D.S. and Mosse, Barbara. (1971). Plant growth responses to vesicular-arbuscular mycorrhiza. I Growth of Endogone-inoculated plants in phosphate-deficient soils. New Phytol. 70: 19-27.

Hayman, D.S. and Mosse, B. (1972). Plant growth responses to vesicular-arbuscular mycorrhiza. III Increased uptake of labile P from soil. New Phytol. 71: 41-47.

Helyar, K.R. and Anderson, A.J. (1970). Responses of five pasture species to phosphorus, lime and nitrogen on an infertile acid soil with a high phosphate sorption capacity. Aust. J. agric. Res. 21: 677-692.

Hemwall, J.B. (1957). The fixation of phosphorus by soils. Adv. Agron. 9: 95-112.

Hendrix, J.E. (1967). The effect of pH on the uptake and accumulation of phosphate and sulphate ions by bean plants. Am. J. Bot. 54: 560-564.

Hewitt, E.J. (1966). "Sand and Water Culture Methods used in the Study of Plant Nutrition". 2nd Ed. Comm. Agric. Bur. Tech. Comm. 22.

Hingston, F.J., Atkinson, R.J., Posner, A.M. and Quirk, J.P. (1967). Specific adsorption of anions. Nature, Lond. 215: 1459-1461.

Hingston, F.J., Atkinson, R.J., Posner, A.M. and Quirk, J.P. (1968). Specific adsorption of anions on geothite. Trans. 9th Intern. Congr. Soil Sci., Adelaide, Aust., 1968. I: 669-678.

Hinman, W.C., Beaton, J.D. and Read, D.W.L. (1962). Some effects of moisture and temperature on transformation of mono-calcium phosphate in soil. Can. J. Soil Sci. 42: 229-239.

Hoagland, D.R. and Arnon, D.I. (1950). The water culture method for growing plants without soil. Calif. Agr. Expt. Sta., Circ. 347.

Hofstee, B.H.J. (1952). On the evaluation of the constants Vm and Km in enzyme reactions. Science 116: 329-333.

Holevas, C.D. (1966). The effect of a vesicular arbuscular mycorrhiza on the uptake of soil phosphorus by Strawberry (Fragaria sp. var. Cambridge Favourite) J. hort. Sci. 41: 57-64.

Hsu, P.H. (1965). Fixation of phosphate by aluminium and iron in acidic soil. Soil Sci. 99: 398-402.

Huffman, E.O. (1962). Reactions of phosphate in soils: recent research by T.V.A. Proc. Fertil. Soc. 71: 5-35.

Huffman, E.O. (1970). Fertilizer-soil reactions and the phosphate status of soils. In "Phosphorus in Agriculture" Bull. Doc. 55: 1-11.

Huffman, E.O., Cate, W.E., Deming, M.E. and Elmore, K.L. (1960). Rates and mechanisms of dissolution of some iron and aluminium phosphates. Trans. 7th Intern. Congr. Soil Sci., Madison, Wisc., 1960. II: 404-412.

Hughes, J.D. and Searle, P.G.E. (1964). Observations on the residual value of accumulated phosphorus in a red loam. Aust. J. agric. Res. 15: 377-383.

Humble, G.D., Leboudi, A. El. and Renig, V.V. (1969). Effect of nitrogen on phosphorus absorption by excised barley roots. Pl. Soil 31: 353-364.

Hunter, A.S. (1965). Effect of silica on uptake of phosphorus from soils by four crops. Soil Sci. 100: 391-96.

Hyde, A.H. (1966). Nature of the calcium effect in phosphate uptake by barley roots. Pl. Soil 24: 328-331.

Ikaya, T., Nisizawa, K. and Miwa, T. (1964). Specificities of several acid phosphatases from plant sources. Nature, Lond. 203: 939-940.

Jackman, R.H. (1965). The uptake of rubidium by the roots of some graminaceous and leguminous plants. N.Z. Jl. agric. Res. 8: 763-777.

Jackman, R.H. and Black, C.A. (1951). Solubility of iron, aluminium, calcium and magnesium inositol phosphates at different pH values. Soil Sci. 72: 179-186.

Jackson, N.E., Franklin, R.E. and Miller, R.H. (1972). Effects of vesicular-arbuscular mycorrhizae on growth and phosphorus content of three agronomic crops. Proc. Soil Sci. Soc. Am. 36: 364-67.

Jackson, R.M., Brown, M.E. and Burlingham, S.K. (1964). Similar effects on tomato plants of Azotobacter inoculation and application of gibberellins. Nature, Lond. 203: 851-852.

Jelley, J.V. (1958). "Cerenkov Radiation and its Application". (Pergamon Press, Oxford)

Jenkins, W.L. (1962). The yield and uptake of P by grasses and clovers. II Effect of species, variety and stage of growth. J. Brit. Grassld. Soc. 17: 198-205.

Jennings, D.H. (1963). "The Absorption of Solutes by Plant Cells". (Oliver and Boyd, Edinburgh and London).

Johnson, H.W. (1959). The solubilization of "insoluble" phosphates. V The action of some organic acids on Fe and Al phosphates. N.Z. Jl. Sci. Technol. 2: 215-218.

Johnston, A.E., Warren, R.G. and Penny, A. (1969). The value of residues from long period manuring at Rothamsted and Woburn. IV The value to arable crops of residues accumulated from superphosphate. Rep. Rothamsted exp. Stn. 1969. 2: 39-68.

Juo, A.S.R. and Ellis, B.G. (1968). Chemical and physical properties of iron and aluminium phosphates and their relation to phosphorus availability. Proc. Soil Sci. Soc. Am. 32: 216-221.

Kaila, A. (1965). Effect of liming on the mobilization of soil phosphorus. Maat. Aikak. 37: 243-254.

Kakie, T. (1969). Effect of phosphorus deficiency on the photosynthetic carbon dioxide fixation-products in tobacco plants. Soil Sci. Pl. Nutr. 15: 245-251.

Kalra, Y.P. (1971). Application of split-root technique in orthophosphate absorption experiments. J. agric. Sci. Camb. 77: 77-81.

Kalra, Y.P. and Soper, R.J. (1968). Efficiency of rape, oats, soyabeans and flax in absorbing soil and fertilizer phosphorus at seven stages of growth. Agron. J. 60: 209-212.

Kamprath, E.J. (1967). Residual effect of large applications of phosphorus on high phosphorus fixing soils. Agron. J. 59: 25-27.

Katznelson, H. and Cole, S.E. (1965). Production of gibberellin-like substances by bacteria and actinomycetes. Can. J. Microbiol. 11: 733-741.

Katznelson, H. and Sirois, J.C. (1961). Auxin production by species of Arthobacter. Nature, Lond. 191: 1323-1324.

Katznelson, H., Peterson, E.A. and Rouatt, J.W. (1962). Phosphate-dissolving micro-organisms on seed and in the root zone of plants. Can. J. Bot. 40: 1181-1186.

Keay, J., Biddiscombe, E.F. and Ozanne, P.G. (1970). The comparative rates of phosphate absorption by eight annual pasture species. Aust. J. agric. Res. 21: 33-44.

Ketcheson, J.W. (1957). Some effects of soil temperature on phosphorus requirement of young corn plants in the greenhouse. Can. J. Soil Sci. 37: 41-47.

Kirby, E.A. and Mengel, K. (1967). Ionic balance in different tissues of the tomato plant in relation to nitrate, urea or ammonium nutrition. Pl. Physiol., Lancaster. 42: 6-14.

Knoll, H.A., Brady, N.C. and Lathwell, D.J. (1964). Effect of soil temperature and phosphorus fertilization on the growth and phosphorus content of corn. Agron. J. 56: 145-147.

Kramer, P.J. and Wiebe, H.H. (1952). Longitudinal gradients of P^{32} absorption in roots. Pl. Physiol., Lancaster. 27: 661-674.

Kurtz, L.T. and Quirk, J.P. (1965). Phosphate adsorption and phosphate fractions in field soils of varying histories of phosphate fertilization. Aust. J. agric. Res. 16: 403-412.

Kylin, A. (1950). A new method for large-scale aseptic cultivation of higher plants. Physiologia Pl. 3: 165-174.

Lance, J.C. and Pearson, R.W. (1969). Effect of low concentrations of aluminium on growth and water and nutrient uptake by cotton roots. Proc. Soil Sci. Soc. Am. 33: 95-98.

Larsen, S. (1952). The use of P^{32} in studies on the uptake of phosphorus by plants. Pl. Soil 4: 1-10.

Larsen, S. (1967). Soil phosphorus. Adv. Agron. 19: 151-210.

Larsen, S., Gunary, D. and Devine, J.R. (1964). Stability of granular dicalcium phosphate dihydrate in soil. Nature, Lond. 204: 1114

Lauchli, Andre (1967). Investigations on the distribution and transport of ions in plant tissue with the X-ray microanalyser. Planta 75: 185-206.

Lauchli, Andre (1969). Radio-assay for B-emitters in biological materials using Cerenkov radiation. Int. J. appl. Radiat. Isotopes 20: 265-270.

Lauchli, Andre and Schwander, H. (1966). X-ray micro-analyser study on the location of minerals in native plant tissue sections. Experimentia. (Basel) 22: 503-505.

Lawton, K., Apostolakis, C., Cook, R.L. and Hill, W.L. (1956). Influence of particle size, water solubility and placement of fertilizers on the nutrient value of phosphorus in mixed fertilizers. Soil Sci. 82: 465-476.

Leaver, J.P. and Russell, E.W. (1957). The reaction between phosphate and phosphate-fixing soils. J. Soil Sci. 8: 113-126.

Leggett, J.E. (1961). Entry of phosphate into yeast cells. Pl. Physiol., Lancaster. 36: 277-284.

Leggett, J.E. (1968). Salt absorption by plants. Ann. Rev. Pl. Physiol. 19: 333-346.

Leggett, J.E., Heald, W.R. and Hendricks, S.B. (1965). Cation binding by baker's yeast and resins. Pl. Physiol., Lancaster. 40: 665-671.

Leggett, J., Raymond, E., Galloway, A. and Gauch, H.G. (1965). Calcium activation of orthophosphate absorption by barley roots. Pl. Physiol., Lancaster. 40: 897-902.

Lehr, J.J. and Van Wesemael, J. Ch. (1952). The influence of neutral salts on the solubility of soil phosphate with special reference to the effect of nitrates of sodium and calcium. J. Soil Sci. 3: 125-135.

Lehr, J.J. and Van Wesemael, J. Ch. (1956). Variations in the uptake by plants of soil PO_4 as influenced by $NaNO_3$ and $Ca(NO_3)_2$. J. Soil Sci. 7: 148-155.

Lehr, J.R. and Brown, W.E. (1958). Calcium phosphate fertilizers. II A petrographic study of their alteration in soils. Proc. Soil Sci. Soc. Am. 22: 29-32.

Lehr, J.R., Brown, W.E. and Brown, E.H. (1959). Chemical behaviour of monocalcium phosphate monohydrate in soils. Proc. Soil Sci. Soc. Am. 23: 3-7.

Leopold, A.C. (1964). "Plant Growth and Development". (McGraw-Hill Book Co., New York).

Lewis, D.G. & Quirk, J.P. (1967). Phosphate diffusion in soil and uptake by plants. III P^{31} Movement and uptake by plants ad indicated by P^{32} autoradiography. Pl. Soil 26: 445-453.

Lilleland, O., Brown, J.G. and Conrad, J.P. (1942). The phosphate nutrition of fruit trees. III Comparison of fruit tree and field crop responses on a phosphate deficient soil. Proc. Am. Soc. hort. Sci. 40: 1-7.

Lindsay, W.L. and Moreno, E.C. (1960). Phosphate phase equilibrium in soils. Proc. Soil Sci. Soc. Am. 24: 177-182.

Lindsay, W.L. and Stephenson, H.F. (1959a). Nature of the reactions of monocalcium phosphate monohydrate in soils. I The solution that reacts with the soil. Proc. Soil Sci. Soc. Am. 23: 12-18.

Lindsay, W.L. and Stephenson, H.F. (1959b). Nature of the reactions of monocalcium phosphate monohydrate in soils. IV Repeated reactions with metastable triple-point solution. Proc. Soil Sci. Soc. Am. 23: 440-445.

Lindsay, W.L. and Taylor, A.W. (1960). Phosphate reaction products in soil and their availability to plants. Trans. 7th Intern. Congr. Soil Sci. Madison, Wisc., 1960. III: 580-589.

Lindsay, W.L., Frazier, A.W. and Stephenson, H.F. (1962). Identification of reaction products from phosphate fertilizers in soils. Proc. Soil Sci. Soc. Am. 26: 446-452.

Lindsay, W.L., Lehr, J.R. and Stephenson, H.F. (1959). Nature of the reactions of monocalcium phosphate monohydrate in soils. III Studies with metastable triple-point solution. Proc. Soil Sci. Soc. Am. 23: 342-345.

Lingle, J.C. and Davis, R.M. (1959). The influence of soil temperature and P fertilization on the growth and mineral absorption of tomato seedlings. Proc. Am. Soc. hort. Sci. 73: 312-322.

Lipsett, J. (1964). The phosphorus content and yield of grain of different wheat varieties in relation to phosphorus deficiency. Aust. J. agric. Res. 15: 1-8.

Loneragan, J.F. and Asher, C.J. (1967). Response of plants to phosphate concentration in solution culture. II Rate of phosphate absorption and its relation to growth. Soil Sci. 103: 311-318.

Loutit, Margaret, W. (1969). Soil micro-organisms and molybdenum concentrations in plants. Trans. 9th Intern. Congr. Soil Sci., Adelaide, Aust. 1969. III: 491-499.

Louw, H.A. (1970). A study of the phosphate - dissolving bacteria in the root region of wheat and lupin. Phytophylactica 2: 21-26.

Louw, H.A. and Webley, D.M. (1959a). The bacteriology of the root region of the oat plant grown under controlled pot culture conditions. J. Appl. Bact. 22: 216-226.

Louw, H.A. and Webley, D.M. (1959b). A study of soil bacteria dissolving certain mineral phosphate fertilizers and related compounds. J. Appl. Bact. 22: 227-233.

Loveday, J. and Farquhar, R.N. (1958). The Soils and Some Aspects of Land Use in the Burnie, Table Cape and Surrounding Districts, North West Tasmania. C.S.I.R.O., Div. of Soils, Soils and Land Use Series No. 26.

Low, P.F. and Black, C.A. (1950). Reactions of phosphate with kaolinite. Soil Sci. 70: 273-290.

Lüttge, U. and Laties, G.G. (1966). Dual mechanisms of ion absorption in relation to long distance transport in plants. Pl. Physiol., Lancaster. 41: 1531-1539.

Luttge, U. and Laties, G.G. (1967). Selective inhibition of absorption and long distance transport in relation to the dual mechanisms of ion absorption in maize seedlings. Pl. Physiol., Lancaster. 42: 181-185.

Lutz, J.F. (1952). Mechanical impedance and plant growth. In "Soil Physical Conditions and Plant Growth", (Shaw, B.T., Ed.), Academic Press Inc., New York. Vol. II. pp 43-71.

Lyness, A.S. (1936). Varietal differences in the phosphorus feeding capacity of plants. Pl. Physiol., Lancaster. 11: 665-688.

MacDonald, I.R. (1967). Bacterial infection and ion absorption capacity in beet discs. Ann. Bot. 31: 163-172.

Mack, A.R. and Sanderson, R.B. (1971). Sensitivity of the nitrate-ion membrane electrode in various soil extracts. Can. J. Soil Sci. 51: 95-104.

Marais, J.S. (1922). The comparative agricultural value of insoluble mineral phosphates of aluminium, iron and calcium. Soil Sci. 13: 355-409.

Marais, P.G., Deist, J., Harry, R.B.A., et al (1970). Ability of different plant species to absorb phosphate. Agrochemophysica 2: 7-12.

Markham, R. (1942). A steam distillation apparatus suitable for micro-Kjeldahl analysis. Biochem. J. 36: 790-791.

Martin, J.K. (1970). Organic phosphate compounds in water extracts of soils. Soil Sci. 109: 362-375.

Martin, J.K. and Cartwright, B. (1971). The comparative availability of ^{32}P myo-inositol hexaphosphate and $K_2H^{32}PO_4$ added to soils. Communs. Soil Sci. Pl. Anal. 2: 375-381.

Martin, J.K. and Wicken, A.J. (1966). Soil organic phosphorus. IV. Fractionation of organic phosphorus in alkaline soil extracts and the identification of inositol phosphates. N.Z. Jl. agric. Res. 9: 529-535.

Martin, W.E., Vlamis, J. and Quirk, J. (1953). Effect of ammoniation on availability of phosphorus in super-phosphates, as indicated by plant response. Soil Sci. 75: 41-49.

Mattson, S. (1966). The ionic relationships of soil and plant. Acta Agriculturae Scand. 16: 135-143.

Mattson, S. (1967). Ionic relationships of soil and plant. II The salt effect on ion uptake. Acta Agriculturae Scand. 17: 78-82.

Mazurak, A.P. and Pohlman, K. (1968). Growth of corn and soybean seedlings as related to soil compaction and matric suction. Trans. 9th Intern. Congr. Soil Sci., Adelaide, Aust., 1968. III: 813-822.

Menary, R.C. and Hughes, J.D. (1967). The effect of sulphate on phosphorus availability of a krasnozem soil. Aust. J. exp. agric. Anim. Husb. 7: 168-173.

Menary, R.C. and Kruger, N.S. (1966). Influence of soil bulk density on nutrition and growth in the tomato. Qld. J. agric. Anim Sci. 23: 359-371.

Menzel, Gisela (1971). Influence of bacteria upon the growth performance of higher plants under conditions of different phosphorus content of the nutrient medium and of phosphorus deficiency. Zbl. Bakt. Abt. II 126: 270-279.

Michaelis, L. and Menten, M.L. (1913). Die kinetik der Ivertinwirkung. Biochem. Z. 49: 333-369.

Miller, M.H. and Ohlrogge, A.J. (1958). Principles of nutrient uptake from fertilizer bands. I Effect of placement of nitrogen fertilizer on the uptake of band-placed phosphorus at different soil phosphorus levels. Agron. J. 50: 95-97.

Miller, M.H. and Vij, V.N. (1962). Some chemical and morphological effects of ammonium sulphate in a fertilizer phosphorus band for sugar beets. Can. J. Soil Sci. 42: 87-95.

Miller, M.H., Mamaril, C.P. and Blair, G.J. (1970). Ammonium effects on phosphorus absorption through pH changes and phosphorus precipitation at the soil-root interface. Agron. J. 62: 524-527.

Mitchell, J., Dehm, J.E. and Dion, H.G. (1952). The effect of small additions of elemental sulphur on the availability of phosphate fertilizers. Scientific Agriculture 32: 311-316.

Mosse, Barbara (1971). Effect of Endogone mycorrhiza on plant growth. Rep. Rothamsted exp. Stn., 1970. 1: 89-90.

Mosse, Barbara and Phillips, J.M. (1971). The influence of phosphate and other nutrients on the development of vesicular-arbuscular mycorrhiza in culture. J. gen. Microbiol. 69: 157-166.

Munns, D. (1965). Soil acidity and growth of a legume. I Interactions of lime with nitrogen and phosphate on growth of Medicago sativa L. and Trifolium subterraneum L. Aust. J. agric. Res. 16: 733-741.

Murdoch, C.L., Jackobs, J.A. and Gerdemann, J.W. (1967). Utilization of phosphorus sources of different availability by mycorrhizal and non-mycorrhizal maize. Pl. Soil 27: 329-334.

Murdock, J.T. and Seay, W.A. (1954). The effect of soil conditioner on uptake of superphosphate by greenhouse wheat. Proc. Soil Sci. Soc. Am. 18: 97-98.

Murrman, R.P. and Peech, M. (1968). Reaction products of applied phosphate in limed soils. Proc. Soil Sci. Soc. Am. 32: 493-496.

Murrman, R.P. and Peech, M. (1969). Relative significance of labile and crystalline phosphates in soil. Soil Sci. 107: 249-255.

McKercher, R.B. and Anderson, G. (1968a). Characterisation of the inositol penta- and hexa-phosphate fractions of a number of Canadian and Scottish soils. J. Soil Sci. 19: 302-310.

McKercher, R.B. and Anderson, G. (1968b). Content of inositol penta- and hexa-phosphates in some Canadian soils. J. Soil Sci. 19: 47-55.

McLean, E.O. (1956). Factors affecting yields and uptake of phosphorus by different crops. II Rock phosphate and superphosphate, separate and in combination under extended cropping. Soil Sci. 82: 181-192.

McLean, E.O. and Hoelsher, J.E. (1954). Factors affecting yields and uptake of phosphorus by different crops. I Previous application to the soil of rock phosphate and superphosphate. Soil Sci. 78: 453-462.

McLean, E.O. and Logan, T.J. (1970). Sources of phosphorus for plants grown in soils with differing phosphorus fixation tendencies. Proc. Soil Sci. Soc. Am. 34: 907-911.

Nassery, H. (1970). Phosphate absorption by plants from habitats of different phosphate status. II Absorption and incorporation of phosphate by intact plants. New Phytol. 69: 197-203.

Nassery, H. (1971). Phosphate absorption by plants from habitats of different phosphate status. III Phosphate fractions in the roots of intact plants. New Phytol. 70: 949-951.

Nicolson, T.H. (1960). Mycorrhiza in the Gramineae. II Development in different habitats, particularly sand dunes. Trans. Br. mycol. Soc. 43: 132-145.

Nicolson, T.H. (1967). Vesicular-arbuscular mycorrhiza - a universal plant symbiosis. Sci. Progr. (Oxford). 55: 561-581.

Nielson, K.F. and Humphries, E.C. (1966). Effects of root temperature on plant growth. Soils Fertil. 29: 1-7.

Noggle, J.C. and Fried, M. (1960). A kinetic analysis of phosphate absorption by excised roots of millet, barley and alfalfa. Proc. Soil Sci. Soc. Am. 24: 33-35.

Norman, A.G. (1959). Inhibition of root growth and cation uptake by antibiotics. Proc. Soil Sci. Soc. Am. 23: 368-370.

Norrish, K. (1968). Some phosphate minerals of soil. Trans. 9th Intern. Congr. Soil Sci., Adelaide, Aust. 1968. II: 713-724.

Nye, P.H. (1966). The effect of the nutrient intensity and buffering power of a soil and the absorbing power, size and root hairs of a root on nutrient absorption by diffusion. Pl. Soil 25: 81-105.

Nye, P.H. and Foster, W.N.M. (1958). A study of the mechanism of soil phosphate uptake in relation to plant species. Pl. Soil. 9: 338-352.

Nye, P.H. and Foster, W.N.M. (1961). The relative uptake of phosphorus by crops and natural fallow from different parts of their root zone. J. agric. Sci. Cambr. 56: 299-306.

Obihara, C.H. and Russell, E.W. (1972). Specific absorption of silicate and phosphate by soils. J. Soil Sci. 23: 105-117.

Olsen, S.R. (1953). Inorganic phosphorus in alkaline and calcareous soils. In "Soil and Fertilizer Phosphorus in Crop Nutrition" (Pierre, W.H. and Norman, A.G., Eds.), Academic Press Inc., New York. pp 89-122.

Olsen, S.R. and Kemper, W.D. (1968). Movement of nutrients to plant roots. Adv. Agron. 20: 91-151.

Olsen, S.R. and Watanabe, F.S. (1963). Diffusion of phosphorus as related to soil texture and plant uptake. Proc. Soil Sci. Soc. Am. 27: 648-53.

Olsen, S.R. and Watanabe, F.S. (1970). Diffusive supply of phosphorus in relation to soil textural variations. Soil Sci. 110: 318-327.

Omotoso, T.I. and Wild, A. (1970). Content of inositol phosphates in some English and Nigerian soils. J. Soil Sci. 21: 216-223.

Otsuka, Kyoji, (1968). Studies on nutritional physiology of grafted plants. II Influence of phosphorus concentration in nutrient medium on growth and phosphorus uptake of grafted plants. Nippon Dojohiryogaku Zasshi, 39: 479-483.

Ozbek, N. (1967). Factors affecting the amount of available soil phosphorus, A value. In "Isotopes in Plant Nutrition and Physiology". Proceed. of a Symposium on the use of Isotopes in Plant Nutrition and Physiology - (I.A.A.A. and F.A.O.), Vienna, 5-9th Sept. 1966. International Atomic Energy Agency Vienna, 1967. pp 35-45.

Passioura, J.B. (1963). A mathematical model for the uptake of ions from the soil solution. Pl. Soil. 18: 225-238.

Patrick, Wm. H. and Mahapatra, I.C. (1968). Transformation and availability to rice of nitrogen and phosphorus in waterlogged soils. Adv. Agron. 20: 323-359.

Patterson, R.P., Grunes, D.L. and Lathwell, D.J. (1972). Influence of root zone temperature and P supply on total and inorganic P, free sugars, aconitate and soluble amino N in corn. Crop Sci. 12: 227-230.

Paul, N.B. and Sundara Rao, W.V.B. (1971). Phosphate dissolving bacteria in the rhizosphere of some cultivated legumes. Pl. Soil. 35: 127-132.

Perkins, A.T. and King, H.H. (1944). Phosphorus fixation by soil minerals. III Particle size. Proc. Soil Sci. Soc. Am. 9: 61-65.

Phillips, R.E. and Kirkham, D. (1962). Mechanical impedance and corn seedling root growth. Proc. Soil Sci. Soc. Am. 26: 319-322.

Phillips, J.W., Baker, D.E. and Clagett, C.O. (1971). Identification of compounds which account for variation in P concentration in corn hybrids. Agron. J. 63: 541-543.

Pierre, W.H. and Parker, F.W. (1927). Soil Phosphorus studies. II The concentration of organic and inorganic phosphorus in the soil solution and soil extracts and the availability of organic phosphorus to plants. Soil Sci. 24: 119-128.

Pigott, C.D. (1971). Analysis of the response of Urtica dioica to phosphate. New Phytol. 70: 953-966.

Place, G.A. and Barber, S.A. (1964). The effect of soil moisture and rubidium concentration on diffusion and uptake of rubidium - 86. Proc. Soil Sci. Soc. Am. 28: 239-243.

Power, J.F., Grunes, D.L., Reichman, G.A. and Willis, W.O. (1964). Soil temperature effects on phosphorus availability. Agron. J. 56: 545-548.

Power, J.F., Grunes, D.L., Reichman, G.A. and Willis, W.O. (1970). Effect of soil temperature on rate of barley development and nutrition. Agron. J. 62: 567-571.

Power, J.F., Grunes, D.L., Willis, W.O. and Reichman, G.A. (1963). Soil temperature and phosphorus effects upon barley growth. Agron. J. 55: 389-392.

Power, J.F., Willis, W.O., Grunes, D.L. and Reichman, G.A. (1967). Effect of soil temperature, phosphorus and plant age on growth analysis of barley. Agron. J. 59: 231-234.

Raghu, R. and Macrae, I.C. (1966). Occurrence of phosphate dissolving micro-organisms in the rhizosphere of rice plants and in submerged soils. J. appl. Bact. 29: 582-586.

Ragland, J.L. and Coleman, N.T. (1962). Influence of aluminium on phosphorus uptake by snap bean roots. Proc. Soil Sci. Soc. Am. 26: 88-90.

Randall, P.J. and Vose, P.B. (1963). Effect of aluminium on uptake and translocation of phosphorus ^{32} by perennial ryegrass. Pl. Physiol., Lancaster. 38: 403-409.

Raupach, M. (1963). Solubility of simple aluminium compounds expected in soils. III Aluminium ions in soil solutions and aluminium phosphates in soils. Aust. J. Soil Res. 1: 46-54.

Raupach, M. and Piper, C.S. (1959). Interaction of silicate and phosphate in a lateritic soil. Aust. J. agric. Res. 10: 818-831.

Reichman, G.A. and Grunes, D.L. (1966). Effect of water regime and fertilization on barley growth, water use and N and P uptake. Agron. J. 58: 513-517.

Rempe, J.K. and Kaltagova, O.G. (1965). Influence of root microflora on the increase, development and activity of physiological processes in plants. In "Plant-Microbes Relationships" (Macura, J. and Vancura, V. Eds.), Publ. Czek. Acad. Sci., Prague. pp 178-185.

Richards, L.A. and Wadleigh, C.H. (1952). Soil water and plant growth. In "Soil Physical Conditions and Plant Growth", (Shaw, B.T., Ed.), Academic Press Inc., New York. pp 73-251.

Richards, S.J., Hagan, R.M. and McCalla, T.M. (1952). Soil temperature and plant growth. In "Soil Physical Conditions and Plant Growth" (Shaw, B.T., Ed.), Academic Press, New York. pp 303-408.

Ridge, E.H. and Rovira, A.D. (1971). Phosphatase activity of intact young wheat roots under sterile and non-sterile conditions. New Phytol. 70: 1017-1026.

Riley, D. and Barber, S.A. (1969). Bicarbonate accumulation and pH changes at the soyabean (Glycine max. (L) Mirr) root-soil interface. Proc. Soil Sci. Soc. Am. 33: 905-908.

Riley, D. and Barber, S.A. (1971). Effect of ammonium and nitrate fertilization on phosphorus uptake as related to root-induced pH changes at the root-soil interface. Proc. Soil Sci. Soc. Am. 35: 301-306.

Riviere, J. (1961). Inter-relations of some phosphorus bacteria and some cultivated plants. Ann. Inst. Pasteur 101: 611-618.

Robertson, R.N. (1968). "Protons, electrons, phosphorylation and active transport". (Cambridge University Press, London and New York).

Robertson, W.K., Neller, J.R. and Bartlett, F.D. (1954). Effect of lime on the availability of phosphorus in soils of high to low sesquioxide content. Proc. Soil Sci. Soc. Am. 18: 184-187.

Robinson, R.R. (1942). Phosphorus fixation as affected by soil temperature. J. Am. Soc. Agron. 34: 301-306.

Robson, A.D., Edwards, D.G. and Loneragan, J.F. (1970). Calcium stimulation of phosphate absorption by annual legumes. Aust. J. agric. Res. 21: 601-612.

Rogers, H.T., Pearson, R.W. and Pierre, W.H. (1940). Absorption of organic P by corn and tomato plants and the mineralizing action of exoenzymes of growing roots. Proc. Soil Sci. Soc. Am. 5: 285-291.

Romsdal, S.D. and Schmehl, W.R. (1964). Effect of water solubility and granule size of phosphorus fertilizers on alfalfa grown in a calcareous soil. Agron. J. 64: 184-186.

Rorison, I.H. (1965). The effect of aluminium on the uptake and incorporation of phosphate by excised sainfoin roots. New Phytol. 64: 23-27.

Rose, R.E. (1957). Techniques for determining the effect of micro-organisms on insoluble inorganic phosphates. N.Z. Jl. Sci. Technol. B. 38: 773-780.

Rovira, A.D. (1965). Interactions between plant roots and soil micro-organisms. Ann. Rev. Microbiol. 19: 241-266.

Rovira, A.D. (1969). Plant root exudates. Bot. Rev. 35: 35-57.

Rovira, A.D. and Bowen, G.D. (1966). Phosphate incorporation by sterile and non-sterile plant roots. Aust. J. biol. Sci. 19: 1167-1169.

Rovira, A.D. and Bowen, G.D. (1968). Anion uptake by plant roots: Distribution of anions and effect of micro-organisms. Trans. 9th Intern. Congr. Soil Sci., Adelaide, Aust., 1968. II: 207-217.

Rovira, A.D. and Bowen, G.D. (1970). Translocation and loss of phosphate along roots of wheat seedlings. Planta 93: 15-25.

Rovira, A.D. and McDougall, Barbara M. (1967). Microbiological and biochemical aspects of the rhizosphere. In "Soil Biochemistry", (McLaren, A.D. and Peterson, G.H., Eds.), Marcel Dekker Inc., New York. pp 417-463.

Russell, E.W. (1961). "Soil Conditions and Plant Growth". 9th Ed.¢ (Longmans, London)

Russell, R.S. and Sanderson, J. (1967). Nutrient uptake by different parts of the intact roots of plants. J. exp. Bot. 18: 491-508.

Russell, R.S., Russell, E.W. and Marais, P.G. (1958). Factors affecting the ability of plants to absorb phosphate from soil. II Comparison of the ability of different species to absorb labile soil phosphate. J. Soil Sci. 9: 101-108.

Sanders, F.E. and Tinker, P.B. (1971). Mechanism of absorption of phosphate from soil by Endogone mycorrhizas. Nature, Lond. 233: 278-279.

Saric̆, Z. (1965). The occurrence of micro-organisms releasing phosphorus from organic and inorganic compounds in the wheat rhizosphere. In "Plant Microbes Relationships", (Macura, J. and Vancura, V. Eds.) Publ. Czek. Acad. Sci., Prague. pp 143-146.

Saunders, W.H.M. and Williams, E.G. (1955). Observations on the determination of total organic phosphorus in soil. J. Soil Sci. 6: 254-267.

Saxena, S.N. (1964). Phytase activity of plant roots. J. exp. Bot. 15: 654-655.

Scaife, M.A. and Jones, D. (1970). Effect of seed weight on lettuce growth. J. hort. Sci. 45: 299-302.

Seatz, L.F. and Stanberry, C.O. (1963). Advances in phosphate fertilization. In "Fertilizer Technology and Usage" (McVickar, M.H., Bridger, G.L. and Nelson, L.B., Eds.), Soil Sci. Soc. Am., Madison, Wisc. pp 155-188.

Shieh, T.R. and Ware, J.H. (1968). Survey of micro-organisms for the production of extra-cellular phytase. Appl. Microbiol. 16: 1348-1351.

Simpson, J.R. and Williams, C.H. (1970). The effects of fluctuations in soil moisture content on the availability of recently applied phosphate. Aust. J. Soil Res. 8: 209-219.

Singh, R.N. and Seatz, L.F. (1961). Alfalfa yield and composition after different times and rates of lime and phosphorus application. Proc. Soil Sci. Soc. Am. 25: 307-309.

Smith, A.N. (1965). Aluminium and iron phosphates in soils. J. Aust. Inst. agric. Sci. 31: 110-126.

Smith, A.N. (1969). Effects of daylength and time of application of phosphorus on growth and grain yield of wheat. Physiologia Pl. 22: 371-378.

Smith, P.F. (1962). Mineral analysis of plant tissues. Ann. Rev. Pl. Physiol. 13: 81-108.

Smith, S.N. (1934). Response of inbred lines and crosses in maize to variations of nitrogen and phosphorus supplied as nutrients. J. Am. Soc. Agron. 26: 785-804.

Smith, R.C. and Epstein, E. (1964). Ion absorption by shoot tissue: kinetics of potassium and rubidium absorption by corn leaf tissue. Pl. Physiol. Lancaster. 39: 992-996.

Sommer, L. Anna (1936). The relationship of the phosphate concentration of solution cultures to the type and size of root systems and the time of maturity of certain plants. J. agric. Res. 52: 133-148.

Soper, R.J. and Kalra, Y.P. (1969). Effect of mode of application and source of fertilizer on phosphorus utilization by buckwheat rape, oats and flax. Can. J. Soil Sci. 49: 319-326.

Sperber, J.I. (1957). Solution of mineral phosphate by soil bacteria. Nature, Lond. 180: 994-995.

Sperber, J.I. (1958a). Release of PO_4 from soil minerals by H_2S. Nature, Lond. 181: 934.

Sperber, J.I. (1958b). The incidence of apatite solubilizing organisms in the rhizosphere and soil. Aust. J. agric. Res. 9: 778-781.

Starkey, R.L. (1958). Interrelations between micro-organisms and plant roots in the rhizosphere. Bacteriol. Rev. 22: 154-172.

Steel, R.G.D. and Torrie, J.H. (1960). "Principles and Procedures of Statistics". (McGraw-Hill Book Co. Inc., New York).

Stephens, C.G. (1937). "The Basaltic Soils of Northern Tasmania". C.S.I.R., Bull., 108.

Steward, F.C. and Sutcliffe, J.F. (1959). Plants in relation to inorganic salts. In "Plant Physiology - A Treatise" (Steward, F.C. Ed.), Vol. II. Academic Press, New York.

Stewart, J.W.B. and McConaghy, S. (1963). Some effects of reaction (pH) changes in a basaltic soil on the mineral composition of growing crops. J. Sci. Fd. Agric. 14: 613-621.

Strauss, J. and Campbell, W.A. (1963). Release of enzymes by plant tissue cultures. Life Sci. 2: 50-63.

Sutton, C.D. and Larsen, S. (1964). Pyrophosphate as a source of phosphorus for plants. Soil Sci. 93: 196-201.

Szember, A. (1960). Influence on plant growth of the breakdown of organic phosphorus compounds by micro-organisms. Pl. Soil 13: 147-158.

Tabatabai, M.A. and Bremner, J.M. (1969). Use of p-nitrophenyl phosphate for assay of soil phosphatase activity. Soil Biol. Biochem. 1: 301-307.

Taha, S.M., Mahmoud, S.A.Z., Halim El-Damaty, A. and Abd El-Hafez, A.M. (1969). Activity of phosphate dissolving bacteria in Egyptian soils. Pl. Soil 31: 149-160.

Talibudeen, O. (1957). Isotopically exchangeable phosphorus in soils. II Factors influencing the estimation of "labile" phosphorus. J. Soil Sci. 8: 86-96.

Tandon, H.L.S., Cescas, M.P. and Tyner, E.H. (1968). An acid-free vanadate-molybdate reagent for the determination of total phosphorus in soils. Proc. Soil Sci. Soc. Am. 32: 48-51.

Tardieux-Roche, Andree (1964). Sur la formation de polyphosphates par diverses bacteries due sol. Annls. inst. Pasteur 107: 565-567.

Tardieux-Roche, Andree and Tardieux, P. (1970). La biosynthise des phosphates condensés par la microflora du sol et son rôle dans la nutrition des végétaux. Ann. agron. 21: 305-314.

Taylor, H.M. and Burnett, E. (1964). Influence of soil strength on the root-growth habits of plants. Soil Sci. 98: 174-180.

Taylor, A.W. and Gurney, E.L. (1965). Precipitation of phosphate by iron oxide and aluminium hydroxide from solutions containing calcium and potassium. Proc. Soil Sci. Soc. Am. 29: 18-22.

Taylor, A.W., Gurney, E.L. and Lehr, J.R. (1963). Decay of fertilizer reaction products in an acid soil. Proc. Soil Sci. Soc. Am. 27: 145-148.

Taylor, A.W., Gurney, E.L. and Lindsay, W.L. (1960). An evaluation of some iron and aluminium phosphates as sources of phosphorus for plants. Soil Sci. 90: 25-31.

Terman, G.L. and Allen, S.E. (1969). Fertilizer and soil P uptake by maize as affected by soil P level, granule size and solubility of phosphate sources. J. agric. Sci., Camb. 73: 417-424.

Terman, G.L., Anthony, J.L., Mortensen, W.P. and Lutz, J.A., Jr. (1956). Crop response to N P K fertilizers varying in granule size and water solubility of phosphorus. Proc. Soil Sci. Soc. Am. 20: 551-556.

Terman, G.L., De Ment, J.D., Clements, L.B. and Lutz, J.A., Jr. (1960). Crop response to ammoniated superphosphate and dicalcium phosphate as affected by granule size, water solubility and time of reaction with soil. J. agric. Fd. Chem. 8: 13-18.

Theodorou, C. (1971). The phytase activity of the mycorrhizal fungus Rhizopogon luteolus. Soil Biol. Biochem. 3: 89-90.

Thien, S.J. and McFee, W.W. (1970). Influence of nitrogen on phosphorus absorption and translocation in Zea mays. Proc. Soil Sci. Soc. Am. 34: 87-90.

Thomas, W. (1930). The feeding power of plants. Pl. Physiol., Lancaster. 5: 443-489.

Thompson, E.J. and Black, C.A. (1970). Changes in extractable organic phosphorus in soil in the presence and absence of plants. III Phosphatase effects. Pl. Soil 32: 335-348.

Thompson, L.M. and Black, C.A. (1947). The effect of temperature on the mineralization of soil organic phosphorus. Proc. Soil Sci. Soc. Am. 12: 323-326.

Thompson, L.M., Black, C.A. and Zoellner, J.A. (1954). Occurrence and mineralization of organic phosphorus in soils, with particular reference to associations with nitrogen, carbon and pH. Soil Sci. 77: 185-196.

Tidmore, J.W. (1930). Phosphate studies in solution culture. Soil Sci. 30: 13-29.

Torii, K. and Laties, G.G. (1966). Dual mechanisms of ion uptake in relation to vacuolation of corn roots. Pl. Physiol., Lancaster. 41: 863-870.

Ulrich, J.M., Luse, R.A. and McLaren, A.D. (1964). Growth of tomato plants in presence of proteins and amino acids. Physiologia Pl. 17: 683-696.

Vancura, V. (1964). Root exudates of plants. I Analysis of root exudates of barley and wheat in their initial phases of growth. Pl. Soil 21: 231-248.

Vancura, V. and Hovadik, A. (1965). Root exudates of plants. II Composition of root exudates of some vegetables. Pl. Soil 22: 21-32.

Van Diest, A. (1968). Biological immobilization of fertilizer phosphorus. II Evaluation of factors involved in phosphorus transformation. Pl. Soil 29: 248-256.

Van Diest, A. and Black, C.A. (1959). Soil organic phosphorus and plant growth. II Organic phosphorus mineralized during incubation. Soil Sci. 87: 145-154.

Vose, P.B. (1963). Varietal differences in plant nutrition. Herb. Abstr. 33: 1-13.

Watanabe, F.S. and Olsen, S.R. (1965). Test of an ascorbic acid method for determining phosphorus in water and NaHCO3 extracts from soil. Proc. Soil Sci. Soc. Am. 29: 677-678.

Watanabe, F.S., Olsen, S.R. and Danielson, R.E. (1960). Phosphorus availability as related to soil moisture. Trans. 7th Intern. Congr. Soil Sci., Madison, Wisc. 1960. III: 450-456.

Webb, J.R., Eik, K. and Pesek, J.T. (1961). An evaluation of phosphorus fertilizers applied broadcast on calcareous soils for corn. Proc. Soil Sci. Soc. Am. 25: 232-236.

Webb, J.R., Pesek, J.T. and Eik, K. (1961). An evaluation of phosphorus fertilizers varying in water solubility. III Oat fertilization. Proc. Soil Sci. Soc. Am. 25: 222-226.

Webster, A.B. (1969). Manuring and spacing experiments on vegetables. N.Z. Jl. agric. Res. 12: 381-416.

Welch, R.M. and Epstein, E. (1968). The dual mechanisms of alkali cation absorption by plant cells: their parallel operation across the plasmalemma. Proc. natn. Acad. Sci. 61: 447-453.

Welte, E. and Trolldenier, G. (1965). Effect of soil microflora and seed microflora on growth of plants. In "Plant Microbes Relationships", (Macura, J. and Vancura, V., Eds.), Publ. Czek. Acad. Sci., Prague, pp 186-192.

Went, F.W. (1953). Effects of temperature on plant growth. Ann. Rev. Pl. Physiol. 4: 347-362.

White, R.E. (1969). On the measurement of soil pH. J. Aust. Inst. agric. Sci. 35: 3-14.

White, R.E. (1972). Studies on mineral ion absorption by plants. I. The absorption and utilization of phosphate by Stylosanthes humilis, Phaseolus atropurpureus and Desmodium intortum. Pl. Soil 36: 427-447.

Wiersum, L.K. (1962). Uptake of nitrogen and phosphorus in relation to soil structure and nutrient mobility. Pl. Soil 16: 62-70.

Wilcox, G.E., Martin, G.C. and Langston, R. (1962). Root zone temperature and phosphorus treatment effects on tomato seedling growth in soil and nutrient solutions. Proc. Am. Soc. hort. Sci. 80: 522-529.

Wild, A. and Oke, O.L. (1966). Organic phosphate compounds in calcium chloride extracts of soil: identification and availability to plants. J. Soil Sci. 17: 356-371.

Williams, C.H. and Anderson, G. (1968). Inositol phosphates in some Australian soils. Aust. J. Soil Res. 6: 121-130.

Williams, C.H. and Simpson, J.R. (1965). Some effects of cultivation and waterlogging on the availability of phosphorus in pasture soils. Aust. J. agric. Res. 16: 413-427.

Williams, C.H. and Steinbergs, A. (1958). Sulphur and phosphorus in some Eastern Australian soils. Aust. J. agric. Res. 9: 483-491.

Williams, E.G., Scott, N.M. and McDonald, M.J. (1958). Soil properties and phosphate sorption. J. Sci. Fd. Agric. 9: 551-559.

Williams, R.F. (1948). The effects of phosphorus supply on the rates of intake of phosphorus and nitrogen and upon certain aspects of phosphorus metabolism in gramineous plants. Aust. J. Sci. Res., Series B. 1: 333-361.

Wilson, J.R. and Haydock, K.P. (1971). The comparative response of tropical and temperate grasses to varying levels of nitrogen and phosphorus nutrition. Aust. J. agric. Res. 22: 573-587.

Woodman, R.M. (1944). The nutrition of vegetables in sand. Ann. appl. Biol. 31: 22-30.

Woodruff, J.R. and Kamprath, E.J., (1965). Phosphorus adsorption maximum as measured by the Langmuir isotherm and its relationship to phosphorus availability. Proc. Soil Sci. Soc. Am. 29: 148-150.

Woolhouse, H.W. (1969). Differences in the properties of the acid phosphates of plant roots and their significance in the evolution of edaphic ecotypes. In "Ecological Aspects of the Mineral Nutrition of Plants" (Rorison, I.H., Ed.), Blackwell Scientific Pub. Oxford pp 357-380.

Wright, B.C. and Peech, M. (1960). Characterization of phosphate reaction products in acid soils by the application of solubility criteria. Soil Sci. 90: 32-43.

Wuenscher, M.L. and Gerloff, G.C. (1971). Growth of Andropogon scoparius (Little Bluestem) in phosphorus deficient soils. New Phytol. 70: 1035-1042.

PRIVATE COMMUNICATIONS

Bowen, G.D., C.S.I.R.O., Division of Soils, Glen Osmond, South Australia.

Menary, R.C., Faculty of Agricultural Science, University of Tasmania, Hobart, Tasmania.

APPENDICES

APPENDIX IA

Dry Weight Yield of Shoots (g/plant) of Four Plant Species Grown in Krasnozem Soil at Five Levels of Applied Phosphate.

Data on which Table IV A 3.1(i) is based.

Applied Phosphate (ug P/g soil)	Plant Species	Replicate 1	2	3	Total
0		1.95	2.47	2.27	6.69
100		5.92	6.18	6.08	18.18
200	Cabbage	6.26	6.92	7.34	20.52
400		6.83	7.38	7.57	21.78
800		6.95	8.07	8.26	23.28
0		0.09	0.10	0.10	0.29
100		0.70	0.74	0.77	2.21
200	Lettuce	1.46	1.36	1.73	4.55
400		3.04	3.17	3.57	9.78
800		3.78	3.50	3.88	11.16
0		0.29	0.30	0.49	1.08
100		1.01	1.02	1.08	3.11
200	Ryegrass	1.15	1.42	1.58	4.15
400		1.53	1.87	1.96	5.36
800		1.65	1.73	2.01	5.39
0		0.16	0.14	0.21	0.51
100		0.59	0.60	0.64	1.53
200	Phalaris	0.82	0.86	0.89	2.57
400		1.23	1.14	1.28	3.65
800		1.09	1.06	1.37	3.52

APPENDIX IA cont.

Dry weight yields of roots (g/plant) of four plant species grown in krasnozem soil at five levels of applied phosphate.

Data on which Table IV A 3.1(i) is based.

Applied Phosphate (ug P/g soil)	Plant Species	Replicate 1	2	3	Total
0		0.30	0.35	0.34	0.99
100		0.70	0.67	0.64	2.01
200	Cabbage	0.81	0.87	0.89	2.57
400		0.86	0.78	0.82	2.46
800		0.80	1.08	0.89	2.77
0		0.03	0.02	0.04	0.09
100		0.12	0.13	0.14	0.39
200	Lettuce	0.30	0.26	0.35	0.91
400		0.55	0.57	0.53	1.65
800		0.42	0.38	0.43	1.23
0		0.10	0.12	0.20	0.42
100		0.35	0.30	0.39	1.04
200	Ryegrass	0.34	0.36	0.42	1.12
400		0.36	0.46	0.46	1.28
800		0.34	0.32	0.48	1.14
0		0.09	0.07	0.11	0.27
100		0.26	0.26	0.38	0.90
200	Phalaris	0.27	0.30	0.39	0.96
400		0.38	0.40	0.40	1.18
800		0.25	0.20	0.35	0.80

APPENDIX IB

Relative total dry weight yields (% of maximum) (a), and the arcsine $\sqrt{}$ transformation of these values (b), for four plant species grown in krasnozem soil at five levels of applied phosphate.

Data on which Figure IV A 3.1(i) is based.

Applied Phosphate (ug P/g soil)	Plant Species	Replicate 1		2		3	
		a	b	a	b	a	b
0		29.0	32.6	30.9	33.8	28.1	32.0
100		85.5	67.6	74.9	59.9	73.3	58.9
200	Cabbage	91.2	72.7	85.3	67.5	89.9	71.5
400		99.2	84.9	89.2	70.8	91.7	73.3
800		100	90.0	100	90.0	100	90.0
0		2.7	9.5	3.0	10.0	3.1	10.1
100		19.4	26.1	22.5	28.3	21.0	27.3
200	Lettuce	42.0	40.4	41.5	40.1	48.2	44.0
400		85.2	67.4	96.4	79.1	95.2	77.3
800		100	90.0	100	90.0	100	90.0
0		19.4	26.1	17.8	25.0	27.7	31.8
100		68.3	55.7	57.1	49.1	58.5	49.9
200	Ryegrass	74.9	59.9	76.7	61.1	80.0	63.4
400		95.1	77.2	100	90.0	97.0	80.0
800		100	90.0	88.2	69.9	100	90.0
0		15.4	23.1	13.8	21.8	18.3	25.3
100		54.5	47.6	55.4	48.1	59.4	50.4
200	Phalaris	69.5	56.5	75.3	60.2	74.9	59.9
400		100	90.0	100	90.0	100	90.0
800		85.5	67.6	82.2	65.1	99.2	84.87

APPENDIX IB cont.

Analysis of variance - arcsine $\sqrt{\quad}$ transformed values of relative total dry weight.

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Blocks	2	63.4	31.7	1.3 n.s.
Treatment	19	35174.8	1851.3	80.5***
Species (S)	3	2498.7	832.9	36.2***
Phosphorus (P)	4	29998.7	7499.6	326.0***
S X P	12	2677.4	223.1	9.7***
Error	38	873.8	23.0	
Total	59	36112.0		

APPENDIX IC

Shoot phosphorus content (ug P/g dry tissue) of four plant species grown in krasnozem soil at five levels of applied phosphate

Data on which Table IV A 3.3(i) is based.

Applied Phosphate (ug P/g soil)	Plant Species	Replicate 1	2	3	Total
0		850	946	938	2734
100		1892	1667	1592	5151
200	Cabbage	2471	2450	2615	7536
400		3484	3283	3108	9875
800		4317	4147	3834	12298
0		3067	2900	3220	9187
100		3108	3329	3179	9616
200	Lettuce	3246	3125	3254	9625
400		3667	3459	3896	11022
800		4717	4905	5396	15018
0		3751	3142	3367	10260
100		3267	3021	3313	9601
200	Ryegrass	3680	3375	3225	10280
400		4505	4142	4250	12897
800		5634	5396	5813	16843
0		2771	2908	3329	9008
100		2553	2134	2792	7479
200	Phalaris	2671	2842	2746	8259
400		3667	3651	3417	10735
800		5050	4700	4534	14284

APPENDIX IC cont.

Root phosphorus content (ug P/g dry tissue) of four plant species grown in krasnozem soil at five levels of applied phosphate.

Data on which Table IV A 3.3(i) is based.

Applied Phosphate (ug P/g soil)	Plant Species	Replicate 1	2	3	Total
0		2300	1938	2413	6651
100		2500	2463	2613	7576
200	Cabbage	3075	3050	3338	9463
400		4063	4350	4050	12463
800		5063	4875	5438	15376
0		3391	3378	3421	10190
100		3286	3400	3488	10174
200	Lettuce	3025	2937	2888	8850
400		3188	3025	3288	9501
800		3688	4213	4625	12526
0		2038	2338	2038	6414
100		1988	2037	2138	6163
200	Ryegrass	2063	2188	2338	6589
400		2838	2600	2838	8276
800		4000	3963	4000	11963
0		1675	1800	2008	5483
100		1438	1575	1800	4813
200	Phalaris	1575	1625	1788	4988
400		1700	1763	2125	5588
800		3338	3025	3238	9601

APPENDIX IC cont.

Analysis of variance - shoot phosphorus contents

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Blocks	2	227898	113949	2.8 n.s.
Treatments	19	66497068	3499846	85.9***
Species (S)	3	18055462	6018487	147.6***
Phosphorus (P)	4	43555648	10888912	267***
S X P	12	4885958	407163	<1 n.s.
Error	38	1549158	40767	
Total	59	68274124		

Analysis of variance - root phosphorus contents

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Blocks	2	407375	203688	8.1***
Treatments	19	53685142	2825534	111.7***
Species (S)	3	20693513	6897838	272.7***
Phosphorus (P)	4	26127682	6531921	258.27***
S X P	12	6863947	571996	22.62***
Error	38	961042	25291	
Total	59	55053559		

APPENDIX II

Period of growth (days) and leaf number at harvest for cabbage and lettuce plants grown in krasnozem soil at twenty levels of applied phosphate.

Applied Phosphate (ug P/g soil)	Cabbage		Lettuce	
	Period of Growth (Days)	Mean Leaf No. at Harvest	Period of Growth (Days)	Mean Leaf No. at Harvest
01	63	15	58	15
02	63	15	54	15
03	63	15	53	15
25	62	15	54	15
50	53	15	51	15
75	57	15	51	15
100	53	15	47	15
125	53	13*	45	15
150	49	15	45	15
175	46	15	42	15
200	46	15	40	15
225	46	15	42	15
250	46	15	40	15
275	42	14*	32	15
300	42	15	32	15
350	39	15	32	15*
400	39	15	32	16*
450	39	14*	28	15
500	39	15	28	15
600	39	15	26	13*
800	35	15	26	15
1000	35	15	26	15

*Data from these treatments not used in interpretation of results.

APPENDIX III

Phosphorus concentrations (ug P/g dry tissue) in phosphate fractions of whole shoot tissue of four plant species.

Data on which Table IV C 3.1 is based.

Treatment	Rep.	Ortho-phos-phate	T.C.A.-soluble	Resi-dual	Total	Ortho-phosphate as a % of total
Cabbage	1	250	290	669	959	26
No applied P	2	240	274	637	911	26
	3	236	277	549	826	29
Cabbage	1	500	553	680	1233	41
100 ug P/g soil	2	524	585	810	1395	38
	3	651	713	899	1612	40
Lettuce	1	803	974	947	1921	42
No applied P	2	855	899	1043	1942	44
	3	848	899	1038	1937	44
Lettuce	1	1200	1291	1382	2673	45
100 ug P/g soil	2	1164	1239	1045	2284	51
	3	1431	1518	1172	2690	53
Ryegrass	1	655	704	1510	2214	30
No applied P	2	776	843	1612	2455	32
	3	698	748	1583	2331	30
Phalaris	1	856	888	1440	2328	37
No applied P	2	861	877	1484	2361	36
	3	871	914	1552	2466	35

APPENDIX III cont.

Phosphorus concentrations (ug P/g dry tissue) in phosphate fractions of young and old leaf tissue of cabbage and lettuce.

Data on which Table IV C 3.1 is based.

Treatment	Rep.	Ortho-phos-phate	T.C.A.-soluble	Resi-dual	Total	Ortho-phosphate as a % of total
Cabbage	1	174	174	504	678	26
No applied P	2	193	193	634	827	23
old leaves	3	168	168	406	574	29
Cabbage	1	437	497	936	1433	30
No applied P	2	417	483	969	1452	29
Young leaves	3	361	405	1012	1417	25
Lettuce	1	791	872	324	1196	66
No applied P	2	687	752	435	1187	58
Old leaves	3	662	722	407	1129	59
Lettuce	1	1081	1128	1758	2886	37
No applied P	2	1368	1463	1648	3111	44
Young leaves	3	994	1046	1300	2346	42
Cabbage	1	224	260	515	775	29
100 ug P/g soil	2	213	249	447	696	31
Old leaves	3	230	276	443	719	32
Cabbage	1	791	847	1370	2217	36
100 ug P/g soil	2	905	978	1271	2249	40
Young leaves	3	886	955	1095	2050	43
Lettuce	1	650	702	685	1387	49
100 ug P/g soil	2	626	685	565	1250	50
Old leaves	3	800	935	568	1503	53
Lettuce	1	2298	2572	1306	3878	59
100 ug P/g soil	2	1708	1913	1723	3636	47
Young leaves	3	1581	1727	1017	2744	58

APPENDIX III cont.

Analysis of variance - orthophosphate content of whole shoots of lettuce and cabbage grown at two levels of applied phosphate expressed as a percentage of total phosphate content.

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Replication	2	18	9	1.8 n.s.
Treatments	3	821	270	54***
Species (S)	1	520	520	104***
Phosphorus (P)	1	271	271	54***
S X P	1	30	30	6 n.s.
Error	6	30	5	
Total	11	869		

Analysis of variance - orthophosphate content of young and old leaf tissue of lettuce and cabbage expressed as a percentage of the total phosphate content.

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Replication	2	22.6	11.3	<1 n.s.
Treatments	7	3543.3	506.2	35.4***
Species (S)	1	145.1	145.1	10.1**
Phosphorus(P)	1	2583.4	2583.4	180.7***
Tissue (T)	1	9.4	9.4	<1 n.s.
S X P	1	63.3	63.3	4.4 n.s.
P X T	1	360.3	360.3	25.2**
T X S	1	273.4	273.4	19.1**
S X P X T	1	331.1	331.1	23.2**
Error	14	200.1	14.3	
Total	23	3766.0		

APPENDIX IV A 1

Hydrolysis of p-nitrophenol phosphate by non-sterile 2 cm root tips.

Data on which Table V A 3.1 is based.

Species	Rep.	Total nitrophenol hydrolysed (ugs/hr/root sample) 1*	2*	Root surface area (cm^2) 1	2	Rate of hydrolysis (ugs nitrophenol/cm^2 root/hr.) 1	2
Cabbage	1	25.3	20.3	10.9	8.2	23.3	24.7
	2	20.0	17.9	10.4	7.9	19.3	22.8
	3	22.3	15.0	10.8	7.8	20.6	19.2
	Total	67.6	53.2	32.1	23.9	63.2	66.7
Lettuce	1	18.7	13.9	17.1	12.1	10.9	11.5
	2	21.1	11.7	19.5	9.4	10.8	12.5
	3	23.5	13.6	19.7	12.3	11.9	11.1
	Total	63.3	39.2	56.3	33.8	33.6	35.1
Ryegrass	1	25.3	15.3	16.7	7.7	15.2	19.9
	2	23.1	18.7	16.5	9.0	14.0	20.8
	3	22.1	14.5	16.5	8.2	13.4	17.7
	Total	70.5	48.5	49.7	24.9	42.6	58.4
Phalaris	1	40.1	28.8	24.6	12.5	16.3	23.0
	2	44.0	33.4	19.8	12.8	22.3	26.1
	3	44.0	37.7	21.4	13.7	20.5	27.5
	Total	128.1	99.9	65.8	39.0	59.1	76.6

*Harvests 1 and 2

Analysis of variance of rates of hydrolysis (above)

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Treatments	7	568.0	81.1	20.8***
Species (S)	3	472.9	157.6	40.4***
Harvests (H)	1	61.1	61.1	15.7***
S X H	3	34.0	11.3	2.9 n.s.
Error	16	62.0	3.9	
Total	23	630.0		

APPENDIX IV A 2

Hydrolysis of p-nitrophenol phosphate by sterile and non-sterile 2 cm root tips

Data on which Table V A 3.2 is based.

Species	Rep.	Total nitrophenol hydrolysed (ugs/hr/root sample)		Root surface area (cm^2)		Rate of hydrolysis (ugs nitrophenol/cm^2 root/hr)	
		Sterile	Non-sterile	Sterile	Non-sterile	Sterile	Non-sterile
Cabbage	1	6.8	6.8	3.1	3.3	22.0	20.4
	2	5.6	7.7	3.6	3.3	15.9	23.4
	3	6.7	6.5	3.5	3.4	19.0	18.8
	Total	19.1	21.0	10.2	10.0	56.9	62.6
Lettuce	1	2.7	3.1	2.8	3.2	9.5	9.7
	2	2.4	4.2	3.1	3.1	7.7	13.7
	3	3.2	3.5	3.0	3.0	10.9	11.6
	Total	8.3	10.8	8.9	9.3	28.1	35.0
Ryegrass	1	1.1	3.0	3.2	3.0	3.5	10.0
	2	0.9	2.4	3.0	3.2	2.9	7.5
	3	0.9	3.9	2.6	3.4	3.4	11.4
	Total	2.9	9.3	8.8	9.6	9.8	28.9
Phalaris	1	5.2	8.1	3.4	3.6	15.0	22.7
	2	3.0	4.6	3.3	3.5	9.0	13.3
	3	4.4	7.1	3.4	3.7	12.9	19.3
	Total	12.6	19.8	10.1	10.8	36.9	55.3

Analysis of variance of rates of hydrolysis above.

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Replications	2	25.0	12.5	2.0 n.s.
Treatments	7	745.5	106.5	16.6***
Species (S)	3	615.0	205.0	32.0***
Micro-organisms (M)	1	104.6	104.6	16.3***
S X M	3	25.9	8.6	1.3 n.s.
Error	14	89.6	6.4	
Total	23	860.1		

APPENDIX IV B 1

Plant dry weight yields (mg) and phosphorus contents (ug) of cabbage (C), lettuce (L), ryegrass (R) and phalaris (P) grown in sterile sand culture with three phosphate sources.

Data on which Table V B 3.1 is based.

Rep.	Control (No P)				Sodium inositol hexaphosphate				Disodium orthophosphate			
	C	L	R	P	C	L	R	P	C	L	R	P
					Dry weight yields (mg/tube)							
1	34	37	21	23	26	24	17	17	35	21	24	19
2	30	24	13	13	36	27	24	17	25	18	16	22
3	32	37	15	13	31	24	22	22	31	28	17	36
4	37	33	16	12	21	30	14	32	-	-	-	-
5	34	33	11	23	30	26	16	18	-	-	-	-
6	31	36	16	25	39	22	16	21	-	-	-	-
Total	198	200	92	109	183	153	109	128	91	67	57	77
					Phosphorus contents (ugs/tube)							
1	77	30	8	15	82	30	10	14	138	70	123	58
2	73	21	7	11	77	28	16	11	118	115	100	88
3	75	29	9	8	79	26	23	10	122	93	112	103
4	89	26	7	11	65	32	15	15	-	-	-	-
5	91	32	5	11	83	28	10	11	-	-	-	-
6	70	21	8	15	90	24	11	13	-	-	-	-
Total	475	159	44	71	476	168	85	74	378	278	335	249

APPENDIX IV B 2

Plant dry weight at harvest (mg/2 plants)

Data on which Table V B 3.2 is based

Species	Rep.	Calcium inositol hexaphosphate		Control (No P)	
		Sterile	Non-sterile	Sterile	Non-sterile
Cabbage	1	85	78	44	68
	2	58	62	59	66
	3	59	47	55	42
	4	66	65	55	48
	Total	268	252	213	224
Lettuce	1	29	28	30	21
	2	27	30	27	27
	3	30	24	27	22
	4	23	33	28	28
	Total	109	115	112	98
Ryegrass	1	35	32	15	21
	2	39	29	25	14
	3	28	27	19	11
	4	38	21	19	9
	Total	140	109	78	55
Phalaris	1	25	29	25	13
	2	28	18	23	15
	3	31	26	22	21
	4	36	19	25	15
	Total	120	92	95	64

APPENDIX IV B 2 cont.

Plant phosphorus content (ugs P/2 plants) and inorganic orthophosphate contents (ugs P/tube) of unplanted tubes at harvest.

Data on which Table V B 3.2 is based.

Species	Rep.	Calcium inositol hexaphosphate		Control (No P)	
		Sterile	Non-sterile	Sterile	Non-sterile
Cabbage	1	177	150	75	38
	2	155	163	48	40
	3	167	121	57	43
	4	168	182	47	45
	Total	667	616	227	166
Lettuce	1	83	75	25	20
	2	73	92	23	22
	3	83	57	27	18
	4	62	75	25	22
	Total	301	299	100	82
Ryegrass	1	43	31	7	10
	2	45	41	17	7
	3	41	44	11	5
	4	49	39	10	6
	Total	178	155	40	28
Phalaris	1	31	26	16	9
	2	37	29	10	10
	3	33	33	14	13
	4	36	30	13	12
	Total	137	118	53	44
No plants	1	12	8	<1	1
	2	14	8	<1	2
	3	13	12	<1	3
	4	14	9	<1	2
	Total	53	37	<4	8

APPENDIX IV B 2 cont.

Analysis of variance - Plant phosphorus contents

Source of variation	D.F.	Sum of squares	Mean Square	Variance ratio
Replications	3	113	38	<1 n.s.
Treatments	15	137820	9188	109.4***
Phosphorus (P)	1	46818	46818	557.3***
Species (S)	3	70473	23491	279.6***
Microbiol (M)	1	594	594	7.1*
P X S	3	19558	6519	77.6***
P X M	1	1	1	<1 n.s.
M X S	3	341	114	1.4 n.s.
P X S X M	3	35	12	<1 n.s.
Error	45	3800	84	
Total	63	141733		

APPENDIX IV C 1

Shoot dry weight yields and tissue phosphate concentrations of four species grown on nine phosphate sources.

Data on which Tables V C 3.2(b)(i) and (ii) are based.

Phosphate Source		Species	Shoot Dry Wt. (g/pot)			Shoot P Content (ppm D.W.)		
			Rep. 1	Rep. 2	Total	Rep. 1	Rep. 2	Total
Control (no P)	(1)	Cabbage	0.122	0.138	0.260	424	399	823
Strengite	(2)		0.270	0.319	0.589	546	580	1126
Pot. iron phosphate	(3)		3.881	3.767	7.648	1722	1796	3518
Pot. taranakite	(4)		4.913	5.007	9.920	2482	2435	4917
Col. ferric phosphate	(5)		6.126	6.701	12.827	2632	2491	5123
Col. aluminium phosphate	(6)		5.042	4.699	9.741	4463	4790	9253
Calcium phytate	(7)		8.570	8.049	16.619	3614	3601	7215
D.C.P.D.	(8)		8.211	8.907	17.118	3966	3501	7467
M.C.P.	(9)		5.800	5.128	10.928	10118	9037	19155
	(1)	Lettuce	0.021	0.020	0.041	870	874	1644
	(2)		0.160	0.144	0.304	738	698	1436
	(3)		1.453	1.279	2.732	2931	3068	5999
	(4)		4.866	4.761	9.627	2182	2595	4777
	(5)		3.952	3.057	7.009	3166	3491	6657
	(6)		5.525	3.812	9.337	3761	4212	7973
	(7)		8.827	8.870	17.697	2612	3035	5647
	(8)		7.558	8.651	16.209	4250	4187	8437
	(9)		5.700	5.349	11.049	9564	9490	19054
	(1)	Ryegrass	0.014	0.014	0.028	565	592	1157
	(2)		0.253	0.198	0.451	1314	1107	2421
	(3)		2.024	2.060	4.084	1082	1104	2186
	(4)		3.672	3.894	7.566	3205	2869	6074
	(5)		4.342	3.848	8.190	3273	3425	6698
	(6)		4.498	3.913	8.411	4519	5053	9572
	(7)		3.708	3.803	7.511	3321	2906	6227
	(8)		3.911	3.706	7.617	5810	5904	11714
	(9)		2.867	2.955	5.822	5979	5955	11934
	(1)	Phalaris	0.013	0.019	0.032	683	539	1222
	(2)		0.137	0.128	0.265	1172	1021	2193
	(3)		1.031	1.143	2.174	1119	1232	2351
	(4)		1.844	1.828	3.672	3425	3182	6607
	(5)		2.829	3.120	5.949	3491	3981	7472
	(6)		2.862	2.681	5.543	5022	5712	10734
	(7)		2.437	2.016	4.453	2999	3199	6198
	(8)		1.936	2.174	4.110	6807	6731	13538
	(9)		1.415	1.298	2.713	6818	7225	14043

APPENDIX IV C 2

Shoot phosphate content as a % of the maximum (excluding M.C.P. treatment) (a) and arcsine $\sqrt{\ }$ transformations of these values (b) for four species grown on eight different phosphate sources.

Data on which Figure V C 3.2(b) is based.

Species	Rep.	Phosphate Sources* 1	2	3	4	5	6	7	8
Cabbage	1 (a)	0.16	0.57	20.5	37.4	49.5	69.1	95.1	100
	(b)	2.29	4.33	26.92	37.70	44.71	56.23	77.21	90.00
	2 (a)	0.18	0.47	21.7	39.1	53.5	72.2	92.9	100
	(b)	2.43	3.93	27.76	38.70	47.01	58.18	74.55	90.00
Lettuce	1 (a)	0.06	0.37	13.3	33.1	39.0	50.0	71.8	100
	(b)	1.40	3.49	21.39	35.12	38.65	45.00	57.92	90.00
	2 (a)	0.05	0.28	10.8	34.1	29.5	57.4	74.3	100
	(b)	1.28	3.03	19.19	35.73	32.90	49.26	59.54	90.00
Ryegrass	1 (a)	0.03	1.46	9.6	51.8	62.5	89.5	54.2	100
	(b)	0.99	7.04	18.05	46.03	52.24	71.09	47.41	90.00
	2 (a)	0.04	1.00	10.4	51.1	60.2	90.4	50.5	100
	(b)	1.15	5.74	18.81	45.63	50.89	71.95	45.29	90.00
Phalaris	1 (a)	0.06	1.12	8.0	43.9	68.7	100	50.9	91.7
	(b)	1.40	6.02	16.43	41.50	55.98	90.00	45.52	73.26
	2 (a)	0.07	0.86	9.2	38.0	81.1	100	42.1	95.6
	(b)	1.52	5.32	17.66	38.06	64.23	90.00	40.46	77.89

*See appendix IV C 1 for code to numbering system.

Analysis of variance - arcsine $\sqrt{\ }$ transformed values

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Replication	1	<1	<1	<1 n.s.
Treatment	31	54661.8	1763.3	507.1***
Phosphorus (P)	7	50060.1	7151.4	2056.8***
Species (S)	3	360.2	120.1	34.5***
P X S	21	4241.5	202.0	58.1***
Error	31	107.8	3.477	
Total	63	54769.7		

APPENDIX V A 1

Shoot dry weights (g/plant) - continuous-flow nutrient culture, Experiment 1.

Data on which Table VI A 3.1b is based.

Species	Phosphate Concentration (uM)	Replicate 1	2	3	Total
Cabbage	0.1	1.443	1.411	1.239	4.093
	0.3	2.379	2.348	2.301	7.028
	0.9	3.849	3.670	4.087	11.606
	2.7	1.860	2.362	2.572	6.794
Lettuce	0.1	0.038	0.030	0.036	0.104
	0.3	0.184	0.229	0.195	0.608
	0.9	0.899	0.990	1.140	3.029
	2.7	0.670	0.828	0.662	2.160
Ryegrass	0.1	0.122	0.150	0.137	0.409
	0.3	0.251	0.250	0.284	0.785
	0.9	0.227	0.296	0.248	0.771
	2.7	0.296	0.254	0.248	0.798
Phalaris	0.1	0.073	0.073	0.084	0.230
	0.3	0.119	0.110	0.123	0.352
	0.9	0.135	0.117	0.123	0.376
	2.7	0.107	0.107	0.096	0.310

APPENDIX V A 1 cont.

Root dry weights (g/plant) continuous-flow nutrient culture, experiment 1.

Data on which Table VI A 3.1b is based.

Species	Phosphate Concentration (uM)	Replicate 1	2	3	Total
Cabbage	0.1	0.322	0.321	0.275	0.918
	0.3	0.459	0.476	0.447	1.382
	0.9	0.437	0.385	0.481	1.303
	2.7	0.224	0.294	0.322	0.840
Lettuce	0.1	0.021	0.019	0.022	0.062
	0.3	0.091	0.100	0.092	0.283
	0.9	0.177	0.211	0.223	0.611
	2.7	0.172	0.215	0.171	0.558
Ryegrass	0.1	0.073	0.093	0.087	0.253
	0.3	0.132	0.131	0.152	0.415
	0.9	0.110	0.150	0.116	0.376
	2.7	0.153	0.133	0.129	0.415
Phalaris	0.1	0.046	0.047	0.049	0.142
	0.3	0.055	0.062	0.066	0.183
	0.9	0.068	0.055	0.061	0.184
	2.7	0.056	0.058	0.048	0.162

APPENDIX V A 1 cont.

Total plant dry weights as a percentage of maximum yields (arcsine $\sqrt{\ }$ transformed values) - continuous-flow nutrient culture, experiment 1.

Species	Phosphate Concentration (uM)	Replicate 1	2	3	Total T
Cabbage	0.1	39.9	40.8	35.0	115.7
	0.3	54.5	56.5	50.9	161.9
	0.9	90.0	90.0	90.0	270.0
	2.7	44.2	54.0	52.8	151.0
Lettuce	0.1	13.6	11.7	12.0	37.3
	0.3	30.4	31.6	27.3	89.3
	0.9	90.0	90.0	90.0	270.0
	2.7	62.2	68.7	51.4	182.3
Ryegrass	0.1	41.2	47.6	45.8	134.6
	0.3	67.5	67.5	90.0	225.0
	0.9	60.1	90.0	66.0	216.1
	2.7	90.0	68.7	68.4	227.1
Phalaris	0.1	50.0	56.7	57.0	163.7
	0.3	67.8	90.0	90.0	247.8
	0.9	90.0	90.0	81.7	261.7
	2.7	63.7	78.3	60.8	202.8

Analysis of variance - dry weight as % of max. (arcsine $\sqrt{\ }$ transformed values)

Source of Variation	D.F.	Sum of squares	Mean square	Variance ratio
Cabbage-Lettuce				
Phosphorus (P)	3	13533		
Species (S)	1	597	597	37.1***
P X S	3	1469	489.7	30.4***
Error	16	258	16.1	
Total	23	15857		
Ryegrass-Phalaris				
Phosphorus (P)	3	3506		
Species (S)	1	223	223	2.04 n.s.
P X S	3	450	150	1.37 n.s.
Error	16	1749	109.3	
Total	23	5928		

APPENDIX V A 2

Shoot phosphorus content (ug P/g dry tissue) - continuous-flow nutrient culture, experiment 1.

Data on which Table VI A 3.1b is based.

Species	Phosphate Concentration (uM)	Replicate 1	2	3	Total
Cabbage	0.1	1992	1912	1963	5867
	0.3	2270	2321	2457	7048
	0.9	4072	3988	4035	12095
	2.7	4880	4961	4952	14793
Lettuce	0.1	1489	1444	1423	4356
	0.3	2608	2625	2619	7852
	0.9	4821	4816	4841	14478
	2.7	5373	5195	5283	15851
Ryegrass	0.1	3658	3554	3533	10745
	0.3	3796	3840	3886	11522
	0.9	4990	5329	5163	15482
	2.7	6913	6862	7098	20873
Phalaris	0.1	3474	3538	3492	10504
	0.3	3662	3377	3499	10538
	0.9	4642	4846	4928	14416
	2.7	7512	8629	8268	24409

APPENDIX V A 2 cont.

Root phosphorus content (ug P/g dry tissue) - continuous-flow nutrient culture, experiment 1.

Data on which Table VI A 3.1b is based.

Species	Phosphate Concentration (uM)	Replicate 1	2	3	Total
Cabbage	0.1	3105	2992	3022	9119
	0.3	3342	3148	3328	9818
	0.9	4536	4333	4484	13353
	2.7	5539	5596	5544	16679
Lettuce	0.1	2132	1745	2098	5975
	0.3	3464	3298	3353	10115
	0.9	5183	4978	4934	15095
	2.7	5252	5418	5335	16005
Ryegrass	0.1	3407	3297	3188	9892
	0.3	3469	3952	3616	11037
	0.9	4667	4833	4578	14078
	2.7	6396	6188	6318	18902
Phalaris	0.1	2647	2772	2855	8274
	0.3	3221	3212	2814	9247
	0.9	4184	4348	4188	12720
	2.7	6380	7204	7109	20693

APPENDIX V A 2 cont.

Analyses of variance - shoot phosphate content

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Cabbage-Lettuce				
Phosphorus (P)	3	46121649		
Species (S)	1	311448	311448	103.7***
P X S	3	1309817	436606	145.3***
Error	16	48071	3004.4	
Total	23	47790985		
Ryegrass-Phalaris				
Phosphorus (P)	3	62100458		
Species (S)	1	64584	64584	1.2 n.s.
P X S	3	2379748	793249	15.2***
Error	16	837619	52351	
Total	23	65382409		

Analyses of variance - root phosphate content

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Cabbage-Lettuce				
Phosphorus (P)	3	31841261		
Species (S)	1	131869	131869	10.1 **
P X S	3	2111762	703921	53.7***
Error	16	209738	13109	
Total	23	34294630		
Ryegrass-Phalaris				
Phosphorus (P)	3	46554563		
Species (S)	1	368776	368776	7.8*
P X S	3	1443536	481179	10.2***
Error	16	756117	47257	
Total	23	49122992		

APPENDIX V B.1

Shoot dry weights (g/plant) - continuous-flow nutrient, experiment 2.

Data on which Figure VI A 3.2(b) is based.

Species	Phosphate Concentration (uM)	Replicate 1	2	3	Total
Cabbage	0.06	0.1235	0.1264	0.1085	0.3584
	0.12	0.3912	0.3992	0.3585	1.1489
	0.24	0.6272	0.7186	0.6033	1.9491
	0.48	1.1961	1.0238	1.1009	3.3209
	0.96	1.1141	1.0429	0.7709	2.9278
	3.84	1.0585	0.9688	1.0923	3.1196
	7.68	1.1519	0.9112	1.1338	3.1969
	30.72	1.1360	0.9092	1.2123	3.2575
Lettuce	0.06	0.0541	0.0577	0.0734	0.1852
	0.12	0.0918	0.1116	0.0763	0.2797
	0.24	0.1358	0.1106	0.1227	0.3691
	0.48	0.2412	0.2335	0.2372	0.7119
	0.96	0.3400	0.3351	0.4057	1.0808
	3.84	0.5902	0.6284	0.5200	1.7386
	7.68	0.4630	0.4035	0.5654	1.4319
	30.72	0.3748	0.3981	0.3083	1.0812

APPENDIX V B.1 cont.

Root dry weights (g/plant) - continuous-flow nutrient culture, experiment 2.

Data on which Figure VI A 3.2(b) is based.

Species	Phosphate Concentration (uM)	Replicate 1	2	3	Total
Cabbage	0.06	0.0303	0.316	0.0278	0.0897
	0.12	0.1016	0.1022	0.0926	0.2964
	0.24	0.1214	0.1350	0.1113	0.3677
	0.48	0.1936	0.1687	0.1769	0.5392
	0.96	0.1675	0.1728	0.1248	0.4651
	3.84	0.1525	0.1396	0.1531	0.4452
	7.68	0.1709	0.1339	0.1735	0.4782
	30.72	0.1472	0.1338	0.1687	0.4496
Lettuce	0.06	0.0214	0.0172	0.0255	0.0641
	0.12	0.0285	0.0382	0.0241	0.0909
	0.24	0.0530	0.0432	0.0519	0.1481
	0.48	0.0998	0.1009	0.100	0.3006
	0.96	0.1116	0.1184	0.1250	0.3550
	3.84	0.1416	0.1344	0.1171	0.3931
	7.68	0.1244	0.1080	0.1478	0.3803
	3072	0.1014	0.1050	0.1118	0.3182

APPENDIX V B.1 cont.

Total plant dry weights as a percentage of maximum yields (arcsine $\sqrt{\ }$ transformed values) - continuous-flow nutrient culture, experiment 2.

Species	Phosphate Concentration (uM)	Replicate 1	2	3	Total
Cabbage	0.06	19.5	21.1	18.3	58.9
	0.12	36.6	39.9	34.9	111.4
	0.24	47.2	56.9	46.0	150.1
	0.48	90.0	82.1	74.1	246.2
	0.96	73.8	90.0	53.7	217.5
	3.84	69.0	72.7	71.8	213.5
	7.68	77.3	68.0	76.7	222.0
	30.72	73.9	67.9	90.0	231.8
Lettuce	0.06	18.7	18.2	21.9	58.8
	0.12	23.9	26.3	22.1	72.3
	0.24	30.5	26.7	29.7	86.9
	0.48	43.0	41.4	43.5	127.9
	0.96	51.8	50.5	59.6	161.9
	3.84	90.0	90.0	70.9	250.9
	7.68	63.7	55.0	90.0	208.7
	30.72	53.8	54.3	50.1	158.2

Analysis of variance - dry weight as % of max. (arcsine $\sqrt{\ }$ transformed values).

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Phosphorus (P)	7	18469		
Species (S)	1	2211	2211	32.3***
P X S	7	2723	389.0	5.7***
Error	32	2192	68.5	
Total	47	25595		

APPENDIX V B.2

Shoot phosphate content (ug P/g dry tissue) - continuous-flow nutrient culture, experiment 2.

Data on which Table VI A 3.2(c) is based.

Species	Phosphate Concentration (uM)	Replicate 1	2	3	Total
Cabbage	0.06	1239	1076	1276	3591
	0.12	2285	2495	2343	7123
	0.24	3317	3430	3491	10238
	0.48	3668	3741	3813	11222
	0.96	4760	4874	4357	13991
	3.84	4945	5134	5294	15373
	7.68	5310	5066	5291	15667
	30.72	5073	5210	5171	15454
Lettuce	0.06	656	604	657	1917
	0.12	652	658	631	1941
	0.24	1216	1292	1190	3698
	0.48	3358	3487	3127	9972
	0.96	4875	4765	4973	14613
	3.84	7240	7537	7342	22119
	7.68	6138	6321	7751	20210
	30.72	5526	5588	5912	5912

Analysis of variance

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Phosphorus (P)	7	180451217		
Species (S)	1	28179	28179	<1 n.s.
P X S	7	23804309	3400616	51.4***
Error	32	2115267	66102	
Total	47	206398972		

APPENDIX V B 2 cont.

Root phosphate content (ug P/g dry tissue) - continuous-flow nutrient culture, experiment 2.

Data on which Table VI A 3.2(c) is based.

Species	Phosphate Concentration (uM)	Replicate 1	2	3	Total
Cabbage	0.06	2279	2797	2358	7434
	0.12	3444	3592	3457	10493
	0.24	4173	4300	4348	12821
	0.48	4429	4519	4504	13452
	0.96	4981	5002	4840	14823
	3.84	6228	6403	6377	19008
	7.68	6885	6667	6761	20313
	30.72	6257	6720	5880	18857
Lettuce	0.06	1327	1303	1319	3949
	0.12	1653	1658	1705	5016
	0.24	2444	2395	2483	7322
	0.48	4000	4108	3809	11917
	0.96	4704	4743	4738	14185
	3.84	6692	7060	6905	20657
	7.68	6926	7056	7027	21009
	30.72	6947	6813	7019	20779

Analysis of variance

Source of variation	D.F.	Sum of squares	Mean square	Variance ratio
Phosphorus (P)	7	158380366		
Species (S)	1	3186306	3186306	135.3***
P X S	7	10487481	1498212	63.6***
Error	32	753526	23548	
Total	47	172807679		

APPENDIX VI A 1

Rate of phosphate absorption by non-sterile excised roots from solutions of various phosphate concentration.

Data on which Figures VI B 3.1, 2 and 3 are based.

Phosphate Concentration (uM)	Rate of Phosphate Absorption (nM/g fresh root/hr)			
	Cabbage*	Lettuce*	Ryegrass+	Phalaris+
0.1	22	5	19	12
0.5	98	20	75	52
1	172	31	154	102
5	457	68	318	316
10	601	76	391	339
25	899	110	515	406
50	951	133	467	469
100	1109	164	597	520
500	1444	241	726	626
1000	1618	301	744	651

	Cabbage+	Lettuce+
0.1	11	5
0.5	42	21
1	70	44
5	151	63
10	153	88
25	197	118
50	222	102
100	289	185
500	403	225
1000	548	252

* Complete excised roots (with laterals)
\+ Excised roots free of laterals

APPENDIX VI A 2

Phosphate absorbed by non-sterile roots of lettuce, cabbage, ryegrass and phalaris for time periods up to twenty minutes.

Data on which Figures VI B 3.4 and 3.5

Phosphate Concentration (uM)	Species	Phosphate Absorbed (nM/g fresh root) in Time (minutes)				
		4	8	12	16	20
500	Cabbage	43	77	135	162	214
500	Lettuce	16	35	49	64	92
0.5	Cabbage	23	48	74	97	110
0.5	Lettuce	19	45	61	84	90

		^{32}P Absorbed (uncorrected c.p.m./0.15 g fresh root) in Time (minutes).		
		3	7	20
100	Ryegrass	1514	2542	3841
100	Phalaris	840	1490	2535
1	Ryegrass	8295	12618	16678
1	Phalaris	4793	8114	12760

APPENDIX VI B

Determination of Vmax and Km values by a mathematical solution based on simultaneous equations.

Now $v = -Km\frac{v}{s} + Vmax$ (Michaelis and Menten, 1913)

i.e. $Vmax = v\left[1 + \frac{Km}{s}\right]$ or $v = \frac{Vmax}{1+\frac{Km}{s}}$

For the two reactions "a" and "b" we get

$$va + vb = \frac{Vmaxa}{1+\frac{Kma}{s}} + \frac{Vmaxb}{1+\frac{Kmb}{s}}$$

$$\text{i.e. } va + vb = \frac{Vmaxa + Vmaxb + \frac{1}{s}(VmaxaKmb + VmaxbKma)}{1 + \frac{Kma}{s} + \frac{Kmb}{s} + \frac{KmaKmb}{s^2}}$$

Putting $vt = va + vb$ we get

$$vt + \frac{vt}{s}(Kma + Kmb) + \frac{vt}{s^2}(KmaKmb) = Vmaxa + Vmaxb + \frac{1}{s}(VmaxaKmb + VmaxbKma)$$

$$\text{i.e. } \frac{vt}{s^2}(KmaKmb) + \frac{vt}{s}(Kma + Kmb) - \frac{1}{s}(VmaxaKmb + VmaxbKma) = Vmaxa + Vmaxb - vt$$

Let $KmaKmb = x$, $Kma + Kmb = y$ and $VmaxaKmb + VmaxbKma = z$

Then $\frac{vt}{s^2}(x) + \frac{vt}{s}(y) - \frac{1}{s}(z) = Vmaxa + Vmaxb - vt$

From the experimental data the values of vt at a number of solution concentrations (s) are known. From a plot of the experimental data (v vs $\frac{v}{s}$) the value of Vmaxa + Vmaxb can also be obtained. By substituting these values into the final equation above, a number of simultaneous equations are obtained which can be solved for x, y and z.

Since KmaKmb = x and Kma + Kmb = y

$$Kma = \frac{x}{Kmb} \qquad \text{i.e.} \qquad \frac{x}{Kmb} + Kmb = y$$

$$\text{i.e. } x + Kmb^2 = Kmb\ y \qquad \text{or } Kmb^2 - Kmby + x = 0.$$

Substitution of x and y in this equation enables the value of Kmb to be obtained. Further substitution enables the determination of Kma and Vmaxa and Vmaxb.

APPENDIX VI C

Rate of phosphate absorption by sterile and non-sterile excised cabbage and lettuce roots from solutions of various phosphate concentration.

Data on which Figures VI B 3.9 - 3.14 are based.

Phosphate Concentration (uM)	Rate of Phosphate Absorption (nM/g fresh root/hr)			
	Sterile		Non-sterile	
	Cabbage	Lettuce	Cabbage	Lettuce
1	16.8	27.5	60.5	38.0
2.5	19.3	43.4	46.2	55.8
5	18.0	61.6	74.2	79.8
10	32.9	62.6	67.3	80.3
25	30.4	84.8	113.2	107.5
50	32.0	101.1	202.8	129.8
100	33.6	168.9	197.5	119.2
250	37.7	197.4	187.5	158.8
500	83.6	162.2	377.2	204.8
1000	102.2	242.1	309.2	231.7

APPENDIX VI D

Solution Phosphate Concentrations in Krasnozem Soil

(Data on which the phosphate concentrations, used in the continuous-flow solution cultures, are based.)

Krasnozem soil, equilibrated at field capacity for 4 weeks at 5 levels of added P as $Ca(H_2PO_4)_2.H_2O$ (0, 100, 200, 400 and 800 ug P/g soil), was used to obtain phosphate sorption isotherms (Fox and Kamprath, 1970) and intensity values for phosphate in the soil solution (Omanwar and Robertson, 1970).

Using the method of Fox and Kamprath (1970) the equilibrium supernatant phosphate concentration obtained after sorption of 100 ug P/g soil was found to be 1uM for the unfertilized soil but approximately 6uM for the soil to which 800 ugP/g soil was applied. To obtain 6uMP in the equilibrium supernatant of the unfertilized soil required the sorption of 800 ugP/g soil while other phosphate treatments required intermediate amounts. Although the results of this experiment do not indicate the actual phosphate concentration in the soil solution they indicate that the soil has a high capacity to sorb applied phosphate thus reducing the concentration of phosphate in the supernatant to low levels.

The phosphate concentration in the supernatant of the five phosphate treated soil samples were also measured at each of five soil: solution ratios (1:1, 1:1.25, 1:2.5, 1:5.0 and 1:10.0) using the method of Omanwar and Robertson (1970). By plotting the phosphate concentration in the supernatant against the extraction ratio and by extrapolation to the appropriate soil:solution ratio the phosphate concentration in the soil solution at field capacity was estimated. Unlike the soils used

by Dmanwar and Robertson (1970) which gave straight-line relationships the krasnozem soil gave a curvilinear plot of extraction ratio against phosphate concentration in the supernatant. This made extrapolation difficult but the results obtained indicated that the phosphate concentration in the soil solution was $<$3uM for the unfertilized soil, approximately 8uM for the 800 ugP/g soil treatment and intermediate values for the remaining treatments.