THE REGULATION OF PHOSPHATE UPTAKE BY INTACT BARLEY PLANTS

by

DANIEL DENIS LEFEBVRE

B.Sc., University of Ottawa, 1977

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Department of Botany)

We accept this thesis as conforming to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

1980

In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the Head of my Department or by his representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of BOTANY

The University of British Columbia
2075 Wesbrook Place
Vancouver, Canada
V6T 1W5

Date June 12, 1980

ABSTRACT

The study of phosphate influx in roots of intact barley (Hordeum vulgare L. var. Bonanza) revealed the presence of two distinct regulatory processes for phosphate absorption.

One of these processes, which was elicited in response to phosphate deprivation, in the form of enhanced phosphate uptake, became evident between 11 and 13 days after germination. At 16 days the uptake rates of these plants had reached a maximum value at 2.43μmol/g.f.wt./hour which compared to a value of 0.39μmol/g.f.wt./hour for phosphate sufficient plants. Simultaneously, differences between the respective treatments were also noted in growth rates and phosphate pools.

A second regulatory process brought about a rapid reduction of phosphate influx upon the provision of orthophosphate to plants previously starved of phosphate during the phase of enhanced uptake. Within hours of supplying inorganic phosphate to these plants influx was reduced by greater than 50% and during this period influx values were linearly correlated with root orthophosphate concentrations. The time scale of this second response is suggestive of an allosteric inhibition of influx by internal orthophosphate levels.

Both regulatory systems studied represented physiological adaptations which would better enable plants, under field conditions,

to obtain a sufficient phosphate supply. Severe phosphorus deprivation eventually resulted in a morphological response such as the production of longer, narrower roots providing the plants a greater surface area, presumably for greater phosphate absorption. At a later time, an increased formation of root-hairs resulted in even greater surface area modification.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (continued)

ACKNOWLEDGEMENTS

I would like to thank my graduate advisor, Dr. Anthony D. M. Glass, Botany Department, University of British Columbia, for his technical assistance and support throughout the course of the graduate work.

Thanks is also extended to my advisory committee consisting of Dr. Beverly R. Green and Dr. Paul J. Harrison.

Special appreciation for the patience, understanding, and help I have received, goes to my dear friend Dr. Lois Shepherd and to my parents.

This work was supported by the Natural Sciences and Engineering Research Council of Canada, through a grant to Dr. Glass.

I. INTRODUCTION

There is now considerable evidence to suggest that the active or energy dependent uptake of distinct inorganic ions by the roots of higher plants, is subject to independent negative feedback control (Cram, 1976). This control is thought to be elicited in response to changes of the internal concentration of the particular nutrient, which ultimately exerts control over the root uptake process.

The investigation of the mechanism underlying the control of uptake would appear, a priori, to be more complicated in the cases of metabolized ions, such as SO_4^{2-}, NO_3^-, $H_2PO_4^-$, etc., by virtue of the diversity of the end products of their metabolism. This may consequently account for the more extensive investigations of this aspect of uptake of non-metabolized ions. Thus, feedback control mechanisms have been proposed for potassium (Humphries, 1951; Johansen et. al., 1970; Young et. al., 1970; Glass, 1976; Jensen and Pettersson, 1978), Na^+ (Pitman et. al., 1968), Cl^- (Cram, 1968; 1973; Mott and Steward, 1972), and Br^- (Sutcliffe, 1954; Cseh et. al., 1970). In the case of an ion which is metabolized, control of influx might be achieved via concentrations of the ion itself or one of its metabolic products. Sulfate uptake for example, has been shown to be reduced by prior feeding with the sulfur-containing amino acids

cysteine and methionine (Hart and Filner, 1969; Ferrari and Renosto, 1972; Cram, 1976). Similar results have been obtained for the influence of NH_4^+ and amino acids upon nitrate uptake (Heimer and Filner, 1970).

Plants grown in low phosphorus regimes consistently display elevated rates of phosphate uptake (Humphries, 1951; Bowen, 1970; Barber, 1972; Cartwright, 1972; Clarkson et. al., 1978) and analysis of total phosphate concentrations in these plants reveals that influx and phosphate content are negatively correlated (Barber, 1972; Clarkson et. al., 1978).

Living organisms might be imagined to possess long- and short-term mechanisms by which they are able to modify their rates of ion uptake through biomembranes. The "carrier" systems responsible for transport across natural membranes are thought to be composed of protein molecules (Mitchell, 1967). On a long-term basis "carrier" degradation or synthesis could effect changes of transport rates. A much more rapid, direct enzymatic control mechanism could be elicited by allosteric control of "carrier" activity or energy supply for the specific transport phenomenon in question. Fluctuation of nutrient levels within plants could act as the signal for nutrient uptake regulation through the mechanisms described above. The present study is directed toward a better understanding of the signals and mechanisms which govern phosphate uptake in barley (Hordeum vulgare (L.) c.v. Bonanza).

In higher plants internal orthophosphate concentration is much more susceptible to fluctuations in phosphorus supply than the organic phosphorus concentration (Bieleski, 1968; Nassery, 1971). Because of the flexibility of the inorganic phosphate level its absolute magnitude within plant cells represents a good candidate for effective regulation of phosphate uptake. With this in mind the analysis of inorganic and organic phosphate levels within the plant was conducted simultaneously with measurements of phosphate uptake rates under different external phosphate regimes. The plant chosen for this study, namely barley, is one of economic value and representative of the cereal crop plants raised in Canada.

II. METHODS

1. Hydroponic Plant Growth

A. Seeds

Seeds of barley (Hordeum vulgare (L.) c.v. Bonanza) purchased from Buckerfields, Vancouver, were surface sterilized for ten minutes in 1% hypochlorite and after several washings with distilled water were germinated in sand at 27±2C.

B. Choice of Phosphate Levels and Growth Regimes

Many workers in previous studies have employed phosphate levels which were unnaturally high (Cartwright, 1972; Clarkson et. al., 1978; Bieleski, 1968; Barber, 1967). It was considered worthwhile in the present study to use phosphorus levels which were more representative of soil solution phosphorus concentrations (Bieleski, 1973). These criteria are met by a 15μM orthophosphate level in hydroponic media. Plants were therefore grown in either full nutrient solution (+P) or nutrient solution in which no phosphorus was supplied (-P, see Table 1). Because of the large volume of circulated growth medium it was necessary to monitor and top up phosphate levels to 15μM only twice daily. Both +P and -P growth media were replaced in full every four days. Plants were grown in an environmental regime which consisted of 16h days at 27±2C and

irradiance of 3.0 mW cm^{-2}, and 8h nights at 19±2C.

Table 1. Composition of hydroponic growth media

- Concentrations are µMolar	
-P 111.2 KNO_3	0.11 $MnSO_4.H_2O$
83.3 $Mg(NO_3)_2$	0.11 $ZnSO_4.7H_2O$
27.8 $MgSO_4$	0.03 $CuSO_4.5H_2O$
55.6 $Ca(NO_3)_2$	0.03 H_2MoO_4
2.7 KCl	0.11 FeEDTA
1.4 H_3BO_3	
Buffered to pH 6.25 with 10µM Na_3Citrate: Citric Acid	
+P as -P plus	
5.0 Na_2HPO_4	
10.0 NaH_2PO_4	

2. Determination of Phosphate Influx Method

A. Root Wash Period

The cell wall or free space of plant cells contain substantial ion reservoirs which possess half-lives for ion exchange of the order of 1-3 min (Walker and Pitman, 1976; Cram, 1973; Dainty and Hope, 1959). Prior to influx experiments of relatively short duration, designed to estimate initial plasmalemma fluxes, it is necessary therefore to standardize the cell wall phosphate status of roots grown in differing phosphate regimes. Otherwise the possible transfer of phosphorus within the root free space to the uptake solution would change the specific activity of phosphate in the uptake solution and introduce uncertainty to the calculated flux.

By loading the root with 15μM ^{32}P-labelled orthophosphate and subsequently transferring these roots to non-labelled 15μM orthophosphate a measure of the half-life of cell wall exchange can be made. This was done by measuring release of ^{32}P to the non-labelled wash medium at intervals of time up to 30 min after transfer. The estimated value of the half-life for cell wall ion exchange can be used to determine the appropriate duration of the standardizing prewash.

To obtain an estimate of plasmalemma influx it is necessary to distinguish the active uptake from passive adsorption in the cell

wall. The half-life for cell wall exchange can be used here to obtain the necessary separation.

B. Phosphate Influx

Rates of orthophosphate uptake were determined after a 5 min prewash in 50μM $CaSO_4$ at 30C. The uptake solution was identical to the +P growth medium with ^{32}P-labelled orthophosphoric acid and/or $^{86}RbCl_2$ added. Either 10 min (short-term) or 24h (long-term) uptake periods were used. In all but one experiment the uptake solution was vigorously stirred and aerated. Uptakes were performed at 30C in solution volumes (1.6 l) where nutrient depletion was negligible. The uptake period was followed immediately by a 5 min desorption period in cold +P solution at 4C. Thereafter plant samples were spun in a basket centrifuge to remove extraneous water. These samples were weighed into glass vials to obtain fresh weights and finally ashed at 500C. The resultant ashed samples were dissolved in 10 ml distilled H_2O and their radioactivity was determined by Cerenkov counting (Lauchli, 1969; Glass, 1978a) with an Isocap/300 liquid scintillation counter.

3. Efflux Analysis

Efflux determination was performed after feeding plants in a constant $^{32}P/P$ +P nutrient solution for five days. These plants were then placed in non-labelled +P solutions at 30C and an irradiance level of 3.0 mW cm^{-2}. In order to estimate efflux uncomplicated by plant reabsorption of isotope, standard procedure is to replace the efflux medium with fresh non-labelled solution at regular intervals. By this methodology isotopic flux from the medium back to the cytoplasm is presumed to be zero. At the end of the efflux analysis total phosphate content as well as isotopic content remaining in the root tissue, was determined. This enabled subsequent calculations of the pattern of efflux according to standard methodology (Walker and Pitman, 1976).

4. Determination of the Plants' Phosphate Concentrations

A. Total Phosphate Concentrations

Fresh root and shoot samples were weighed, ashed, dissolved in 10ml distilled water, and assayed for total phosphorus by the method of Eibl and Lands (1969). Values expressed throughout the text are in µmol/g.f.wt.

B. Inorganic Phosphate Concentrations

Inorganic phosphorus pools of both shoots and roots were obtained by a variation of the method of Daley and Vines (1977). Samples were plunged into boiling water for two min, then were rapidly frozen, thawed, and placed in boiling baths for 5 min, twice in succession. Using samples of glucose-6-P and ATP, it was established that this method,as claimed by Hulett (1970), caused no hydrolysis of organic P bonds.

5. Double Labelled Uptake Determination

^{32}P and ^{86}Rb were used simultaneously to determine phosphate and potassium influxes (Lauchli and Epstein, 1970) respectively. The separation of these isotopes was obtained by anion-exchange chromatography of the ashed samples. Dowex-1x8-100 was primed in one hundred times its volume of 5M NaOH. 2 ml Pasteur pipette columns were then poured and washed three times with deionized water. 2 ml of wet Dowex-1x8-100 resin has an exchange capacity of 2.8 milli-equivalents. By the means described in Table 2 a suitable regime was defined for use on biological samples. The separation of ^{86}Rb and ^{32}P by this method was equally effective whether these isotopes were present in carrier-free solutions or in solutions more representative of the natural distribution of potassium and phosphate. Samples were counted before and after exposure to the defined regime. Initial counts gave estimates of $^{86}Rb + ^{32}P$ activity. Counting of the eluate obtained after anion-exchange chromatography gave ^{86}Rb activity. ^{32}P counts were obtained by subtracting the latter activity from the combined $^{86}Rb + ^{32}P$ radioactivity.

Table 2. Determination of regime required for elution of all ^{86}Rb and retention of all ^{32}P from anion-exchange columns. 10 ml of solutions shown (A to F) were applied to the columns and eluate collected 1. without further column washing. 2. after elution with 10 ml H_2O. 3. after elution with a further 10 ml of H_2O.

Efficiency of elution of ^{86}Rb or ^{32}P expressed as % of known counts applied to column			
		^{86}Rb	
Treatment	A. Carrier-free	B. +0.1M K	C. +0.1M K + ^{32}P + 2.0mM P
1.	75.5	80.7	81.6
2.	24.0	20.3	18.4
3.	0.5	nil	nil
		^{32}P	
	D. Carrier-free	E. +2.0mM P	F. +2.0mM P + ^{86}Rb + 0.1M K
1.	nil	nil	nil
2.	nil	nil	nil
3.	nil	nil	nil

6. Determination of Root External Protein

The eosin protein staining technique of Williams (1962) was used to determine root external protein. The procedure is presented in Table 3.

Table 3. Eosin staining technique

Treatment	Duration (min)
1. Weighing of root samples	
2. 2 distilled water rinses	
3. 0.1 N HCl	1.0
4. 5 distilled water rinses	
5. 0.2% eosin	0.5
6. 5 distilled water rinses	
7. 0.1 M KCl + KOH (pH = 13.0) 3 ml. volume	0.5
8. Optical Density measurement at 520 nm.	

III. RESULTS AND DISCUSSION

1. Effect of Phosphate Level on Growth

Fresh weights of barley plants were monitored for a 20 day growth period (see Figure 9). There were no differences between +P and -P seedlings up to day 11 ($\alpha = 0.05$), beyond which stage the fully nourished plants exhibited exponential growth rates while the phosphate starved plants increased their mass at a linear rate (Treatments significantly different at 12 days and older, $\alpha = 0.05$, see Table 4). By day 20 the ratio of +P to -P plant weights was in excess of two but no qualitative morphological differences were apparent between the treatments. The diameters of roots in the +P and -P plants are shown in Figures 1 to 8. Phosphate stress has been previously shown to result in decreased root diameters (Bowen et. al., 1974). Increased root-hair development became apparent in -P roots only well after day 20 (see Figures 7 and 8).

Beyond day 13 -P plants demonstrated statistically significant ($\alpha = 0.05$) root:shoot ratio increases (see Figure 10) when compared to +P plants. By day 20 for example, -P plants had favoured root growth to such an extent that the root:shoot ratio equalled 2 compared to 0.5 for +P plants. This preferential growth enabled the -P plants to form almost as much root mass as the fully nourished plants (Figure 11). In the soil environment where phosphate supply may be limited and depletion zones sharply localized due to the

Table 4. +P and -P growth equations

A. +P exponential growth equation from day 5 to 20.

$y = 0.1072e^{0.1743x}$, $r = 0.9967$

B. -P linear growth equation from days 12 to 20.

$y = -1.1667 + 0.13983x$, $r = 0.9679$

y = plant size (g.f.wt./plant)

x = age of plants (days)

relative immobility of phosphate, increased surface area and soil exploration would be an adaptive advantage (Harley, 1969).

Growth studies performed with numerous species under various phosphorus regimes have shown positive correlations between growth and P_i supply. The magnitude of growth response is dependent upon the species involved (Clarkson, 1967; Piggott, 1971; Rorison, 1968; Asher and Loneragan, 1967; Bradshaw et. al., 1960). Root to shoot ratios often increase in plants grown in low phosphate environments (Hackett, 1968; Asher and Loneragan, 1967), but this is not always the case (Troughton, 1977). Increased root growth, however, might have occurred if Troughton had used lower levels of phosphorus (Bradshaw et. al., 1960). The root:shoot ratios of a number of grasses grown at different P-levels are comparable to the barley data obtained (Figure 10). Because root-hair formation is retarded in hydroponic culture (Bole, 1973), it is of particular interest that root-hairs were observed to develop under severe P-deficiency and only once has this phenomenon been previously reported (Brewster et. al., 1976). Root-hairs enable plants to increase their P-uptake from the soil environment where available phosphate is strongly localized and diffusion is often limiting the uptake process (Barley and Rovira, 1970).

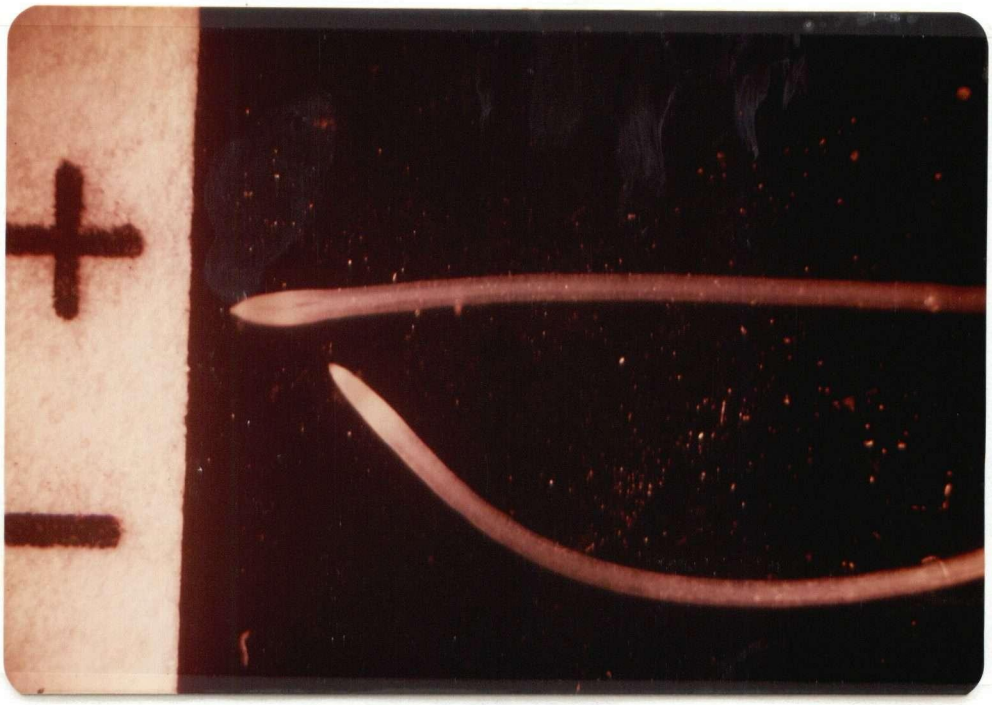


Figure 1. 5 day old root tips (8 times life-size)

Figure 2. 10 day old root tips (8 times life-size)

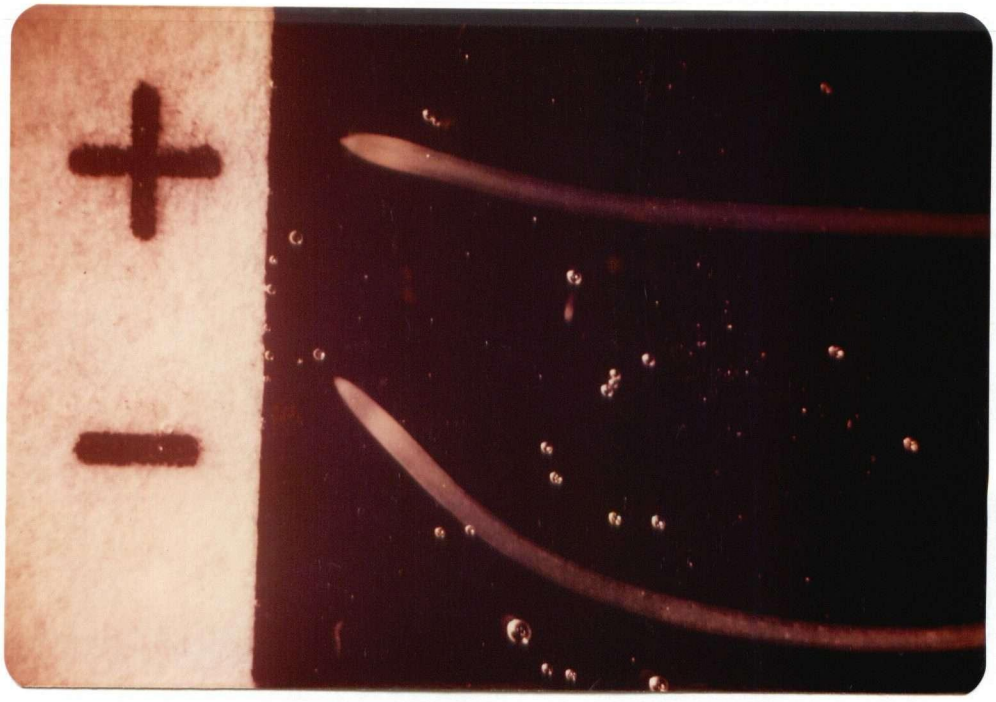


Figure 3. 15 day old root tips (8 times life-size)

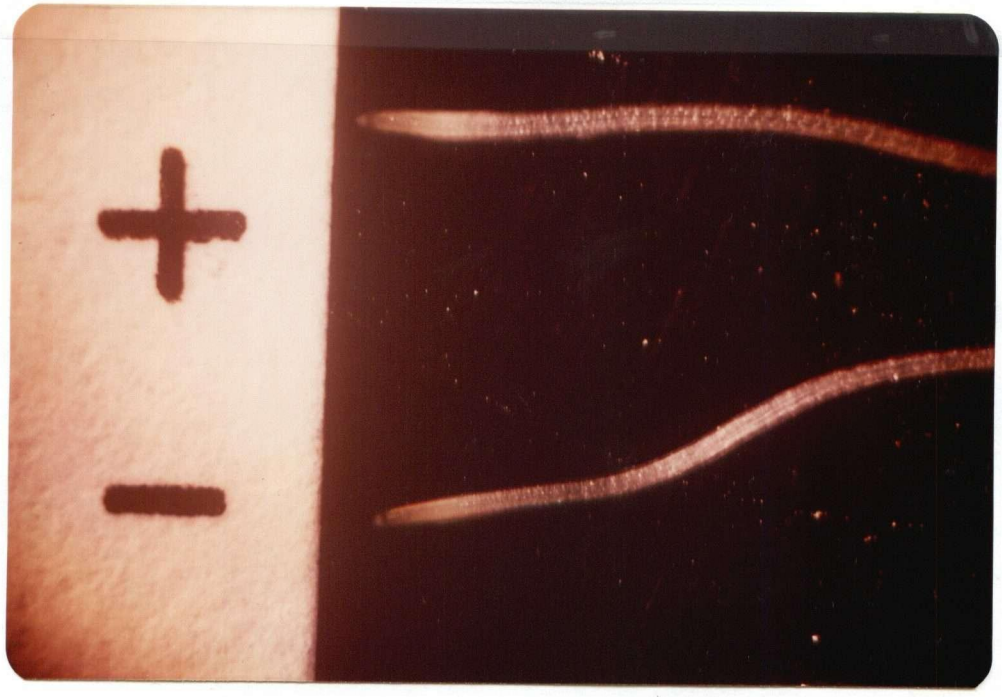


Figure 4. 20 day old root tips (8 times life-size)

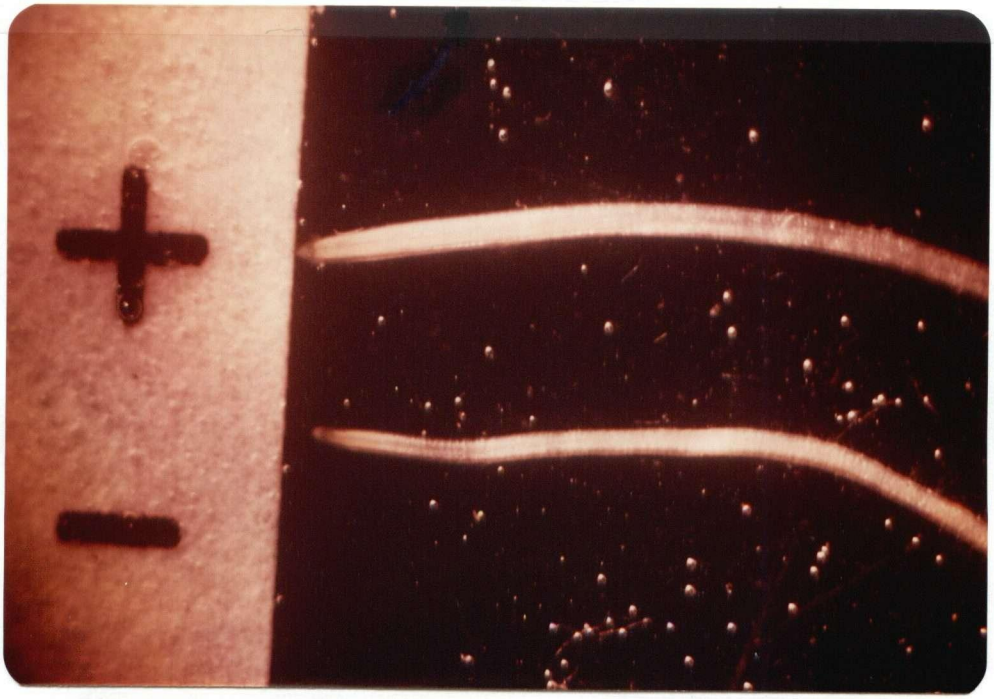


Figure 5. 25 day old root tips (10 times life-size)

Figure 6. 30 day old root tips, +P above, -P below (26 times life-size)

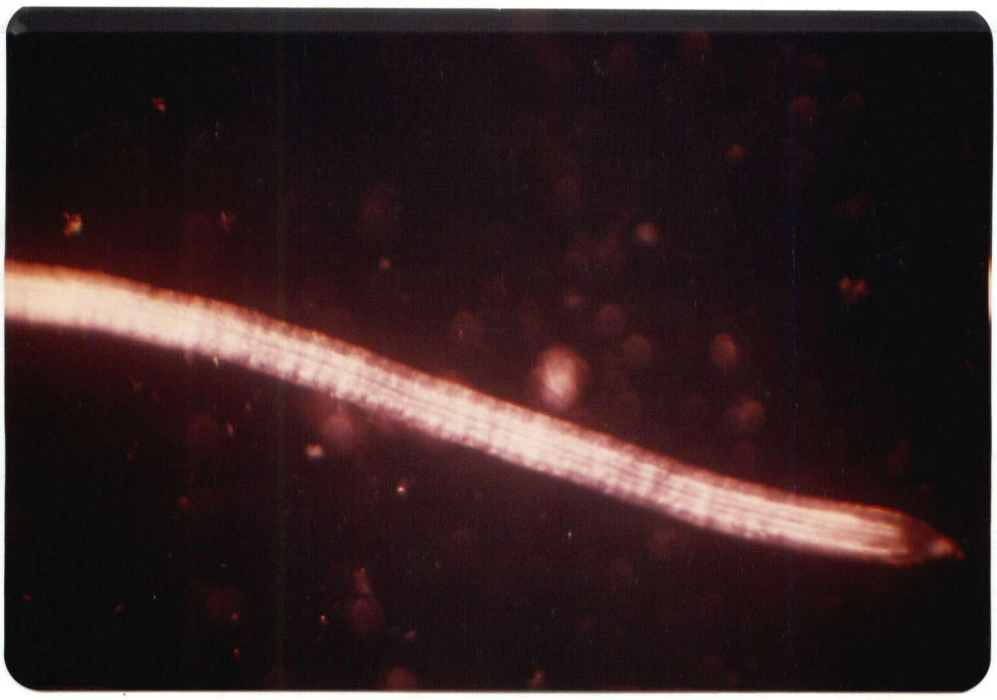


Figure 7. 40 day old +P root tip (16 times life-size)

Figure 8. 40 day old -P root tip (30 times life-size)

Figure 9. Plant growth vs. age

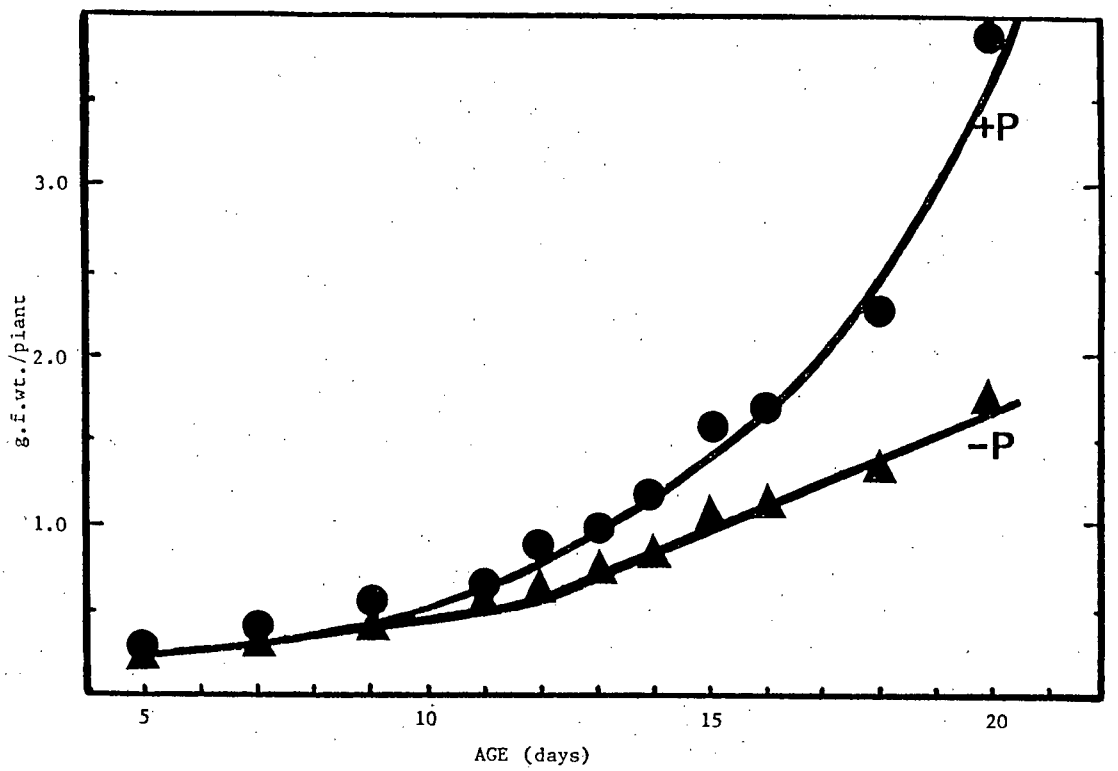


Figure 10. Root: shoot ratio vs. plant age

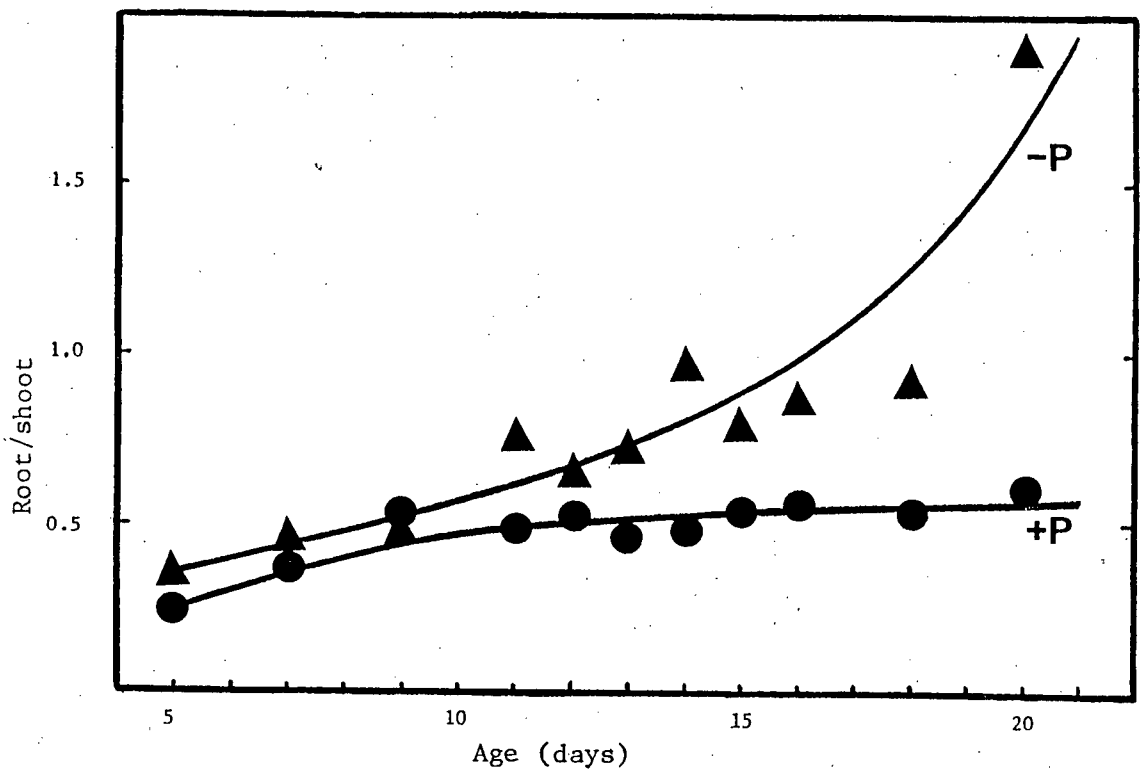
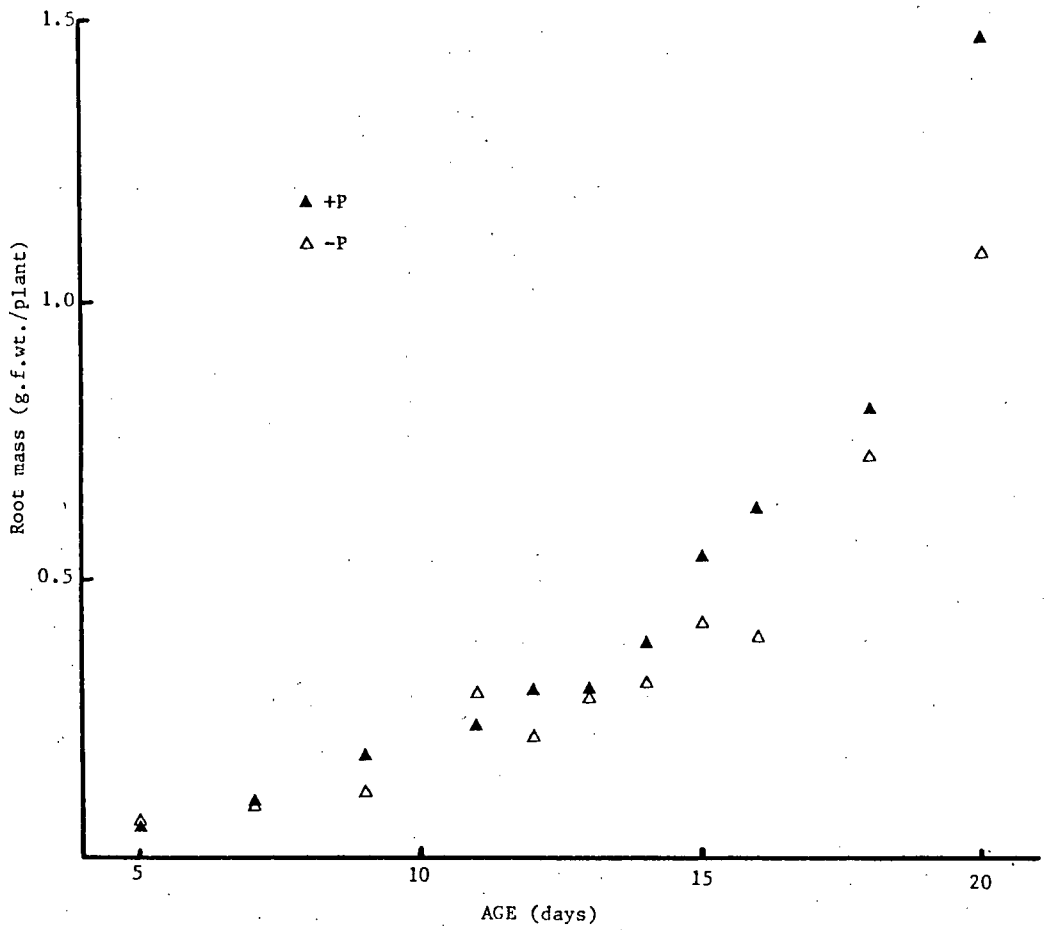


Figure 11. Growth of roots

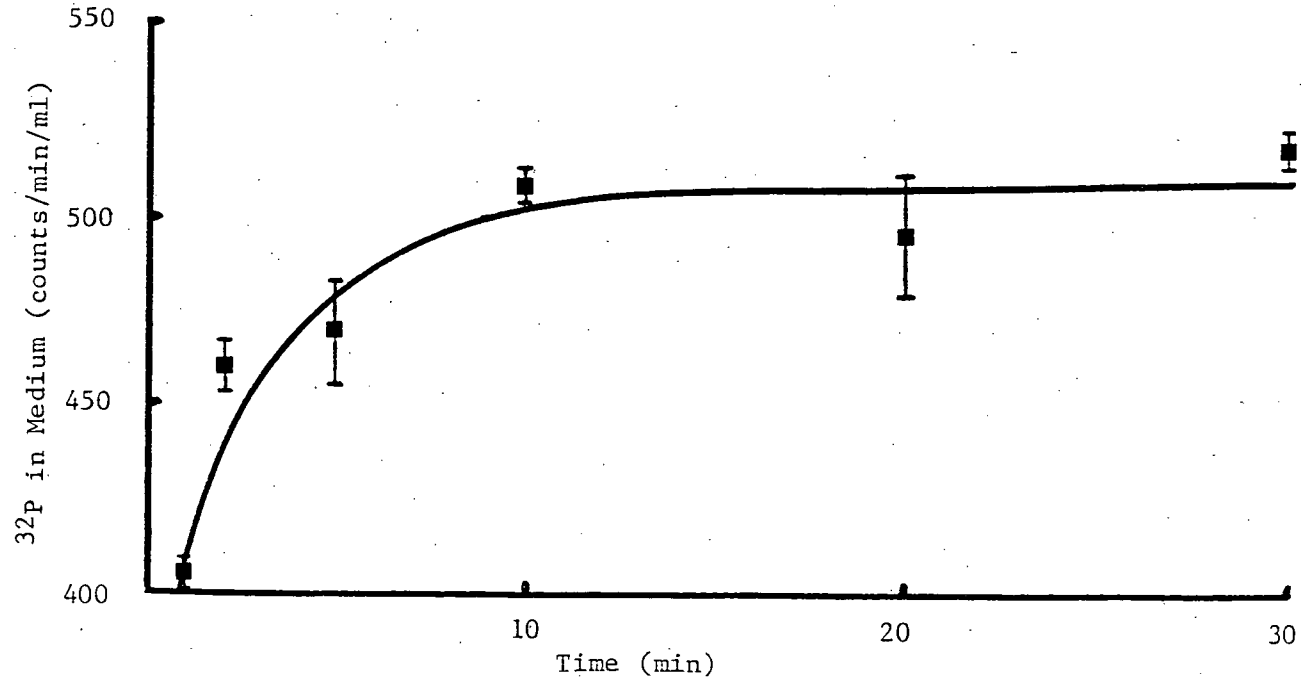


2. Characterization of Phosphate Influx

A. Determination of Root Wash Period

The kinetics of ^{32}P release from roots exposed to labelled medium for 10 min are shown in Figure 12. Greater than 96% of the rapidly-exchanging ^{32}P fraction had effluxed by 5 min. In subsequent experimentation therefore, a 5 min wash period was adopted as standard procedure wherever it was necessary to estimate intracellular, as opposed to extracellular phosphate or ^{32}P-phosphate content.

Figure 12. Efflux of ^{32}P from Rapid Exchange Phase



B. Temperature and pH Optimums.

Transport of phosphate into the symplasm from the environment is probably an enzymatic process (Epstein, 1976) and the "carriers" which catalyze vectorial phenomena are therefore susceptible to changes in environmental temperature and pH. These physical parameters could feasibly alter the rate of phosphate uptake by modifying the molecular configuration of specific enzymes or by an effect on the general energy metabolism in toto (Boyer, 1970). The temperature at which barley roots express their optimum P-uptake rate is approximately 30C (see Figure 13). The optimum pH for phosphate uptake within a reasonable soil range of 5.0 to 7.5 was 6.25 (Figure 14). Plants in general preferentially absorb monovalent ions over divalent or trivalent ions (Epstein, 1976) and Hagen and Hopkins (1955) have hypothesized that phosphate uptake is directly proportional to the amount of monovalent orthophosphate present in the medium as determined by medium pH. Although pH does control $H_2PO_4^-$:HPO_4^{2-} ratios, this relationship did not appear to be the only effect of pH upon orthophosphate uptake in the present study. Dunlop and Bowling's (1978) study with white clover indicated that pH may affect phosphate uptake in ways other than its control on the absolute amount of available monovalent orthophosphate.

All phosphate flux determinations were subsequently performed at 30C in media buffered to pH = 6.25 by 10μM Na_3citrate and citric acid.

Figure 13. Temperature Curve of Phosphate Uptake at pH 6.25

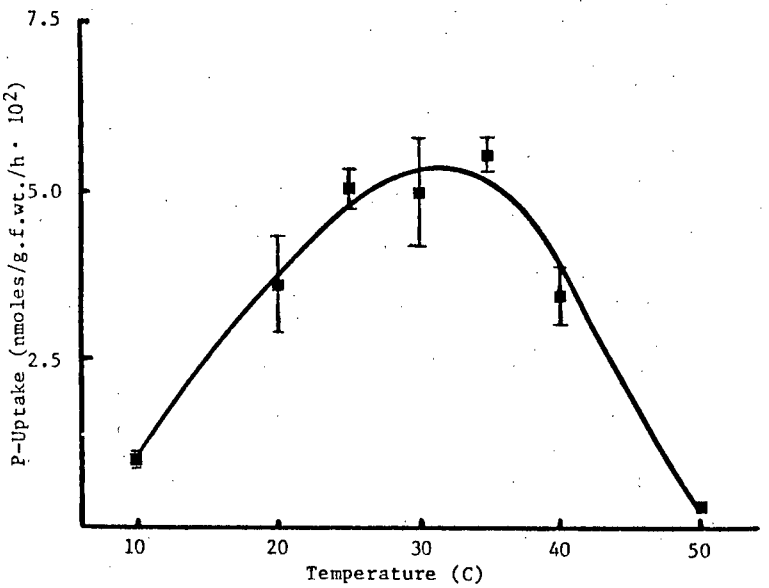
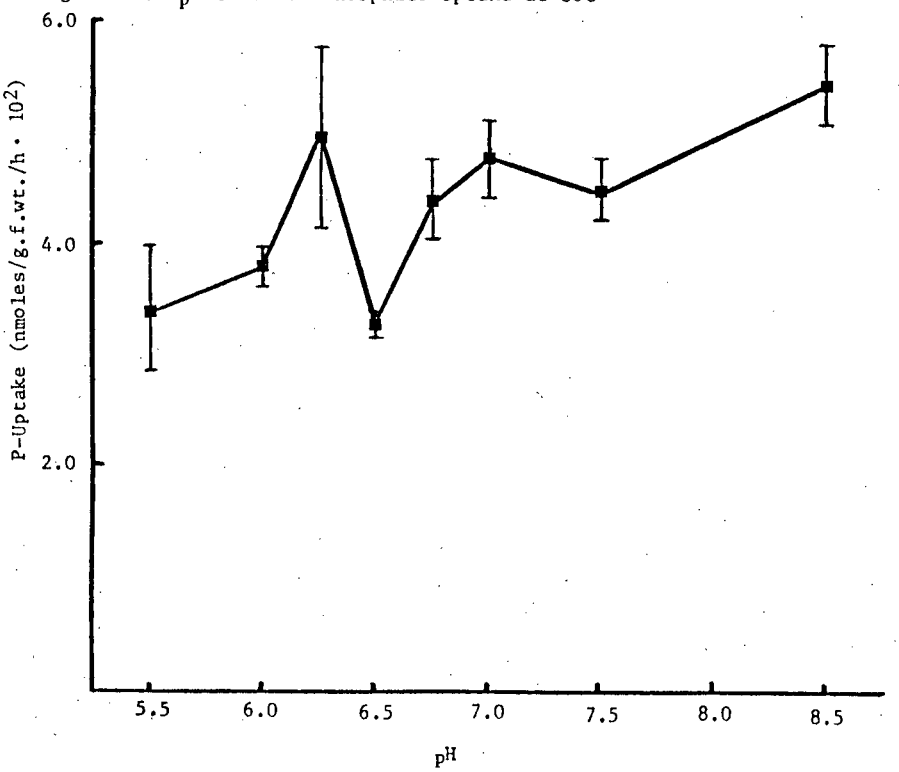


Figure 14. pH Curve of Phosphate Uptake at 30C



3. Phosphate Efflux Kinetics

The efflux kinetics of well nourished five day old Bonanza barley plants are shown in Figure 15. The data revealed a rather straightforward separation into three phases of efflux. Considerations of the size and the kinetic constants of exchange for these phases have led workers to the conclusion that these phases represent a series arrangement of the cell wall, cytoplasm, and vacuolar fractions (Walker and Pitman, 1976). Most work in root ion exchange previously published, however, has dealt with non-metabolized ions such as K^+, Na^+, Cl^-, etc. (Cram, 1973; Walker and Pitman, 1976). A priori it was anticipated that the kinetics of phosphate efflux would be complex. Not only would the P_i fraction be expected to show the standard triphasic pattern but organic fractions could be expected to overlay this basic form.

By contrast the striking similarity to previously reported kinetics lead to the conclusion that the observed kinetics describe the exchange of a single P species - most probably inorganic phosphate since under the present conditions of growth it would represent the major P-fraction. Furthermore the enzymatic reactions which would release labelled orthophosphate from metabolized forms are probably not limiting exchange. If such reactions were restricting efflux the exchange process would almost certainly be much more complex. As such it is reasonable to believe that the three phases represent cell wall, cytoplasmic and vacuolar exchange of P_i.

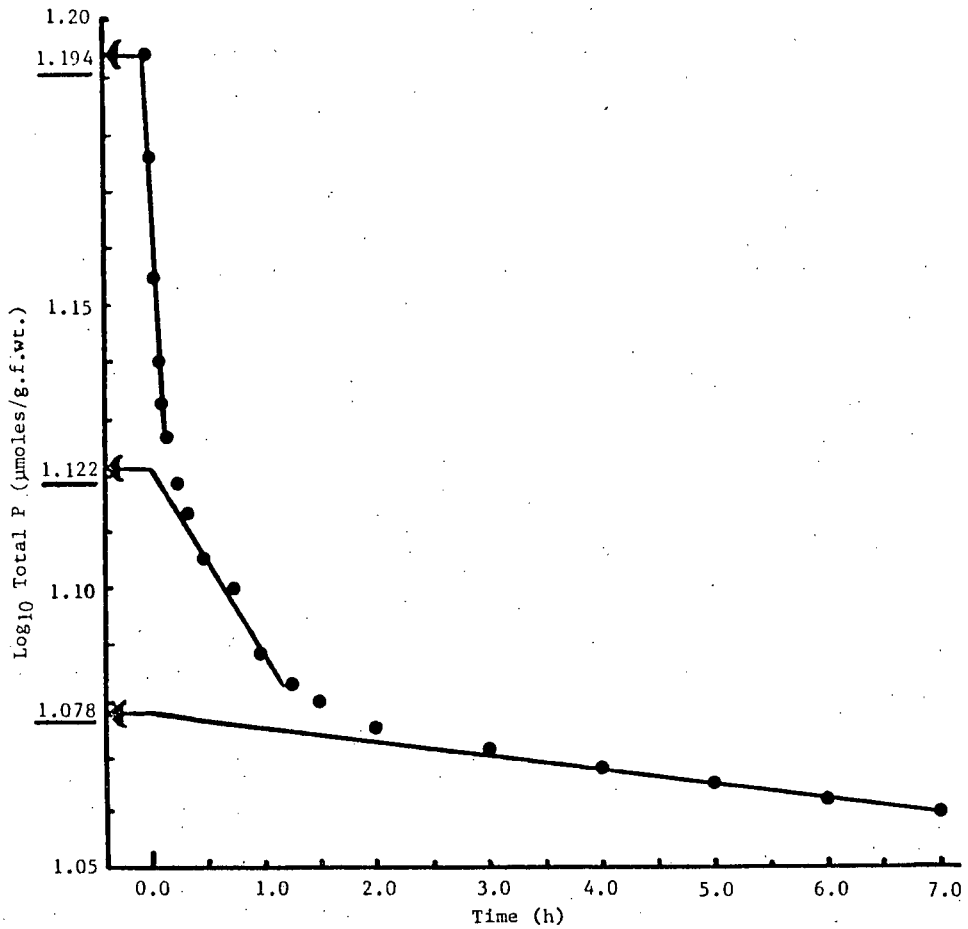
Calculation of the P-content of the fraction with the lowest exchange rate gave a maximum pool size of 11.97μmol/g.f.wt. (see Figure 15). Although this value includes non-exchanging components such as stable DNA (Bieleski, 1973), the majority of the exchanging phosphate probably arises from the vacuoles of cells and the root xylem if a comparison can be made to non-metabolized ions (Jeschkle, 1973). This estimate is entirely consistent with the literature values which place vacuolar P-levels in this range (Bieleski, 1973).

The second fastest exchanging phase has a magnitude of 1.27μmol/g.f.wt. and an exchange half-life of approximately 45 min which is comparable to the half-life of cytoplasmic exchange for other ions (Walker and Pitman, 1976).

The free space phase contained 2.38μmol/g.f.wt. and had a half-life of approximately 2 min which is again similar to values obtained for other ions (Walker and Pitman, 1976). These data provide confirmation that a five min wash period is sufficient to release the bulk of free space $^{32}P_i$ as well as validating the use of a 10 min flux period to estimate plasmalemma influx.

These three phases are consistent with the belief that only a single phosphate species is being exchanged and that compartmental barriers are the plasmalemma and tonoplast. The terms "pool" and "compartment" as defined by Oaks and Bidwell (1970) denote the situation where different portions of a compound are metabolically

Figure 15. Total phosphate efflux kinetics



isolated from one another, whether or not this is due to their physical separation in cell organelles. Smith (1966) has shown that in excess of 90% of the soluble phosphate-esters such as sugar-phosphates are present in the cytoplasm. The terminal P-groups of ATP turn over with a half-life of 2-20 sec and most P-esters have half-lives of less than 30 min (Bieleski, 1968; Johnson and Bluff, 1967; Loughman, 1960; Weigl, 1963). In addition if as has been suggested, the greater proportion of phosphate is in the form of orthophosphate, it is not unreasonable to assume that the magnitude of P-esters would not limit the exchange of P_i at the membrane level. Very little is known about the turnover of Plipids and RNA which contain over 75% of the metabolized phosphate in plants (Bieleski, 1973). Although the half-lives of these compounds may vary considerably, if the magnitude of ^{32}P-ester to $^{32}P_i$ conversion is many fold higher than that of ^{32}P-lipids or ^{32}P-RNA, then the latter's contribution to the cytoplasmic exchange phase would be negligible. DNA is not expected to contribute any exchangable ^{32}P. RNA and P-lipid turnover could be of importance in the slowest exchange phase, however the large vacuolar P_i-content (Bieleski, 1973) would render this unlikely.

It is encouraging to observe essentially similar patterns of phosphate efflux to those describing the efflux of non-metabolized ions. The half-life of cytoplasmic exchange may be of importance in short-term regulation of phosphate uptake particularly in severely

P-deficient plants where the vacuole is no longer able to supply P_i to the cytoplasm (Bieleski, 1968; Crossett and Loughman, 1966; Greenway and Klepper, 1968).

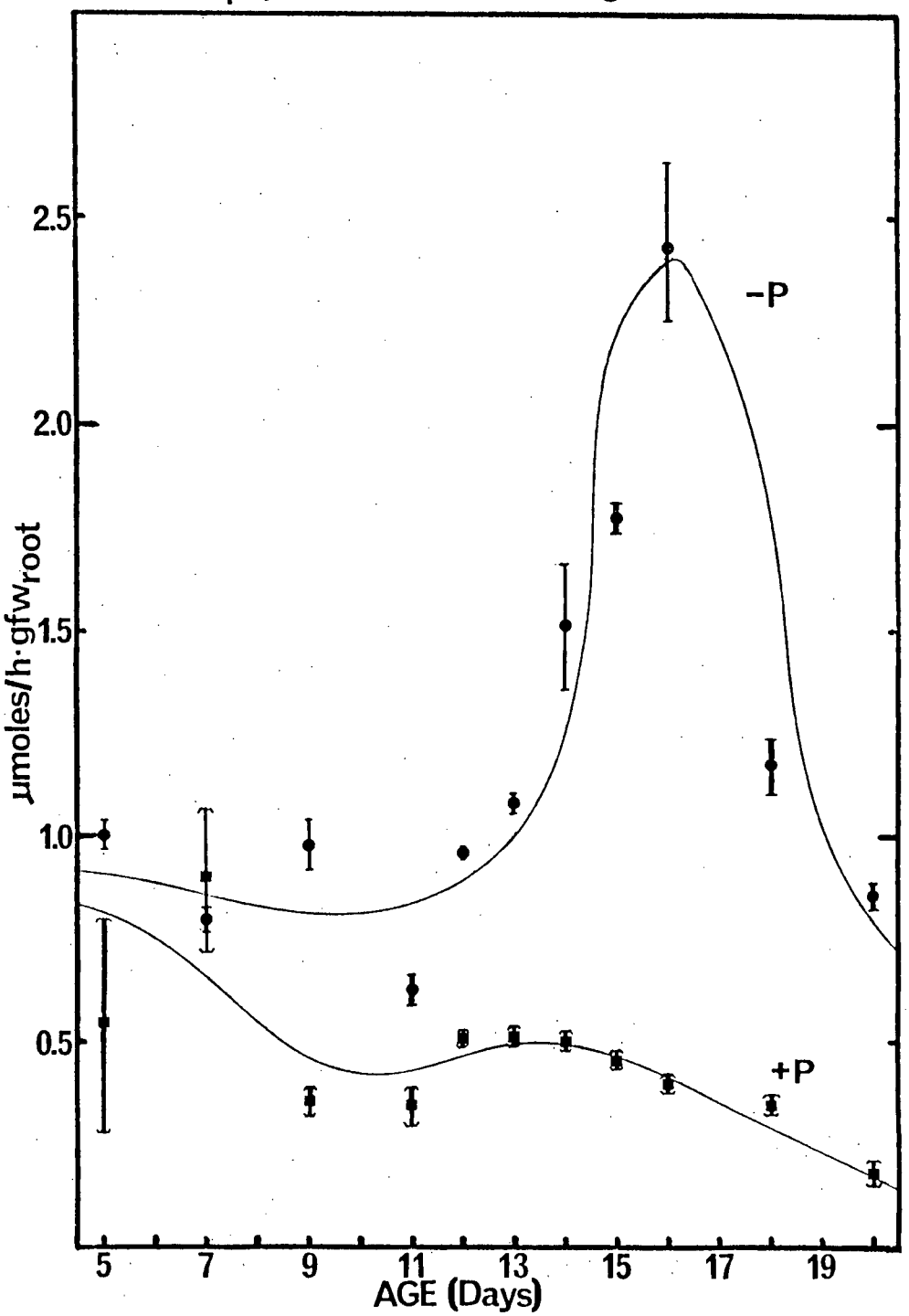
4. Enhancement of Phosphate Uptake Rate by Phosphate Deprivation

A. Phosphate Uptake

As fully nourished barley seedlings aged their phosphate uptake rates on a per gram fresh weight basis declined. A similar trend occurred in -P grown plants until they were 12 days old (see Figure 16), at which time a rapid increase or enhancement of inorganic phosphate uptake was initiated in the low P plants. By day 16 the uptake rate of -P plants was 6.3 times that of +P plants, however within two days a dramatic decline became evident.

Plants exposed to low amounts of phosphate have often been shown to express enhanced uptake rates (Barber, 1972; Cartwright, 1972; Clarkson et. al., 1978), however the phosphate levels employed previously were high in comparison to this study. Clarkson and his colleagues (1978), for example, supplied barley plants with 150μM phosphate for a period of seven days before transferring his -P plants to phosphate minus solutions. As such, uptake rates obtained by these researchers were in some instances an order of magnitude lower than the present results. +P plants in the present studies could almost be described as -P by comparison with the former studies. These results indicate the extent of the regulatory response to P-status. The present choice of 15μM P, as previously stated, was an attempt to simulate more natural soil conditions.

FIGURE 16. P_i Uptake Rate vs. Plant Age.

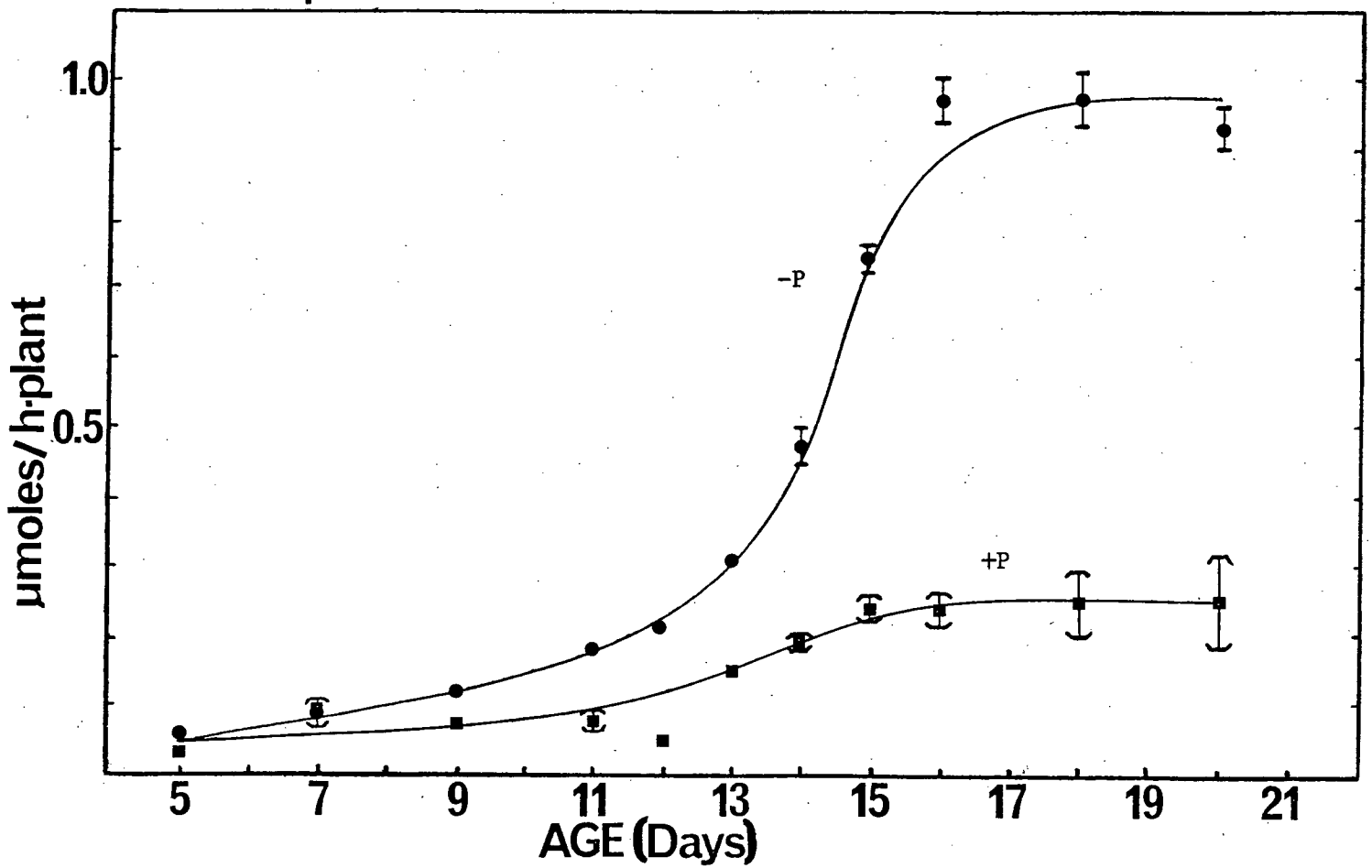


Clarkson et. al. (1978) obtained four fold differences in uptake between P-deficient and fully nourished treatments on a per gram fresh weight basis. Similarly Cartwright's (1972) work yielded a 2.5 fold increase in the uptake rate of -P plants.

When P_i-uptake per plant is plotted (see Figure 17) similar rates are witnessed for +P and -P plants at younger ages, but at day 13 dramatic increases in uptake abilities were initiated in both treatments. On a per plant basis reduction in uptake rates as seen in the per gram fresh weight presentation (after day 16) did not occur. Rather the rates reached a maximum value at day 16, which was sustained until the termination of the experiment at day 20. Throughout the period from day 16 to 20 the ratio of uptake rates (-P/+P) remained steady at 4.

The response of barley plants to low phosphorus levels (-P) in hydroponic culture clearly shows their ability to regulate phosphate uptake rates in relation to phosphate status. The enhancement curve of the -P plants' uptake rates on a per gram fresh weight basis can be divided into three regions; a lag stage, an enhancement stage, and a decline stage. The length of the lag and rapidity of the enhancement stage might be dependent on the balance of nutrient stores within the seeds used. Changes in the phosphate uptake rates can be attributed to physiological properties of the plant since the uptakes were performed under conditions where phosphate diffusion was not limiting the phosphorus supply to the roots (Polle and Jenny, 1971).

FIGURE 17. P_i Uptake vs. Plant Age



It is interesting to compare the differences in the time scale of response in this experiment to those exhibited by K-uptake rates in barley in response to K-deprivation (Glass, 1975). In the latter experiments transfer of plants from K-sufficient to K-minus solutions produced increased influx within hours. This may be attributed to the major osmotic function of potassium. Enzyme activation requirements have been postulated to be in the range of 5-10μM, whereas osmotic requirements necessitate up to 100-200mM K. This large demand for K by contrast to the relatively low levels of P required may account for the extreme responsiveness in the control of K-uptake. The rapidity of regulation in the case of K has led to the proposal of an allosteric control (Glass, 1976; Pettersson and Jensen, 1978). The time scale of the P-response makes it difficult to assess the importance of allosteric as opposed to transcriptional regulation.

On a per gram fresh weight basis there was a decline stage in P-uptake rates. This may be attributed to a rapid decline in vigour of the plants resulting from P-deprivation although at this stage no obvious signs of P-deficiency, other than reduced growth by comparison to +P plants, were apparent. The mechanism involving this response might be either physiological e.g. a termination of "carrier" synthesis, etc., or morphological e.g. an increased relative root growth. Fresh weight analysis indicates that the roots of -P plants were actively growing during that time

period after P-uptake per plant had reached its maximum level. This suggests that the vigour of the plant was not severely retarded and that metabolic energy was available for numerous biochemical processes. It is unlikely that there would have been an energy limitation upon the active uptake processes and therefore the decline in P-uptake per gram fresh weight was probably the result of a cessation in net "carrier" synthesis.

On a per plant basis the lag, enhancement, and levelling off stages are of identical duration in both +P and -P treatments. These simultaneous occurrences reveal their developmental origin as distinct and independent of the phosphorus status. The magnitude of the enhancement of P-uptake, however, could be attributed to a critical level of P_i and/or one or more of its numerous metabolites.

Growth patterns already presented showed divergence between +P and -P grown plants at the period between days 11 and 13 which is concurrent with the divergence in phosphate uptake rates between the two treatments. Therefore the morphological responses revealed through distinct growth patterns are paralleled by physiological differences in orthophosphate uptake. Although phosphate deficiency has often been shown to result in elevated P-uptake rates (Humphries, 1951; Barber, 1972; Cartwright, 1972; Bowen, 1970; Clarkson et. al., 1978), such simultaneous physiological and morphological effects of phosphate status have not been previously reported.

B. Total Phosphate and Inorganic Phosphate Levels within the Plants.

A decline in total phosphate content of -P roots on a per gram fresh weight basis, as expected, did occur as the barley seedlings aged (Figure 18). Such a decline was not evident in the inorganic phosphate pool (confidence level $\alpha = 0.05$), although this pool represented but a small percentage of the total P. The shoots of P-deficient plants dropped in phosphorus content by over one half between days 11 and 12, and a subsequent steady decline followed (see Figure 19). The inorganic phosphorus amounts in -P shoots also dropped significantly ($\alpha = 0.05$), but this occurred between days 9 and 11.

The inorganic phosphate content of -P roots remained constant while a steady decline in total phosphate was present throughout the investigation period. At as early as day 5 the P_i-content was at its minimum level and since P_i is considered the most flexible of P-pools in higher plants (Bieleski, 1973) it is not surprising that the P_i-level had reached a minimum before the decline in the root organic phosphate was evident. The subsequent decline in organic phosphate could be responsible for the enhancement of phosphate uptake, however it is impossible to discern to what extent allosteric and transcriptional processes were involved. Obviously no allosteric control could be attributed to the internal orthophosphate whose amount was essentially constant throughout the experiment. Discounting the

possibility of developmental uptake processes which would be independent of the plants' P-status, the timing of the enhancement of phosphate uptake must of necessity be controlled by the levels of one or more organic phosphates. Although positive correlations between phosphate absorption and ATP levels have been reported in higher plants (Lin and Hanson, 1974) negative correlations between uptake and organic phosphate content of roots have not. On the other hand, as already discussed, there does appear to be a developmental control over P-uptake and as such the absolute magnitude of a phosphate species, P_i included, could govern the extent of P-uptake enhancement.

The total amount of phosphorus per gram fresh weight decreased slowly in the roots of P-sufficient plants while a subsequent increase in P_i-content ensued (see Figure 20). Total phosphate in the +P shoots increased rapidly between days 5 and 7, and then decreased gradually throughout the duration of the experiment (Figure 20). A possible signal for the regulation of P-uptake is the dramatic 17 fold increase in orthophosphate levels within the +P shoots which occured between days 11 and 12. This was followed by a levelling off of the P_i-concentration at approximately 8µmol/g.f.wt. The lack of such a shift in phosphate pool composition could be the signal for increased P-uptake in -P roots.

In the present study, where all determinations of P-uptake were performed with intact plants, the shoot's phosphate status could effect change in the roots' phosphate uptake rates. -P shoots did

Figures 18 to 21. P_i and total phosphorus of aging barley plants

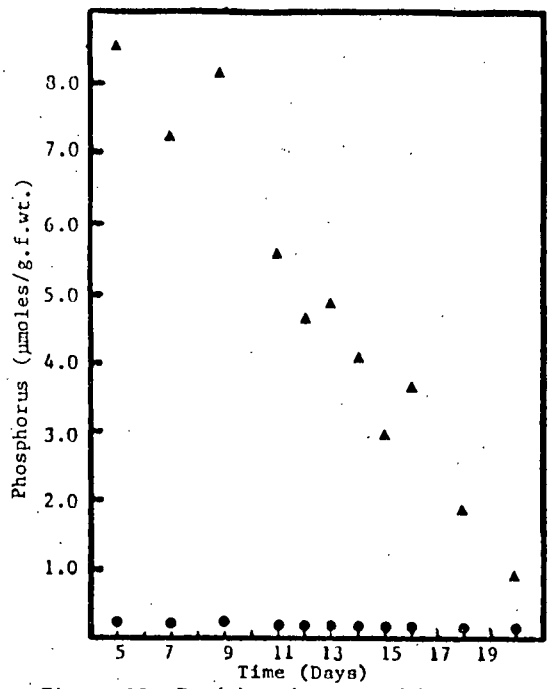


Figure 18. P_i (●) and P_{total} (▲) of -P roots vs. Age.

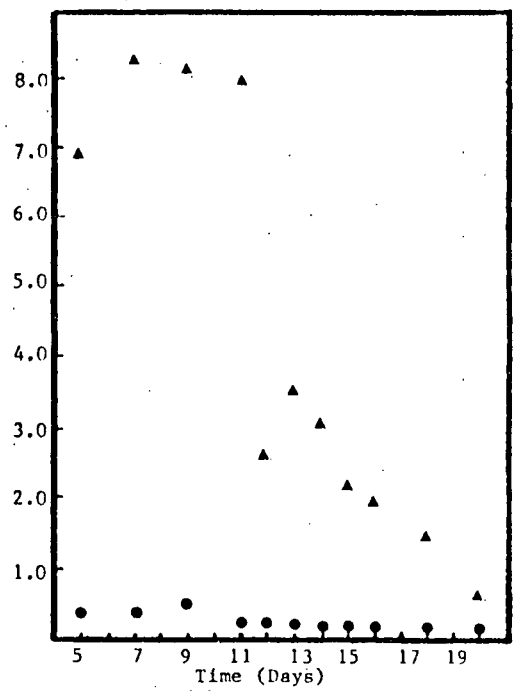


Figure 19. P_i (●) and P_{total} (▲) of -P shoots vs. Age.

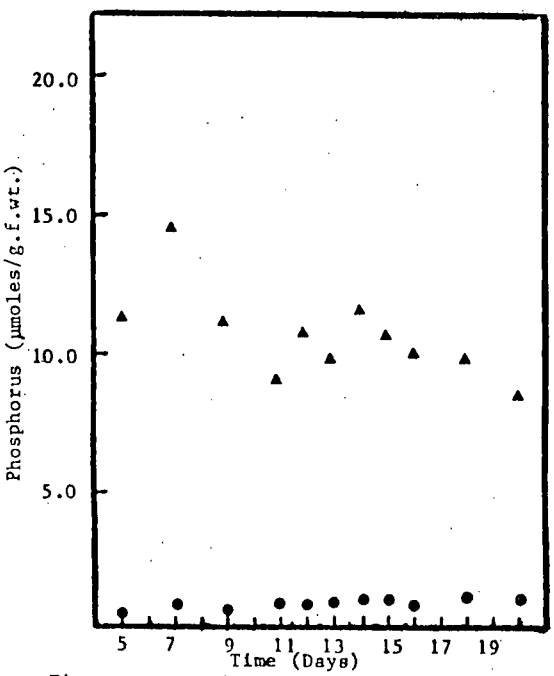


Figure 20. P_i (●) and P_{total} (▲) of +P roots vs. Age.

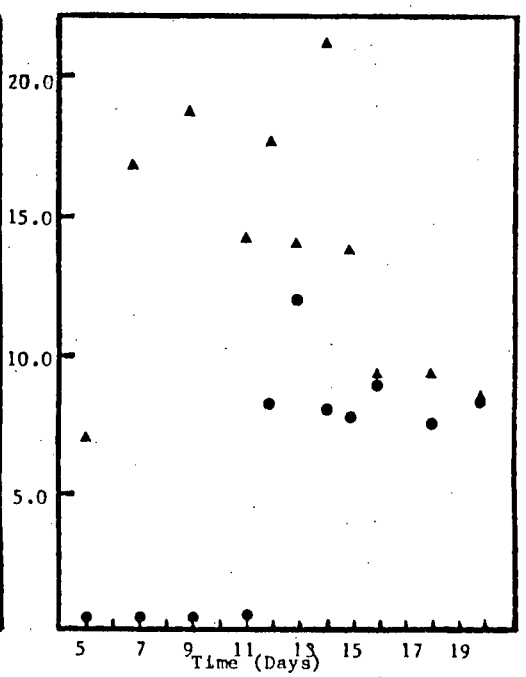


Figure 21. P_i (●) and P_{total} (▲) of +P shoots vs. Age.

show a large decrease in total phosphorus at the time of initiation of enhanced P-uptake (Days 11 and 12). There is also a drop in these shoots' P_i-levels between days 9 and 11. These occurrences could trigger control through translocation of hormones from the shoot to the uptake organ. Several studies have shown that hormones applied to tissues increase their ion uptake rates (Luttge and Higinbotham, 1979). Excised maize roots, however, did not increase in ^{86}Rb or ^{36}Cl uptake when auxin was applied (van Steveninck, 1974), and auxin induced only a 20% stimulation of SO_4^{2-} uptake in beetroot slices (Neirinckx, 1968). Pursuits in the hormonal control of root P_i-uptake, per se, have been neglected.

Where hormones have been externally applied to tissues it is not clear whether increased ion influx was the cause or the result of increased growth rates. -P plants demonstrated a preferential root growth at the time of initiation of increased P_i-uptake rates. However, the -P growth media contained no phosphate, and therefore increased growth could not be due to orthophosphate accumulation. Plant growth is thought to be mediated through interactions between the major plant hormones. Since growth implies a greater demand for all nutrients including inorganic ions, it is not surprising to find that increased growth rates in response to hormonal treatments are associated with higher rates of inorganic ion uptake. In this manner, the hormonal influence upon ion absorption is an indirect, general effect providing no opportunity for the control of

uptake of specific ions. Nevertheless on a short-term basis there are clear indications that hormones such as IAA and ABA may influence specific ion transport systems e.g. H^+ extrusion (Cleland, 1973) and K^+ transport into guard cells (van Steveninck, 1976). ^{86}Rb and ^{32}P double-labelled experiments performed at various plant ages indicated complete independence of their influx (see Table 6) and it would be difficult to attribute this to the general hormonal effects discussed.

In this study the shoot was designated to comprise the seed and green tissue of the seedling. The rather large orthophosphate pool which was suddenly formed in the +P shoots is more representative of the P_i in higher plants than are the levels witnessed at the younger barley ages (Bieleski, 1973). The breakdown of phytin, stable orthophosphate uptake and translocation, and a net conversion of shoot organic-P could all contribute to this increase in orthophosphate concentration. Phosphate stored as phytin could have broken down to release large amounts of phosphorus available for the photosynthesizing tissue in the translocated form of orthophosphate (Morrison, 1965; Selvendran, 1970). Correlations between phytin hydrolysis and P_i increases have been demonstrated in seedlings starved for phosphorus (Mukherji et. al., 1971; Ergle and Guinn, 1959). The action of phytase is retarded when phosphate is supplied to the seedlings (Biachetti and Sartirana, 1967; Sartirana and Biachetti, 1967) and although these studies were terminated at 6 days growth,

phytin levels declined gradually during this period until at the termination of the study they were almost exhausted. Hence it is unlikely that a sudden breakdown of phytin could have occurred at day 11 of the present study (Figure 21). P_i uptake from the 15μM P growth media could have accounted for approximately one third of the increase, i.e. if all P_i taken up was translocated unchanged to the leaves. Polyphosphates have been found in relatively low levels in a few higher plants (Miyachi, 1961; Nassery, 1969; Tewari and Singh, 1964; Vagabov and Kulaev, 1964) and therefore they could be only a minor source for orthophosphate. Organic P sources are the best candidates for breakdown to supply the observed P_i-levels in the +P shoots beyond day 11.

Expression of inorganic and total phosphate levels on a per plant basis gives further insight into phosphorus nutrition. Although the total phosphorus and inorganic phosphate amounts within the -P plants remained relatively constant throughout the first 18 days of growth, a drop in total phosphorus which was not paralleled in the inorganic pool occurred between day 18 and 20 (see Figure 22). This implies that phosphorus had effluxed into the environment and this may have been due to minor losses of plasmalemma integrity. Indirect evidence, recently put forward by Menge et. al. (1978), suggests that leakage of root materials in phosphorus deficient plants may be a prerequisite for the initiation of root-mycorrhizal fungal associations. The fact that the number of these associations in a

given soil environment does correlate negatively with the available phosphate level may also be explained, however, by the reduced survival rates of mycorrhizal spores under high levels of P-application in modern agricultural practices (Ducey, 1980). These rhizosphere associations, although apparently impossible to form in hydroponic culture are believed to be essential for proper plant phosphorus nutrition in low phosphorus soils (Gerdemann, 1968). Plant phosphate compensation points are often in excess of 0.5μM (Bieleski, 1973) and could therefore account for some of the -P plants' phosphate loss (-P medium was changed every 4 days). A rapid increase in the levels of inorganic phosphate occurred in the +P treatment between days 11 and 12 (see Figure 23), which can be attributed to the shoot phenomenon which has already been discussed at length.

The increase in phosphate influx in both +P and -P plants on a per plant basis started and finished at the same time (see Figure 17). These phenomena per se cannot be attributed to nutritional status and therefore appear to be developmental in nature. Crop plant studies have indicated that their P as well as N and K levels vary in a manner which can be timed to distinct morphological stages within the plants' development (Mengel, 1969; Mengel and Kirkby, 1978). It therefore follows that the initiation of elevated phosphate uptake in the present study might also be of a predetermined nature, however the extent of this increase appears to be negatively related to internal phosphorus levels. A possible candidate for the control

Figure 22. Phosphate concentration in -P barley plants

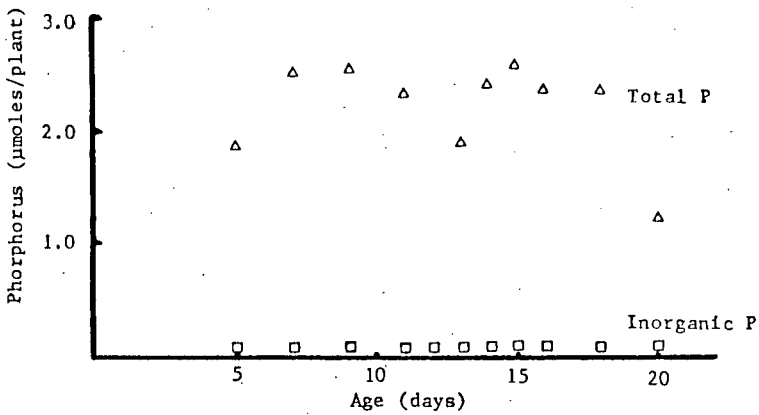
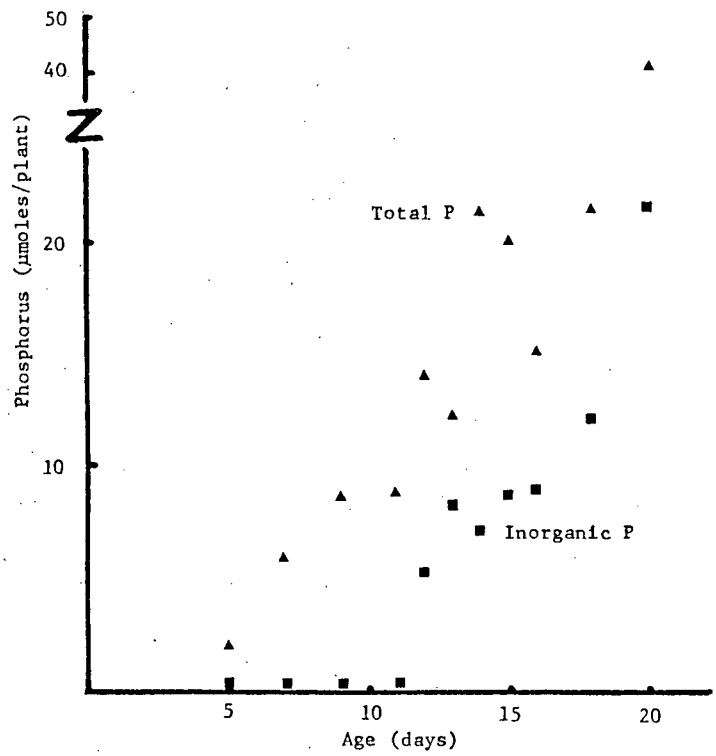


Figure 23. Phosphate concentration in +P barley plants



of the extent of enhancement is the concentration of the roots' internal orthophosphate or organic phosphate at the beginning and during the P-influx enhancement period. The ratios of P_i ($+P_{roots}/-P_{roots}$) are 10.4 and 15.7 at days 11 and 16 respectively; the same ratios for organic phosphate are 1.5 and 2.4. Clarkson et. al. (1978) obtained a +P/-P root total phosphorus ratio of 2.4 and a -P/+P influx ratio of 3.9. Because P_i is much more flexible in magnitude than the organic phosphates it could act as a more finely tuned regulatory signal for P-uptake in roots.

C. Physiological Characteristics of Enhanced P-uptake.

i. Uptake Isotherms

The kinetic plots, either Michaelis-Menten or Eadie-Hofstee (see Figures 24 and 25) indicate that the phosphate uptake Vmax of -P plants had increased (-P Vmax = 1.25μmol/.g.f.wt./hour; +P Vmax = 0.32μmol/g.f.wt./hour), whereas there was no significant ($\alpha = 0.05$) increase in the -P roots affinity for phosphate, i.e. no significant decrease in the Km (-P Km = 2.37μM; +P Km = 3.00μM) of the phosphate uptake system occurred.

The ratio of -P to +P V max's is approximately 4 and the -P plants in these isotherm experiments had not reached total P-uptake enhancement (see Figure 16). The high P-concentration used by many researchers (Nissen, 1973) and the multiphasic nature of the orthophosphate uptake isotherms (Barber, 1972; Nissen, 1973) make it difficult to compare Km and Vmax values. Because of the multiphasic uptake pattern in plants, only natural soil P-concentrations were used in the present study for influx isotherm determinations. In this natural range (circa 10μM), Farrar (1976) working with lichens obtained comparable Km and Vmax values to the -P treated barley roots of this study, whereas Carter and Lathwell (1967) working with corn found values similar to the +P treatment. These workers' results can be attributed to the phosphorus nutrition of their experimental plants. Barber, using excised barley roots (analysis by Nissen, 1973)

Figure 24. Orthophosphate uptake kinetics: Michaelis-Menten plot

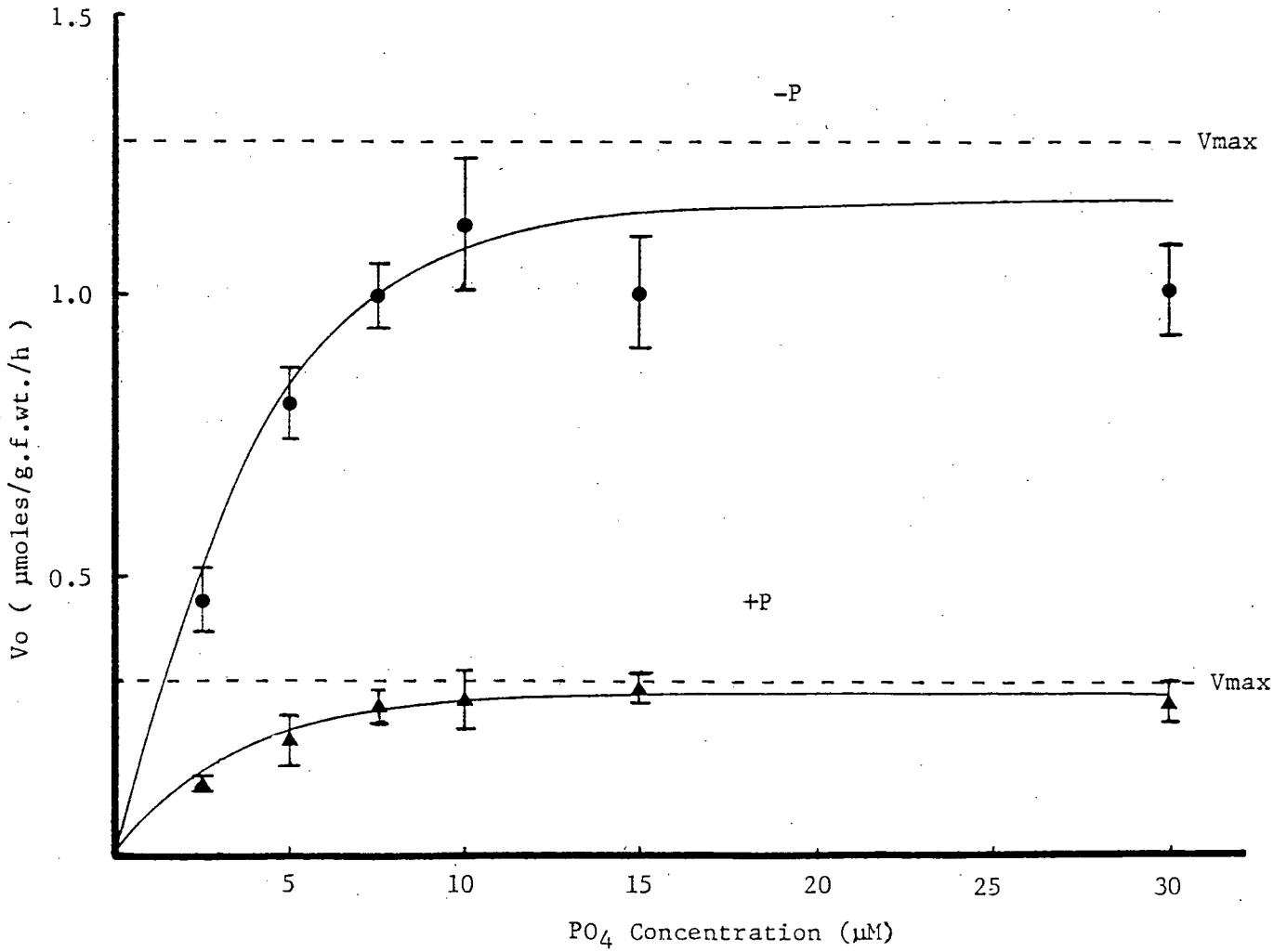
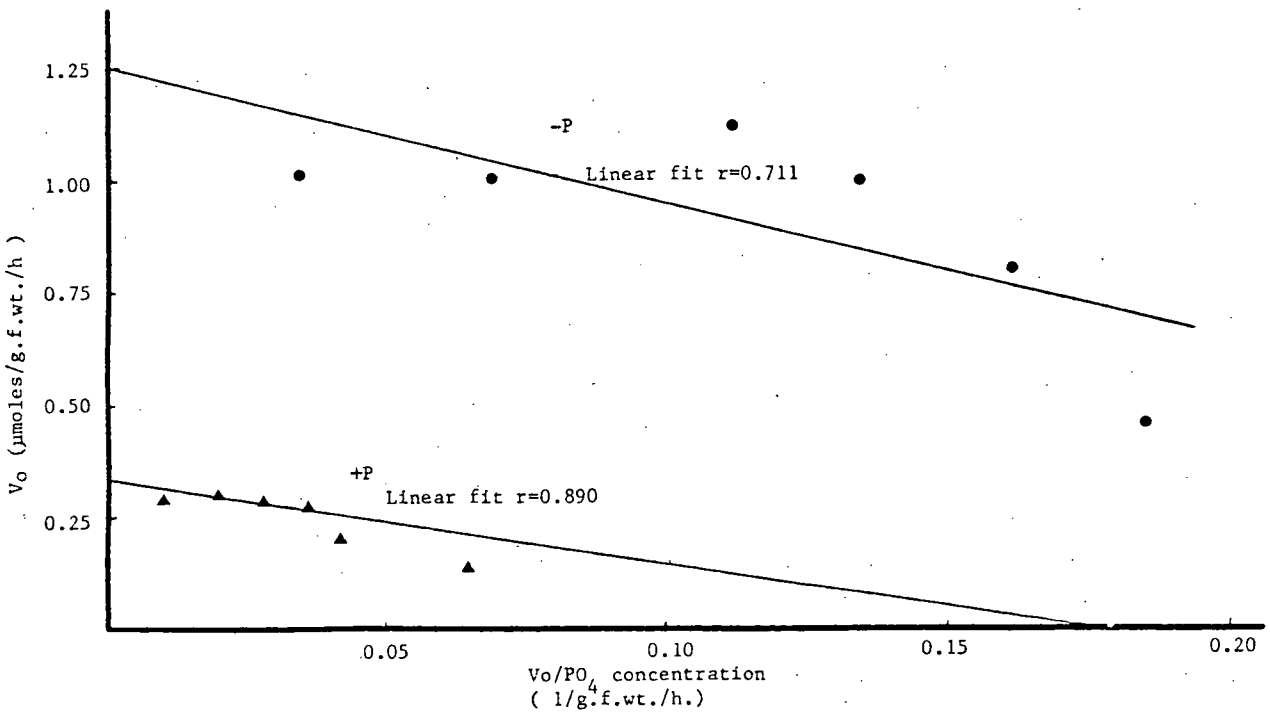


Figure 25. Orthophosphate uptake kinetics: Eadie-Hofstee plot



obtained, in phosphate deficient plants, approximately a 4 fold Vmax increase over that of well nourished plants and his -P Km values were lower than in his +P treatments, though as in the present study the differences of Km were not statistically significant. Cartwright (1972) also showed an increase in the affinity for phosphate by -P barley plants, although no changes in Vmax were reported. This could be due to the nutrient status of the plants employed as well as the P-concentration range over which influx determinations were performed. At higher external concentrations of inorganic ions differences in uptake rates between plants which are apparent at lower concentrations, may be lost (Glass and Dunlop, 1978). This combined with the multiphasic nature of ion uptake in plants would suggest that an isotherm determined over a greater range of ion concentration might overlay the pattern of uptake which occurs at the lower P-concentrations such as those used in the present study.

The present study together with those cited now clearly show that increased P-status may be associated with both decreases of Vmax values for P-uptake as well as increases of Km although in the present study significant Km differences were not observed.

ii. Uptake from Stirred and Non-stirred Media

All uptake determinations so far described were performed in well stirred media and therefore the diffusional limitation on phosphate supply to the root was reduced to a minimum. Diffusion is the limiting factor for ion uptake when roots are bathed in non-stirred solutions of concentration below 10μM (Polle and Jenny, 1971; Bole, 1977). In such an environment an increase in the root area relative to its weight would contribute to an increased uptake rate. By comparison of uptake rates in a diffusion and a non-diffusion limiting system insight can be gained into physiological and morphological contributions to enhanced P_i-uptake rates (see Table 5).

The fact that the -P to +P uptake ratios were consistently lower under the non-stirred compared to the stirred conditions might be explained by presuming that increased uptake rates in -P plants arise from increased "carrier" synthesis and incorporation into the plasmalemma. Under conditions where uptake rates are diffusion limited (the non-stirred treatment) the overlap of depletion zones (in -P plants) would be anticipated to more adversely reduce uptake rates than in the +P plants. An analogy can be drawn to the phenomenon of gas exchange at the stomata of leaves (Heath, 1975). As Raven (1977) has pointed out, however, speculations regarding "carrier" synthesis will remain tenuous until the isolation and characterization of these putative molecules.

Table 5. Uptake rates from 2.5μM P solution with (S) stirring or without (WS) stirring. (μmoles/g.f.wt./h.) Ratios are shown.

Day		S	ratio -P/+P	WS	ratio -P/+P
8	-P	744.14	2.22	304.30	1.46
	+P	335.46		207.75	
11	-P	693.33	2.47	225.12	1.27
	+P	281.49		176.70	
14	-P	548.16	2.73	161.84	1.24
	+P	201.61		130.52	

The seedlings employed in this and the remaining experiments of the thesis attained optimum P-uptake rates at eight days of age, not 16 as in the previous plantlets. Differences in the age needed to reach optimum uptake rates does not appear to be solely dependent upon phosphate stores (see Figures 18, 19, 27 and 28), and other nutrients, although not investigated, may be involved.

iii. Double-labelled Uptake Experiment

Phosphorus deprivation can alter the metabolic cycling of energy through a limitation upon energy coupling which occurs via the synthesis and hydrolysis of ATP. The concentrations of both the ATP precursors ADP and metabolically available orthophosphate rely on a source of phosphorus which could be within the cell as a storage pool and/or within the roots' external environment. Even if these sources were depleted it is plausible that an increase in the rapidity of phosphate cycling through perhaps an increase in phosphatase activity could prolong the plants ability to maintain high levels of energy coupling. In fact high phosphatase activities commonly occur in P-deficient plants (Bieleski, 1973).

In the event that plant ATP supplies became lowered as a result of phosphate deprivation, the relative effect upon all the organism's endergonic ion uptake processes might be expected to be of similar magnitude barring allosteric changes within the "carriers" or changes in the number of specific "carriers" during the course of P-starvation. In order to specifically ascertain whether the decline in P- uptake during P-deficiency (Figure 16) was due to limitation upon energy resources for active transport it was decided to examine the uptake of another actively transported ionic species, namely K^+. By means of double-labelled uptake experiments the uptake of ^{86}Rb and ^{32}P was determined simultaneously during this period of P-uptake decline in -P plants. ^{86}Rb is the isotope of choice for

Table 6. Uptake rates of P from a 15μM P solution and K from a 111μM K solution. (nmoles/g.f.wt./h.) K concentrations of the roots are also presented. (mmoles/g.f.wt.)

Day		P uptake	K uptake	K concentration
8	-P	2678.12	889.12	0.092±0.021
	+P	886.48	1080.64	0.067±0.008
11	-P	2247.08	410.27	0.080±0.005
	+P	867.75	974.55	0.079±0.003
14	-P	1259.39	618.56	0.077±0.013
	+P	699.80	901.59	0.085±0.006

the determination of potassium uptake in higher plants (Lauchli and Epstein, 1970). While the well nourished plants gradually reduced their uptake rates of both ions over the time period investigated, there was no such relationship between the respective ion uptakes in the -P plants. From day 8 to day 14 phosphate uptake rates in the -P plants were halved, and while the potassium uptake decreased considerably by day 11, it had increased again by day 14.

Because of the independence of the two ion uptake rates it appears that energy supply to the "carriers" which might be expected to act similarly upon active uptake processes does not seem to be the overriding cause for reduction in phosphate uptake rates and therefore a more likely cause of the P-uptake decline is a net loss of active P-"carriers" on the root surface.

In both the +P and -P plants a relationship between P-uptake and potassium content of the roots is difficult to ascertain. Cram (in press), on the other hand showed decisively that KCl fed to carrot discs resulted in a decreased phosphate absorption. Franklin (1969) showed that adsorbed cations increased phosphate uptake by barley roots, whereas conversely, applied phosphate has never been reported to augment K-uptake rates. In the present study +P plants consistently showed greater K-uptake rates than -P plants and this may be attributed to the greater growth rates of the +P plants.

iv. Determination of Root External Protein

Because of the ubiquitous metabolic roles in which phosphorus is involved it was of interest to determine if deprivation of this essential nutrient would result in the reduced synthesis of protein and in particular proteins which would be exposed to the exterior of the root. Proteins oriented within the root plasmalemma could be responsible for mineral uptake and for the structural integrity of the membrane itself.

Eosin is a protein specific stain which penetrates biological membranes at a very slow rate and as such can be used to measure relative amounts of root external protein (Williams, 1962). At 10 days of age the staining value for -P plants' roots was 20.82±5.04 $O.D._{520nm}$ units/g.f.wt. and that of +P roots was 35.28±9.06. The diameters of the main roots of both +P and -P plants were 0.45±0.05mm (see Figure 2). At 30 days of age protein values were 16.61±3.86 and 16.53±1.78 for the -P and +P roots respctively and while the +P root diameter had not changed from 0.45±0.05mm, the -P root width had decreased to 0.20±0.02mm (Figure 6).

The primary root's surface area to volume ratio might be taken as representative of the entire root system, in which case the calculation of surface area per g.f.wt. root revealed that per unit area at both days 10 and 30 there was approximately 0.6 times as much external protein on -P as on +P roots. P-deficient plants seem

to have produced and sustained much less plasmalemma and cell wall protein. This combined with the apparent increase in density of phosphate uptake sites (see stirred vs. non-stirred experiment) would suggest that the -P plants possessed a substantial enrichment in the membrane proteins responsible for phosphate uptake. Such an enrichment could be exploited as a source for obtaining a purification of the phosphate "carrier" in question, a feat not yet accomplished in plants. The low protein level may also be partly responsible for the loss of phosphate from the roots of the 20 day old -P plants (see Figure 22). A leakage of phosphate could have resulted from a decreased root integrity caused by an inadequate production of structural membrane proteins.

5. Regulation of Rapid Decline of Enhanced P_i-uptake

A. Short-term versus Long-term Uptake

-P plants which demonstrated enhanced P-uptake rates in short-term determinations (10 min influx periods) did not show as pronounced an elevated uptake rate when a 24h uptake period was used (see Table 7). Depletion of ^{32}P-labelled uptake media was negligible in both short- and long-term experiments and cannot therefore have been responsible for this effect. When -P plants were exposed to +P media for 24h prior to short-term uptake determination the values of P-uptake revealed that the supplied 15μM phosphate had resulted in a considerable reduction in their P-influx, dropping the rate to a level similar to that of the +P plants. Short-term studies of +P and -P plants at days 7 and 8 indicated that no developmental decline in uptake had occurred. The low uptake rates which Clarkson and his colleagues (1978) obtained in their low phosphate plants may be attributed, at least in part, to the 24h influx determinations employed. Differences in the values obtained for P-influx through the use of either short- or long-term assays indicated that the regulation of phosphate uptake in -P plants was extremely sensitive to internal P-levels. This control mechanism, by virtue of the rapidity of its response, could be further investigated only through the use of the short-term assays in which minimal exposure to phosphate results in a measure of the initial P-uptake rate.

Table 7. Short-term (s.t.) vs. long-term (l.t.) uptake rate determination

Plant Status at Beginning of Assay	Assay Employed	Uptake (μmol./g.f.wt./h)*
A. 7 day -P	s.t.	2.047±0.226
B. 7 day +P	s.t.	0.699±0.144
C. 8 day -P	s.t.	2.587±0.481
D. 8 day +P	s.t.	0.616±0.104
E. 7 day -P plus 1 day +P	s.t.	0.651±0.016
F. 7 day -P	l.t.	0.732±0.052
G. 7 day +P	l.t.	0.579±0.039

*Mean and standard deviation of four replicates.

B. P-uptake Rates and Plant Phosphorus Levels Following Exposure of Plants to 15μM Phosphate

In order to determine the rapidity of the -P plants' response to the 15μM P supplied, short-term influx estimates were obtained after these plants had been fed 15μM P for increasing time periods. Figure 26 presents the time course of the effect of 15μM phosphate upon -P plants' uptake rates. The rates began decreasing within 1h which incidentally is close to the half-life of cytoplasmic P-exchange (estimated at 45 min, see Efflux Kinetics). The decline was rapid until 6 hours, beyond which no further reduction was evident. The uptake determinations for times 10 to 24h were carried out on the second day of the 2 day experiment, however the elevated values at 12, 16 and 20h cannot be due to the plants' diurnal cycle since all procedures were performed within the 6 central hours of the light period. Rate determination of non-treated +P and -P plants at 0 and 24h revealed only minimal changes.

The total phosphorus content per gram fresh weight of the treated plants did not show a net gain over the 24h feeding period (Figures 27 and 28). Rapid growth, causing dilution of absorbed P and thus maintenance of P at a relatively constant level, best explains this phenomenon. The fact that P-content decreased in the non-treated -P plants over the same time period is in accord with this explanation. The inorganic phosphorus component of both the shoots and roots changed considerably as phosphate was supplied to -P plants

Figure 26. Phosphate uptake rate vs. time of root exposure to 15μM orthophosphate

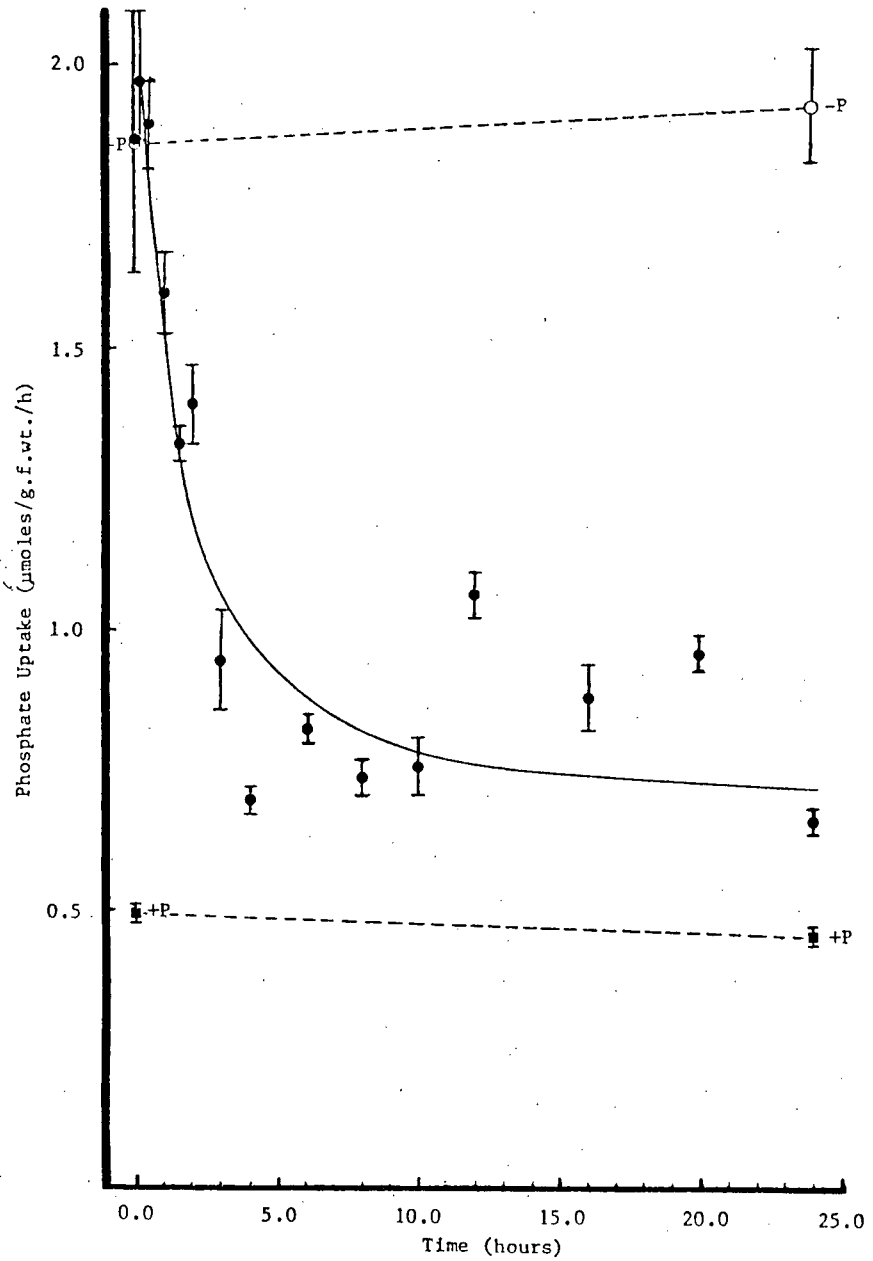


Figure 27. Total phosphorus concentration during the period of phosphate loading of -P grown barley plants

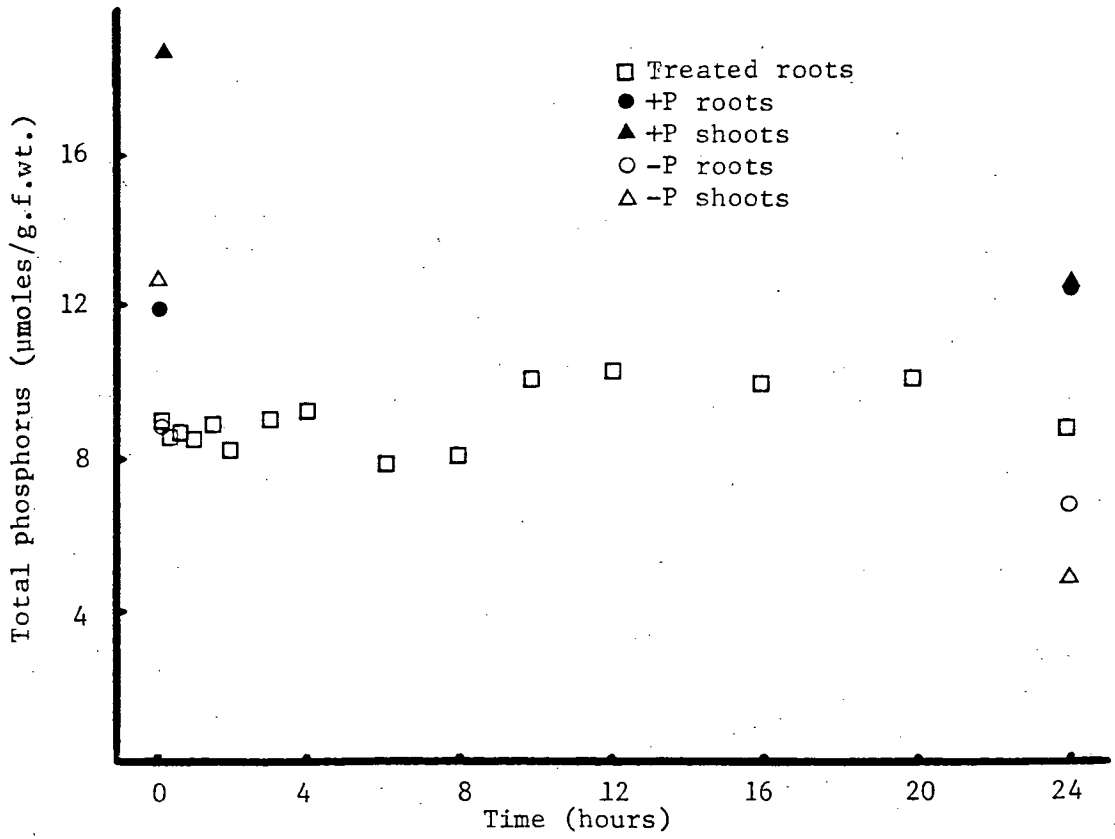
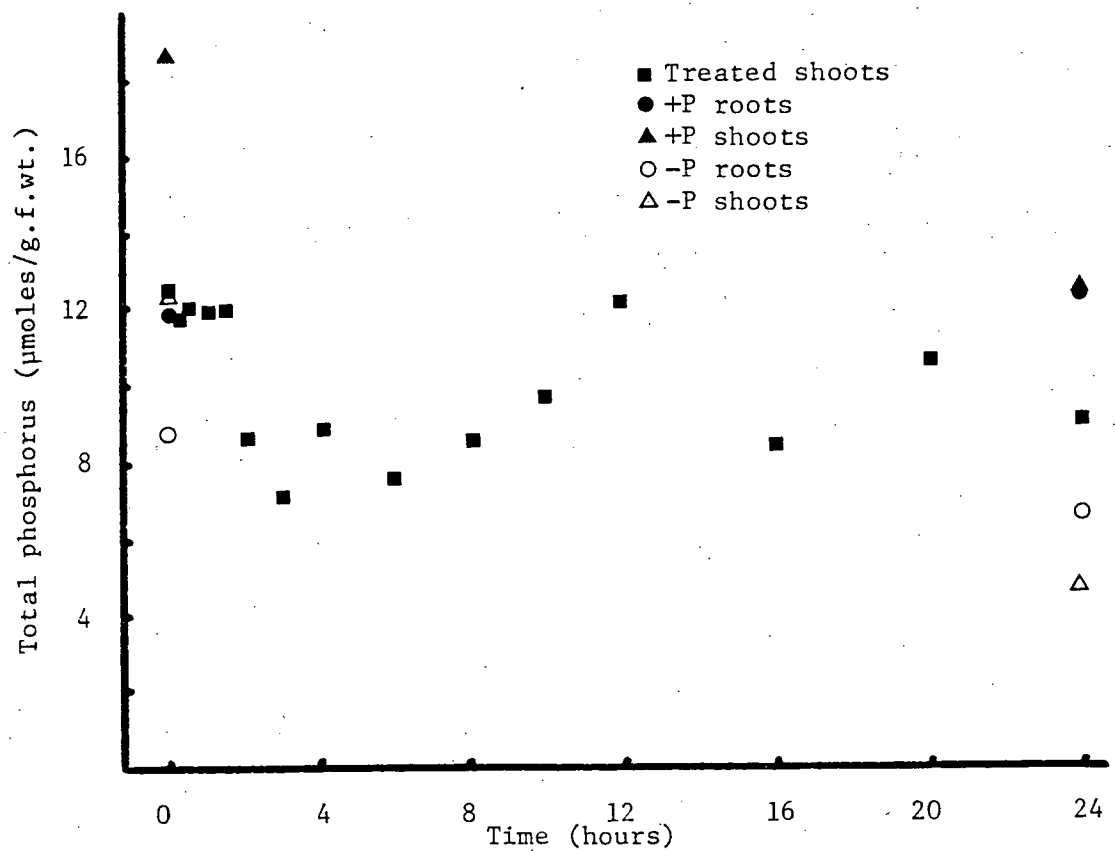


Figure 28. Total phosphorus concentration during the period of phosphate loading of -P grown barley plants



and although no obvious P-uptake control signal can be attributed to the shoots, there appears to be a relationship between the P_i-content of the root and the P-influx (see Figure 29). The uptake rate plotted against the root's internal orthophosphate concentration (Figure 30, feeding curve) brings to light a negative correlation which was maintained until 6h of exposure to 15µM P. After 6h the internal phosphate showed a decline to approximately 0.75µmol/g.f.wt. (not shown in Figure 30) while the P-uptake rates remained at relatively low values. This suggests that beyond 6h some control mechanism other than direct P_i feedback upon uptake had also presented itself. Moreover, the inorganic phosphate level of the +P roots at the end of the 24h feeding period was lower than in the treated roots and regulatory processes may still at this time be actively stabilizing the better nourished root's physiology. Evidence therefore supports the possibility of two control mechanisms:

1) in which a rapid feedback occurs at the early stage, and

2) a slower mechanism which might act through the degradative reduction in the number of active phosphate "carriers".

Also presented in Figure 30 are the root inorganic phosphate levels and P-uptake rates of 11 to 16 day old -P plants during the -P phosphate uptake enhancement phase (see Figure 16). In this case internal P_i-levels did not correlate negatively with the enhanced uptake and as such internal orthophosphate could not have acted as an allosteric regulator of the influx process. The

Figure 29. Inorganic phosphate concentration during the period of phosphate loading of -P barley plants

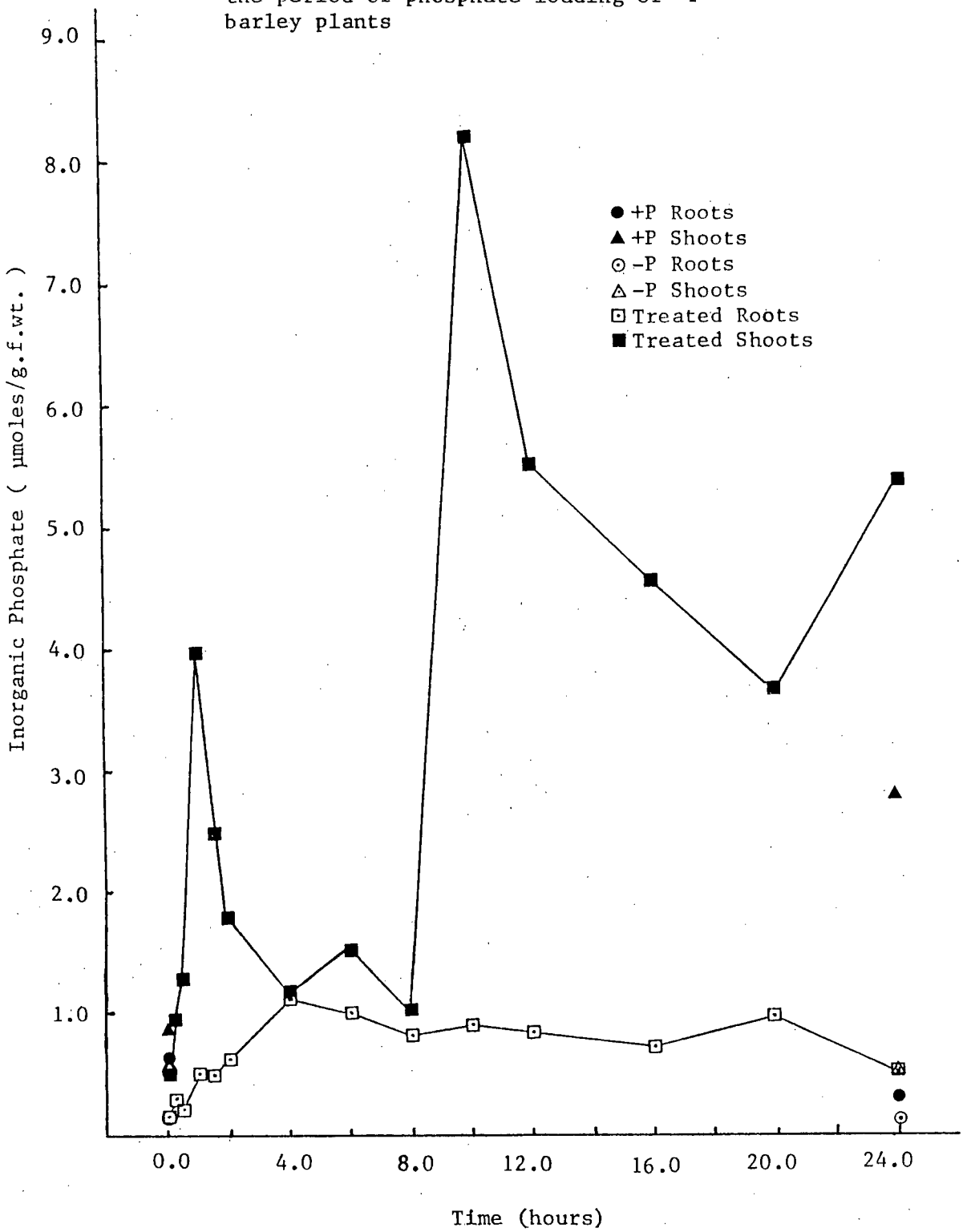
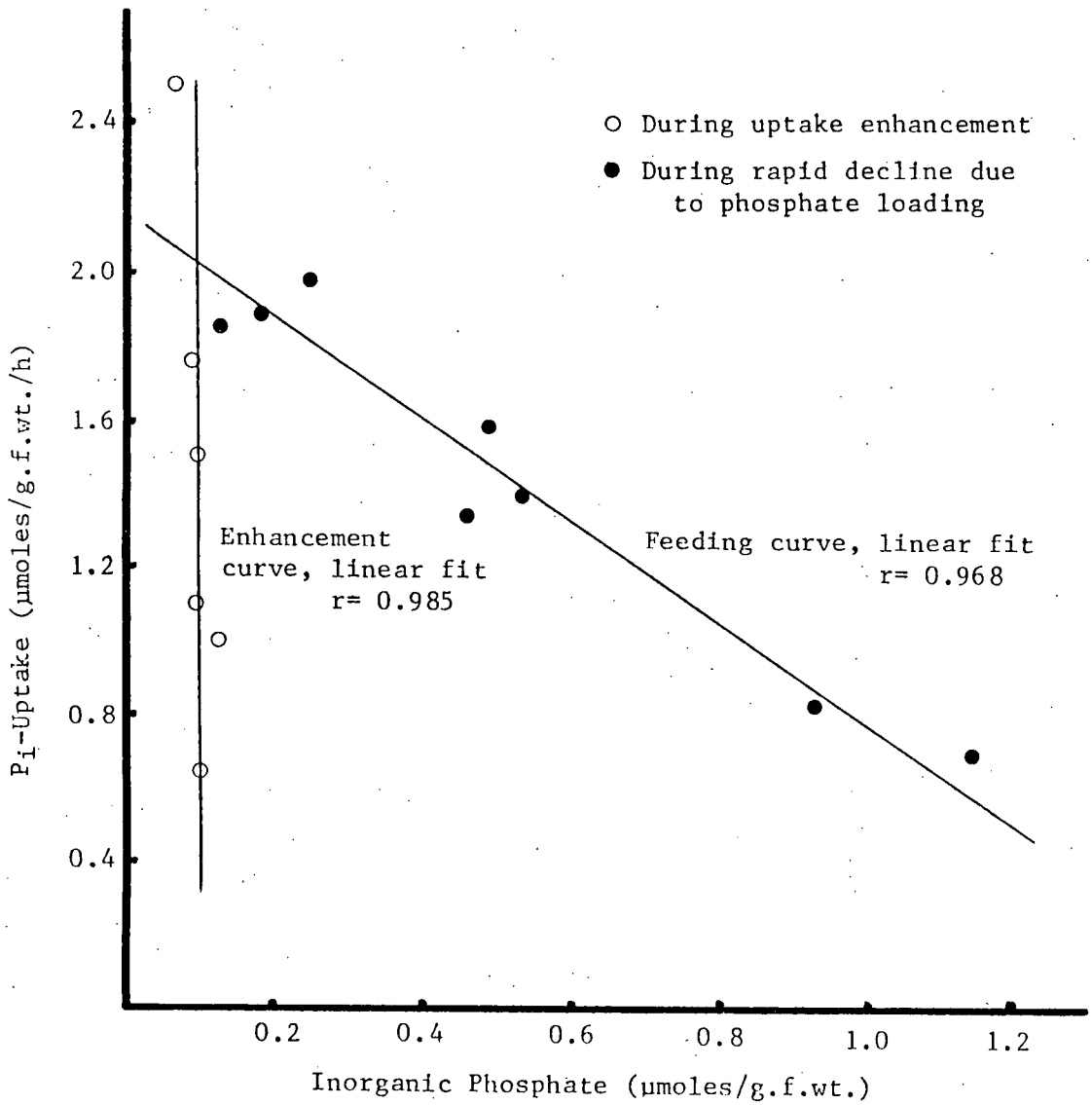


Figure 30. Phosphate uptake rate vs. P_i concentration



internal P_i-concentration remained at a minimum level throughout the enhancement stage and hence inhibition of uptake by internal orthophosphate would, as such, also be at a minimum. It follows that a possible mechanism for the control of enhanced P-uptake is through the addition of new "transporter proteins", each of which was free from allosteric inhibition. This agrees with the argument, already put forward, that phosphate uptake enhancement resulted from an increased density of "carriers" on the root surface. It does remain possible that during the enhancement phase of P-uptake a concomitant decline in one or more organic phosphates resulted in the release of an allosteric inhibition of the uptake process. This was not further investigated in the present study.

Several studies involving ions which are not metabolized have demonstrated negative relationships between the rate of uptake of a given ion and its internal concentration. The ion itself would in these cases be the most efficient direct feedback signal for uptake.

Rapid declines in Rb^+ uptake occurred when corn was supplied with potassium (Leigh and Wyn Jones, 1973) and increasing potassium nutrition caused decreasing Rb^+ uptake rates in sunflower (Pettersson, 1975) and Lemna minor (Young et. al., 1970). Allosteric control of rubidium and potassium uptake has been reported in barley and sunflower (Glass, 1976; 1977; 1978b; Pettersson and Jensen, 1978; 1979; Jensen and Pettersson, 1978). Cl^- uptake decreases as internal

Cl^- levels increase in carrot tissues and barley roots (Cram, 1973) and Br^- is absorbed more slowly in Br^- fed beets (Sutcliffe, 1954) and wheat (Cseh et. al., 1970).

By virtue of their metabolism the study of the maintenance of sulfur, nitrogen, and phosphate levels in plants is complicated. Ivan Smith's work (1975) with cultured tobacco cells indicated that sulfate uptake rates correlated negatively with internal sulfate levels. If methionine and cysteine were applied externally, sulfate uptake rates declined in tobacco (Hart and Filner, 1969) and barley (Ferrari and Renosto, 1972), however conversion of the S-compounds to sulfate may precede the given effect (Smith, 1975). Very little evidence has been reported to suggest that internal NO_3^- levels regulate NO_3^- uptake in higher plants (Smith, 1973; Cram, 1973) and such studies are rendered more difficult because of the ability of nitrate to induce nitrate reductase activity (Jackson et. al., 1976).

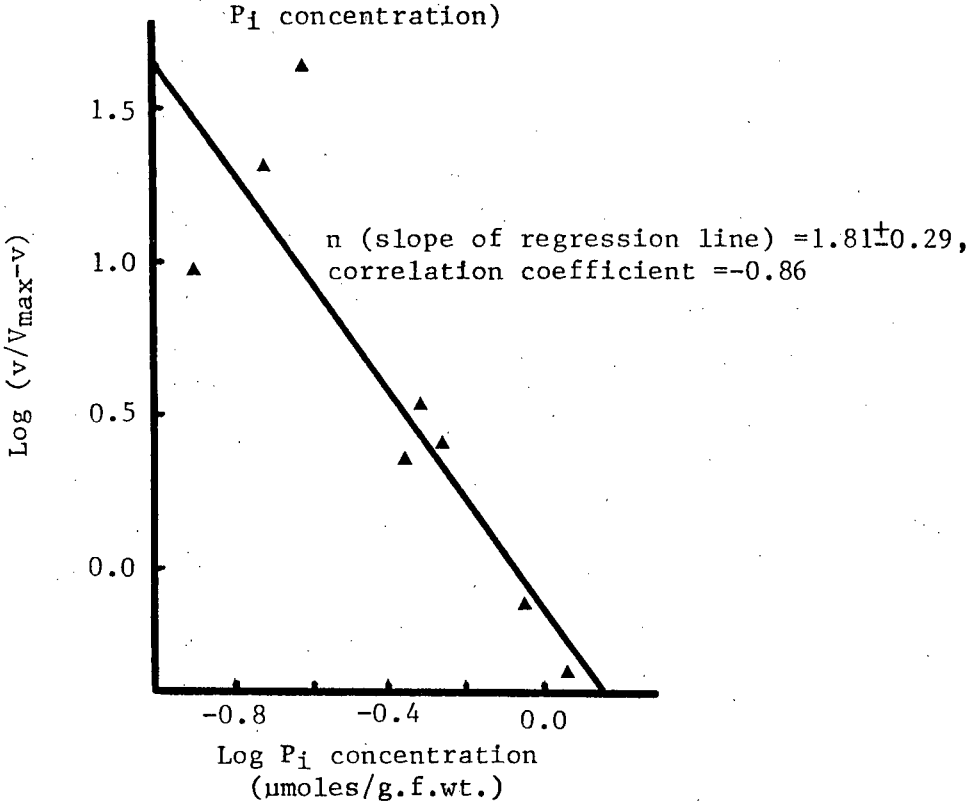
The linear relationship between root P_i-level and P-uptake rates points to an allosteric control mechanism. The sigmoidal curve characteristic of allosteric mechanisms (Ferdinand, 1976) may not present itself in the case of inorganic metabolites within the plant. Minimum and maximum P_i-levels may be governed by reversible conversions into organic forms and hence control may appear only as a linear relationship. The linear transformation of the Hill equation:

$$\log K' = \log \frac{Vmax-v}{v} + n \log |S|$$

was employed to evaluate the Hill coefficient (n) or the degree of cooperativity present in the uptake process (Glass, 1976). An n value of 1.81 ±0.29 (significantly different from 1.0 at $\alpha = 0.01$ level) was obtained from the slope of the linear relationship shown in Figure 31. If no cooperativity were present n would have a value of 1. n values have erroneously been claimed, by enzymologists, to represent the number of allosteric modifier sites when it is actually a measure of the degree of cooperativity possessed by the enzymes involved (Ferdinand, 1976). As such, although the number of regulatory sites per P-"carrier" was not ascertained, control of orthophosphate uptake appeared to be a cooperative process with respect to the roots' internal orthophosphate level.

The rapid 'shutdown' of phosphate uptake could also be explained by more complicated physiological processes. These mechanisms would of necessity involve indirect feedback occurring through intermediates such as hormones and/or organic phosphates. Because of the need for added biochemical steps in such regulatory processes their occurrence might seem unlikely, especially in light of the possibility of a more direct orthophosphate feedback upon phosphate uptake.

Figure 31. Hill plot (v/Vmax - v against internal P_i concentration)



IV. CONCLUSION

Analysis of short-term phosphate uptake in intact barley c.v. Bonanza has provided considerable insight into two processes which appear to be elicited by distinct control mechanisms.

The enhancement of potential phosphate uptake rates through P-deprivation takes place over a period of days which would be ample time for metabolic processes such as protein synthesis or degradation to occur. There is evidence for an enrichment in P-uptake sites on the -P plants' root surface. This enrichment may be the end effect of numerous biochemical processes which ultimately result in an adaptive response to P-deprivation. Control signals may be elicited through growth patterns, or inorganic or organic phosphate levels of either the plants' shoots or roots. Because of the apparent developmental process governing P-uptake rates, the root absolute P_i-concentration could control the extent of enhanced uptake.

The decline in potential phosphate uptake rates revealed when phosphate was supplied to plants possessing elevated influx rates, suggests the occurrence of two regulatory systems. The rapid decline process starts within one hour, and protein degradation therefore is not likely to have been its cause. This process appears to be allosterically controlled by the roots' internal orthophosphate concentration. The time necessary to elicit decline is

similar to the cytoplasmic P-exchange half-life and hence vacuolar P-fluxes may be involved in the triggering of P-uptake decreases. The process which occurs after 8 hours pretreatment with orthophosphate is distinct from that of the previous time period because in the latter period the reduction of P-uptake by P_i failed to demonstrate proportionality to P_i-levels as in the former period. At the longer exposure times to phosphate supply reduced P-uptake may be achieved by "carrier" degradation.

The flexibility of the physiological component of phosphate absorption rates enables homeostatic control of phosphate concentrations within barley plants.

In the soil environment available phosphate activity is buffered by adsorption to soil particles. This physical association is also responsible for the limited mobility of P in soils. The growth of plant roots into a region of high phosphate such as in the drilling and banding of P in agricultural practice might lead to excessive P-absorption with subsequent deleterious effects such as 'burning' of aerial parts. This applies not only to plants adapted to low-P soils but even in agriculturally important crop plants (Bhatti and Loneragan, 1970a, b; Green et. al., 1973a, b; Siddiqi, 1978). Therefore the capacity to 'shutdown' P-uptake fairly rapidly in response to increased P-availability is decidedly important under natural conditions. Those cases cited of tissue damage from excess P-uptake may reflect either an inability to regulate, as in plants

adapted through evolution to low-P environments (e.g. heath plants), or an inability to respond rapidly enough as in the case of crop plants acclimated to low-P regimes.

Physiological adaptations for increased P-uptake are less energy consuming than morphological adaptations which of necessity, require growth. In nutrient limited conditions this difference may be critical. Barley plants grown in -P media revealed increases in main root surface area only well after the physiological uptake rates had maximized on a per plant basis. Increased root-hair development occurs at a still later age as perhaps a last resort. Morphological adaptations may surface only after severe P-deprivation, enabling the roots to, in effect 'search' for localized soil phosphorus sources, however there remains the possibility that the hydroponic environment used in this study unnaturally retarded root morphogenesis.

The work presented in this thesis has tended to concentrate upon the physiological basis of plant adaptation to P-deprivation. It is apparent nevertheless that dependent upon the duration or severity of the stress, morphological changes may also be initiated. In order to appreciate the full capacity for adaptation to nutrient stress in plants it is essential to give equal consideration to both levels of response.

REFERENCES

Asher, C. J. and Loneragan, J. F. 1967. Response of plants to phosphate concentration in solution culture. 1. Growth and phosphorus content. Soil Sci 103, 225-33.

Barber, D. A. 1972. 'Dual isotherms' for the absorption of ions by plant cells. New Phytologist 71, 255-262.

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

Bhatti, A. S., and Loneragan, J.F. 1970a. The effect of early superphosphate toxicity on the subsequent growth of wheat. Australian J. agric. Res. 21, 881-92.

---------- 1970b. Phosphorus concentrations in wheat leaves in relation to phosphorus toxicity. Agron. J. 62, 288-90.

Bianchetti, R. and Sartirana, M. L. 1967. The mechanism of the repression by inorganic phosphate of phytase synthesis in the germinating wheat embryo. Biochim. Biophys. Acta 145, 485-490.

Bieleski, R. L. 1968. Levels of phosphate esters in Spirodela. Plant Physiol. 43, 1297-1308.

---------- 1973. Phosphate pools, phosphate transport, and phosphate availability. Ann. Rev. Plant Physiol. 24, 225-52.

Bole, J. B. 1973. Influence of root hairs in supplying soil phosphorus to wheat. Can. J. Soil Sci. 53, 169-175.

Boyer, P. D. 1970. The Enzymes: Structure and Control, Kinetics and Mechanisms. Academic Press, Cambridge.

Bowen, G. D. 1970. Early detection of phosphate deficiency in plants. Soil Sci. and Plant Anal. 1, 293-298.

---------- , Cartwright, B. and Mooney, J. R. 1974. Wheat root configuration under phosphate stress in Mechanisms of Regulation of Plant Growth, Bieleski, R. L., Ferguson, A. R. and Creswell, M. M. eds. Bulletin 12, The Royal Society of New Zealand, 121-125.

Bradshaw, A. D., Chadwick, M. J., Jowett, D., Lodge, R. W. and Snaydon, R. W. 1960. Experimental investigations into the mineral nutrition of several grass species. Part III. Phosphate Level. J. Ecol. 48, 631-37.

Brewster, J. L., Bhat, K. K. S. and Nye, P. H. 1976. The possibility of predicting solute uptake and plant growth response from independently measured soil and plant characteristics. IV. The growth and uptake of rape in solutions of different phosphorus concentrations. Plant and Soil 44, 279-293.

Carter, O. G. and Lathwell, D. J. 1967. Effect of temperature on orthophsphate absorption by excised corn roots. Plant Physiol. 42, 1407-1412.

Clarkson, D. T. 1967. Phosphorus supply and growth rate in species of Agrostis. J. Ecol. 55, 111-8.

Cleland, R. 1973. An auxin-induced hydrogen ion pump in Avena coleoptiles. Plant Physiol., Suppl. 51, 2.

----------, Sanderson, J. and Scattergood, C. B. 1978. Influences of phosphate-stress on phosphate absorption and translocation by various parts of the root system of Hordeum vulgare L. (Barley). Planta 139, 47-54.

Cram, W. T. 1968. The control of cytoplasmic and vacuolar ion contents in higher plants. Abhandl. Deut. Akad. Wiss. Berl. 117-26.

---------- 1973. Internal factors regulating nitrate and chloride influx in plant cells. J. Exptl. Bot. 24, 328-41.

---------- 1976. Negative feedback regulation of transport in cells in Encyclopedia of Plant Physiology, Vol. 2, Part A, Luttge, U. and Pitman, M. G. eds., Springer-Verlag, N. Y., 284-316.

Crossett, R. N. and Loughman, B. C. 1966. The absorption and translocation of phosphorus by seedlings of Hordeum vulgare (L). New Phytologist 65, 459-68.

Cseh, E., Boszormenyi, Z. and Meszes, G. 1970. Characterization of some parameters of ion transport and translocation. II. The effect of the excision, pretreatment with nutrient elements on bromide and potassium transport

and translocation. Acta Botan. Acad. Sci. Hung. 16, 267-278.

Dainty, J. and Hope, A. B. 1961. The electric double layer and the donnan equilibrium in relation to plant cell walls. Australian J. Biol. Sci. 14, 541-51.

Daley, L. S. and Vines, H. M. 1977. Diurnal fluctuations of inorganic orthophosphate in pineapple (Ananas comusus L., Merr.) leaves and a possible role of ATPase. Plant Science Letters 10, 289-98.

Dunlop, J. and Bowling, D. J. F. 1978. Uptake of phosphate by white clover. II. The effect of pH on the electrogenic phosphate pump. J. Exptl. Bot. 29, 1147-53.

Eibl, H. and Lands, W. E. M. 1969. A new, sensitive determination of phosphate. Anal. Biochem. 30, 51-57.

Epstein, E. 1976. Kinetics of ion transport and the carrier concept in Encyclopedia of Plant Physiology, Vol. 2, Part B, Luttge, U. and Pitman, M. G. eds., Springer-Verlag, N. Y., 70-94.

Ergle, D. R. and Guinn, G. 1959. Phosphorus compounds of cotton embryos and their changes during germination. Plant Physiol. 34, 476-481.

Farrar, J. F. 1976. The uptake and metabolism of phosphate by the lichen Hypogymnia physates. New Phytologist 77, 127-134.

Ferdinand, W. 1976. The Enzyme Molecule. John Wiley and Sons, Toronto.

Ferrari, G. and Renosto, F. 1972. Regulation of sulfate uptake by excised barley roots in the presence of selenate. Plant Physiol. 49, 114-116.

Franklin, R.E. 1969. Effect of adsorbed cations on phosphorus uptake by excised roots. Plant Physiol. 44, 697-700.

Gerdemann, J. W. 1968. Vesicular-arbuscular mycorrhiza and plant growth. Ann. Rev. Phytopathol. 6, 397-418.

Glass, A. D. M. 1975. The regulation of potassium absorption in barley roots. Plant Physiol. 56, 377-380.

---------- 1976. Regulation of potassium absorption in barley roots: an allosteric model. Plant Physiol. 58, 33-37.

Glass, A. D. M. 1977. Regulation of K^+ influx in barley roots: evidence for direct control by internal K^+. Australian J. Plant Physiol. 4, 313-18.

---------- 1978a. An improved method of sample preparation for the radioassay of β-particle emitters by Cerenkov counting. Int. J. Appl. Radiation Isotopes. 29, 75-6.

---------- 1978b. The regulation of potassium influx into intact roots of barley by internal potassium levels. Can. J. Bot. 56, 1759-64.

---------- and Dunlop, J. 1978. The influence of potassium content on the kinetics of potassium influx into excised ryegrass and barley roots. Planta, 141, 117-9.

Green, D. G., Ferguson, W. S., and Warder, F. G. 1973a. Accumulation of toxic levels of phosphorus in phosphorus-deficient barley. Can. J. of Pl. Sci. 53, 241-6.

----------, and Warder, F. G. 1973b. Accumulation of damaging concentrations of phosphorus by leaves of selkirk wheat. Plant and Soil 38, 567-72.

Greenway, H. and Klepper, B. 1968. Phorphorus transport to the xylem and its regulation by water flow. Planta 83, 119-36.

Hackett, C. 1968. A study of the root system of barley I. Effects of nutrition on two varieties. New Phytologist 67, 287-99.

Hagen, C. E. and Hopkins, H. T. 1955. Ionic species in orthophosphate absorption by barley roots. Plant Physiol. 30, 193-9.

Hart, J. W. and Filner, P. 1969. Regulation of sulfate uptake by amino acids in cultured tobacco cells. Plant Physiol. 44, 1253-9.

Harley, J. L. 1969. The Biology of Mycorrhiza. Hill, London.

Heath, O. V. S. 1975. Stomata, Oxford Biology Readers No. 37, Oxford University Press, Oxford.

Heimer, Y. M. and Filner, P. 1971. Regulation of nitrate assimilation pathway in cultured tobacco cells. III. The nitrate uptake system. Biochim. Biophys. Acta 230, 362-72.

Hulett, H. R. 1970. Non-enzymatic hydrolysis of adenosine phosphates. Nature 225, 1248-9.

Humphries, E. C. 1951. The absorption of ions by excised root systems. II. Observations on roots of barley grown in solutions deficient in phosphorus, nitrogen, or potassium. J. Exptl. Bot. 2, 344-79.

Jackson, W. A., Kwik, K. D. and Volk, R. J. 1976. Nitrate uptake during recovery from nitrogen deficiency. Physiol. Plant. 36, 174-81.

Jensen, P. and Pettersson, S. 1978. Allosteric regulation of potassium uptake in plant roots. Physiol. Plant. 30, 24-9.

Jeschkle, W. D. 1973. K^+-stimulated Na^+ efflux and selective transport in barley roots. in Ion Transport in Plants. Anderson, W. P. ed., Academic Press, London-New York, 285-96.

Johansen, C., Edwards, D. G. and Loneragan, J. F. 1970. Potassium fluxes during potassium absorption by intact barley plants of increasing potassium content. Plant Physiol. 45, 601-3.

Johnson, E. J. and Bruff, B.S. 1967. Chloroplast integrity and ATP-dependent CO_2 fixation in Spinacia oleracea. Plant Physiol. 42, 1321-28.

Kucey, R. 1980. The role of vesicular-arbuscular mycorrhizae in plant growth. Ph.D. thesis, University of Saskatchewan, Saskatoon, Canada.

Lauchli, A. 1967. Untersuchungen uber Verteilung and Transport von Ionen in Pflanzengeweben mit der Rontgen-Mikrosonde. I. Versuche an vegetativen Organen von Zea Mays. Planta 75, 185-206.

---------- 1969. Radioassay for β-emitters in biological materials using Cerenkov radiation. Int. J. appl. Radiation Isotopes 20, 265-70.

---------- , and Epstein, E. 1970. Transport of potassium and rubidium in plant roots: the significance of calcium. Plant Physiol. 45, 639-41.

Leigh, R. A. and Wyn Jones, R. G. 1973. The effect of increased ion concentration upon the ion uptake isotherms of excised maize root segments. J. Exptl. Bot. 24, 787-95.

Lin, W. and Hanson, J. B. 1974. Phosphate absorption rates and adenosine 5'-triphosphate concentrations in corn root tissue. Plant Physiol. 54, 250-56.

Loughman, B. C. 1960. Uptake and utilization of phosphate associated with respiratory changes in potato tuber slices. Plant Physiol. 35, 418-24.

Luttge, U. and Higinbotham, N. 1979. Transport in plants. III. Regulation and Control of transport processes by cell metabolism. Springer-Verlag, N. Y., 203-81.

Menge, J. A., Steirle, D., Bagyaraj, D. J., Johnson, E. L. V. and Leonard, R. T. 1978. Phosphorus concentrations in plants responsible for inhibition of mycorrhizal infection. New Phytologist 80, 575-8.

Mengel, K. 1969. Factors Limiting Maximum Yield in Transition from Extensive to Intensive Agriculture with Fertilizers. International Potash Institute, Berne, 27-33.

---------- and Kirby, E. A., 1978. Principles of Plant Nutrition. International Potash Institute, Berne, p. 22.

Miyachi, S. 1961. Inorganic polyphosphate in spinach leaves. J. Biochem. 50, 367-71.

Mitchell, P. 1967. Translocations through natural membranes. Adv. Enzymol. 29, 33-87.

Morrison, T. M. 1965. Xylem sap composition in woody plants. Nature 205, 1027.

Mott, R. L. and Steward, F. C. 1972. Solute accumulation in plant cells. I. Reciprocal relations between electrolytes and non-electrolytes. Ann. Bot. (London) N. S. 36, 621-39.

Mukherji, S., Day, B., Paul, A. K. and Siriar, S. M. 1971. Changes in phosphorus fractions and phytase activity of rice seeds during germination. Physiol. Plant. 25, 94-7.

Nassery, H. 1969. Polyphosphate formation in the roots of Deschampsis flexuosa and Urtica dioica. New Phytologist 68, 21-3.

---------- 1971. Phosphate absorption by plants from habitats of different phosphate status. III. Phosphate fractions in the roots of intact plants. New Phytologist 70, 949-51.

Neirinckx, L. J. A. 1968. Influence de quelque substance de croissance sur l'absorption du sulfate par le tissu radiculaire de beterave rouge (Beta vulgaris L. ssp vulgaris var rubra L.). Ann. Physiol. Veg. Bruxelles 13, 83-108.

Nissen, P. 1973. Multiphasic uptake in plants. I. Phosphate and sulfate. Physiol. Plant. 28, 304-16.

Oaks, A. and Bidwell, R. G. S. 1970. Compartmentation of intermediary metabolites. Ann. Rev. Plant Physiol. 21, 43-66.

Pettersson, S. 1975. Ion uptake efficiency of sunflower roots. Physiol. Plant. 34, 281-85.

---------- and Jensen, P. 1978. Allosteric and non-allosteric regulation of rubidium influx in barley roots. Physiol. Plant. 44, 110-14.

---------- 1979. Regulation of rubidium uptake in sunflower roots. Physiol. Plant. 45, 83-7.

Piggott, C. D. 1971. Analysis of the response of Urtica dioica to phosphate. New Phytologist 70, 953-66.

Pitman, M. G., Courtice, A. C. and Lee, B. 1968. Comparison of potassium and sodium uptake by barley roots at high and low salt status. Australian J. Biol. Sci. 21, 871-81.

Polle, E. O. and Jenny, H. 1971. Boundary layer effects in ion absorption by roots and storage organs of plants. Physiol. Plant. 25, 219-24.

Raven, J. A. 1977. Regulation of solute transport at the cell level. in Society for Experimental Biology Symposium XXXI: Integration of Activity in the Higher Plant 73-99.

Rorison, I. H. 1968. The response to phosphorus of some ecologically distinct plant species. I. Growth rates and phosphorus absorption. New Phytologist 67, 913-23.

Sartirana, M. L. and Bianchetti, R. 1967. The effects of phosphate on the development of phytase in the wheat embryo. Physiol. Plant. 20, 1066-75.

Selvendran, R. R. 1970. Changes in the composition of the xylem exudate of tea plants (Camellia sinensis L.) during recovery from pruning. Ann. Bot. London 34, 825-33.

Siddiqi, M. Y. 1978. Growth and mineral nutrition in Stylosanthes fruticosa (Retz) Alston. Australian J. Ecol. 3, 67-78.

Smith, F. A. 1966. Active phosphate uptake by Nitella translucens. Biochim. Biophys. Acta 126, 94-9.

---------- 1973. The internal control of nitrate uptake into excised barley roots with differing salt contents. New Phytologist 72, 769-82.

Smith, I. K. 1974. Sulfate transport in cultured tobacco cells. Plant Physiol. 55, 303-7.

Steveninck, R. F. M. van, 1974. Hormonal regulation of ion transport in parenchyma tissue. in Membrane Transport in Plants. Zimmerman, U. and Dainty, J. eds., Springer-Verlag, N. Y. 450-6.

---------- 1976. Effect of hormones and related substances on ion transport. in Encyclopedia of Plant Physiology, Vol. 2, Part B, Luttge, U. and Pitman, M. G., eds., Springer-Verlag, N. Y. 308-42.

Sutcliffe, J. F. 1954. The exchangeability of potassium and bromide ions in cells of red beetroot tissue. J. Exptl. Bot. 5, 313-26.

Tewari, K. K. and Singh, M. 1964. Acid soluble and acid insoluble inorganic polyphosphates in Cuscuta reflexa. Phytochemistry 3, 341-7.

Troughton, A. 1977. The effect of phosphorus nutrition upon the growth and morphology of young plants of Lolium perenne L. Ann. Bot. London 41, 85-92.

Vagabov, V. M. and Kulaev, I. S. 1964. Inorganic polyphosphates of corn roots. Dokl. Akad. Nauk. S.S.S.R. 158, 307-309.

Walker, N. A. and Pitman, M. G. 1976. Measurement of fluxes across membranes in Encyclopedia of Plant Physiology, Vol 2, Part A. Luttge, U. and Pitman, M. G. eds., Springer-Verlag, N. Y., 93-126.

Weigl, J. 1963. Die Bedeutung der Energiereichen Phosphate bei der Ionenaufnahme durch Wurzeln. Planta 60, 307-21.

Williams, D. E. 1962. Anion-exchange properties of plant root surfaces. Science 138, 153-4.

Young, M., Jefferies, R. L. and Sims, A. P. 1970. The regulation of potassium uptake in Lemna minor L. Abhandl. Deut. Akad. Wiss. Berl. 67-82.