

PHOSPHATE UPTAKE ALONG ATTACHED AND EXCISED WHEAT ROOTS MEASURED BY AN AUTOMATIC SCANNING METHOD

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Summary

A method has been developed for automatic scanning of plant roots to record accurately and with good resolution sites of uptake and accumulation of radioactively labelled nutrients. This technique was applied to a comparison of phosphate uptake by excised and attached wheat roots. In periods of 2 and of 15 min attached roots absorbed 40–60% more phosphate than did roots excised immediately prior to uptake. These differences could not be ascribed to transpiration nor to translocation in whole plants and hence rapid physiological changes upon excision are suggested. The lower uptake of excised roots occurred in both the apical and the mid-root portions.

This scanning method showed that one major peak occurred in the apical 3 cm and another in the basal portion of the root. This high uptake in the basal region corresponded to lateral root primordia.

I. INTRODUCTION

Although excised roots are used extensively in plant physiology to study nutrient uptake there are few studies in which excised and attached roots have been directly compared in this regard. Only Hoagland and Broyer (1936) appear to have made such a comparison; they found that the uptake of potassium and nitrate by excised roots of barley grown under "high salt" conditions was 31 and 27% respectively of that by attached roots in an uptake period of 7 hr. Only small differences between attached and excised roots occurred with plants of "low salt" status. They suggested that differences between attached and excised roots were due to translocation allowing further uptake in attached roots. No attempt was made to determine whether the differences in uptake in the "high salt" plants [grown in half-strength plant nutrient solution of Hoagland and Arnon (1938)] were due to a general reduction in root activity when excised or whether the reduction was confined to certain sectors of the roots.

Nutrient uptake studies with plant roots are complicated by differences in uptake along the root. Previous studies of activities of different parts of the root have been conducted by selectively feeding relatively large root segments (Wiebe and Kramer 1954), or cutting roots into segments before or after exposure to nutrient solutions (Prevot and Steward 1936; Kramer and Wiebe 1952). Such methods are laborious, especially when considerable replication is required. The examination of the contributions made by different parts of a root to uptake kinetics of the whole root has been hampered by the lack of a fast, accurate, quantitative technique. Such a method has now been developed to automatically record with high resolution the distribution of ^{32}P in different parts of excised and attached roots of wheat after short exposure to potassium dihydrogen phosphate containing [^{32}P]phosphate.

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II. MATERIALS AND METHODS

Seed of wheat (*Triticum vulgare* cv. Gabo) graded to 45–50 mg was surface-sterilized with 7% calcium hypochlorite and germinated on agar at 25°C. Seeds which germinated within 24 hr were transferred to sterile, cotton-plugged, 20 by 3 cm tubes containing stainless steel gauze over 40 ml of sterile plant nutrient solution (Hoagland and Arnon 1938); two seeds were planted per tube. After 4 days in a constant-environment cabinet (12-hr day at 3000 lumens per square foot, 16°C night and 22°C day) the roots, which had not yet developed laterals, were placed in 5×10^{-4} M calcium sulphate for 2 hr at 20°C, prior to uptake from a solution of 5×10^{-4} M calcium sulphate and 5×10^{-6} M potassium dihydrogen phosphate with [32 P]phosphate added at 400 μ c per litre. After uptake for 2 and for 15 min with intermittent agitation the roots were washed in rapidly running tap water for 5 min. Preliminary experiments with 2- and 15-min uptake periods had shown no differences in phosphate uptake between roots shaken intermittently as above and roots in solution stirred by aeration. Dainty (1963) has pointed out that despite vigorous stirring, quite wide unstirred layers usually occur in biological experiments. The 5-min washing had been proved effective in removing the non-absorbed phosphate from the "free space" in roots treated with potassium cyanide. Excised roots were severed immediately prior to immersion in the phosphate solution and the attached roots were severed immediately following washing in the tap water. Adhering water was removed and the roots laid end to end along a continuous strip of 4 cm wide Whatman No. 1 chromatography paper and secured to it by covering with a continuous strip of cellulose tape. The preparations were dried at 50°C for 30 min. Measurements of radioactivity along roots before and after this drying treatment showed that no changes in phosphate distribution occurred during drying. Radioactivity along roots was measured by passing through a gas flow chromatogram scanner and recorder (Nuclear Chicago Actigraph III). Scanning speed, collimator width, integration time, and scale range were adjusted for maximum resolution for the levels of radioactivity in the roots. The scans selected in Figures 3 and 4 were with machine settings of 10,000 counts/min for a full scale deflection, collimator width 1.5 mm, integration time 10 sec, and chart speed 15 cm/hr. In practice, preliminary scans were made at 240 cm/hr with 1.5 mm slit and a shorter integration time with good resolution. The instrument gives an exact 1 : 1 ratio between the recording chart and paper strip holding the roots.

III. RESULTS AND DISCUSSION

(a) Assessment of Method

The quantitative accuracy of the technique was determined with 140 separate roots by correlating areas under the curves with radioactivity subsequently measured with an end window Geiger-Müller tube. Hagen and Hopkins (1955) have shown that in counting of radioactive phosphorus in young barley roots self absorption was negligible and no correction was necessary. The observed correlation of $r = 0.977$ between the areas under the curves, estimated by the weight of paper, and the radioactivity of corresponding roots (Fig. 1) shows that this technique provides a reliable measurement of total uptake of [32 P]phosphate by plant roots.

The resolution was assessed by correlating the radioactivity of individual 2-mm segments of a 9.4 cm unbranched root with the areas under corresponding portions of the curve obtained for that root with the chromatogram scanner. The high correlation ($r = 0.953$) between the radioactivity of 2-mm segments of a root and corresponding areas under the curve (Fig. 2) established that this root-scanning technique can be used to indicate the sites of radioactivity in roots with considerable precision. The resolution has been shown to be better than 2 mm, except when there was more than one-fifth full scale deflection within 2 mm due to sudden changes in radioactivity along the root. In such cases the peak in the curve lagged from 2 to 4 mm behind the peak of radioactivity in the root.

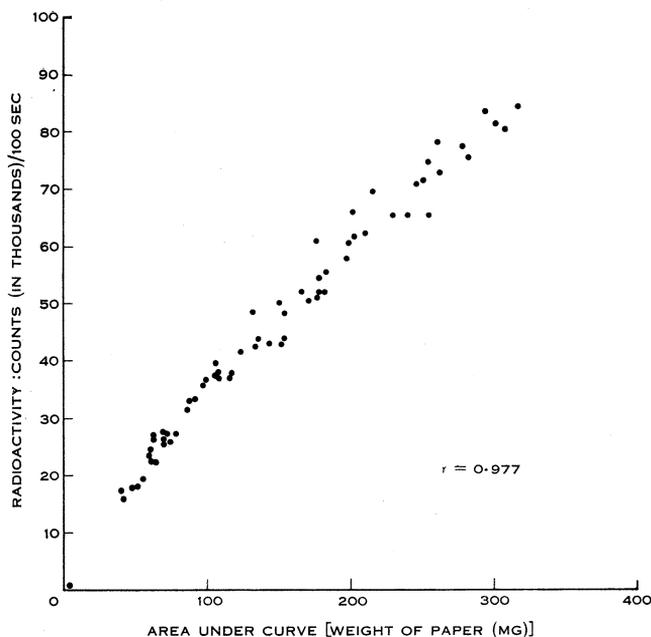


Fig. 1.—Relationship between radioactivity of whole roots estimated by end window Geiger-Müller counting and areas under curves. The graph is a composite of values obtained for excised and attached roots immersed for 15 min in [^{32}P]phosphate in $5 \times 10^{-6}\text{M}$ potassium dihydrogen phosphate and $5 \times 10^{-4}\text{M}$ calcium sulphate.

The method has also been successful with sodium-24, but has its limitations as a quantitative method for low energy radioisotopes, e.g. carbon-14, in which radial distribution of radioactivity within the root and self-absorption of emissions become important or where differences in root thickness lead to differences in absorption of radiation. Furthermore, following scanning it is possible to stain for root hairs, apices, and microbial colonization thus enabling an assessment of the significance of these features in the uptake of plant nutrients.

(b) *Uptake by Excised and Attached Roots*

With short uptake periods of 2 and 15 min and excision immediately before uptake, the total uptake by attached roots was significantly greater than by excised roots (Table 1). The results in Table 1 were obtained by counting with an end window Geiger-Müller tube although measurement of areas under root scan records gave similar results.

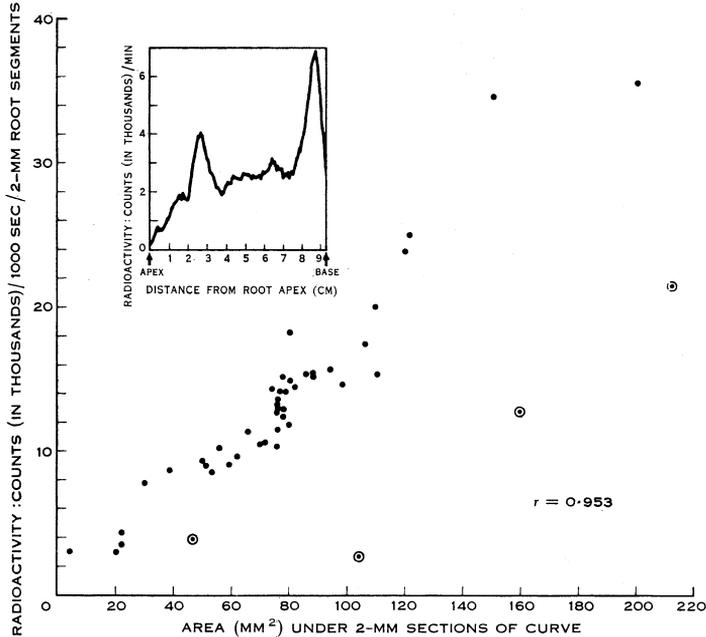


Fig. 2.—Relationship between the radioactivity of 2-mm segments of one excised root and corresponding areas under the curve. The four outlying points marked ⊙ represent the tail after the high activity near the base of the root had passed the detector. Inset shows the scan of the root from which the above relationships were obtained.

The uptake by attached roots compared with excised was relatively greater after 15 min (61%) than after 2 min (43%) but an analysis of variance of logarithmically transformed data failed to show a significant interaction between time and condition of roots.

In an experiment on 4-day-old tomato plants, attached roots took up 123% more phosphate than did excised roots in 15 min. In the zone of maximum uptake, which occurred 1–2 cm behind the apex, the mean peak height for the attached roots was 124% greater than that of excised roots. Both of these increases were statistically significant at the 0.1% level.

The greater nutrient uptake by attached roots of wheat and tomato are consistent with the findings of Hoagland and Broyer (1936) for “high salt” plants and longer uptake periods. With plants grown in phosphate-deficient Hoagland and

Arnon solution attached roots absorbed 15% more phosphate than did excised over 15 min [see Section III(d)]. This is not inconsistent with the results of Hoagland and Broyer (1936) with "low salt" barley; an examination of their data shows that in two experiments uptake of attached over excised was 4 and 21% greater with potassium and 5 and 19% greater with nitrate.

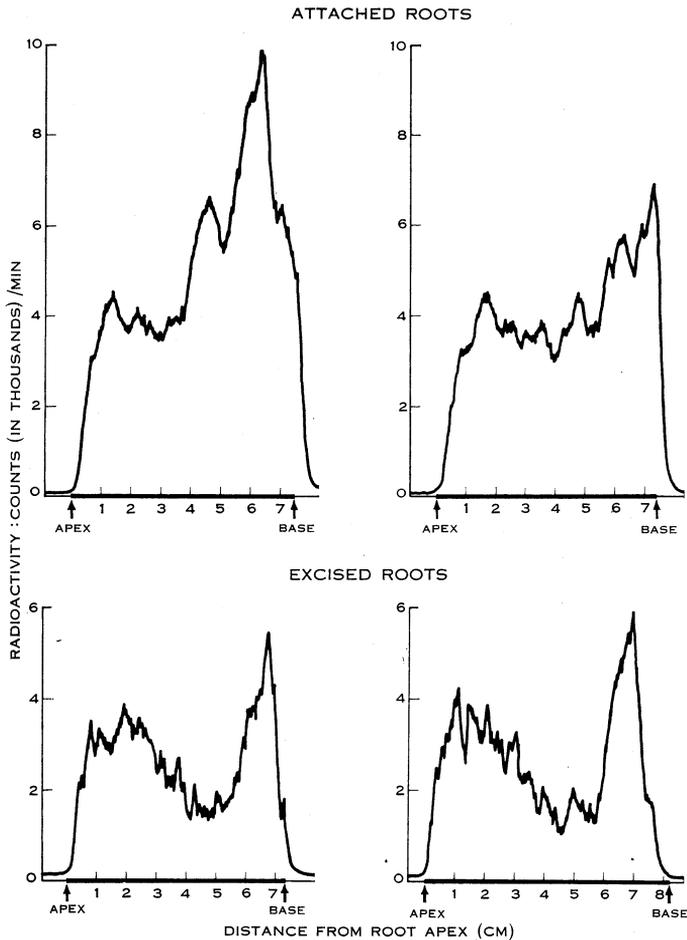


Fig. 3.—Radioactivity along morphologically similar attached and excised wheat roots. Exposure for 15 min to $[^{32}\text{P}]$ phosphate in $5 \times 10^{-6}\text{M}$ potassium dihydrogen phosphate and $5 \times 10^{-4}\text{M}$ calcium sulphate.

(c) *Patterns of Uptake along Roots*

The root scanning technique showed different patterns of radioactivity along attached and excised roots. Sites of activity corresponded to sites of uptake [see Section III(e)]. The charts in Figure 3, which are typical examples of morphologically similar roots, show that both types of root had a high uptake in the apical 3 cm and

also at the base of the root. The high phosphate levels in the basal portions of these unbranched roots coincided with lateral root primordia which would have emerged

TABLE 1
PHOSPHATE UPTAKE BY ATTACHED AND EXCISED WHEAT ROOTS
Uptake from $5 \times 10^{-6}M$ KH_2PO_4 in $5 \times 10^{-4}M$ $CaSO_4$ for 2 and 15 min

Condition of Roots	Uptake Period 2 Min		Uptake Period 15 Min	
	No. of Replicates	Mean Phosphate Uptake per Centimetre of Root \pm Standard Error (10^{-12} moles)	No. of Replicates	Mean Phosphate Uptake per Centimetre of Root \pm Standard Error (10^{-12} moles)
Attached	20	45.0 ± 2.4	20	259.8 ± 10.8
Excised	15	$31.6 \pm 2.7^{***}$	13	$160.0 \pm 13.4^{***}$

*** Difference significant at 0.1% level.

TABLE 2
PHOSPHATE UPTAKE* BY THE APICAL REGION OF ATTACHED AND EXCISED WHEAT ROOTS

Condition of Roots	Uptake Period 2 Min		Uptake Period 15 Min	
	No. of Replicates	Mean Peak Height (cm) \pm Standard Error	No. of Replicates	Mean Peak Height (cm) \pm Standard Error
Attached	12	4.28 ± 0.41	12	8.8 ± 0.55
Excised	11	$2.61 \pm 0.43^{***}$	12	$6.9 \pm 0.55^{***}$

* Assessed by the height of the peak within the apical 3 cm.

*** Difference significant at 0.1% level.

TABLE 3
INFLUENCE OF TRANSPIRATION ON PHOSPHATE UPTAKE BY WHEAT ROOTS
Uptake from $5 \times 10^{-6}M$ KH_2PO_4 in $5 \times 10^{-4}M$ $CaSO_4$ for 15 min

Condition of Roots	No. of Replicates	Transpiration	Mean Phosphate Uptake per Centimetre of Root \pm Standard Error (10^{-12} moles)
Attached	30	Normal	440 ± 13.2
	30	Reduced	423 ± 13.2
Excised	28	—	$375 \pm 13.8^{**}$

** Uptake by excised roots significantly less at 1% level.

within the following 24–48 hr. There was considerable variability between what appeared to be identical roots — an observation made also by Kramer and Wiebe

(1952) and Lundberg (1961). However, the overall patterns for attached and excised roots were reasonably consistent with these 4-day roots.

An obvious contributing factor to the lower total uptake by excised roots was the lower activity in the central portion when compared with the activity of the corresponding portion of attached roots. When the activity of the apical region of the root was assessed by measuring the height of the peak within 3 cm of the apex, it was found that the uptake by this region was also very markedly reduced by excision in both the 2- and the 15-min uptake treatments (Table 2).

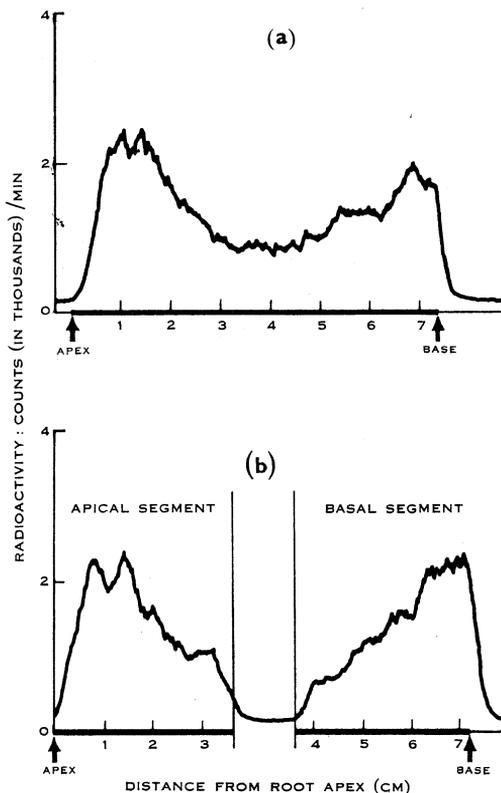


Fig. 4.—Phosphate uptake by whole and cut excised roots. Exposure for 15 min to $[^{32}\text{P}]$ phosphate in $5 \times 10^{-6}\text{M}$ potassium dihydrogen phosphate and $5 \times 10^{-4}\text{M}$ calcium sulphate: (a) whole during immersion; (b) cut into two segments before immersion.

Scans of excised roots cut in two before immersion in $[^{32}\text{P}]$ phosphate showed negligible phosphate entry through the cut ends (Fig. 4). Hence the basal peak of excised roots reflects a real uptake in that portion of the root.

(d) Role of Transpiration in Uptake

The effect of transpiration on uptake by attached roots was investigated by placing the tops in a humid chamber for 2 hr before and during the uptake period of 15 min. This reduced transpiration by 75%. Table 3 shows that the decrease in

transpiration did not significantly affect phosphate uptake, although in the same experiment excision very significantly decreased uptake. Therefore, differences

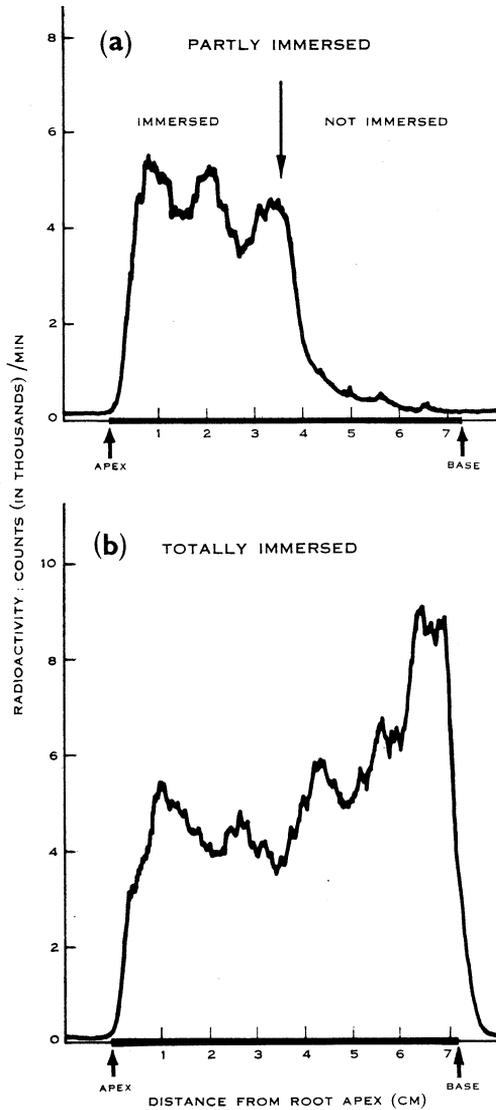


Fig. 5.—Radioactive phosphate distribution along (a) partly immersed root; (b) totally immersed root. Exposure for 15 min to $[^{32}\text{P}]$ phosphate in $5 \times 10^{-6}\text{M}$ potassium dihydrogen phosphate and $5 \times 10^{-4}\text{M}$ calcium sulphate.

between excised and attached roots were not due to a possible greater entry of phosphate to attached roots induced by mass flow of water caused by transpiration.

The plants used in this experiment had been grown for 5 days in phosphate-free plant nutrient solution [prepared according to the 1950 revision of Hoagland and Arnon (1938)]. Thus, excision affects the phosphate uptake of phosphate-deficient as well as phosphate-rich wheat roots. The uptake of phosphate per centimetre of roots grown in phosphate-deficient solution was greater than the uptake by roots grown in complete nutrient solution.

(e) *Role of Translocation*

Experiments with partly immersed roots (Fig. 5) show that there was little translocation of phosphate during 15-min exposure periods so that radioactivity along the roots essentially recorded the sites of uptake for both excised and attached roots. During exposures of 2 and 15 min only 5–10% of the phosphate taken up was translocated to the tops. Hence it is impossible for the much greater uptake by attached roots to be due to translocation as suggested by Hoagland and Broyer (1936).

(f) *Inhibition of Metabolism*

The contribution of non-metabolic absorption to the patterns of uptake was assessed in studies with roots treated with $1 \times 10^{-3}M$ potassium cyanide and with roots held at 2°C. The inhibitor treatments were imposed for 1 hr before uptake (i.e. during the calcium sulphate treatment) and maintained during an uptake period of 30 min. The uptake of phosphate by both excised and attached roots was completely eliminated by the potassium cyanide treatment and reduced by 90–95% at 2°C. Uptake was therefore linked almost completely with metabolic processes.

IV. CONCLUSIONS

Hoagland and Broyer (1936) suggested that the greater uptake of potassium and nitrate by attached roots over that of excised roots was due to translocation. The present studies have shown that similar large differences in phosphate uptake cannot be accounted for by translocation nor by a greater movement of phosphate from solution to roots induced by transpiration.

Excision effects were detectable within 2 min and it is concluded that severance of roots from tops induces immediate physiological changes in the roots. However, there was a trend which indicated that the effects of excision on total phosphate uptake were relatively greater after 15 min than after 2 min, but major changes occurred within 2 min.

Differences due to excision were greater with wheat grown in complete nutrient solution than with those grown in phosphate-deficient solution, although occurring with plants grown under both sets of conditions. These results agree with the data of Hoagland and Broyer (1936) remarkably well considering the differences in test plants, test nutrients, uptake times, and somewhat different growth media. Effects of excision therefore appear to be general.

Measurements of radioactivity along roots by the scanning technique showed that uptake by all regions of the root was retarded following excision.

V. ACKNOWLEDGMENTS

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