THE UNIVERSITY OF MANITOBA

UPTAKE, TRANSLOCATION, AND LOCALIZATION OF PHOSPHATE IN RAPE

by

ALLAN JAMES MURPHY

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PLANT SCIENCE

WINNIPEG, MANITOBA

October 1975

"UPTAKE, TRANSLOCATION, AND LOCALIZATION

OF PHOSPHATE IN RAPE"

by

ALLAN JAMES MURPHY

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

To Mina

TABLE OF CONTENTS

		PAGE
LIST OF TABLES AND FIGURES		iii
ABSTRACT		v
CHAPTER I.	INTRODUCTION	1
CHAPTER II.	LITERATURE REVIEW	3
CHAPTER III.	METHODS AND MATERIALS	21
	Toxicity Effects	21
	Growth Studies	22
	Analytical Procedures	23
	Kinetic Studies	24
	Growth Studies at Low Phosphorus Concentrations	25
CHAPTER IV.	RESULTS	26
	Emergence	26
	Growth Experiments	33
	Analytical Results	37
	Kinetic Studies	37
	Yields at Low Phosphorus Concentrations	48
CHAPTER V.	DISCUSSION	52
	Toxicity Effects	58
	Growth	61
	Analytical Experiments	63
ACKNOWLEDGEMENTS		69
BIBLIOGRAPHY		70
APPENDIX		77

LIST OF TABLES AND FIGURES

		PAGE
FIGURE 1	Per cent emergence of Arlo seedlings following pre-treatment with potassium phosphate and Tris-HCl buffers over a concentration range of 0 to 0.4M	28
FIGURE 2	Per cent emergence of Echo seedlings following pre-treatment with potassium phosphate and Tris-HCl buffers over a concentration range of 0 to 0.4M	30
FIGURE 3	Abnormalities observed in rape seedlings pre-treated in 3.5M phosphate buffer at pH 6.5	32
FIGURE 4	Growth response of the cultivars Arlo and Echo as determined by dry weight analysis to varying concentrations of phosphate	35
TABLE 1	Root/shoot ratios of Arlo and Echo as determined on a dry weight basis	36
TABLE 2	Results of phosphate analyses showing values for total phosphorus, inorganic phosphorus, and inorganic as a percentage of total phosphorus	38
FIGURE 5	Phosphate uptake by excised roots of Arlo as a function of solution concentration in the low range (0.01 to 0.2 mM)	41
FIGURE 6	Phosphate uptake by excised roots of Echo as a function of solution concentration in the low range (0.01 to 0.2 mM)	43
FIGURE 7	Phosphate uptake by excised roots of Arlo as a function of solution concentration in the high range (0.5 to 50 mM)	45
FIGURE 8	Phosphate uptake by excised roots of Echo as a function of solution concentration in the high range (0.5 to 50 mM)	47

PAGE

TABLE 3 Vmax and Km values for phosphate absorption by excised roots of two cultivars of *Brassica campestris* based on kinetic data presented in Figs. 5 to 8..... 49

TABLE 4 Yield (g dw) of two cultivars of *Brassica campestris* grown under low phosphate concentration in dilute Hoagland solutions 50

ABSTRACT

Murphy, Allan James, Ph.D., The University of Manitoba, July, 1975. UPTAKE, TRANSLOCATION, AND LOCALIZATION OF PHOSPHATE IN RAPE.

Major Professor: Lucien J. LaCroix, Department of Plant Science.

Several aspects of a differential response to potassium phosphate by two cultivars of turnip rape (*Brassica campestris* L.) are described. Effects of seedling emergence were studied by pre-soaking seeds of both cultivars, Arlo and Echo, in potassium phosphate buffered solutions ranging from 0 (control) to 0.4 M potassium phosphate. Emergence rates of Echo were decreased slightly while those of Arlo were drastically reduced at higher phosphate concentrations. Growth studies were conducted in nutrient solution culture using varying phosphate concentrations. Highest dry matter yields were observed with 7.5×10^{-4} M and 1.25×10^{-3} M phosphate for Arlo and Echo, respectively. Toxicity symptoms occurred beyond these concentrations in both cultivars. The kinetics of phosphate uptake was studied in two concentration ranges, 0.01 to 0.2 mM phosphate and 0.2 to 50 mM phosphate. In the low range, Arlo exhibited a Vmax of 0.3 µmole P/g/h and a Km of 0.04 µmole P/l. Corresponding values for Echo were 0.5 µmole P and 0.1 µmole P/l, respectively. In the high range Arlo exhibited a Vmax of 1.6 µmole P/g/h

and a Km of 4.2 µmole P/l. Corresponding values for Echo were 0.9 µmole P/g/h and 1.6 µmole P/l. Root and shoot tissue of mature plants which had been grown in nutrient solutions containing a range of phosphate concentrations were analyzed for total P, P_i, and P_i calculated as a percentage of total P. Both P_i and P_i as a percentage of total P was found to be highest in root tissue of Arlo. Further growth studies were conducted in nutrient solutions with below normal phosphate concentrations. Yields (dw) of Echo were consistently higher than those of Arlo.

It is concluded that there are genetic variabilities within the species in regard to both the mechanism of phosphate uptake and the plant reaction to varying tissue levels of this nutrient.

CHAPTER I

INTRODUCTION

In recent years, rapeseed has assumed a position of major economic importance as an oilseed crop in various parts of the world. Of the major crops grown in Western Canada, it is probably the one which responds most dramatically to applications of fertilizer phosphorus. While much empirical data has been collected in such areas as yield response to phosphate and preferred modes of application, relatively little is known regarding basic uptake and transport phenomena within the plant or why certain strains respond to treatment more readily than others.

There is evidence that high phosphate concentrations may affect germination of rapeseed. During the course of their investigations into the effects of the mutagen ethyl methanesulfonate on rapeseed, Fowler and Stefansson (1972) observed that seeds which had been soaked in control solutions buffered with potassium phosphate showed reduced germination compared to equivalent soakings in distilled water controls. They further observed that some strains were affected more than others, suggesting a differential strain response to potassium phosphate.

Racz *et al.* (1965) investigated the effect of the addition of phosphorus to field plantings of rape, flax, and wheat. It was found that the amount of phosphorus taken up

by rape was greater than that utilized by flax or wheat. It was for these and other reasons that an investigation which would provide basic information on the physiology of phosphorus nutrition in rapeseed seemed desirable.

Preliminary work involved the use of four cultivars; Arlo, Echo, Nugget, and Target. For practical reasons, the investigation was confined to the study of two of these, Arlo and Echo. The fact that both are cultivars of turnip rape (*Brassica campestris*), lent further interest to the study.

Since it was felt that differential strain response may have been due in some way to the relative amounts of phosphorus taken up, the problem was approached from the points of view of uptake rates, growth response at various phosphate concentrations, and total accumulation of phosphorus in the mature plant.

The primary objective of this study was to determine the cause or causes of differential strain response to potassium phosphate by two cultivars of turnip rape, Arlo and Echo.

All of the experiments were conducted using nutrient solution culture techniques. While this method has certain drawbacks, it is one which allows a high degree of control over experimental conditions and was deemed the most suitable to the problem at hand.

CHAPTER II

LITERATURE REVIEW

Phosphorus has been implicated in biological processes since the latter part of the eighteenth century when Gahn, a Swedish chemist, showed phosphorus to be an essential constituent of bones (Legal and Myrick, 1968). The requirement for phosphorus by plants was recognized by Justus von Liebig about a century later and shortly thereafter by J. B. Dawes and J. A. Gilbert who in 1855 established the necessity of this element for crop production (Tisdale and Nelson, 1966).

The recognition of phosphorus as being necessary for plant growth and development led quickly to the commercial production of phosphate fertilizer. Indeed, it was Dawes who in 1842 obtained a patent at Rothamsted on the manufacture of superphosphate (Farber, 1966). An excellent review of the establishment and technology of this particular branch of the fertilizer industry was published recently (Slack, 1968).

Once the requirement for phosphate by plants had been demonstrated, and that animals obtained their phosphates from digested plant materials, the attention of physiologists and chemists was focused on the task of finding the actual state in which phosphorus compounds were present in organisms. In the middle of the nineteenth century, Théophile Juste Pelouze showed that phosphoric acid would combine with glycerol to form an ester in a similar fashion to the esterification of

alcohols, a process which had been known for some time (Farber, 1966). Since glycerol had been shown by Michel Chevreul as the substance released by fats on soap boiling, the significance of this "new" compound and its unique properties was not lost to contemporary workers (ibid.). Lecithin was discovered shortly thereafter by Nicolas Gobley (1845) by means of an ether and alcohol extraction of egg yolk. It was soon found that similar substances could be extracted from other sources such as nerve and brain tissue but that upon hydrolysis, they yielded fatty acids different from those found in lecithin and also that amino-ethanol was present instead of choline (Farber, 1966). The term "phosphatides" was coined by Ludwig Thudicum in 1901 and is still currently used to designate phosphorus-containing lipids (Wittcoff, 1951).

A peculiar substance obtained from the nuclei of cells of pus and salmon sperm by Mieschner in 1868 which he termed nuclein, was easily shown to contain phosphorus as well as other components (Fruton, 1972). Much work by many investigators was necessary before Robert Feulgen in 1918 proposed the following scheme for the structure of a nucleic acid: phosphoric acid-carbohydrate-base, four of these groups constituting a "tetranucleotide" which was thought to be repeated over and over again to form a molecule of nucleic acid (Farber, 1966). Although the tetranucleotide hypothesis was shortly abandoned, this is essentially the structure of nucleotides as determined by later workers using more sophisticated techniques which enabled them to establish the nature of the

carbohydrate moiety as well as the precise location of the convalent bonds which link the three components. In particular, the work of Todd and his associates during the period 1940 to 1955 should be mentioned (Fruton, 1972).

During the decade preceding 1930, a compound of adenine, ribose, and phosphoric acid was found in yeast, blood, and in skeletal muscles of animals (Farber, 1966). It was named adenosine triphosphate, usually abbreviated by the symbol ATP. The corresponding derivatives of guanine, cytosine and uracil were also found.

With the establishment of reliable techniques for the isolation of phosphorus-containing compounds, the way was paved for more critical investigations into the crucial role which phosphorus plays in cell metabolism, and the gradual elucidation of this role forms a large part of the history of modern biochemistry.

Harden and Young (1906), published a paper which implicated phosphorus in the alcoholic fermentation of yeast,and this discovery may be regarded as a landmark in the study of the biochemistry of phosphorus. They showed that the addition of phosphate to mixtures of glucose and yeast juice enhanced the fermentation process as measured by carbon dioxide evolution. For each mole of phosphate added, one mole of carbon dioxide was evolved. They also showed that orthophosphate was incorporated as the phosphate ester fructofuranose - 1, 6 - diphosphate. Consecutive events now known to occur in fermentation and glycolysis were established one by one with

the identification of enzymes, intermediates, and cofactors involved. Many of the intermediates were shown to be phosphorylated compounds and the energy required to drive the reactions derived from the terminal phosphate group of ATP (Van Wazer, 1961). Outstanding contributions were made by Embden, Myerhoff, Parnas, Warburg, Cori, and Lipmann, to mention only a few. Similarly, research into the biochemistry of photosynthesis revealed an equally important role played by phosphorylated intermediates in the overall process, albeit an anabolic rather than a catabolic one (Benson *et al.*, 1950). The biochemical pathways established for fermentation and glycolysis have proven to be models for a host of biological processes. A more thorough treatment of these fundamental areas of biochemistry is beyond the scope of this review, but a voluminous amount of literature is available in current biochemistry texts and review articles.

Any discussion of the physiological aspects of phosphorus and plant growth should begin with consideration of the source, viz., the soil. The low concentrations of available phosphate in most soil solutions are due in part to the lack of abundance of elemental phosphorus in the lithosphere relative to other elements required in the biological process (Halmann, 1972) and also to the low solubility products of compounds yielding phosphate ions. Concentrations of phosphorus seldom exceed 10 μM even in fertile soils (Fried and Shapiro, 1961).

The ultimate source of phosphorus found in the biosphere is that class of compounds present in igneous rock known as

apatites, $CaX_2.3Ca_3(PO_4)_2$, where X may be fluoride, chloride, or hydroxyl anions (Halmann, 1972). Small amounts of phosphate ion are released to the soil by the physical processes of leaching and weathering, but the strong tendency of these ions to interact with certain cations normally present in soils prevents a significant accumulation of forms available to plants. Under acid conditions insoluble precipitates of iron and aluminum are found, while at pH values greater than 7.0 precipitates of calcium and magnesium predominate (Tisdale and Nelson, 1966). Lewis and Racz (1969) showed that movement of phosphorus in noncalcareous soils was much greater than in calcareous soils when phosphorus was added to the soil in the form of monoammonium phosphate or diammonium pellets. Many factors influence the movement and availability of soil phosphorus, the best known of which are source, soil type, and pH. Bouldin and Sample (1959), demonstrated that the relative effectiveness of different sources of water-soluble phosphates may vary with the soil type. Diammonium phosphate was shown to be superior to monocalcium phosphate monohydrate in a Hartsells soil, while in a Webster soil, the reverse was true.

Plants may absorb phosphorus as either the monobasic or dibasic form of the phosphate ion and the relative amounts present in solution are intimately related to pH (Tisdale and Nelson, 1966). Since the monobasic form of the ion predominates under slightly acid conditions, a condition which is generally conducive to plant growth, it has long been

assumed that this form of the ion is taken up by plants in amounts relative to its concentration. But Hagen and Hopkins (1955), have pointed out the dangers of this assumption. Their kinetic studies on phosphate absorption by excised barley roots indicated that separate uptake sites exist for the two ionic species and that the much greater affinity of the site of uptake for dibasic orthophosphate renders it at least equal in importance to the monobasic form of the ion, assuming the concentration of orthophosphate is high enough to be nonrate-limiting. These factors assume great importance when related to practical situations.

The feeding habits of plants grown under similar conditions may also vary between different species and even between different varieties of the same species. As early as 1898, it was reported that the influence of rock phosphate on plant growth varied with the species (Merrill, 1898), and more recently by Racz *et al.* (1965), who found that the yield increase of rape was greater than that of wheat or flax when fertilizer phosphorus was added. Varietal differences have been demonstrated as well. Smith (1934), working with four inbred lines of maize and six of their crosses, found that the differential growth response of some lines to varying levels of phosphorus in the growth medium was under genetic control and that when a phosphorus efficient line was crossed with a less efficient one, dominance of phosphorus efficiency was exhibited by the F_1 hybrid. This he ascribed to inherited difference in root types, the more efficient plants having a

higher ratio of secondary to primary roots. Similar results were obtained by Lyness (1936) for three varieties of Reids Yellow Dent Corn. Here again, a high correlation was found between phosphate absorbing power and the relative number of secondary roots.

There is general agreement regarding the manner in which phosphate ions are released from colloidal clay particles through anion exchange into the soil solution, from which they move via diffusion and bulk flow into the vicinity of the root (Brady, 1974; Fried and Shapiro, 1961). But the actual mechanism by which ions are transported into the living cell against a concentration gradient (and therefore energy-requiring), is still an area of intensive research.

It is convenient at this point to introduce the terminology and concepts of root anatomy as proposed by Munch in 1932 (Salisbury, 1969) which allows one to conceive of the root as being composed of two distinct regions. One he terms the apoplast which comprises the cell walls, intercellular spaces and xylem elements; and the other the symplast, made up of the cytoplasmic portion of cells of the cortex and stele, these cells being contiguous with one another by means of the plasmadesmata. Ions are regarded as having free access to the cortical region of the apoplast by diffusion but are prevented from entering the stele by the endodermal layer. Diffusion access to the symplast is prevented by the cell membranes (ibid.).

The region of the root freely permeable to ions by diffusion is called "free space." In initial studies, attempts

were made to calculate the volume of free space by experiments in which the change in concentration of a solution of a known molarity was measured after root tissue had been immersed and allowed to come to equilibrium. These experiments indicated that the so-called free space exceeded the volume accounted for by cell wall material and inter-cellular spaces calculated from anatomical studies (Briggs and Robertson, 1957). This led to the belief that a portion of the cell inside the outer membrane was freely permeable to ions by diffusion and the term "apparent free space" was adopted. The obvious possibilities for experimental error in this type of study left many workers in doubt as to the accuracy of the proposed values. Levitt (1957), challenged some of the work which had led to this hypothesis and concluded that protoplasm is not available for the free diffusion of ions and, therefore, is not a component of apparent free space (AFS). The values reported by Levitt for AFS represent about eight per cent of the root volume, or roughly two-thirds of the space occupied by cell wall material. This value is significantly lower than those reported by Butler (1953) and Epstein (1955), which ranged from 20 to 30 per cent.

Numerous monographs and review articles on the subject of ion uptake are available (Kotyk and Janacek, 1970; Higinbotham, 1973; Rains, 1972).

Important contributions to the study of ion uptake have been made by Epstein and co-workers. It was Epstein and Hagen

(1952) who first applied the concepts of enzymes kinetics to the study of ion uptake which have since proven so useful in uptake studies. They reported that when the rate of absorption of rubidium by barley roots (used as an analogue for potassium), was plotted as a function of the external concentration, the isotherm obtained showed saturation kinetics according to the Michaelis-Menten relation. It was also shown that when potassium and rubidium were both present, competition effects were observed, indicating that both ions were competing for the same uptake site. This, and other evidence, lent further support to the hypothesis that ions are transported across membranes by carriers which form transitory complexes with the ions for which they are specific.

A further implication of this work was the proposal that two sets of carrier sites may exist for the uptake of a given ion, one operating at low concentrations (0.002 to 0.2 mM) and the other at relatively higher concentrations (0.5 to 50 mM). Experiments of this sort have since been extended to other plant species using different combinations of divers tissue types and inorganic ions, and it would appear that this dual pattern of uptake is a general aspect of cellular physiology (Epstein, 1966).

The demonstration of the existence of two distinct mechanisms for ion uptake led quite naturally to an attempt to localize them within the cell, generating a lively debate which continues to this day. Two schools of thought have emerged. One, led by Laties and co-workers, maintains that

both mechanisms, usually referred to as mechanism one and mechanism two, operative at low and high substrate concentrations respectively, reside at different locales in the cell. Their model associates mechanism one, that is, the mechanism having high substrate affinity, with the plasmalemma, and mechanism two which has relatively low substrate affinity, with the tonoplast. In other words, the two mechanisms must operate in series. This concept is based mainly on evidence gathered from experiments with corn roots in which uptake isotherms were obtained for rubidium and chloride (Torii and Laties, 1966). The results indicated that only vacuolated cells having both plasmalemma and tonoplast membranes exhibit the dual mechanism phenomenon. Cells closest to the root tip which are essentially nonvacuolated, exhibited only the first mechanism. While intuitively attractive and based on persuasive experimental evidence, this hypothesis makes the assumption that at high concentrations ions are able to move freely through the plasmalemma, an assumption which is open to question (Laties, 1959).

A different stand, taken by Epstein and his group, holds that both mechanism one and mechanism two are located at the plasmalemma, operating in parallel. Their conclusions are based upon experiments dealing with the mutual interactions of potassium and sodium during absorption by barley roots, ion exchange experiments with barley roots, and exudation studies with barley and corn roots. In all three cases the results obtained led to the conclusion that both mechanisms

are operative at the outer membrane of the cell (Welch and Epstein, 1968).

The evidence presented by both groups is convincing but contradictory. More recent evidence suggests that uptake mechanisms may be multiphasic, and a review article on multiphasic mechanisms has been published recently by Nissen (1974). This concept was first suggested by Nissen (1971), based on the results of a detailed study of uptake versus concentration of sulfate in roots and leaf slices of barley. Several important observations were made; among them that uptake of sulfate can be described by a single, multiphasic isotherm and that the phases are separated by sharply defined inflection points, each phase obeying Michaelis-Menten kinetics. The isotherm describing sulfate uptake in roots was resolved into eight distinct phases and that for leaf slices into five. On the basis of this and similar data, the existence of a single structure (site or carrier) which changes characteristics at discreet external concentrations of sulfate was postulated. By reanalysis of the data of Edwards (1968), Nissen (1973) has shown that the uptake of phosphate by excised roots of subterranean clover can be represented by three phases in the range 5 X 10^{-8}M to 10^{-3}M. The kinetics of phosphate uptake in *Elodea densa* were studied by Grünsfelder (1971), who showed that in the range 5 x 10^{-6}M to 5 X 10^{-2}M, uptake of phosphate during a time period of five minutes can be represented by five phases in the light and four phases in the dark. An enhancement effect on uptake by light was also observed.

It is now well established that ATPases are involved in ion transport in animal tissues, but their role in ion transport in plants remains controversial. Ratner and Jacoby (1973) compared the effects of organic monovalent cations with salts of mineral monovalent cations on the activity of Mg^{2+} - dependent ATPase in membrane fractions of *Avena sativa*, *Hordeum vulgare* and *Zea mays*. They concluded that monovalent salt effects on ATPase from these plant roots were not cation specific and were not related to the ability of root cells to absorb cations.

Since much of the research work done in this area has been concentrated on the use of excised root tips as experimental material, the interpretation of results is somewhat complicated by the fact that one is dealing with a variety of discreet tissue types, the individual cells of which are in varying stages of development. This has led to the use of giant algal cells of species such as *Nitella translucens*, *Chara corallina*, and *Hydrodictyon africanum* for uptake studies (MacRobbie, 1971). The kinetics of phosphate uptake in excised roots and leaves of corn hybrids were studied by Phillips, Baker *et al.* (1971) and were found to conform to the dual uptake pattern outlined above.

Lateral transport of ions into the xylem of roots is generally conceded to occur mainly along the terminal 10 cm of root, a region where cells of the cortex are fully vacuolated and the Casparian strip has been formed along the radial and transverse walls of those cells making up the endodermis

(Laüchli, 1972; Esau, 1965). Anderson and House (1967), in an attempt to correlate structure and function in roots of *Zea mays*, demonstrated that excised roots possess the ability to absorb potassium and chloride ions over a distance extending at least 10 cm from the tip but with absorption rates decreasing in a manner quantitatively correlated with distance from the apex. Results from both electron and light microscope studies used in the same investigation indicated that those xylem vessels nearest the root tip contained membrane-bounded cytoplasm with organelles. The number of mature xylem vessels was found to increase in sections taken farther from the tip and at a distance of 10 cm, all xylem vessels were completely mature, i.e., lacking cytoplasm.

The use of microautoradiography and, more recently, electron probe analysis, has enabled workers to explore the correlation of anatomical structure with ion distribution in an effort to trace the lateral pathway of ions. These techniques may be used separately or in combination, and the high degree of resolution attainable by electron probe analysis, which enables the investigator to obtain quantitative data on individual cell compartments, promises to yield much information on intercellular transport. The application of these two methods to plant physiology is outlined in detail in a book by Luttge (1972).

Structural studies centred on examining the theory of symplastic transport of ions in roots as proposed by Crafts and Broyer (1938), have led to the conclusion that plasmadesmata provide a symplastic pathway in tissue, since a

mutual cytoplasmic continuum has been demonstrated between cells of cortex, endodermis, and pericycle (Bonnett, H. T. Jr., 1968). Laüchli (1967) has shown that a barrier to lateral transport of ions across the roots of *Zea mays* is located in the plasmalemma of the outermost cells of the cortex. This necessitates transport across the barrier after which ions move in the symplasm to the vascular tissue.

It has long been thought that the endodermis provides a barrier to passive movement of ions between cortex and stele, thus delimiting the inner boundary of AFS. This view is based on structural studies which have shown the endodermal cells to be contiguous with one another on the radial and transverse walls by means of the Casparian strip. This is a narrow band of hydrophobic material composed of lignin, suberin, or both (Esau, 1965), forming part of the primary cell wall. Plasmolytic studies by Bonnett (1968) and earlier workers have shown the plasmalemma to be securely attached to the cell wall in the region of the Casparian strip. However, as Laüchli (1972) points out, structural evidence alone is not enough and other evidence is required to assess the efficiency of the endodermis as a barrier to passive transport. Dumbroff and Peirson (1971) have shown that during the early stages of growth of branch roots, there is a period during which formation of the Casparian strip lags behind the division of new endodermal cells. This may allow a temporary pathway for the passive movement of water and ions to the stele of the parent root. In addition to vascular tissue,

the stele normally contains parenchyma cells which are in close proximity to the outermost metaxylem elements. Although concrete evidence is lacking at present, it is felt that these cells, located as they are between xylem and pericyle, likely play some role in lateral ion transport.

Phosphorus is transported in the xylem mainly in the inorganic form. When sap is obtained by suction from the xylem of woody stems, little or no organic phosphate is present (Morrison, 1965). Bleeding sap on the other hand, may have as much as 25 per cent of total phosphorus in the organic form. Maizel *et al.* (1956) showed that phosphoryl choline is the major phosphate ester found in plant sap. Although membrane transport processes for inorganic phosphorus are well documented, mechanisms for uptake of organic phosphorus, if they occur, have not been demonstrated. It has been suggested by Maizel *et al.* (ibid.) that because of its zwitterion structure and organic solubility, phosphoryl choline may act as a phosphorus carrier capable of penetrating plant membranes. There is some evidence to indicate that phloem tissue may also be involved in translocation of nutrients. Biddulph (1956) injected ^{32}P labelled phosphoric acid into red kidney bean plants through the leaf-flap injection method. Autoradiographs showed that ^{32}P moved downwards in the phloem of the vascular traces from the treated leaf.

Phosphorus may be stored as the phosphate ion in the cell vacuole or may occur in the cell as a constituent of organic or inorganic storage compounds. Inorganic polyphosphates have been shown to occur widely in lower plants such

as moulds, bacteria and algae (Miyachi, 1961), but they have been identified only occasionally in higher plants. Wiame (1949) demonstrated the presence of two distinct metaphosphate fractions in yeast, one considerably more active metabolically than the other. He also observed that when yeast cultures, which had previously been grown in a phosphorus deficient medium, were fed normal concentrations of phosphate, the total metaphosphate accumulated by the starved yeast was greater than that formed in normal yeast. Wintermans (1955) has shown that polyphosphate formation in *Chlorella* may be related in some way to photosynthesis. He found that in the absence of carbon dioxide, the greater part of phosphate taken up was converted to polyphosphates.

The presence of polyphosphates in seeds of *Cuscuta* has been demonstrated by Rahman and Krishnan (1971). Inorganic polyphosphate has also been found in spinach leaves (Miyachi, 1961). More research is needed to properly assess the role of polyphosphates as a storage form of phosphorus in angiosperms, but further progress must await the development of more unequivocal identification procedures. In the examples cited above, techniques similar to those of Wintermans (1955), which rely on solubility, acid lability, and metachromatic staining, were used. Chromatographic techniques have been developed by Ebel (1952) which may add a further degree of reliability to results from traditional methods.

Phytic acid has been clearly established as a major phosphorus reserve in seeds. Ergle and Guinn (1959) have

shown that more than 80 per cent of phosphorus in the embryos of cotton seeds is present as phytic acid. They found that during germination the rapid dephosphorylation of phytin is accompanied by the simultaneous accumulation of relatively large amounts of inorganic phosphorus. Mukherji *et al.* (1971) elucidated the changes undergone by phosphorus compounds during germination of rice seeds. In ungerminated seeds, 76 per cent of total phosphorus appeared in phytin. During the experimental period of five days, there was good correlation between phytin breakdown and the corresponding increase in amounts of inorganic phosphorus. Phytin has also been found in small amounts in actively growing plant tissue. In a study on the levels of phosphate esters in *Spirodela*, Bieleski (1968) found that phosphatidyl inositol was present in significant amounts in the phospholipid fraction.

The phenomenon of phosphorus toxicity, as manifested by necrotic areas of leaf tissue and reduced growth rates, has been studied by several workers. Howell (1954), examined the effects of high and low levels of phosphorus on three varieties of soybeans: Lincoln, Chief, and Adams. It was found that the variety Chief continued to respond favorably to concentrations as high as 112 ppm, whereas the other two were adversely affected by levels of 50 ppm. Foote and Howell (1964) investigated the response of two soybean varieties to varying concentrations of phosphate. They found that increasing the phosphate supply stimulated uptake by the sensitive variety (Lincoln) more than by the tolerant variety (Chief).

When tops of Lincoln were grafted to roots of Chief, no toxicity symptoms appeared at concentrations normally toxic to entire Lincoln plants. They concluded that the critical genotypic difference in phosphorus nutrition appeared to reside in the roots.

Phosphorus toxicity as it relates to yield has been investigated by Asher and Loneragen (1967), who studied the effect of phosphate concentration on eight annual pasture species representative of several genera. They found a wide variation in the levels at which each species reached its maximum yield. Toxicity symptoms appeared as necrotic areas commencing at the tips of older leaves and progressing towards the base. Upon analysis, it was found that those plants which showed toxicity symptoms had high internal levels of phosphate in both shoot and root. Bhatti and Loneragen (1970), studied the relationship between phosphorus concentration and the development of toxicity symptoms in leaves of young wheat plants. The appearance of necrotic areas in the tips of the first leaves was found to coincide closely with regions of high phosphate accumulation. They suggested that the observed injury to the leaves may have been due to the disturbance of water relations in the leaf brought about by low osmotic potential in the cell sap. Warren and Benzian (1959) have shown that an excess of monocalcium phosphate can cause die-back in yellow lupins. Phosphorus content in the leaves of severely damaged plants ranged between one and two per cent of total dry matter.

CHAPTER III

METHODS AND MATERIALS

Toxicity Effects

Seeds of two turnip rape (*Brassica campestris*) cultivars, Arlo and Echo, were used in these experiments. Seeds were screened to ensure uniformity of size and only seeds in the range of 0.21 to 0.16 cm in diameter were used. Fifty seeds constituted a single treatment unit. Seeds were soaked in five ml of a potassium phosphate buffer solution at pH 7.2 for 12 hr at 20 to 25C. Concentrations ranged from 0 to 0.4M.

At the end of this period, the buffer solution was removed and seeds were washed twice with tap water and then allowed to soak in distilled water for one hour prior to planting. Seeds were sown in flats at a rate of 50 seeds per row with 10 cm spacings between rows. The medium consisted of equal parts of loam, peat moss and sand. Flats were placed in a greenhouse receiving a daily photoperiod of 16 hr, and natural light was supplemented by a cool white fluorescent source at approximately 1,000 foot candles.

Flats were watered at regular intervals, and ten days after sowing, counts were taken of those seedlings which had emerged. In all, three replicates of the entire experiment were performed. As a control, a duplicate series of experiments was conducted simultaneously, identical in all respects

to the above with the exception that a Tris-HCl buffer was used. Tris-HCl buffer treatments equivalent in molarity to those of the phosphate buffer were adjusted to equal ionic strength by the addition of sodium chloride.

Growth Studies

Seeds of the cultivars Arlo and Echo were germinated in plastic trays containing moistened "Turface" and watered daily with one litre of Hoagland solution diluted tenfold with distilled water. Light conditions were as described previously. When the first true leaves began to appear, approximately ten days after sowing, the seedlings were transferred to five litre glazed containers, nine plants per container, and grown in a one-half strength nutrient solution identical to the above with the exception of potassium phosphate, the concentration of which was varied from 3×10^{-4} to 1.5×10^{-3}M. A pH of 6.0 was maintained by the addition of 1N KOH. The solutions were continuously aerated and kept at constant volume by the daily addition of distilled water. The entire solution in each container was replaced twice weekly with freshly prepared nutrient solution throughout the growing period. Plants were harvested when the first florets began to appear. The harvesting procedure was as follows: plants were removed from solution and the roots rinsed in 0.01N hydrochloric acid for one minute, followed by a brief rinse in distilled water. Each plant was then separated into root and shoot sections by cutting at the

cotyledonary node. The tissue was immediately frozen in liquid nitrogen, placed in plastic trays and freeze-dried. The dried tissue was weighed, ground in a Wiley mill (60 mesh) and then stored in a desiccator at -10C for further analytical investigations.

Analytical Procedures

Total phosphorus content was determined for root and shoot tissue after the method of Koenig and Johnson (1942). A standard solution containing 0.1 mg of phosphorus per ml was prepared by dissolving twice recrystallized potassium dihydrogen phosphate in water. Tissue samples weighing 0.1 g were wet-ashed by boiling in five ml of a 10:4:1 (v/v) mixture of nitric, perchloric, and sulfuric acids in 100 ml volumetric microkjeldahl flasks. When digestion was complete, the digest was allowed to cool and brought to volume by the addition of distilled water. Ten ml aliquots of the dilute digest were transferred to 50 ml volumetric flasks containing 10 to 15 ml of distilled water. Four drops of 2-4-dinitrophenol were added followed by the dropwise addition of 5N ammonium hydroxide until a faint yellow color appeared. Ten ml of vanadomolybdate reagent were added. The flasks were brought to volume with distilled water and mixed thoroughly. Optical density of the yellow solutions was determined at 470 nm against a reagent blank using a Zeiss spectrophotometer.

Inorganic phosphate was determined for root and shoot tissue by the method of Berenblum and Chain (1938) as modified

by Pons and Guthrie (1946). The procedure involves a trichloroacetic acid extraction followed by colorimetric evaluation of the molybdenum blue complex developed in isobutyl alcohol.

Kinetic Studies

Seeds were germinated in plastic trays and watered with a one-tenth-strength nutrient solution as before. After ten days, plants were transferred to plastic containers (capacity 10 litres) and grown in a one-half-strength solution for a period of ten days in the greenhouse. All other growth conditions were as described previously. On the tenth day after transplanting, they were transferred to a 10^{-4}M solution of calcium sulfate.

After 48 hours, plants were removed from solution and rinsed briefly in distilled water at 20C. The root section of each plant was removed, blotted gently, and 0.5 g portions were weighed out. These were placed in 50 ml of a holding solution of distilled water until required. The time from excision of the roots until the beginning of the experiment was not allowed to exceed one hour.

At time zero, roots were removed from the holding solution and placed in flasks containing 500 ml of a solution of potassium phosphate adjusted to pH 6.5 with potassium hydroxide. Solution concentrations ranged from 0.01 to 0.2 mM. $H_3{}^{32}PO_4$ had been added to each treatment solution to give a final specific activity of approximately 4 X 10^{10} cpm/mole

of phosphorus. Solutions were held at 28C in a water bath and continuously aerated during the absorption period of three hours. At the end of this time roots were removed, rinsed briefly in distilled water and placed in a solution of cold potassium phosphate and maintained as before for a period of one hour. Finally, the tissue was removed, rinsed in distilled water and blotted dry. Each treatment was then digested in acid by the method described previously. Ten ml aliquots of the dilute digest were assayed for radioactivity in a Nuclear Chicago liquid scintillation counter using the Cerenkov counting procedure (Parker and Elrick, 1970). An experiment designed to investigate uptake rates at higher phosphate concentrations was also performed, using a series of solutions in the range of 0.2 to 50 mM phosphorus. The procedures were otherwise identical to the above.

Growth Studies at Low Phosphorus Concentrations

Further growth studies were conducted under conditions described previously with the exception of phosphate concentrations. Plants were grown under two phosphorus regimes, 1×10^{-4} and 5×10^{-5} M phosphate in glazed porcelain containers. Each container held eight plants, and three replicates of the experiment were conducted. The nutrient solution was changed daily in order to maintain a constant phosphate concentration. Plants were harvested when the first florets began to appear, dried in a forced-air drying oven for 36 hours at 100C and dry weights were recorded.

CHAPTER IV

RESULTS

Emergence

Emergence rates of seeds pre-soaked in phosphate and Tris-HCl buffers were compared and it was found that as buffer concentration increased, emergence decreased (Figs. 1 and 2). Earlier investigations had shown that a high percentage of seeds in each treatment unit germinated regardless of buffer concentration, but as concentration increased, there was a tendency for the seedlings to abort after the radicle had attained a length of three to four mm (Fig. 3). Accordingly, emergence, rather than germination, was used as the criterion upon which to base evidence of buffer damage to the seedlings. Although considerable variability occurred within treatments, there was an increase in injury as buffer concentration increased and the relationship was essentially linear. Deviation from linearity in the response may have been due to seed source, or perhaps to slight differences in growing conditions. Nevertheless, two important observations may be made from the graphs in Figures 1 and 2.

First, a differential response occurred between cultivars after pre-treatment, regardless of the buffer system employed. Emergence rates of Echo were not seriously affected by buffer pre-treatment. A decrease in emergence of approximately 10 per cent was observed at the highest concentration of

FIGURE 1

Per cent emergence of Arlo seedlings following pre-treatment with potassium phosphate and Tris-HCl buffers over a concentration range of 0 to 0.4M.

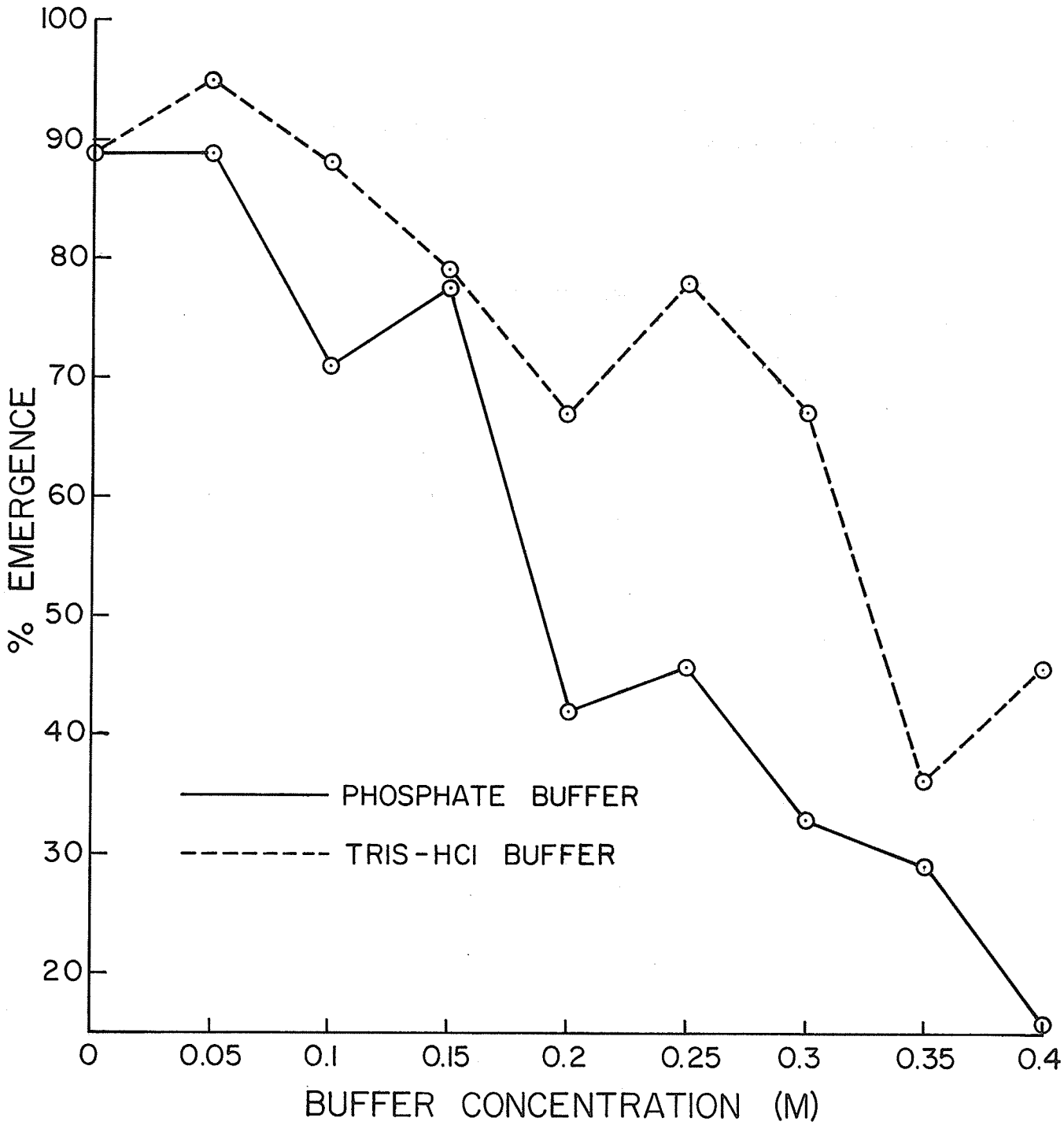

100
90
80
70
60
50
40
30
20
% EMERGENCE
PHOSPHATE BUFFER
TRIS-HCl BUFFER
0
0.05
0.1
0.15
0.2
0.25
0.3
0.35
0.4
BUFFER CONCENTRATION (M)

Fig. 1

FIGURE 2

Per cent emergence of Echo seedlings following pre-treatment with potassium phosphate and Tris-HCl buffers over a concentration range of 0 to 0.4M.

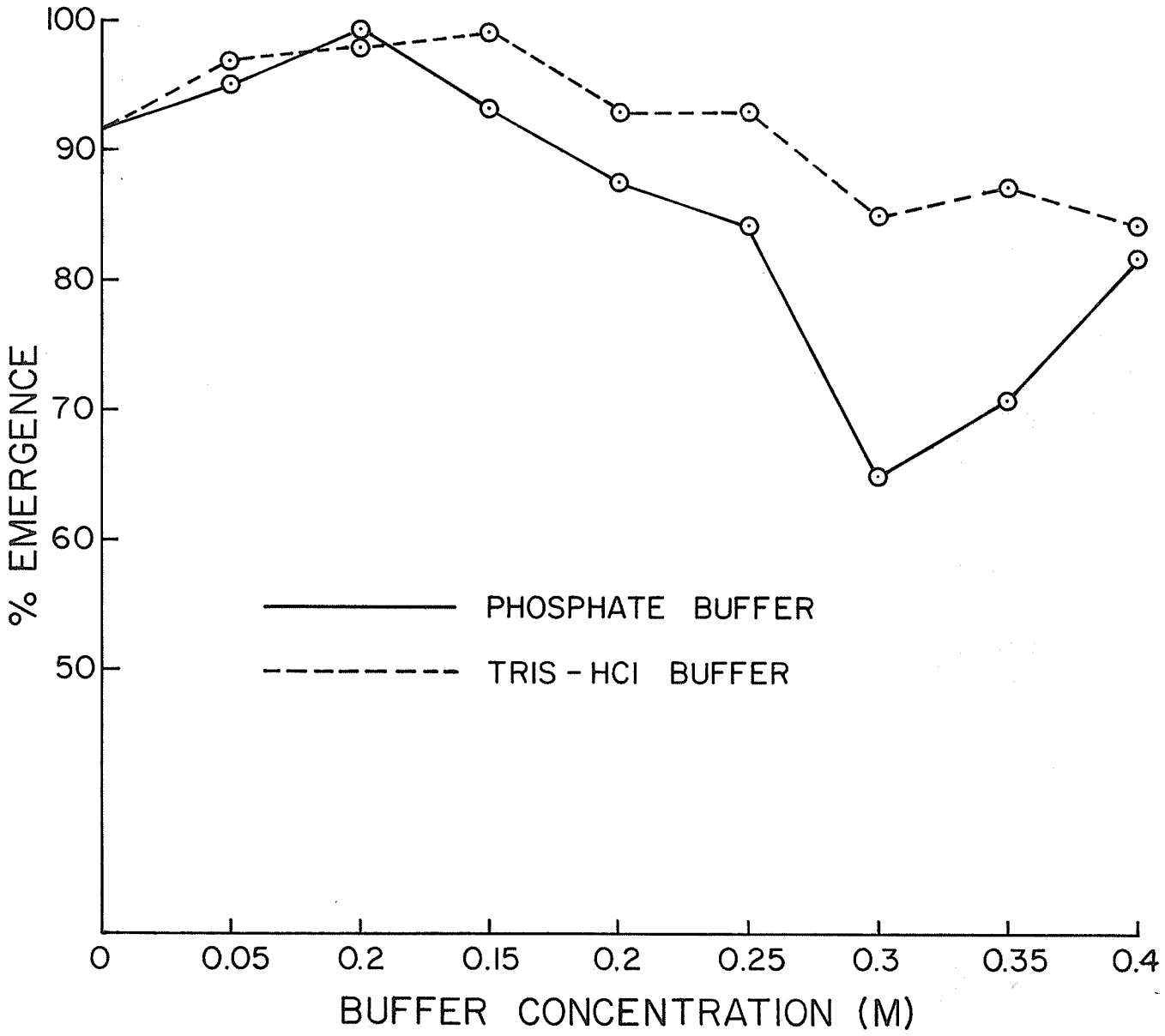
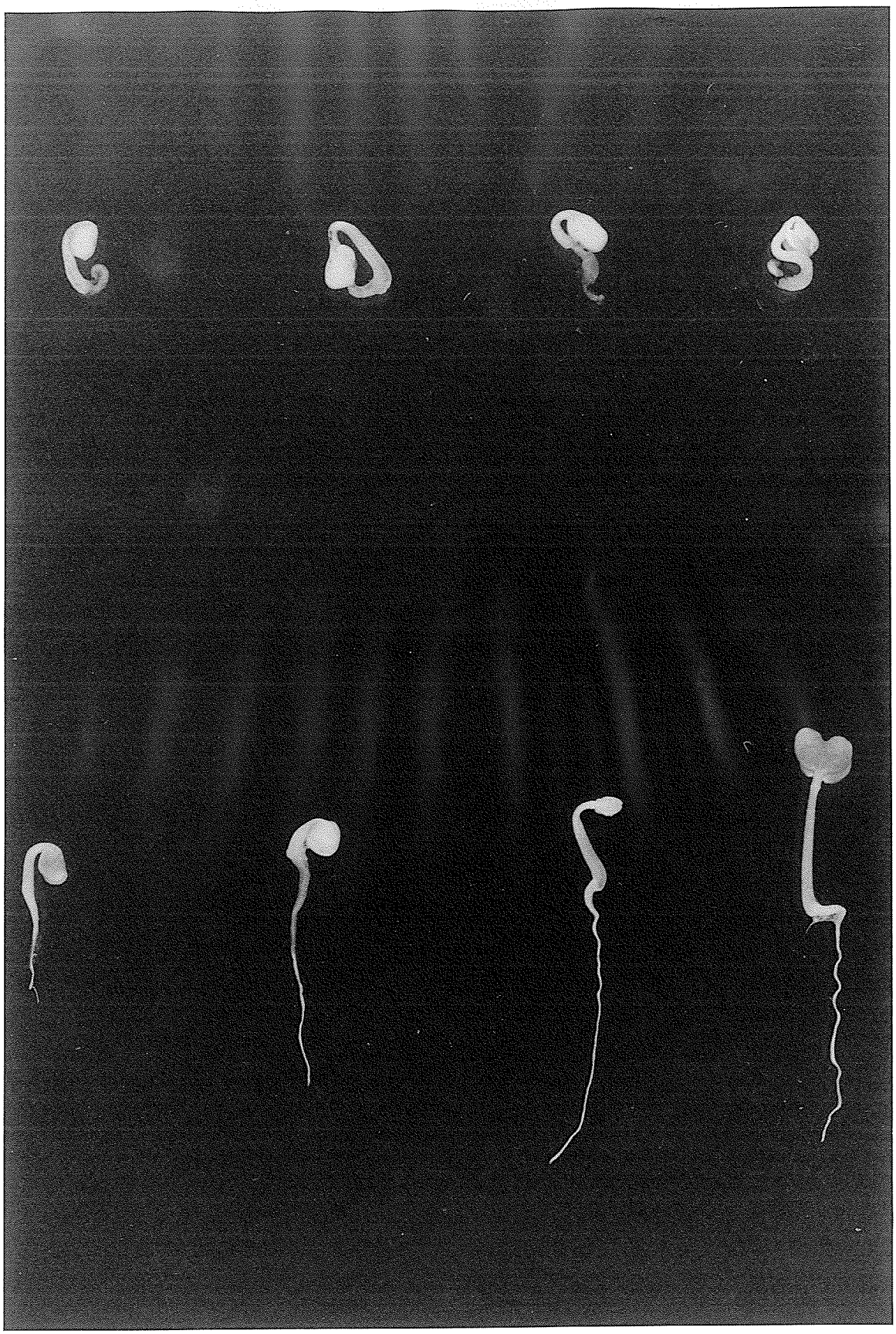

100
90
80
70
60
50
% EMERGENCE
PHOSPHATE BUFFER
TRIS - HCl BUFFER
0
0.05
0.2
0.15
0.2
0.25
0.3
0.35
0.4
BUFFER CONCENTRATION (M)

Fig. 2

FIGURE 3

Abnormalities observed in rape seedlings pre-treated in 3.5M phosphate buffer at pH 6.5. Top row: pre-treated seedlings 10 days after sowing. Bottom row: untreated seedlings 2, 4, 6, and 8 days after sowing.



phosphate buffer. On the other hand, the emergence of Arlo was severely affected even at lower buffer concentrations. For example, pre-treatment of Arlo seeds by 0.25M phosphate buffer resulted in an approximate decrease in seedling emergence of 40 per cent, while the same treatment of Echo seeds lowered seedling emergence by roughly 5 per cent.

Secondly, while higher concentrations of both buffer systems tended to lower emergence rates, the effect of the phosphate treatment was consistently greater than that of the Tris-HCl treatment, particularly with respect to Arlo. The results would suggest that the responses observed may have been due to a combination of osmotic effects and some form of phosphorus toxicity.

Growth Experiments

When growth rates of the two varieties were compared on the basis of dry weight determination, it was found that the maximum yield for Arlo was reached at a phosphate concentration of 7.5×10^{-4}M, whereas the maximum yield for Echo was attained at a significantly higher phosphate concentration, 1.25×10^{-3}M. Beyond these concentrations, yields of both varieties decreased (Fig. 4). When root-shoot ratios were determined, again on a dry weight basis, it was found that those of Echo were consistently higher than the corresponding ratios for Arlo (Table 1).

FIGURE 4

Growth response of the cultivars Arlo and Echo as determined by dry weight analysis to varying concentrations of phosphate. Shaded area represents root tissue.

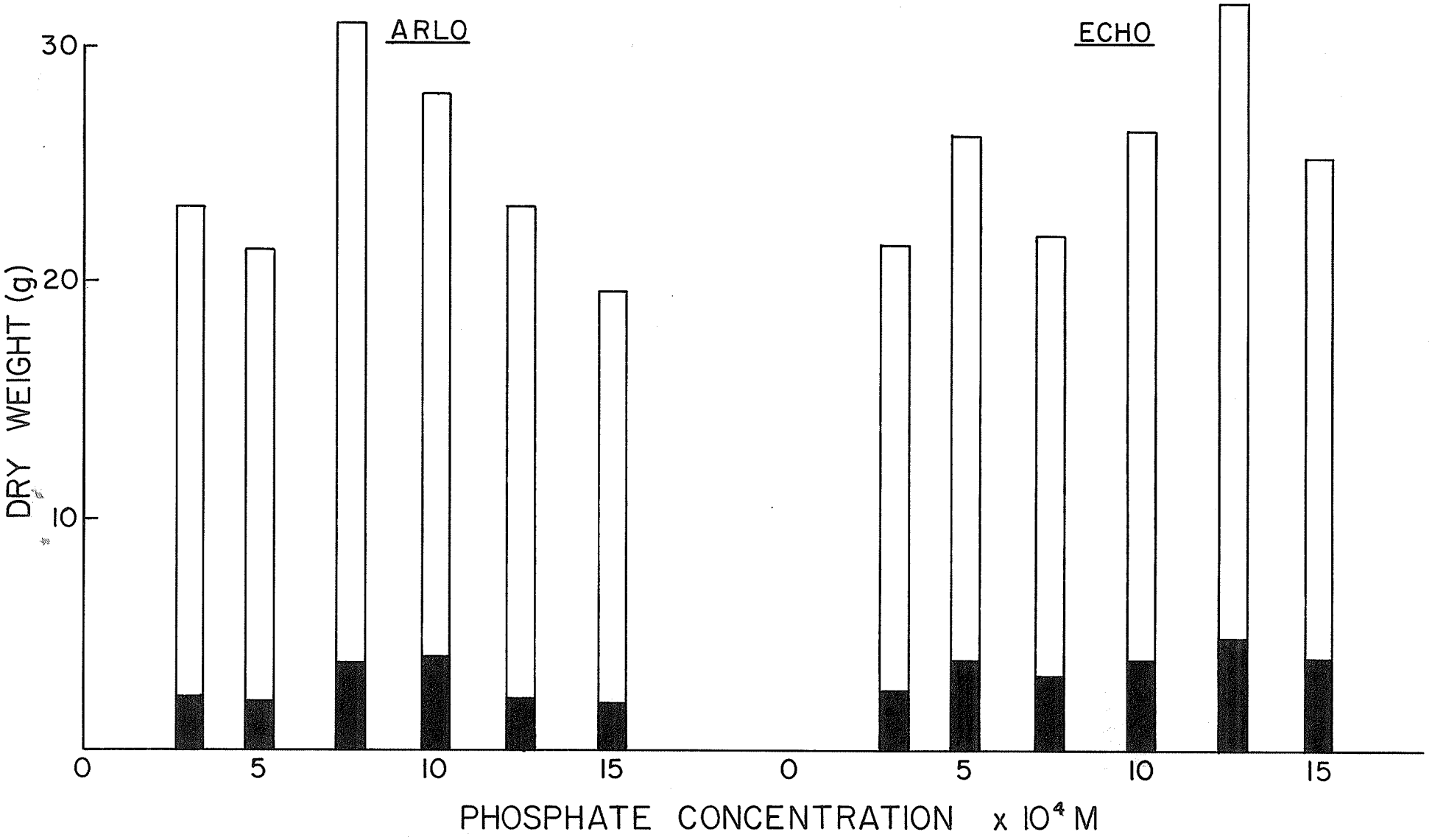

ARLO
ECHO
30
20
10
DRY WEIGHT (g)
0
5
10
15
0
5
10
15
PHOSPHATE CONCENTRATION x 10⁴ M

Fig. 4

TABLE 1

Root/shoot ratios of Arlo and Echo as determined on a dry weight basis.

Phosphate conc. (M)	Arlo	Echo
3×10^{-4}	0.14	0.15
5×10^{-4}	0.14	0.17
7.5×10^{-4}	0.13	0.16
1×10^{-3}	0.15	0.16
1.25×10^{-3}	0.13	0.18
1.5×10^{-3}	0.15	0.16

Analytical Results

The results of phosphorus determinations for root and shoot tissue are given in Table 2, where values for total phosphorus and inorganic phosphorus are expressed in μg/g on a dry weight basis. It was observed that values for total phosphorus found in root tissue increased in direct proportion to the concentration of phosphorus in nutrient solution, while the total phosphorus content in shoot tissue remained relatively constant over the entire range of phosphate concentrations in nutrient solution. These observations apply to both varieties. However, when inorganic phosphate was expressed as a percentage of total phosphorus, it was found to be significantly higher in the root tissue of Arlo than in Echo.

Kinetic Studies

As in customary in studies of the kinetics of ion uptake, the assumption was made that the absorption of phosphorus involves the formation of reversibly dissociable intermediates, and the rate of reversible association and dissociation of the active intermediate (k_1 and k_2 in equation 1) was assumed to be very rapid when compared with the reaction rate of the breakdown of the intermediate (Epstein, 1953). These assumptions are expressed by the following equation.

$$R + P \text{ (outside)} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} PR \underset{k_4}{\overset{k_3}{\rightleftharpoons}} R + P \text{ (inside)} \qquad (1)$$

TABLE 2

Results of phosphate analyses showing values for total phosphorus, inorganic phosphorus, and inorganic as a percentage of total phosphorus.

Treatment (Phosphate M)		Total P mg/g dw		P_i mg/g dw		P_i %	
		Arlo	Echo	Arlo	Echo	Arlo	Echo
	Root						
3	$\times 10^{-4}$	9.3	8.4	5.0	3.4	53	40
5	$\times 10^{-4}$	10.9	8.9	6.0	4.1	55	46
7.5	$\times 10^{-4}$	12.0	10.9	7.3	5.4	60	49
1.0	$\times 10^{-3}$	12.2	11.9	6.9	6.1	56	51
1.25	$\times 10^{-3}$	13.4	11.7	8.0	5.7	59	49
1.5	$\times 10^{-3}$	14.2	12.1	7.1	5.8	50	47
	Shoot						
3	$\times 10^{-4}$	6.2	6.1	2.9	2.3	47	38
5	$\times 10^{-4}$	6.4	6.4	2.8	2.4	43	38
7.5	$\times 10^{-4}$	6.6	6.7	2.3	2.9	35	43
1	$\times 10^{-3}$	6.2	6.7	3.2	3.0	52	45
1.25	$\times 10^{-3}$	7.1	6.3	3.3	3.0	45	48
1.5	$\times 10^{-3}$	7.1	6.4	3.1	2.8	43	44

Where P is the ion,

R is the carrier containing a specific binding site or sites,

PR is the active intermediate, and

k the rate constant for the reaction.

If k_4 is negligible, the rate-limiting step of absorption is k_3 and the overall process is essentially irreversible. Such assumptions have been shown to be valid for the uptake of phosphate by barley roots (Hagen and Hopkins, 1955).

A velocity equation may be derived from equation (1) which is analogous to the analysis of kinetic studies of enzyme reactions described by Michaelis and Menten (1913). It was pointed out by Lineweaver and Burk (1934) that the velocity equation is made linear in form by taking the reciprocal of both sides, as shown by equation (2);

$$\frac{1}{v} = \frac{Km}{Vmax\ [S]} + \frac{1}{Vmax}$$

In the present context, v denotes the observed absorption of phosphate, [S] the concentration of phosphate, Vmax the maximum absorption at infinite substrate concentration, and Km the apparent dissociation constant of an activated phosphate intermediate, PR.

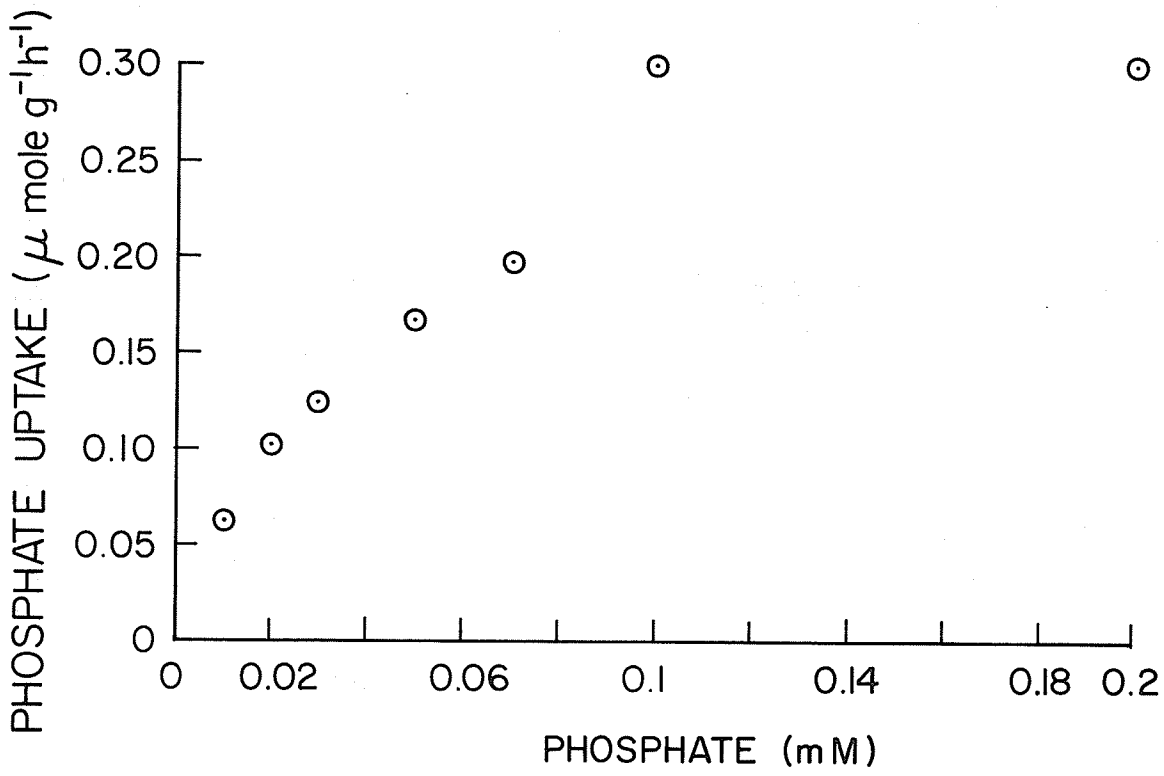
Km and Vmax values may be obtained by making double reciprocal plots of v vs. S and extrapolation of the straight lines thus obtained to the intercept of both axes.

The data obtained in the present study were treated in this manner. Figures 5 to 8 illustrate the effect of phosphate

FIGURE 5

Upper: Phosphate uptake by excised roots of *Brassica campestris* cv. Arlo as a function of solution concentration in the low range (0.01 to 0.2 mM) after an imbibition period of three hours.

Lower: Double reciprocal plot showing effect of phosphate concentration on uptake by excised roots.


PHOSPHATE UPTAKE (μ mole $g^{-1}h^{-1}$)
0.30
0.25
0.20
0.15
0.10
0.05
0
0
0.02
0.06
0.1
0.14
0.18
0.2
PHOSPHATE (mM)

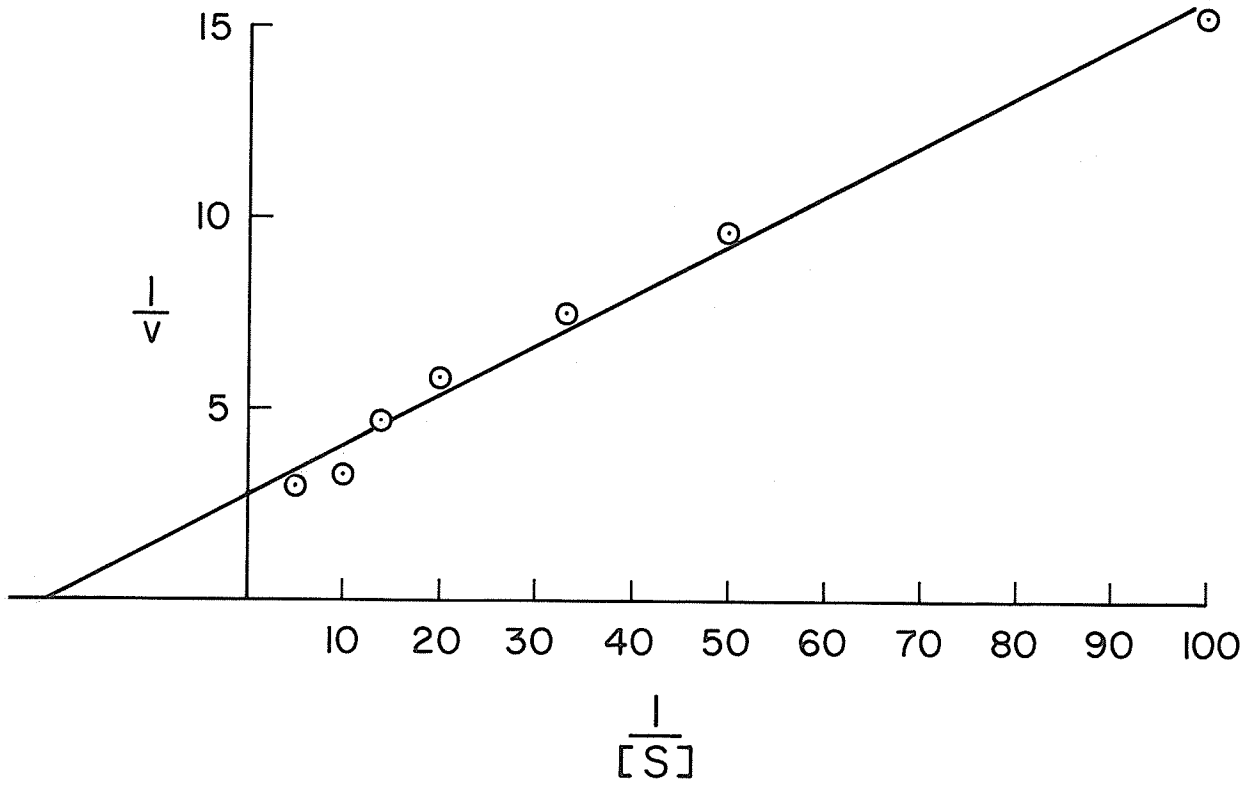
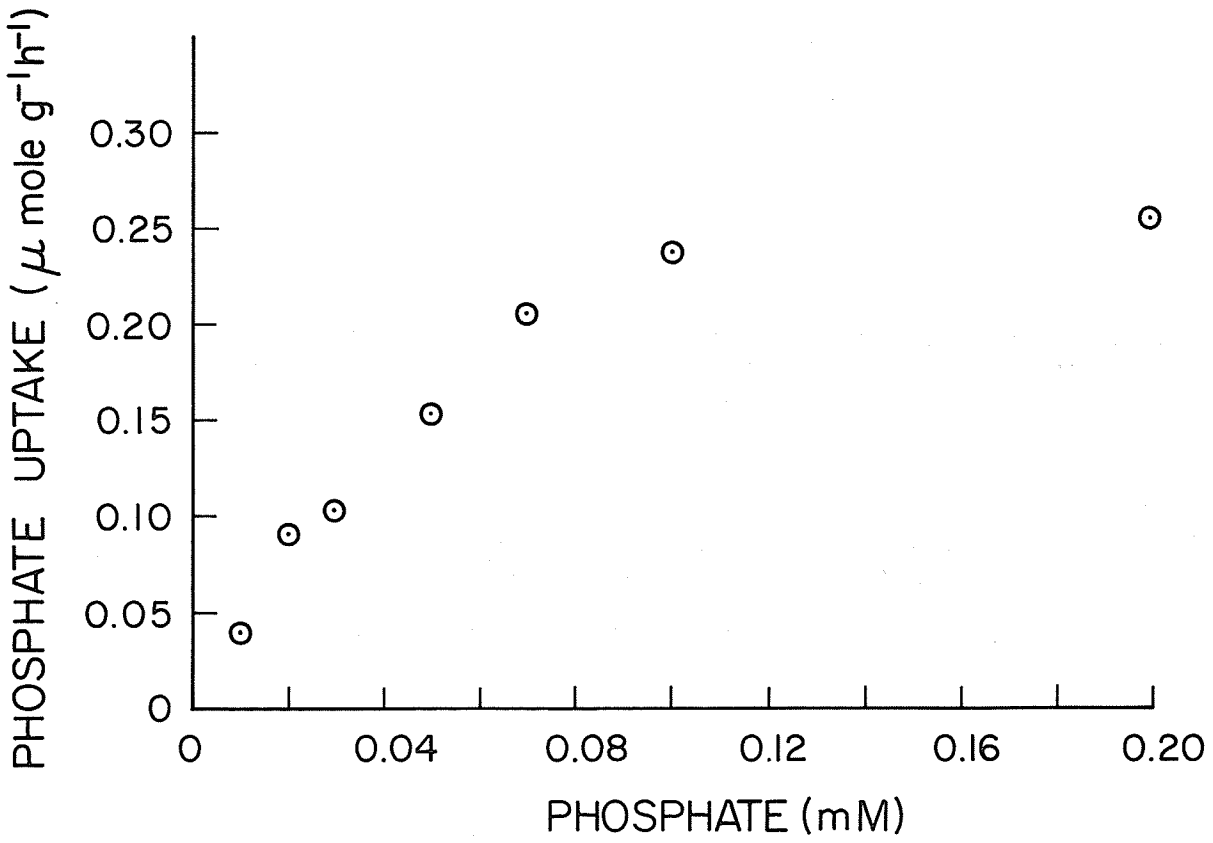

15
10
5
$\frac{1}{V}$
10
20
30
40
50
60
70
80
90
100
$\frac{1}{[S]}$

Fig. 5

FIGURE 6

Upper: Phosphate uptake by excised roots of *Brassica campestris* cv. Echo as a function of solution concentration in the low range (0.01 to 0.2 mM) after an imbibition period of three hours.

Lower: Double reciprocal plot showing effect of phosphate concentration on uptake by excised roots.


PHOSPHATE UPTAKE (μ mole g⁻¹h⁻¹)
0.30
0.25
0.20
0.15
0.10
0.05
0
0
0.04
0.08
0.12
0.16
0.20
PHOSPHATE (mM)

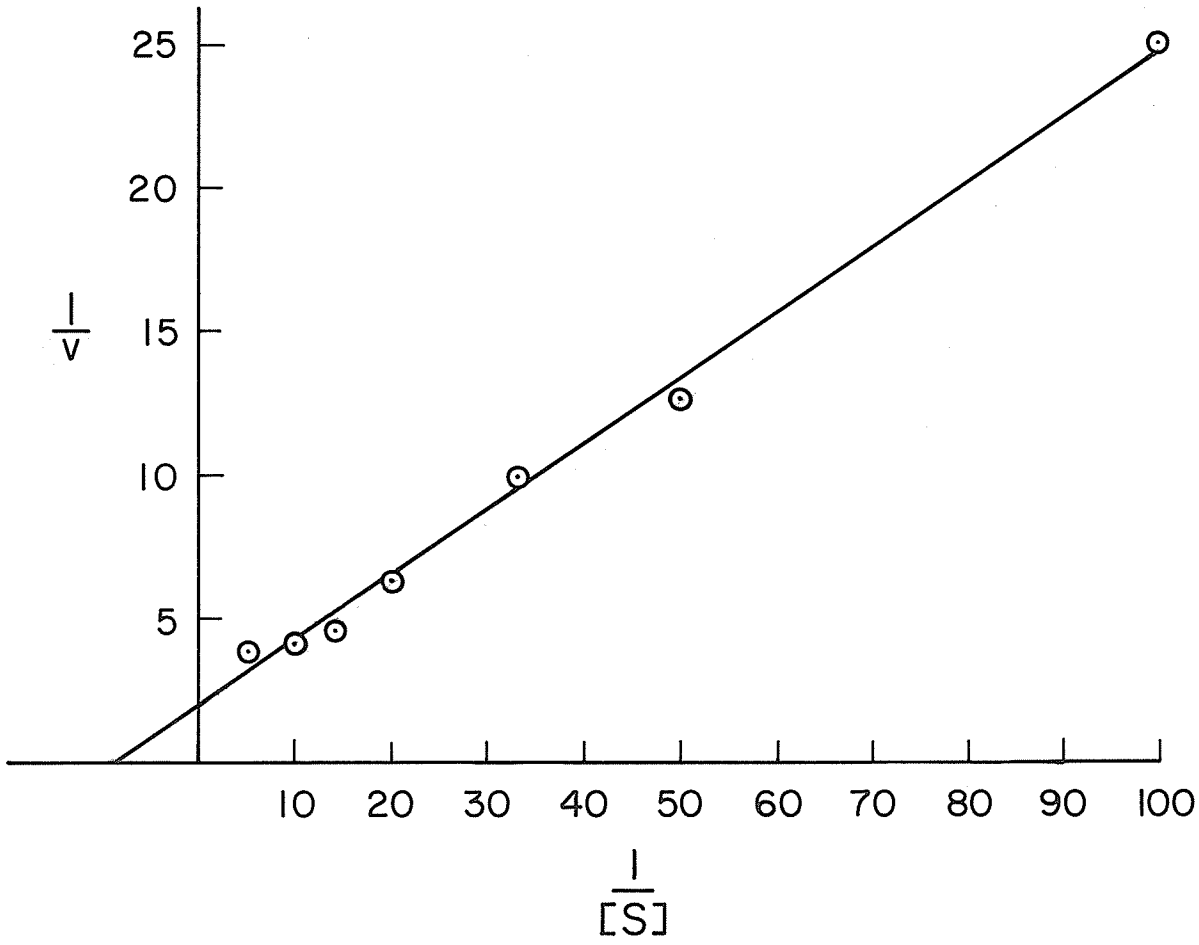

25
20
1/v
15
10
5
10
20
30
40
50
60
70
80
90
100
1/[S]

Fig.6

FIGURE 7

Upper: Phosphate uptake by excised roots of *Brassica campestris* cv. Arlo as a function of solution concentration in the high range (0.5 to 50 mM) after an imbibition period of three hours.

Lower: Double reciprocal plot showing effect of phosphate concentration on uptake by excised roots.

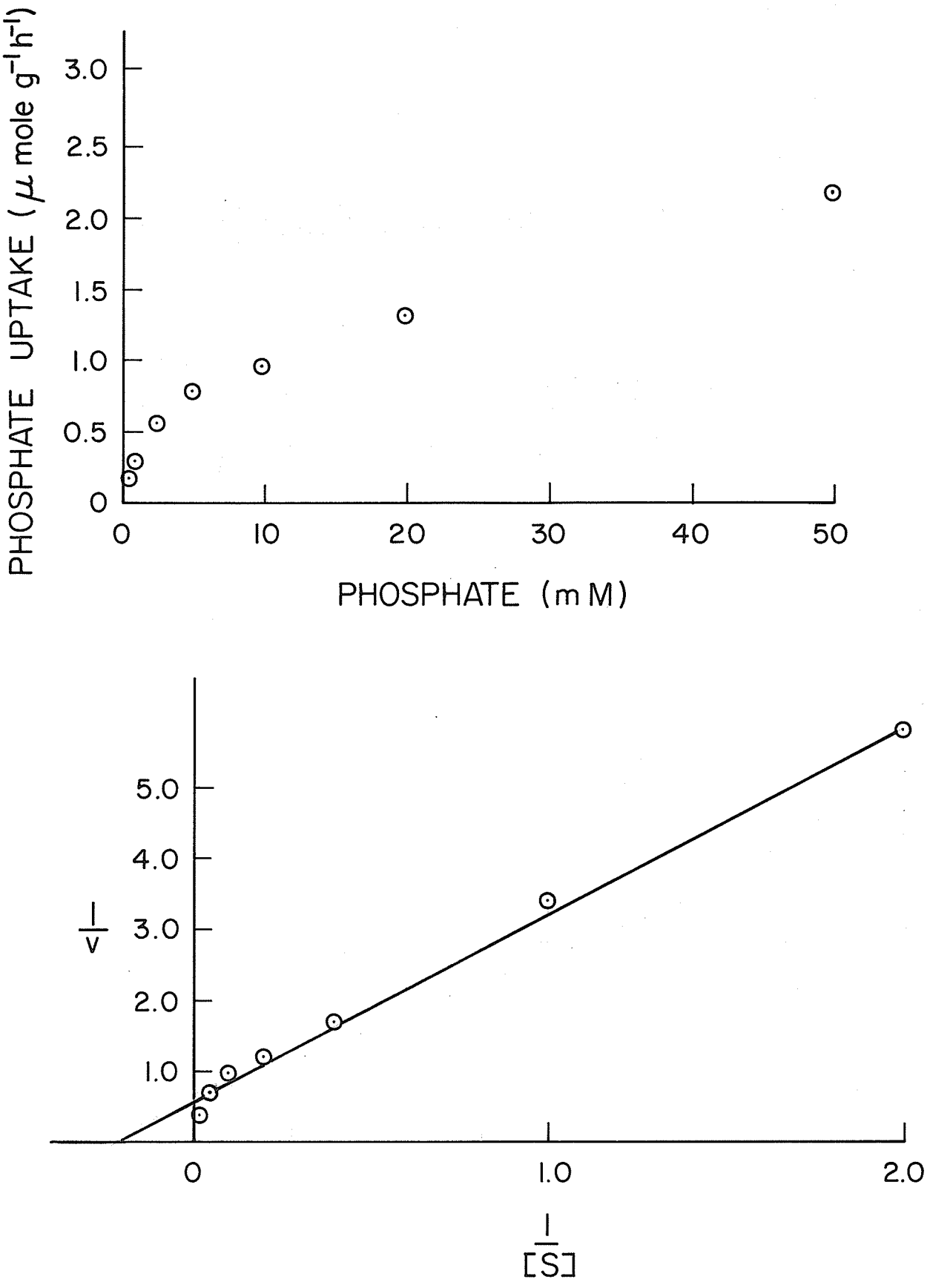
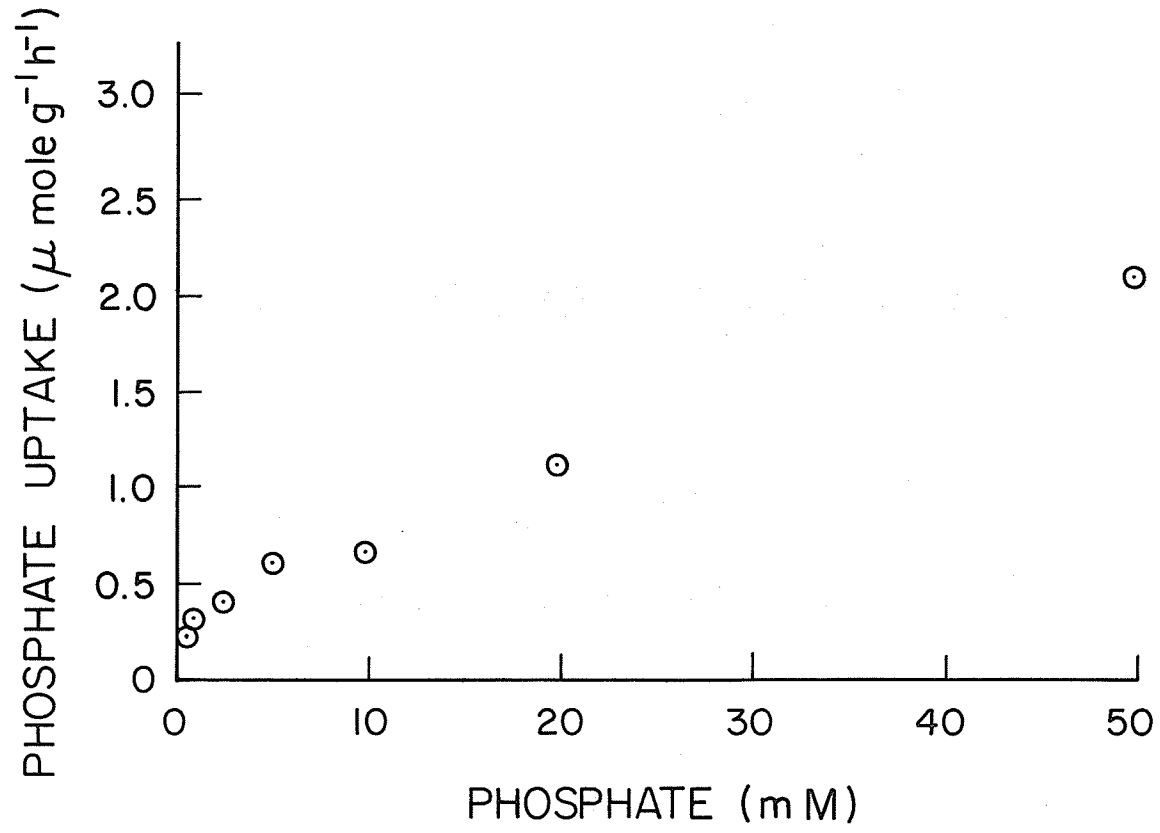

PHOSPHATE UPTAKE (μ mole $g^{-1}h^{-1}$)
3.0
2.5
2.0
1.5
1.0
0.5
0
0
10
20
30
40
50
PHOSPHATE (m M)
$\frac{1}{V}$
5.0
4.0
3.0
2.0
1.0
0
1.0
2.0
$\frac{1}{[S]}$

Fig.7

FIGURE 8

Upper: Phosphate uptake by excised roots of *Brassica campestris* cv. Echo as a function of solution concentration in the high range (0.5 to 50 mM) after an imbibition period of three hours.

Lower: Double reciprocal plot showing effect of phosphate concentration on uptake by excised roots.


PHOSPHATE UPTAKE (μ mole g⁻¹h⁻¹)
3.0
2.5
2.0
1.5
1.0
0.5
0
0
10
20
30
40
50
PHOSPHATE (m M)

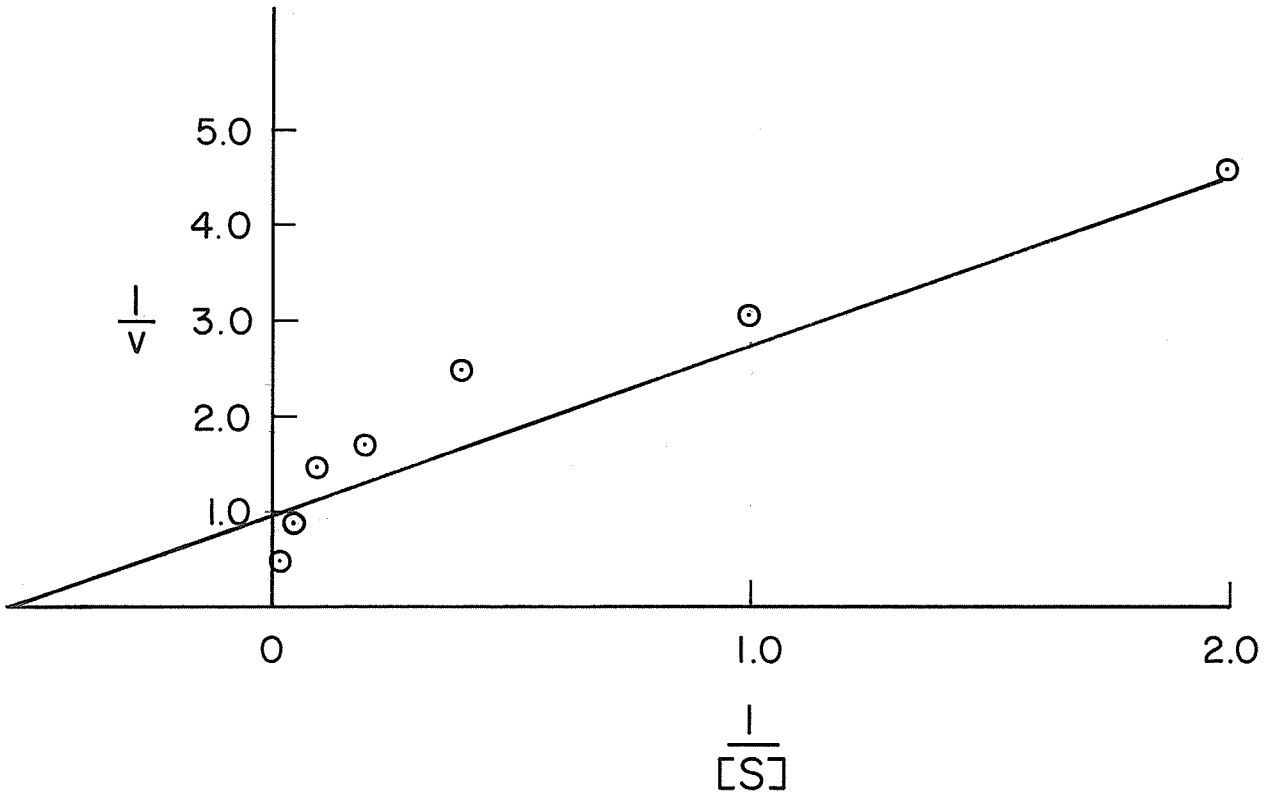

5.0
4.0
1/v
3.0
2.0
1.0
0
1.0
2.0
1/[S]

Fig. 8

concentration on uptake by excised roots and include double reciprocal plots obtained from the same sets of data. The method of least squares was used to obtain the straight line giving the best fit for each plot, and using the slope of the line and the point of interception along the ordinate, Km and Vmax values were obtained (Table 3).

Yields at Low Phosphate Concentrations

Although there is some objection to the use of Km values to denote affinity constants in enzyme studies, such usage is common in kinetic studies of ion uptake. Based on Km values as shown in Table 3, the higher affinity exhibited by Arlo for phosphate at low concentrations suggested that under conditions where phosphorus was in short supply, Arlo might well possess an advantage over Echo which would be reflected in yield as determined by total dry weight. The results of the present experiment (Table 4) indicate that the relationship is a complex one and that the two components, phosphorus concentration and yield, cannot be analyzed without taking into consideration other factors such as the relative concentration of other nutrients in solution.

When the plants were grown in a one-tenth strength Hoagland solution, the yields of Echo were similar at both phosphate concentrations, while yields of Arlo differed considerably and inversely to phosphate concentration. When the experiment was carried out in a one-half strength Hoagland solution (with the exception of phosphate concentration which

TABLE 3

Vmax and Km values for phosphate absorption by excised roots of two cultivars of *Brassica campestris* based on kinetic data presented in Figs. 5 to 8.

Concentration range	Cultivar	Vmax µmole/g/h	Km µmole P/l
Low	Arlo	0.3	0.04
	Echo	0.5	0.1
High	Arlo	1.6	4.2
	Echo	0.9	1.6

TABLE 4

Yield (g dw) of two cultivars of *Brassica campestris* grown under low phosphate concentrations in dilute Hoagland solutions.

Cultivar	Phosphate concentration	Yield		
		Root	Shoot	Total
Hoagland solution, one-tenth strength				
Arlo	1×10^{-4}	0.11	0.41	0.52
	5×10^{-5}	0.19	0.65	0.85
Echo	1×10^{-4}	0.16	0.56	0.73
	5×10^{-5}	0.16	0.55	0.71
Hoagland solution, one-half strength				
Arlo	1×10^{-4}	0.48	2.7	3.2
	5×10^{-5}	0.54	2.6	3.2
Echo	1×10^{-4}	0.59	3.0	3.6
	5×10^{-5}	0.71	3.2	4.0

was maintained as before), an overall increase in yield for both varieties was observed. Yields for Arlo remained almost constant at both phosphate concentrations, while the yields for Echo were slightly higher, the highest yield being observed at the lowest phosphate concentration.

These observations admit of no simple explanation and are difficult to account for on the basis of previous experimental data. Some form of phosphorus toxicity may be ruled out since the levels of phosphate present were well below those previously shown to evoke a negative growth response. The well known tendency of phosphate to interact with other ions and hence become unavailable for plant nutrition, was considered but the results were the opposite of what one might expect, i.e., at the higher concentration of other nutrients where more interaction would be likely to occur, yields were higher for both varieties and at both phosphate concentrations.

CHAPTER V

DISCUSSION

The pathway followed by phosphate from the time it leaves the soil solution until it becomes an integral part of the plant involves a series of orderly, well-defined steps; some are fairly well understood while others remain highly speculative. Removal of phosphate from the soil solution by adsorption to root cell wall material is the first and, in a sense, the most important in the sequence of events which are summed up in the term plant nutrition.

Adsorption is followed by uptake into the cytoplasm of the outermost cells of the cortex. This involves transport across the plasmalemma, a process which is still far from being understood despite a growing accumulation of empirical data. The process of membrane transport with respect to phosphorus, may be further complicated by the fact that phosphorus plays a functional role in the membranes which it must cross as a component of phospholipid molecules. In addition, one must also consider the possible involvement of ATPases in membrane transport. Although convincing evidence for the existence of ATPase activated carrier mechanisms in plants is lacking at present (Ratner and Jacoby, 1973), many investigators are confident that their participation will eventually be firmly established. Should this prove to be the case, phosphate concentrations in the

immediate vicinity of this or similar mechanisms may be crucial in determining the direction in which reactions involving the hydrolysis of ATP are likely to proceed.

Uptake of phosphate by excised roots of rapeseed in both the low and high ion concentration ranges was investigated and the data analyzed according to the method of Michaelis and Menten for kinetic studies of enzyme reactions.

In interpreting these data, several considerations must be borne in mind. First, the experiments were carried out on excised roots, and one may legitimately question the propriety of extrapolating results obtained under the experimental conditions described to what might be the expected behavior of whole plants grown under normal conditions.

Secondly, experiments of this type are not a true measure of ion flux, that is, assays do not yield values which can properly be expressed in units such as moles cm^{-2} s^{-1}, units one would consider normal for defining ion flux across a membrane.

Thirdly, both cultivars under investigation are derived from the same botanical species, *Brassica campestris*.

With regard to the first consideration, results obtained by those using entire plants grown in nutrient solution reflect the same selectivities and uptake patterns as do short term kinetic experiments with excised tissues (Collander, 1941; Smith and Epstein, 1964). Results obtained from uptake experiments with excised roots may thus be taken to approximate whole plant behavior in this aspect of mineral

nutrition. Indeed, information gathered by this method would be of much less practical value if such was not the case, although relative values might still be used as an index of uptake patterns.

The task of obtaining true measures of ion flux is a technical problem of seemingly insurmountable proportions, at least with techniques presently available. One is forced to surmise that anatomical differences between varieties are sufficiently slight that any observed differences in uptake rates are due to reasons other than total membrane surface available for ion transport.

The common ancestry of the two varieties under study, coupled with their dissimilar patterns of phosphate uptake, provoke speculation as to how this situation came about and serves as a reminder that during the selection process for obvious, more desirable agronomic characters, other subtle changes in genotype will inevitably occur, such as those affecting uptake mechanisms.

A marked difference in uptake for the two varieties under investigation was observed in the low range (Figs. 5 and 6). The basis for this difference might conceivably be due to a fundamental difference in the nature of the carrier mechanism itself or to a difference in the number of uptake sites (or carrier concentration) per unit area of membrane, or to a combination of both.

The Km for Arlo in the low range was found to be 0.04 μM while that for Echo was 0.1. If we interpret the Km as

being equal to the apparent dissociation constant of an activated intermediate of phosphate PR (see eq 1), then it becomes apparent that the affinity of Arlo for substrate is much greater than that of Echo. The disparities in potential for phosphate transport revealed by the Km values are conceivably the result of fundamental differences in the nature of the transport mechanisms themselves.

A significant difference in Vmax values in the low range was also observed, these being 0.3 and 0.5 μmoles $g^{-1}hr^{-1}$ for Arlo and Echo respectively. Since Vmax values yield information on K_3 (eq 1), this may be interpreted as further evidence for the existence of a distinct mechanism, characteristic of each variety and operative at low concentrations.

Uptake rates were also studied in the high range, i.e., at phosphate concentrations from 0.5 to 50 mM and the data treated as described previously.

The Km of Arlo in the high range was found to be 4.2 μM compared to 1.7 μM for Echo, indicating the presence of another uptake mechanism operative in the high range but once again distinct for each variety. Similarly, different Vmax values were obtained as well, these being 1.6 and 0.9 μmoles $g^{-1}h^{-1}$ for Arlo and Echo respectively. It will be noted that these values are the converse of those observed in the low range. The fact that the high substrate affinity exhibited by Arlo in the low ranges does not confer upon it a net uptake efficiency may simply indicate that the rate of breakdown of the carrier-ion complex (eq 1) which releases phosphate into the cell occurs at a much faster rate in Echo

in the low range; but it may also indicate a difference in concentration of carrier.

It is generally accepted that factors governing ion uptake are under genetic control, as illustrated by the work of Weiss (1943) who showed that uptake of iron by soybeans was under the control of a single gene pair. Since both varieties under investigation are derived from the same botanical species, *B. campestris*, we may assume that these different mechanisms are a by-product of the selection process.

It will be recalled that the concept of uptake sites is purely an operational one and the entire process of ion transport presumably involves a complex series of individual steps involving numerous chemical entities. Since the biosynthesis of this system is utimately dependant on genetic information passed on from generation to generation, it is possible to conceive of events occurring in the selection process, whether it be natural or artificial, which would be highly disruptive to the orderly transfer of information necessary for the exact replication of the chemical components required. This would invariably reflect on the relative uptake efficiencies of the derived genotypes. It seems likely that events similar to those alluded to above may have occurred during the selection process and are at least partially responsible for the observations noted.

The concept of carrier concentration was briefly alluded to above. If one accepts the hypothesis that ion uptake

occurs at discreet sites or is a function of concentration of carrier molecules associated with the cell membrane, then one must also accept that these sites are present in finite numbers, characteristic of cell type and cultivar, and that differences in carrier concentration will be reflected in uptake rates. While this may seem to add yet another dimension to an already complex problem, it is one which cannot be ignored and may be a contributing factor to the observations noted above.

On the basis that uptake mechanisms are under genetic control and may be altered during the selection process as the results of these experiments seem to infer, it is suggested that the tailoring of uptake mechanisms to suit the requirements of specific situations is a distinct possibility, one which could be of enormous practical value to the plant breeder.

The uptake mechanism which enables Echo to take up phosphate at a faster rate than Arlo in the low range, might be expected to confer upon it an advantage which would be reflected in higher yields at very low phosphate concentrations. However, one might also make the *a priori* assumption that if phosphate levels were decreased sufficiently, the higher affinity of Arlo for phosphate, which has been shown to be roughly three times that of Echo, would come into play and override the practical advantage of a higher Vmax.

When experiments were conducted to determine which might be the case, it was found that Echo continued to show higher

growth rates under all experimental conditions on a dry weight basis than did Arlo (Table 4), even at phosphate concentrations as low as 5 X 10^{-4}M. While a hypothetical point may exist at which sufficiently low phosphate concentrations would render substrate affinity the determining factor in net uptake, it would appear to have no practical significance since all plants in both treatments showed a substantial loss of vigor at a phosphate concentration of 1 X 10^{-4}M or less. It may be concluded that in experiments of this type where one is trying to predict yield from kinetic data, that Vmax values are the criteria upon which predictions should be made.

Uptake of phosphate by the outer cortical cells of roots is followed by lateral transport to the stele via the symplasm and endodermal layer. Once in the stele, it is taken up by the xylem cells of the vascular system and transported upwards from the roots to the various plant organs. Transport of phosphate to the shoot as it relates to this investigation will be discussed more fully below.

From the Vmax values obtained experimentally using excised roots, we may predict that the variety Arlo will absorb phosphate at a much greater rate than Echo at high phosphate concentrations. This prediction would seem to be valid when viewed in the light of the results of experiments discussed below.

Toxicity Effects

It has been shown that soaking rapeseed in potassium phosphate buffered solutions ranging from 0.05 M to 0.40 M

for 12 hr at 20 to 25C immediately prior to planting can result in a reduction in seedling emergence. In addition, significant interactions between buffer concentrations and those cultivars studied indicate that one cultivar (Arlo) was affected to a greater extent than the other (Echo) by this pre-planting treatment. This response was interpreted as being due to some form of phosphorus toxicity.

Phosphorus toxicity in plants has been widely recognized, but no firm explanation of its mode of action as it occurs at the sub-cellular level has been found in the literature by the author. It must be further emphasized that under certain circumstances the term "toxicity" might be quite inappropriate; that is, the response of the plant to high levels of phosphate may be the result of secondary effects rather than direct interference with some vital function within the plant cells or tissues.

Under the former heading would be included those phenomena which may be considered to be essentially extra-cellular in origin. This includes alteration of pH, osmotic potential, and interactions with other ions (although it should be noted that a phosphate imbalance within the cell could have much the same result). Due to its structural role in plant cells as an essential component of cell membranes, involvement in energy transfer reactions, and participation in an infinite number of other reactions within the cell, either as substrate or product, any significant change in the concentration of phosphate within the cytoplasm is likely to have far-reaching

effects. For example, Ku *et al.* (1968), found that the addition of 0.5 M phosphate buffer to a suspension of isolated mitochondria from tomato fruit, lowered the respiratory control ratio by a factor of three compared with the control.

In some cases, the effects of large amounts of phosphorus in the growing medium are quite obvious, giving rise to readily observable symptoms such as necrotic areas on leaves. In others, the effects may be less obvious and one must assess the degree of damage using other criteria as was done in the present investigation. In a broad sense, the term "phosphorus toxicity" may simply be taken to indicate a negative response by the plant which could be offset by decreasing the supply of available phosphate.

The underlying cause of the apparent difference in seedling emergence can only be theorized on, but several possibilities present themselves. The possibility that the initial observations made by Fowler and Stefansson were due to osmotic phenomena were investigated as reported earlier and, while it was found that pre-soaking seeds in Tris-HCl buffer of equal osmotic potential tended to lower emergence rates, the effect was consistently less than when seeds were pre-soaked with potassium phosphate buffer. Since only the external osmotic potential was under experimental control, it is quite possible that damage may have occurred within the cell due to differences in osmotic potential from one region of the cell to another. Phosphate, as well as most other ions necessary for plant growth, are normally taken up against a concentration

gradient, resulting in high internal concentrations relative to the surrounding medium. Abnormally high intracellular concentrations would be likely to have harmful effects upon cell organelles. If this were the case, one would have to assume the presence of an uptake mechanism present in seeds capable of acting in the earliest stages of germination. It should be emphasized that the word "mechanism" is used here in a very broad sense and denotes all uptake mechanisms, both active and passive.

It was further observed during these emergence tests that Echo germinated much more quickly than Arlo. At the end of the 12 hr pre-soak period, the radicle had normally ruptured the seed coat whereas those of Arlo were invariably intact. This more advanced state of growth might be connected in some way with the ability of the seedling to regulate the influx of phosphate or to incorporate it into cell material.

Regardless of what the underlying cause might be, these results indicate that phosphorus metabolism in the two cultivars differs in some fundamental way. The different uptake mechanisms described earlier are most certainly a contributing factor.

Growth

Any discussion of comparative growth rates must begin with a definition of the term "growth" and the criteria by which it is to be measured.

Plant physiologists have been unable to establish a

rigid definition of growth applicable to all phases of plant growth and development, although numerous mathematical formulas and models have been proposed. Growth may be measured in several ways: as an increase (or decrease) in size, fresh weight, dry weight, or by comparing different stages of development, a normal concomitant of growth, and by various other means. But since no simple correlation exists between these and other parameters it becomes necessary to choose the one which seems most suitable to the problem at hand. In actual practice, measurement of growth as a function of dry weight is probably the one most frequently employed, since it relates most readily to practical situations and was the one chosen for this study.

The relationship of plant growth to phosphate levels is dramatically illustrated in Fig. 4. The fact that Arlo shows a maximal growth response at 7.5×10^{-4} potassium phosphate, while Echo responds maximally at 1.25×10^{-3}M, almost a two-fold increase in phosphate concentration, is further evidence of fundamental differences in phosphate metabolism between the two cultivars. The negative growth responses observed beyond these levels would seems to indicate once again some form of phosphorus toxity. Despite the wide variation in yield, the root/shoot ratios of both cultivars remained remarkably consistent both with respect to variety and difference in phosphate concentration (Table 1).

Analytical Experiments

Tissue analyses were conducted on root and shoot tissue separately and values for total and inorganic phosphate were determined (Table 2). In the discussion that follows, this information will be used in an attempt to correlate plant response to varying phosphate concentrations with their respective uptake mechanisms.

An examination of the analytical data for shoot tissue reveals little difference both with respect to treatment concentration and variety although there is a slight tendency in the case of Arlo for total phosphorus to increase as a function of external concentration. It seems doubtful that this increase would be sufficient to account for the observed decrease in yield. Similarly, inorganic phosphorus as a percentage of total phosphorus in the shoot is essentially the same in both varieties although once again the values for Arlo are slightly higher. This was taken to imply that transport of phosphate from root to shoot proceeds independently of concentration within the range of 3×10^{-4} to 1.5×10^{-3}M phosphate and would appear to rule out the possibility that events responsible for toxicity symptoms take place in the shoot portion of the plant.

When similar data for root tissue were examined, a different pattern emerged. It has been noted previously that maximum growth response for Arlo occurred at an external phosphate concentration of 7.5×10^{-4}M while that for Echo was reached at a concentration of 1.25×10^{-3}M phosphate.

However, an examination of Table 2 reveals that at these widely different external concentrations, the uptake of phosphorus on a dry weight basis was essentially the same (12.0 mg/g for Arlo and 11.7 mg/g for Echo), an indication that the internal concentration of phosphate required for maximum yield is essentially the same for both cultivars. The difference in uptake rates necessary to achieve this internal concentration can of course be accounted for by the relative difference in Vmax values. It will also be noted that the range for total phosphate uptake by Arlo is considerably greater than the range for Echo, once again reflecting the greater relative efficiency of the uptake mechanism present in Arlo for phosphate uptake in the high range.

When inorganic phosphate is calculated as a percentage of total phosphate in root tissue, a further important observation may be made. Inorganic phosphate as a percentage of total phosphate is consistently higher in the root tissue of Arlo than in Echo. Interpretation of these results poses considerable difficulties, and it becomes necessary to invoke some form of hypothesis regarding uptake mechanisms and compartmentation.

Current views on the location of uptake mechanisms have been reviewed in Chapter II. The observations made in this study would appear to be explicable in terms of the multiphasic seris model proposed by Nissen (1974). This involves a single, multiphasic mechanism located at the plasmalemma and a similar mechanism located at the tonoplast. At low

external ion concentrations the high affinity mechanism located at the plasmalemma is primarily responsible for ion uptake into the cytoplasm. At higher concentrations a similar mechanism, located at the tonoplast becomes active, transporting ions into the vacuole. It will be recalled that the Vmax for Arlo in the high range was found to be 1.6 μmole/g/h compared with 0.9 for Echo. It was also found that the percentage of inorganic phosphate found in the root tissue of Arlo was consistently higher than in root tissue of Echo. It is suggested that the higher values for inorganic phosphorus in root tissue of Arlo are a consequence of a much more active uptake mechanism operative in the high range at both the plasmalemma and tonoplast giving rise to high concentrations of inorganic phosphate in both vacuole and cytoplasm. An abnormally high concentration of inorganic phosphate in the cytoplasm may well be the basis of toxicity symptoms through interference with normal metabolic activities as was suggested earlier.

Nissen (ibid.) points out that models of this type have inherent limitations. One of the objections to the multiphasic model is the fact that in plots of uptake vs. concentration no sigmoidal isotherms have been reported. It would also appear from the results of this study that phosphate uptake is not subject to some form of feedback control mechanism; that is, the roots continue to absorb phosphate beyond levels required for optimum plant growth. These two points illustrate the dangers inherent in too literal an application

of the concepts of enzyme kinetics to the study of ion uptake in plants. It must also be borne in mind that models such as those referred to are limited in that they only provide information on the rate-limiting step and do not indicate rate limitations by organelles, vesicles, etc., which must certainly participate in ion transport in various ways.

Mention might be made here of the high concentration of phosphate required in solution cultures to achieve maximum growth compared to phosphate concentrations found in fertile soils. This is true not only of the cultivars used in these experiments but is generally true of all plants grown in nutrient solution, although it should be noted that high phosphate requirements are characteristic of the genus Brassica (Salisbury, 1969). Asher and Loneragan (1967) have suggested that this may be due in part to the considerable technical difficulties in maintaining low phosphate concentrations in solution culture due to interactions with other ions. One must bear in mind, therefore, that because of these interactions, only a small percentage of the total phosphorus in solution may be available for plant uptake at any given time, thus avoiding the tendency to overestimate the concentration of phosphate necessary for healthy plant growth. Nevertheless, the same investigators demonstrated through simultaneous field trial and solution culture experiments, that data obtained from solution culture studies are a reliable index of the relative phosphate requirements of different species.

It was observed by the author during this investigation that root hair development is severely impaired by solution culture methods, and it is suggested that this may also account for the high concentrations of nutrients necessary for optimum yield in solution culture experiments.

Laties (1969) has raised the extremely valid point that it is difficult to conceive of the evolutionary pressure for the development of a parallel absorption system with an ion affinity almost three orders of magnitude less than that for system one. While Laties was referring specifically to the parallel model as opposed to the series model, the question is still a valid one regardless of the model in question. An explanation of this phenomenon was put forth previously by Epstein (1966) who suggested that dual uptake mechanisms enable plants to face a dual problem in coping with diverse mineral substrates. He resolves the problem by suggesting that high affinity mechanisms in the low range enable plants to take up ions which may be present in the external solution in low concentrations but which must be absorbed in substantial amounts. He sees the elaboration of a mechanism of the second type (low affinity, operative at high concentrations), having come about due to the necessity for plants to maintain a high internal osmotic pressure when faced with high ion concentrations in the environment.

During the course of this work, the writer has noted several possible avenues of further research related to this topic, but among them, two may be singled out as being of

primary importance. One of these is the need for a thorough investigation of the phenomenon of phosphorus toxicity as it occurs at the sub-cellular level. The other is the need for clarification of the storage forms of phosphorus found in higher plants during various stages of the life cycle, particularly with respect to the genus Brassica whose members exhibit a high requirement for phosphorus. While research into these areas might seem to be more appropriately relegated to the realm of the biochemist, progress in recent years in the fields of plant physiology and biochemistry has rendered any division of the two a purely arbitrary one, justifiable on practical grounds only. In reality they are simply convenient terms for separate points on a continuum of scientific endeavor.

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to all members of the Department of Plant Science including Faculty, Staff, and fellow graduate students who gave freely of their time and assistance throughout the course of this study. A great many people helped in many ways but special thanks is extended to Dr. L. J. LaCroix for his constant encouragement and to Miss A. Wilson for her help in preparation of the manuscript.

BIBLIOGRAPHY

1. ANDERSON, W. P. and HOUSE, C. R. 1967. A correlation between structure and function in the root of *Zea mays*. J. Exp. Bot. *18*: 544-555.

2. ASHER, C. J. and LONERAGAN, J. F. 1967. Response of plants to phosphate concentration in solution culture: I. Growth and phosphorus content. Soil Sci. *103*: 225-233.

3. BENSON, A. A., BASSHAM, J. A., CALVIN, M., GOODALE, T. C., HAAS, V. A. and STEPKA, W. 1950. The path of carbon in photosynthesis. V. Paper chromatography and radioautography of the products. J. Am. Chem. Soc. *72*: 1710-1718.

4. BHATTI, A. S. and LONERAGAN, J. F. 1970. Phosphorus concentrations in wheat leaves in relation to phosphorus toxicity. Agron. J. *62*: 288-290.

5. BIDDULPH, Susann F. 1956. Visual indications of ^{35}S and ^{32}P translocation in the phloem. Am. J. Bot. *43*: 143-148.

6. BIELESKI, R. L. 1973. Phosphate pools, phosphate transport, and phosphate availability. Ann. Rev. Plant Physiol. *24*: 225-252.

7. BIELESKI, R. L. 1968. Levels of phosphate esters in *Spirodela*. Plant Physiol. *43*: 1297-1308.

8. BONNETT, H. T. Jr. 1968. The root endodermis: fine structure and function. J. Cell Biol. *37*: 199-205.

9. BOULDIN, D. R. and SAMPLE, E. C. 1959. Laboratory and greenhouse studies with monocalcium, monoammonium and diammonium phosphates. Soil Sci. Soc. Amer. Proc. *23*: 338-342.

10. BRADY, Nyle C. 1974. The nature and properties of soils, 8th Edition. MacMillan Publishing Co., Inc., New York.

11. BRIGGS, G. E. and ROBERTSON, R. N. 1957. Apparent free space. Ann. Rev. Plant Physiol. *8*: 11-30.

12. BUTLER, G. W. 1953. Ion uptake by young wheat plants. II. The "apparent free space" of wheat roots. Physiol. plant. *6*: 617-624.

13. CRAFTS, A. S. and BROYER, T. C. 1938. Migration of salts and water into the xylem of the roots of higher plants. Am. J. Bot. *25*: 529-535.

14. COLLANDER, R. 1941. Selective absorption of cations by higher plants. Plant Physiol. *16*: 691-720.

15. DUMBROFF, E. B. and PEIRSON, D. R. 1971. Probable sites for passive movement of ions across the endodermis. Can. J. Bot. *49*: 35-38.

16. EBEL, J. P. 1952. Recherches sur les polyphosphates çontenus dans diverse cellules vivante. II. Étude chromatographique et potentiométrique des polyphosphates de levure. Bull. Sté. Chim. Biol. *34*: 330-335.

17. EDWARDS, D. G. 1968. The mechanism of phosphate absorption by plant roots. Trans. 9th Int. Congr. Soil Sci. Adelaide. *2*: 183-190 [as cited in Nissen, 1974 (56)].

18. EPSTEIN, E. 1955. Passive permeation and active transport of ions in plant roots. Plant Physiol. *30*: 529-535.

19. EPSTEIN, E. and HAGEN, C. E. 1952. A kinetic study of the absorption of alkali cations by barley roots. Plant Physiol. *27*: 457-474.

20. EPSTEIN, Emanuel. 1966. Dual pattern of ion absorption by plant cells and by plants. Nature *212*: 1324-1327.

21. EPSTEIN, E. 1953. Mechaniam of ion absorption by roots. Nature *7*: 83-84.

22. ERGLE, David R. and GUINN, Gene. 1959. Phosphorus compounds of cotton embryos and their changes during germination. Plant Physiol. *34*: 476-481.

23. ESAU, Katherine. 1965. Plant anatomy. John Wiley and Sons, Inc., New York.

24. FARBER, Eduard. 1966. History of phosphorus. Contributions from the museum of history and technology, Bulletin 240: 178-200.

25. FISHER, Donald B. 1972. Artifacts in the embedment of water-soluble compounds for light microscopy. Plant Physiol. *49*: 161-165.

26. FOOTE, Beverly D. and HOWELL, Robert W. 1964. Phosphorus tolerance and sensitivity of soybeans as related to uptake and translocation. Plant Physiol. *39*: 610-613.

27. FOWLER, D. B., and STEFANSSON, B. R. 1972. Effects of the mutagenic agent ethyl methanesulfonate on the M_1 generation of rape (*Brassica napus*). Can. J. Plant Sci. *52*: 53-62.

28. FRIED, Maurice and SHAPRIO, Raymond E. 1961. Soil-plant relationships in ion uptake. Ann. Rev. Plant Physiol. *12*: 91-112.

29. FRUTON, Joseph S. 1972. Molecules and life. Wiley-Interscience, John Wiley and Sons, Inc., New York.

30. GOBLEY, Nicolas. 1845. Comptes-Rendus. Hebdomadaires des séances de l'académie des sciences, Paris. *21*: 718.

31. GRÜNSFELDER, M. 1971. Die kinetik der phosphataufnahme bei Elodea densa. Ph.D. thesis. Univ. Würzburg, 134 pp [as cited in Nissen, 1974 (56)].

32. HALMANN, M. 1972. Analytical chemistry of phosphorus compounds. Wiley-Interscience. A Division of John Wiley and Sons, Inc., New York.

33. HARDEN, Arthur and YOUNG, William John. 1906. The alcoholic ferment of yeast-juice. Proc. Roy. Soc. (London), B. *77*: 405-420.

34. HIGINBOTHAM, N. 1973. Electropotentials of Plant Cells. Ann. Rev. Plant Physiol. *24*: 25-46.

35. HOWELL, Robert W. 1954. Phosphorus nutrition of soybeans. Plant Physiol. *29*: 477-483.

36. KITSON, R. E. and MELLON, M. G. 1944. Colorimetric determination of phosphorus as molybdovanadophosphoric acid. Ind. Eng. Chem., A.E. *16*: 379-383.

37. KOENIG, Ruth Adele and JOHNSON, C. R. 1942. Colorimetric determination of phosphorus in biological materials. Ind. Eng. Chem., A.E. *14*: 155-156.

38. KOTYK, Arnošt and JANÁČEK, Karel. 1970. Cell membrane transport. Principles and techniques. Plenum Press, New York.

39. KU, Hau San, HARLAN, K. Pratt, SPURR, Arthur R., and HARRIS, William M. 1968. Isolation of active mitochondria from tomato fruit. Plant Physiol. *43*: 883-887.

40. LATIES, George G. 1959. Active transport of salt into plant tissue. Ann. Rev. Plant Physiol. *10*: 87-109.

41. LATIES, George G. 1969. Dual mechanisms of salt uptake in relation to compartmentation and long-distance transport. Ann. Rev. Plant Physiol. *20*: 89-116.

42. LÄUCHLI, A. 1972. Translocation of inorganic solutes. Ann. Rev. Plant Physiol. *23*: 197-218.

43. LÄUCHLI, A. 1967. Investigation on the distribution and transport of ions in plant tissues with the X-Ray microanalyzer. I. Experiments on vegetative organs of *Zea mays*. Planta *75*: 185-206.

44. LEGAL, C. C. and MYRICK, O. D. Jr. 1968. History and status of phosphoric acid. Phosphoric acid, *1*: 1-90. Marcel Dekker, Inc., New York.

45. LEVITT, J. 1957. The significance of "Apparent Free Space" (A.F.S.) in ion absorption. Plant Physiol. *10*: 882-888.

46. LEWIS, E. T. and RACZ, G. J. 1969. Phosphorus movement in some calcareous and noncalcareous Manitoba soils. Can. J. Soil Sci. *49*: 305-312.

47. LÜTTGE, U., Ed. 1972. Microautoradiography and electron probe analysis. Springer-Verlag, Berlin.

48. LÜTTGE, U. and WEIGL, J. 1962. Mikroautoradiographische untersuchungen der aufnahme und des Transportes von $^{35}SO_4$ und ^{15}Ca in Keimwurzeln von *Zea mays* L. und *Pisum sativum*. Planta. *58*: 113-126.

49. LYNESS, A. S. 1936. Varietal differences in the phosphorus feeding capacity of plants. Plant Physiol. *11*: 665-688.

50. MacROBBIE, E. A. C. 1971. Fluxes and compartmentation in plant cells. Ann. Rev. Plant Physiol. *22*: 75-96.

51. MAIZEL, J. V., BENSON, A. A. and TOLBERT, N. E. 1956. Identification of phosphoryl choline as an important constituent of plant saps. Plant Physiol. *31*: 407-408.

52. MERRILL, L. M. Box experiments with phosphoric acid from different sources. Annual Report of Maine Agr. Exp. Station. 64-74. 1898. As found in soil and fertilizer phosphorus in crop nutrition. IV. 55. 1953.

53. MICHAELIS, L. and MENTEN, M. L. 1913. Die kinetik der invertinwirkung. Biochem. Zeits. *49*: 333-369.

54. MIYACHI, Shigetoh. 1961. Inorganic polyphosphate in leaves. Journal of Biochemistry *50*: 367-371.

55. MUKHERJI, S., DEY, B., PAUL, A. K. and SIRCAR, S. M. 1971. Changes in phosphorus fractions and phytase activity of rice seeds during germination. Plant Physiol. *25*: 94-97.

56. NISSEN, Per. 1971. Uptake of sulfate by roots and leaf slices of barley: mediated by single, multiphasic mechanisms. Plant Physiol. *24*: 315-324.

57. NISSEN, Per. 1974. Uptake mechanisms: inorganic and organic. Ann. Rev. Plant Physiol. *25*: 53-79.

58. NISSEN, Per. 1973. Multiphasic uptake in plants. 1: Phosphate and sulfate. Plant Physiol. *28*: 304-316.

59. PARKER, R. P. and ELRICK, R. H. 1970. Cerenkov counting as a means of assaying β-emitting radionuclides, pp. 110-122. *In* The Current Status of Liquid Scintillation Counting. Ed. by Bransome, Edwin D., Jr. Grune and Stratton, New York.

60. PHILLIPS, James W., BAKER, D. E. and CLAGETT, C. O. 1971. Kinetics of P absorption by excised roots and leaves of corn hybrids. Agron. J. *63*: 517-520.

61. PONS, W. A. Jr. and GUTHRIE, John D. 1946. Determination of inorganic phosphorus in plant materials. Ind. Eng. Chem., A.E. *18*: 184-186.

62. RACZ, G. J., WEBBER, M. D., SOPER, R. J. and HEDLIN, R. A. 1965. Phosphorus and nitrogen utilization by rape, flax and wheat. Agron. J. *57*: 335-337.

63. RAHMAN, Tamar and KRISHNAN, P. S. 1971. Phosphorus, nitrogen and carbohydrate composition of the seeds of Cuscuta. Phytochemistry *10*: 1751-1757.

64. RAINS, D. W. 1972. Salt transport by plants in relation to salinity. Ann. Rev. Plant Physiol. *23*: 367-388.

65. RATNER, Ariel and JACOBY, B. 1973. Non-specificity of salt effects on Mg^{2+}-dependent ATPase from grass roots. J. Exp. Bot. *24*: 231-238.

66. SAKAI, A. and OTSUKA, K. 1967. Survival of plant tissue at super-low temperatures. V. An electron microscope study of ice in cortical cells cooled rapidly. Plant Physiol. *42*: 1680-1694.

67. SALISBURY, Frank B. 1969. Plant Physiology. Wadsworth Publishing, Inc., Belmont, California. Chap. 7.

68. SLACK, A. V. 1968. Phosphoric Acid. Marcel Dekker, Inc., New York.

69. SMITH, Richard C. and EPSTEIN, Emanual. 1964. Ion absorption by shoot tissue: Technique and first findings with excised leaf tissue of corn. Plant Physiol. *39*: 338-341.

70. SMITH, S. N. 1934. Response of inbred lines and crosses in maize to variations of nitrogen and phosphorus supplied as nutrients. J. Amer. Soc. Agron. *26*: 785-804.

71. 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.

72. TISDALE, Samuel L. and NELSON, Werner L. 1966. Soil fertility and fertilizers. The MacMillan Company, New York (2nd edition).

73. TORÜ, Jenji and LATIES, George G. 1966. Dual mechanisms of ion uptake in relation to vacuolation in corn roots. Plant Physiol. *41*: 863-870.

74. VAN WAZER, John R. 1961. Phosphorus and its compounds. Vol. II. Interscience Publishers, Inc., New York.

75. WARREN, R. G. and BENZIAN, B. 1959. High levels of phosphorus and die-back in Yellow Lupins. Nature *184*: 1588.

76. WEBBER, M. D. 1963. The phosphorus feeding habits of flax, cereals and rape. M.Sc. Thesis, Univ. of Manitoba, Winnipeg.

77. WELCH, R. M. and EPSTEIN, Emanuel. 1968. The dual mechanisms of alkali cation absorption by plant cells: their parallel operation across the plasmalemma. Proc. Nat. Acad. Sci., U.S.A. *61*: 447-453.

78. WIAME, J. M. 1949. The occurrence and physiological behavior of two metaphosphate fractions in yeast. J. Biol. Chem. *178*: 919-929.

79. WINTERMANS, J. F. G. M. 1955. Polyphosphate formation in chlorella in relation to photosynthesis. Mededelingen Van de Landbouwhoceschool te Wageningen/Nederland. *55*(2): 69-126.

80. WITTCOFF, Harold. 1951. The phosphatides. Reinhold Publishing Corporation, New York. p. 5.

APPENDIX

An attempt was made to follow the lateral pathway of inorganic phosphate across the root by feeding whole plants with $H_3{}^{33}PO_4$ followed by microautoradiography of the specimens using the method of Fisher (1972).

Seeds were germinated and grown in nutrient solution as before. After two weeks, plants were transferred to a 1×10^{-4}M solution of calcium sulfate and cultured for 24 hours. The root portion of each plant was then immersed in a test tube containing 80 ml of a solution of 5 mM potassium phosphate adjusted to pH 6.5 and containing 0.05 mc of ^{33}P. The solution was continuously aerated throughout the absorption period and an artificial light source consisting of a mixture of cool white fluorescent tubes and incandescent bulbs yielding approximately 800 ft-c was provided. The roots remained in solution from one to 10 minutes after which they were removed and rinsed for one minute in 0.01M potassium phosphate at pH 6.5 followed by one minute in distilled water.

Two methods for quick-freezing the tissue were evaluated. Copper-constantin thermocouples were inserted into sections of stem tissue 10 mm long by four mm thick. In method (1), the tissue was immersed directly in liquid nitrogen. A continuous recording of tissue temperature was obtained with a Honeywell strip-chart recorder. In method (2), the tissue was immersed in a bath of isopentane chilled to -155C. The bath consisted of an 80 ml test tube filled with isopentane

which was suspended by means of a clamp and stand inside a Dewar flask containing liquid nitrogen. In method (1), the temperature of the tissue fell from 0C to -50C in 19 seconds while in method (2), a similar drop in temperature occurred in only two seconds. Consequently, the latter method was employed thereafter since the more rapid cooling rate attained by the use of chilled isopentane would lessen the likelihood of damage to cell membranes.

Segments of each plant previously fed ^{33}P were removed and frozen for 30 seconds according to method (2). Each segment comprised that tissue bounded by points 5 mm below and 10 mm above the branch point of the root. They were then transferred to 25 ml screw-cap vials filled with twice distilled acetone precooled to -70C and held under these conditions for five days. A transfer to fresh dry acetone followed and after 48 hours, the vials were removed from the cold and allowed to come gradually to room temperature. Following two more transfers to fresh acetone (2 x 1 hour) at room temperature, root and hypocotyl regions were separated by cutting at the root branch point under acetone. Individual specimens were embedded in Epon by infiltrating with Spurr's embedding mixture. The tissue and resin were transferred to silicone rubber embedding molds and polymerized at 60C for 12 to 15 hours.

Sections of embedded material 5 μ thick were cut on a glass knife and transferred to glass slides where they were floated on 95% ethyl alcohol. Adhesion of the sections to the

slides was effected by warming the slides gently on a hot plate until the alcohol had completely evaporated.

Microautoradiographs were prepared by dipping the slides with attached sections in Kodak NTB_2 liquid emulsion. After a suitable exposure time they were developed in D-19 developer for 3 minutes, washed for 10 seconds in distilled water and fixed for 3 minutes in Kodak rapid fix. A constant temperature of 18C was maintained during all steps of the photographic procedure. The slides were then washed, dried and stored for microscopic examination.

Examination under the light microscope failed to reveal the presence of exposed granules other than those attributable to background as determined by comparison with blank controls. One exception was the presence of high density areas in the vicinity of the root primordium of one specimen in which the apical meristem of a branch root had reached a point in the cortex approximately three-quarters of the distance between pericycle and epidermis.

The reasons for the failure of this technique are difficult to determine but several possibilities exist. The size and nature of the tissue may have been contributing factors. The relatively large amount of tissue used and its heavily cutinized layer of mature epidermis may have prevented both the complete removal of water during the freeze-substitution period and/or thorough penetration of the embedding medium. The possibility of membrane damage at some stage of the procedure may also have been a factor. Since

localization of inorganic solutes by the freeze-substitution method is dependent on the maintenance of the integrity of all membranes in order to prevent subsequent leakage, disruption of the membrane would result in the loss of cellular contents. The appearance of exposed granules in the region of the secondary root meristem is significant since it suggests that inorganic phosphate may have been fixed into organic compounds due to the intense metabolic activity taking place in that region of the tissue.

Regardless of the cause or causes of failure, the use of the freeze-substitution method for localization of inorganic phosphate in this type of plant tissue is not recommended.