EFFECT OF LIGHT UPON THE TRANSLOCATION OF PHOSPHORUS BY SEEDLINGS OF *HORDEUM VULGARE* (L.)

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Summary

In vivo measurements of the radioactivity in the root and shoot of single barley seedlings have been made in an attempt to determine the effect of environmental variables upon the penetration of the root by phosphorus. When a seedling was placed in a nutrient solution which contained ^{32}P-labelled, $10^{-6}M$ KH_2PO_4, radioactivity was absorbed at a constant rate, but its rate of movement into the shoot increased during the first 5 hr of absorption after which time it was constant. If the shoot was placed in darkness after translocation had attained a constant rate, there was an immediate fall in the rate of translocation, followed, in continuing darkness, by a very slow increase to its former constant rate. Experiments involving changes between labelled and unlabelled nutrient solutions and placing the shoot in darkness showed that the initial observation was compatible with the hypothesis, put forward in an earlier paper, that phosphorus entering the root mixes with only a small proportion of its total content before moving into the shoot. The results indicated that changes in the environment of the shoot could modify the turnover rate of the small phosphorus "pool" in the root which lay in the path of translocation. Possible mechanisms for the mediation of this response are discussed.

I. Introduction

The movement of phosphorus from the environment to the xylem of a vascular plant appears to be an active process, i.e. it occurs against a gradient of electrochemical potential (Bowling, Macklon, and Spanswick 1966). The simplest models of the root visualize an extension of the ionic environment through channels in the tissue to a permeability barrier, the site of active transport. Beyond the permeability barrier, ion movement is supposed to be diffusive through a cytoplasmic continuum which extends to the lumen of the xylem vessels. The position of the permeability barrier is controversial, and suggested locations vary between the epidermis (Sandström 1950) and the walls of the xylem vessels (House and Findlay 1966). The spectrum of opinion between these two extremes has been reviewed by Oberländer (1966).

In an earlier paper (Crossett and Loughman 1966) three steps were identified in the transfer of phosphorus from a nutrient solution containing $10^{-6}M$ orthophosphate to the shoot of a barley seedling. These were:

(1) Movement of phosphorus into a root "pool", which contained only a small proportion of the total phosphorus in the root and did not change in size with increasing time.

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(2) Movement of phosphorus from the root pool to the vascular system.

(3) Movement of phosphorus through the vascular system to the shoot.

This paper reports experiments which were carried out in an attempt to find factors which could change the size of the root pool so that it could be located by biochemical methods and in autoradiographs.

II. METHODS

Seedling Culture

Barley seedlings [*Hordeum vulgare* (L.) cv. Proctor] were grown using the methods described by Crossett (1966). The nutrient solutions, pH5·5, contained 1 μ-equiv/l KH_2PO_4 and also the following components (m-equiv/l): K^+ 6, Ca^{2+} 3, Mg^{2+} 3, Na^+ 2, NO_3^- 10, SO_4^{2-} 4. They were aerated continuously and changed each day. The seedlings were used in experiments 10–14 days after the start of soaking, at which stage there were two leaves and well-developed secondary roots, although no lateral roots had appeared. The dry weight of the shoots lay within the range 30–50 mg and those of the root systems between 10 and 20 mg (seed remains excluded).

Measurement of the Accumulation of ^{32}P and Its Movement into the Shoot

Only an outline is given since the method was described and its accuracy discussed by Crossett (1966). The accumulation of ^{32}P in single seedlings was estimated from the depletion that they caused in radioactive nutrient solutions flowing past their root systems. The movement of ^{32}P into the shoot of each seedling, or "translocation", was estimated from direct measurements, with a Geiger–Müller detector, of the radioactivity of the shoot. The environment of the seedling was carefully controlled, with the root in darkness at 25°C, and the shoot at 25°C in a current of air which had been equilibrated with dry silica gel, a condition which ensured that transpiration was not limited by high humidity. The shoot was illuminated by two 100-W tungsten lamps which gave a light intensity of 500 f.c. at the leaf surface. A water-cooled jacket protected the shoot from the radiant heat of the lamps.

The radioactive solutions used in the experiments differed from the nutrient solutions in which the seedlings were grown only in the addition of between 3 and 5 μc carrier-free ^{32}P. Radioactivity has been expressed as micrograms of carrier phosphorus, using the specific activity of each nutrient solution as a conversion factor from the equivalent "count" rate. Although the possibility of isotopic dilution inside the plant means that this notation is incorrect, it facilitates the comparison of results since there was some variation in the specific activity of the nutrient solutions used in the various experiments.

The advantages of the above technique are as follows:

(1) It is possible to make frequent measurements of the amount of radioactivity in both the root and the shoot of the plant without disturbing it in any way.

(2) A well-defined and easily controlled environment is obtained with simple equipment.

(3) Rapid changes can be made between labelled and inactive nutrient solutions which make it easy to study the movement of radioactive substances within the plant after their absorption has ceased.

The major disadvantage of the technique is the impossibility of any simultaneous replication of experiments. Each experiment was repeated at least twice and similar results were obtained on all occasions.

III. RESULTS

Figure 1(*a*) gives the results of an experiment to determine the effect of environmental changes on the translocation of ^{32}P in barley seedlings. A seedling was placed in the apparatus and, after an overnight settling-down period, the

inactive nutrient solution flowing through the root chamber was replaced by the ^{32}P-labelled nutrient solution. The ^{32}P was absorbed at a constant rate throughout the experimental period, but translocation attained a stable rate only after 6 hr of

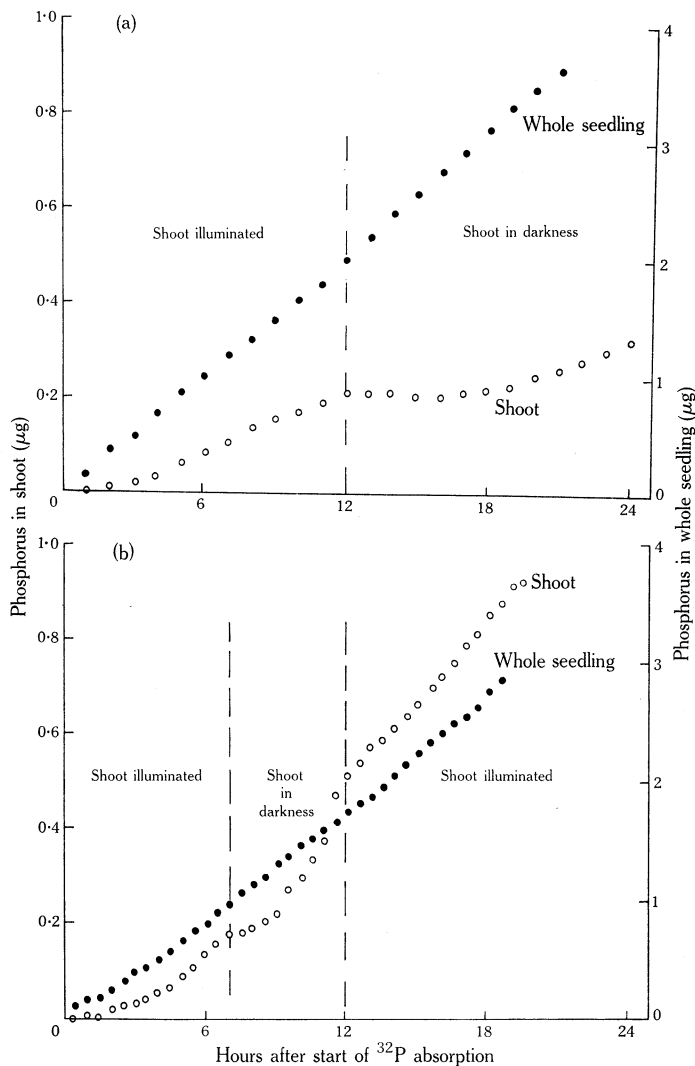Fig. 1.—Effect of illumination of the shoot on the absorption and translocation of ^{32}P by a barley seedling. Radioactivities calculated from the specific activity of the nutrient solution (which contained 10^{-6}M KH$_2$PO$_4$) used in each experiment. (a), (b) Results for two separate experiments, respectively.

absorption. After 12 hr of ^{32}P absorption a black cloth was placed over the shoot water-jacket, simulating conditions of complete darkness. (At this time the shoot had been continuously illuminated for the previous 28 hr.)

Within 5 min of darkening, rate of translocation of ^{32}P in the shoot had fallen
to less than half its former rate. The initial decrease was followed by a slow rise in
the translocation rate until after about 7 hr of darkness it was stable and equal to
that attained while the shoot was illuminated. A similar pattern of behaviour was
observed in many seedlings, although there was some variation in the time that was
required for the translocation rate to "recover" during a period of darkness. No
correlation could be found between the size of the root, or its ^{32}P absorption rate,
and the time required for the translocation rate to recover in darkness. However,
seedlings which took a long time to attain a stable translocation rate in darkness
also required a long time for stabilization at the start of ^{32}P absorption.

Figure 1(b) shows the results of a second experiment, the initial part of which
was similar to the first experiment [Fig. 1(a)]. The response of the seedling was
similar although faster. The shoot was placed in complete darkness after absorption
of ^{32}P for 7 hr. After the translocation rate became stable in the dark (5 hr later)
the shoot was illuminated. At once the rate of translocation increased but quickly
reverted to the stable level which it had reached in both light and dark.

On the basis of its eventual fate, Crossett and Loughman (1966) recognized
two arbitrary categories of phosphorus in the root; that which accumulates in the
root, and that which lies in the path of translocation. It was suggested that the
delay in the attainment of maximal ^{32}P translocation rate was caused by a dilution
of the isotope with phosphorus already in the root. The present results indicate that
the phosphorus "pool" which lies in the pathway of translocation is only a very
small part of the total in the root and that its amount does not change appreciably
with time.

The putative phosphorus pool in the root may be represented as follows:

$$\mathrm{d}A/\mathrm{d}t = I - kA, \tag{1}$$

where A is the mass of phosphorus in the pool (μg), I the rate at which phosphorus
enters the root pool (μg hr^{-1}), k a rate constant for the loss of phosphorus from the
pool (hr^{-1}).

The results reported by Crossett and Loughman (1966) suggested that there
was no change in the size of the root pool ($\mathrm{d}A/\mathrm{d}t = 0$, i.e. $I = kA$), provided that
the plant environment remained constant. The results reported here show that
changes in the plant environment may produce transient changes in the translocation
rate, suggesting that under certain conditions $I \neq kA$.

The present observations may be interpreted in terms of the simple model
[equation (1)] as a rapid drop in the rate constant from k to k' at the start of a period
of darkness. Provided that the rate at which phosphorus enters the root pool, I,
remains unchanged it would be greater than the translocation rate $k'A$. Unless there
is an alternative phosphorus "sink" the pool would increase in size, tending to a new
equilibrium $I = k'A'$. If the reduction k to k' was reversible there would be a
transient increase in the translocation rate, kA', upon re-illumination of the shoot.
Since I would then be smaller than kA' the pool would decrease in size, the system
tending to the pre-darkness equilibrium, $I = kA$.

The validity of the interpretation given above was investigated in experiments which used changes between radioactive and inactive nutrient solutions to measure k and A under various environmental conditions.

At isotopic equilibrium, equation (1) is valid for ^{32}P movement:

$$dA^*/dt = I^* - kA^*$$

(an asterisk denotes the radioactive species). If the radioactive solution is replaced by an inactive solution $I^* = 0$ so that

$$dA^*/dt = -kA^*$$

which may be integrated with respect to time to give

$$A_t^* = A_0^* e^{-kt},$$

or, taking logarithms,

$$\log_e A_t^* = \log_e A_0^* - kt, \tag{2}$$

where A_0^* is the amount of ^{32}P in the root pool at the time of a change to an inactive solution and A_t^* the amount in the pool at time t after the change.

Work reported by Crossett and Loughman (1966) showed that the phosphorus fluxes between root and environment and between shoot and root were negligible at the low concentrations used in these experiments. A_0^* is therefore equal to the total amount of ^{32}P translocated after a change from a radioactive to an inactive nutrient solution. It is possible to calculate A_t^* by subtraction of the ^{32}P content of the shoot at the various times t after ^{32}P absorption had ceased from the maximal content attained when ^{32}P translocation had ceased (the time at which A_0^* is measured). The rate constant k may be obtained from the gradient of a plot of $\log_e A_t^*$ against t [equation (2)].

The hypothesis was tested in three experiments, A, B, and C, which differed only in the illumination of the shoot (the root was kept in darkness throughout). In each experiment a seedling was placed in the apparatus and, after acclimatization overnight, was subjected to an alternation of ^{32}P-labelled and unlabelled nutrient solutions. The pattern of the first 21 hr after acclimatization was common to all three experiments (labelled nutrient solution for 9 hr followed by unlabelled nutrient solution for the next 12 hr, with continuous illumination) and provides a basis for comparison of the individual seedlings. During the second 21-hr period after acclimatization, variations were made which allowed the calculation of A_0^* and k for the illuminated shoot or the shoot in darkness: labelled nutrient solution for 9 hr (between 21 and 30 hr) with the shoot illuminated in experiment A, but with shoots in the dark in experiments B and C, followed by 12 hr (between 30 and 42 hr) with shoots illuminated in experiments A and B, but with the shoot in the dark in experiment C.

The results of experiments A, B, and C are given in Figure 2 and in Table 1, and show the decrease in A^* after the change from a flow of labelled nutrient solution to one of unlabelled solution. Table 1 also shows the total ^{32}P content of the roots at various stages in the experiments and values of A_0^* and k.
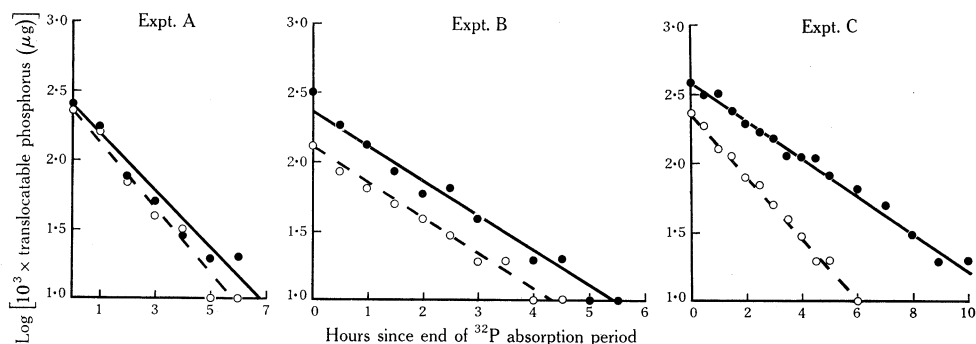

Fig. 2.—Results of three experiments (A, B, and C) showing the decrease in the amount of translocatable ^{32}P in the root after the end of two separate periods (O, ●) of ^{32}P absorption. For further details of experiments see text, p. 229.

The small variation in A_0^* between the two periods of experiment A confirmed the earlier observation (Crossett and Loughman 1966) that the size of the phosphorus pool which lies in the pathway of translocation was independent of the total content

TABLE 1

RESULTS OF EXPERIMENTS A, B, AND C

Expt.	Absorption Period	Total ^{32}P Content of Root*	Amount of ^{32}P Translocated†	k (hr^{-1})‡
A	1	0·76	0·23	0·54
	2	1·14	0·25	0·48
B	1	0·64	0·13	0·58
	2	1·17	0·27	0·61
C	1	0·87	0·23	0·52
	2	1·42	0·38	0·32

  * Expressed as μg carrier phosphorus content measured at the end of each absorption period.

  † Expressed as μg carrier phosphorus amount which moved to the shoot measured after the termination of absorption.

  ‡ Rate constant for loss of ^{32}P from the root pool which lies in the pathway of translocation.

of the root, and that its size showed little change with time. The experiment also showed that the rate constant for loss of ^{32}P from the root pool did not change with time if conditions remained the same.

In experiments B and C the amount of ^{32}P translocated after the end of an absorption period during which the shoot had been in darkness was greater than that translocated after absorption while the shoot was illuminated. These results may be interpreted as an increase in the pool size during darkness.

The values found for k in experiment C indicate that the turnover rate of the root pool of translocatable phosphorus is lower while the shoot is in darkness.

The results of the experiments A, B, and C were therefore in accord with the earlier interpretation of the transient changes in the ^{32}P translocation which followed variation in the illumination of the shoot.

IV. Discussion

The results clearly show that the movement of phosphorus from the environment to the root pool differs from its movement from the pool to the vascular system, at least in terms of sensitivity to periods of darkness. It therefore seems likely that either the two processes are different or they are spatially separated so that each functions in a different cellular environment.

In the absence of measurements of electrical potential difference between the environment, the root pool, and the vascular system, it was impossible to determine whether either of the inward phosphorus fluxes was an active process. The results raised two interrelated questions which merit speculation: What mechanisms of restriction of translocation are compatible with the observations? What is the mechanism whereby the shoot apparently controls the metabolism of the root?

The effect of environmental changes upon the root phosphorus pool may be interpreted either in terms of a permeability or "carrier" mechanism or in terms of an alteration of phosphorus metabolism. The permeability–carrier interpretation is most easily explained by analogy. The agreement between the experiments and the system described by equation (2) means that ^{32}P in the root pool behaves in a similar fashion to a gas in a permeable container surrounded by a vacuum. The frequency of collisions between the gas molecules and the container walls depends upon their number, provided that there is no change in the energy of the system. The number of gas molecules leaving the container is the product of collision frequency and the ratio of the total area of the "holes" to the total area of the container walls.

The rate constant k of equations (1) and (2) is therefore analogous to a permeability constant. Changes in k may therefore represent either changes in the permeability of a membrane, or, in terms of a carrier theory of ion transport (Epstein 1965), a change in the rate of active site availability on the appropriate side of a permeability barrier.

The alternative "metabolic" interpretation is as follows: The earlier findings, that the ^{32}P in the root pool is in equilibrium with a number of organic phosphorus compounds (Crossett and Loughman 1966), and that mannose both inhibits ^{32}P translocation and modifies phosphorus metabolism (Loughman 1966), implicate organic phosphorus compounds in the translocation system. If the root phosphorus

pool contains an equilibrium mixture of phosphorus compounds only some of which could be utilized by the translocation system, a shift in that equilibrium would bring about a change in the translocation rate. For example, if the root pool contains only two species of phosphorus A_m and A_n, only A_m being utilized by the translocation system, any shift in the equilibrium to give more A_n would decrease the amount of the translocatable form and bring about an instantaneous decrease in the translocation rate. If the rate at which phosphorus enters the pool remains unchanged, the pool size would increase until the efflux balanced the rate of entry.

It will be noticed, of course, that the two interpretations are formally identical, the only difference being whether the limiting system is dispersed in the pool, or localized in its walls.

It should be possible to distinguish between the two interpretations by making a detailed analysis of the amounts and turnover rates of the various phosphorus compounds in the root. However, as Loughman (1966) has already pointed out, this would be a very difficult task.

The transpiration stream is a possible method by which the shoot could control phosphorus translocation.

Although the transpiration stream responds quickly to changes in illumination of the shoot (Virgin 1956) the orthophosphate concentration in the nutrient solutions used in the present experiments is well below that required for there to be any relation between translocation and transpiration (Russell and Barber 1960). Simple measurements of the weight lost by seedlings in the growth chamber* show that the transpiration rate decreased from $0 \cdot 11$ g hr^{-1} per seedling to $0 \cdot 04$ g hr^{-1} per seedling when the lights were turned out. The decrease in transpiration rate was thus of the same order as the initial decrease in the rate of ^{32}P translocation, so that there could have been little change in the phosphorus concentration in the xylem. Since the rate of arrival of ^{32}P in the shoot must be equal to the product of the water flux in the xylem and its ^{32}P concentration, the observations could explain the initial drop in translocation rate, but, since the transpiration rate remained constant in the dark there is no obvious explanation of the "recovery" of the ^{32}P translocation during periods of darkness. It is therefore probable that water movement does not limit ^{32}P translocation under the conditions of the present experiments.

An alternative control mechanism is through regulation of the energy supply to an active process, either by an alteration of the intracellular environment or through changes of the substrate supply. Control by an alteration of the cellular environment embraces such a diversity of possible mechanisms that speculation cannot be profitable at this stage. The involvement of organic phosphorus compounds in the control of phosphorus translocation has already been discussed both in this paper and by Loughman (1966). It has also been shown (Geiger and Swanson 1965) that the rate of translocation of photosynthetic products from sugar-beet leaves responds very rapidly to changes in their illumination. If recently translocated photosynthate was used preferentially in the formation of the organic phosphorus compounds supposed to be utilized by the translocation system, there

* Described in Section II.

would be a means for control of upward phosphorus translocation by the supply of organic material from the shoot.

The above suggestions are intended only as a basis for future experiment. If the mechanism which controls the translocation of phosphorus is found to be any of those that have been proposed here, it will obviously apply to only the one element and perhaps be confined to the conditions of the present experiments.

V. Acknowledgments

VI. References

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