

NUTRIENT UPTAKE AND DISTRIBUTION IN SUBTERRANEAN CLOVER DURING RECOVERY FROM NUTRITIONAL STRESSES

I. EXPERIMENTS WITH PHOSPHORUS

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[*Manuscript received November 4, 1966*]

Summary

Young subterranean clover plants (*Trifolium subterraneum* L. cv. Mt. Barker), raised at three phosphorus levels in culture solutions, were transferred to complete solutions and to solutions without phosphorus and grown for a further 7 days.

After transfer to complete solutions, the roots retained approximately the same proportion of absorbed phosphorus at all pretreatment levels. In previously deficient plants most of the phosphorus translocated to the shoots was distributed to leaves existing at transfer, while in non-deficient plants most was distributed to leaves formed after transfer.

In a similar experiment with ^{32}P , phosphorus translocated to existing shoots during the first few days of recovery was less readily available the greater the previous phosphorus stress. By contrast, phosphorus accumulated in the roots was more available for subsequent translocation to new shoots the greater the previous stress.

After transfer of plants in the first experiment to solutions without phosphorus, no net losses of root phosphorus were found for any of the pretreatments. Phosphorus in new leaves and petioles formed after the transfer had all been derived from existing shoots. Export from these shoots was relatively greater the higher the phosphorus status of the plant at transfer.

I. INTRODUCTION

In a study of the growth changes in young subterranean clover plants during the early stages of recovery from phosphorus and sulphur stresses Bouma (1967) found that the relative rates of leaf area expansion were less dependent on previous levels of nutrient supply than the relative rates of increase in dry weight of the plant. The early recovery in leaf expansion after the removal of stresses was related to a preferential distribution of assimilates to new leaf tissue. The markedly lower relative growth rates of previously deficient plants compared with healthy plants were due to reduced net assimilation rates as well as to lower leaf area ratios. There was strong evidence that sulphur stresses had a more drastic effect on the assimilatory capacity of the leaves than did phosphorus stresses.

The present series of papers reports the uptake and distribution of phosphorus and sulphur that accompanied the growth changes in some of the above experiments. Further experiments were carried out with labelled phosphorus and sulphur to permit a more quantitative description of differences in uptake and distribution patterns during recovery from different stress levels. The results for phosphorus are presented in this paper.

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II. METHODS

Young subterranean clover plants (*Trifolium subterraneum* L. cv. Mt. Barker) were germinated in river sand and transferred to nutrient solutions 1 week after sowing.

In the first experiment, plants were grown at three phosphorus pretreatment levels (0.3, 0.7, and 4 p.p.m.). These levels are referred to as P₁, P₂, and P₃ respectively, and were intended to give a deficient, a moderately deficient, and an optimum phosphorus supply. The plants remained in pretreatment solutions for a period of 16 days. They were then transferred to complete solutions (4 p.p.m. phosphorus) and to solutions without phosphorus, in which they were grown for a further 7 days. Culture methods during pretreatment and after transfer were as described earlier (Bouma and Dowling 1966; Bouma 1967). Leaves existing at transfer were identified by placing a small white paper collar around the petioles. Harvests were carried out on the day of transfer (day 0) and 3, 5, and 7 days afterwards. Plants were separated into roots, petioles, and leaves; shoots were further separated into leaves and petioles existing at transfer and those formed afterwards. The experiment was carried out in a glasshouse kept at 24°C during the day and 17°C at night (6 p.m.–6 a.m.).

Dry weights were adjusted by covariance analysis on leaf areas at day 0 (McIntyre and Williams 1949; Bouma and Dowling 1966). Chemical analyses were carried out after bulking the eight replicates in the experiment in two groups of four. Subsamples were ashed in a mixture of nitric and perchloric acids. Phosphorus was estimated by the molybdenum blue method (Truog and Meyer 1929).

The second experiment was carried out in one of the growth rooms of the Institute for Atomic Sciences in Agriculture, Wageningen, The Netherlands. This room was kept at 23°C during a photoperiod of 16 hr and at 20°C during the dark period. Illumination was provided by banks of fluorescent tubes (Philips TL33, 25,000 lux). Seeds were sown in river sand and 7 days later uniform seedlings were placed in nutrient solutions contained in 1 litre glass jars. There were six seedlings per jar, which were thinned to four after 1 week. Plants remained in the pretreatment solutions for a period of 16 days. During this period three phosphorus levels were applied (1, 2, and 4 p.p.m. phosphorus). All nutrients were given at half strength during the first week and this was increased to full strength on the seventh day of pretreatment. After 16 days the plants were transferred to complete nutrient solutions and grown for a further 7 days. Radioactive phosphorus (³²P, 2 μc/l) was added during this period as follows. One group of plants was grown with ³²P for the first day, a second group for the first 2 days, and a third group for the first 3 days after transfer to complete solutions. At the change from radioactive to tracer-free solutions six plants were harvested for counting and two plants for autoradiography. Another six plants were grown for the remaining 6, 5, or 4 days respectively in tracer-free solutions. A fourth group of plants was grown in complete solutions without ³²P for the first 4 days after transfer and then for another 3 days with ³²P in complete solutions. The transfer from radioactive solutions was carried out after washing the roots in distilled water, followed by rinsing in a phosphate solution of approximately

the same strength as the nutrient solution and finally by further washing in distilled water. The same procedure was followed at harvest.

Plants harvested for autoradiography were immediately placed between layers of dry ice and freeze-dried before they were prepared for exposure on Kodak no-screen X-ray film (Levi 1962 and unpublished data). For counting, plant samples were ashed in 10 ml of a mixture of sulphuric, nitric, and perchloric acids in the proportions 0.25 : 10 : 1. A 3-ml aliquot was used for liquid scintillation counting (Tri-Carb, Packard) using a dioxane mixture as scintillation liquid.

Further experimental details were as described before (Bouma and Dowling 1966; Bouma 1967).

III. RESULTS

(a) *Experiment I*

(i) *Uptake and Distribution of Phosphorus after Transfer to Complete Solutions*

Figures 1 and 2 show the relative and absolute contents of total phosphorus in the different plant parts for the 7-day period after transfer to complete solutions of plants raised at three phosphorus levels (P_1 , P_2 , P_3).

The removal of the phosphorus stress in the P_1 and P_2 plants caused five- and sixfold increases in the relative phosphorus contents of existing leaves during the first 3 days after transfer. Relative phosphorus on day 3 in these leaves was as high as in the new leaves and similar to that of the existing leaves of P_3 plants. Relative phosphorus of old and new leaves at all pretreatment levels decreased steadily between day 3 and day 7.

The old petioles and the roots of P_1 and P_2 plants also showed significant increases in relative phosphorus during the first 3 days after transfer. However, the increases were small compared with those for the existing leaves and the values remained well below those of the corresponding parts of the P_3 plants. Relative phosphorus in the old petioles of P_1 and P_2 plants was considerably lower than for new petioles.

The marked increases in absolute phosphorus of the old leaves of P_1 and P_2 plants between day 0 and day 3 were followed by small but significant net losses during the following 4 days (Fig. 2). These losses coincided with the development of new shoots (Bouma 1967) and it appears likely that part of the demand for phosphorus was met by a redistribution of some of the large amounts of phosphorus translocated to older leaves before day 3.

Absolute phosphorus in the old petioles of P_1 and P_2 plants increased slightly during the first 3 days after transfer but changed little after that. The roots showed steady increases in absolute phosphorus with all pretreatments.

Table 1 shows the distribution of phosphorus for the periods 0-3 and 0-7 days after transfer to the various plant parts expressed as a percentage of the intake by the whole plant. The percentage distributed to the roots differed relatively little between pretreatments and was between one-quarter and one-third of the intake by the whole plant. Marked differences were found in the distribution of phosphorus to existing and new shoots between the different pretreatments. During the first 3 days after transfer new leaves of plants raised at an adequate phosphorus level received

somewhat more phosphorus than the existing leaves. The new petioles of these plants also received a considerable proportion of the phosphorus taken up during this period while the older petioles showed a small loss. By contrast, relatively small quantities of phosphorus were distributed to new leaves and petioles of plants

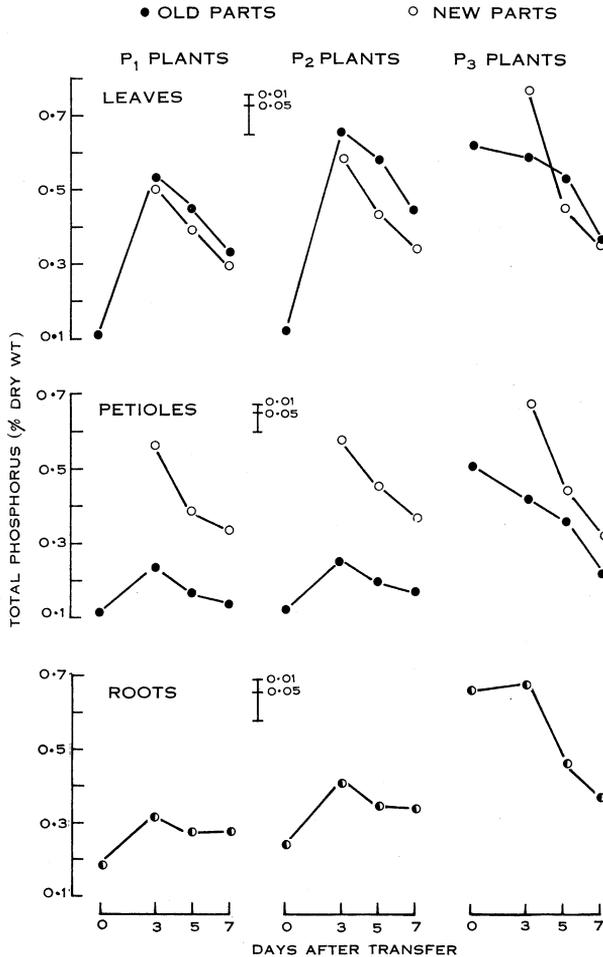


Fig. 1.—Changes in relative phosphorus contents after transfer of plants raised at three phosphorus levels (P_1 , P_2 , P_3) to complete nutrient solutions (expt. I). Minimum differences for significance at $P < 0.05$ and $P < 0.01$ are shown.

recovering from phosphorus stresses (P_1 and P_2) and more than half of the phosphorus taken up by these plants was distributed to existing leaves. During the remainder of the recovery period older leaves of P_1 and P_2 plants lost some phosphorus and considerably more phosphorus was distributed to new leaves than before. Considered over the 7-day period after transfer, the proportions of phosphorus retained by the

roots differed relatively little between pretreatments. In previously deficient plants considerably more phosphorus was distributed to old than to new leaves, while in plants that were not deficient at transfer the reverse was the case.

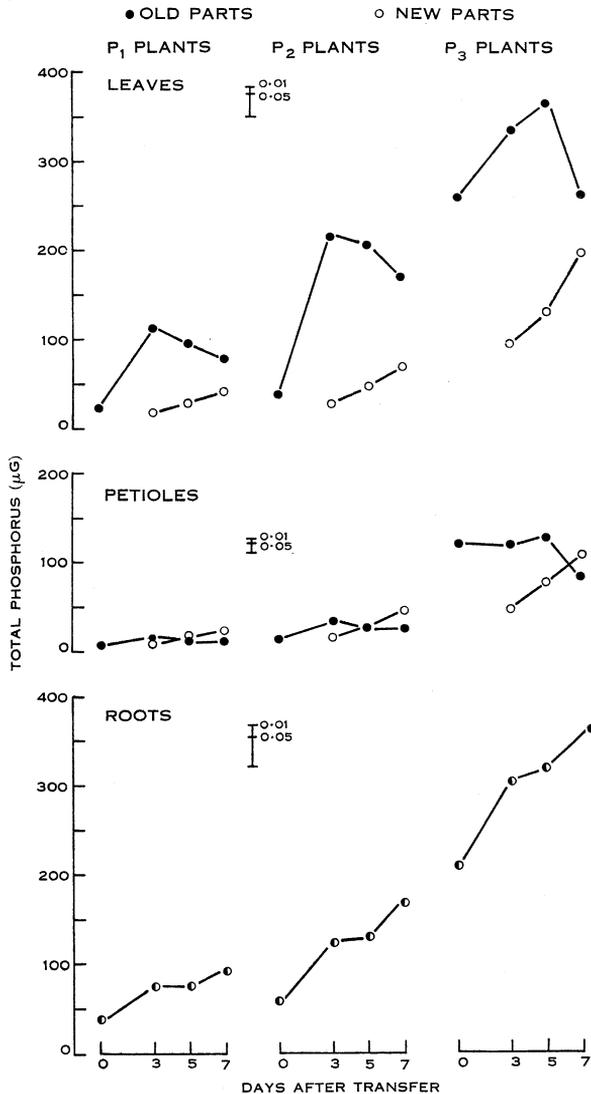


Fig. 2.—Changes in absolute phosphorus contents after transfer of plants raised at three phosphorus levels (P₁, P₂, P₃) to complete solutions (expt. I). Minimum differences for significance at $P < 0.05$ and $P < 0.01$ are shown.

(ii) *Redistribution of Phosphorus after Transfer to Solutions without Phosphorus*

Table 2 shows the redistribution of phosphorus after transfer of P₁, P₂, and P₃ plants to solutions without phosphorus. This had the effect of accentuating an existing stress (P₁, P₂) or of imposing a stress on non-deficient plants (P₃).

At all pretreatment levels the existing leaves and petioles lost a considerable portion of their phosphorus to new shoots. The old leaves at P_1 and P_2 lost approximately one-third of their phosphorus and the old petioles nearly half. At P_3 the old leaves lost nearly 60% of their phosphorus and the old petioles more than 70%. No phosphorus was lost from the roots in any of the pretreatments, but there were some losses from the plant at P_2 and P_3 .

TABLE I
DISTRIBUTION OF PHOSPHORUS TO DIFFERENT PLANT PARTS FOR THE PERIODS 0-3 AND 0-7 DAYS AFTER TRANSFER TO COMPLETE SOLUTIONS, EXPRESSED AS A PERCENTAGE OF THE INCREASE FOR THE WHOLE PLANT

Experiment I*

Plant Part	Period 0-3 Days			Period 0-7 Days		
	P_1 Plants	P_2 Plants	P_3 Plants	P_1 Plants	P_2 Plants	P_3 Plants
New leaves	10	9	29	23	19	47
New petioles	7	4	16	12	12	26
Old leaves	53	58	25	30	35	-1
Old petioles	5	6	-1	1	3	-10
Roots	24	23	31	33	31	38

* The indices for the period 3-7 days are difficult to interpret due to net losses from old parts and are therefore not shown.

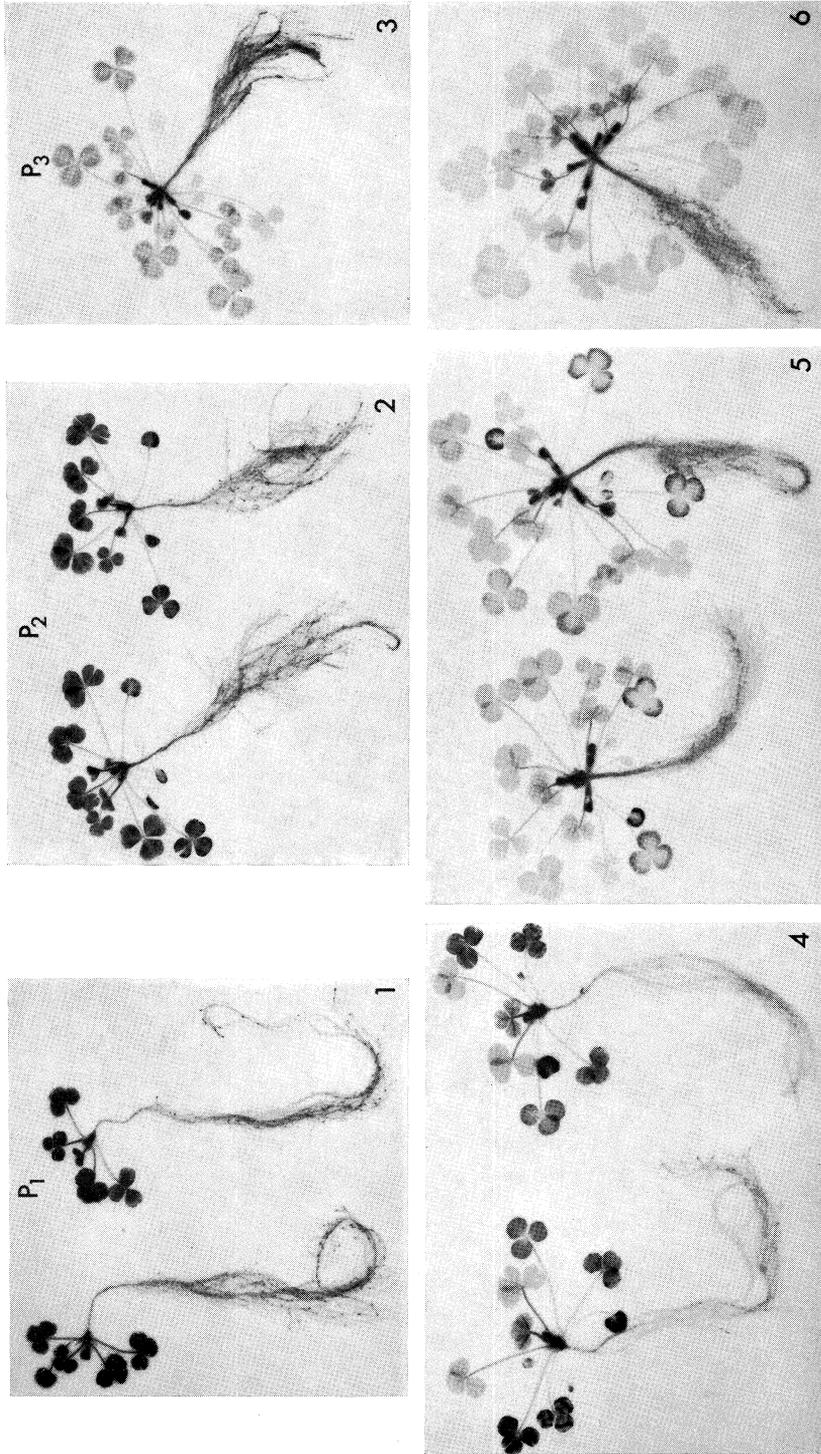
(b) Experiment II

Figure 3 shows the uptake and distribution of ^{32}P on the first, second, and third day after transfer and the redistribution that had occurred during the remainder of the 7-day period after transfer. The dry weights of the shoots and their parts for the different harvest occasions are also shown.

At the end of the first day after transfer to complete solutions the shoots of the P_1 plants contained nearly as much ^{32}P as those of the P_3 plants. The dry weight of P_1 leaves on day 1 was less than half of that at P_3 so that the relative content of ^{32}P was considerably greater at P_1 than at P_3 . The autoradiographs of Plate I, Figures 1, 2, and 3, clearly illustrate the decrease in concentration of ^{32}P between P_1 and P_3 . The amount of radiophosphorus translocated to the shoots of P_1 plants changed little between day 1 and day 3, but there was a 40% increase in the amount translocated to the shoots of P_3 plants.

Of the ^{32}P translocated to existing shoots in the first 24 hr after transfer a considerable part was retranslocated to new leaves and petioles during the subsequent 6 days in tracer-free solutions. This occurred at all pretreatment levels, but the amounts were smaller the greater the phosphorus stress at transfer. The losses

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Figs. 1-3.—Autoradiographs of plants raised at three phosphorus levels (P₁, P₂, P₃) after 1 day in complete solutions containing 2 μC ³²P per litre. Figs. 4-6.—Autoradiographs of similarly treated plants after further growth for 6 days in tracer-free complete solutions. Exposure period 6 hr for all plants.

amounted to 30, 41, and 58% at P₁, P₂, and P₃ respectively. This is illustrated in Plate 1 by the comparison of plants harvested on day 1 with those of day 7. In the P₁ plants harvested on day 7 there was a clear distinction between the four trifoliolate leaves present on day 1 and the three leaves which developed subsequently. That some translocation of ³²P from old leaves between day 1 and day 7 occurred is evident from the somewhat decreased blackening of these leaves on the two autoradiographs concerned. The autoradiograph of P₁ plants on day 7 also suggests that phosphorus in existing leaves fell with their age. The unifoliolate leaf remained blackest while the youngest trifoliolate leaf present on day 1 was considerably lighter on day 7. The

TABLE 2
ABSOLUTE PHOSPHORUS CONTENTS OF PLANT PARTS 0, 3, AND 7 DAYS AFTER TRANSFER OF PLANTS
RAISED AT THREE PHOSPHORUS LEVELS TO SOLUTIONS WITHOUT PHOSPHORUS

Phosphorus Pretreatment Level	Days after Transfer	Phosphorus Content of Old Leaves (μg)	Phosphorus Content of Old Petioles (μg)	Phosphorus Content of New Leaves (μg)	Phosphorus Content of New Petioles (μg)	Phosphorus Content of Roots (μg)
P ₁	0	21	8	—	—	35
	3	15	5	4	2	35
	7	13	4	5	3	37
Total loss or gain		-8	-4	5	3	2
P ₂	0	36	15	—	—	54
	3	30	9	4	2	54
	7	23	8	9	5	56
Total loss or gain		-13	-7	9	5	2
P ₃	0	258	120	—	—	207
	3	167	61	54	33	207
	7	109	34	92	66	247
Total loss or gain		-149	-86	92	66	40

autoradiograph of the P₂ plants shows less difference between older and new leaves than at P₁, indicating a less restricted mobility of phosphorus previously accumulated in older leaves. Only the unifoliolate leaf and the two oldest trifoliolate leaves (closest to the roots) are identifiable by their somewhat greater blackness on the autoradiograph for day 7. The autoradiograph for P₃ plants on day 7 shows little or no difference between old and new expanded leaves, indicating, in line with Figure 3, an even distribution between old and new shoots of phosphorus taken up during the first day.

Existing shoots of P₁ plants grown with ³²P for the first day after transfer to complete solutions contained as much tracer on day 7 as the corresponding P₁ plants grown in tracer solutions for 2 or 3 days after transfer (Fig. 3). The amounts taken up by the old shoots at P₁ during the first 2 or 3 days after transfer remained the same during the following period in tracer-free solutions. It appears, therefore,

that apart from the retranslocation of some of the phosphorus taken up during the first day, no further retranslocation of phosphorus occurred to or from existing shoots of P_1 plants. As a consequence, the considerable increases in ^{32}P in the new shoots of the P_1 plants between day 2 and day 7, or between day 3 and day 7, were the result of translocation to new leaves and petioles of ^{32}P previously accumulated in the roots. The increases in ^{32}P in the new shoots of P_2 or P_3 plants between day 2

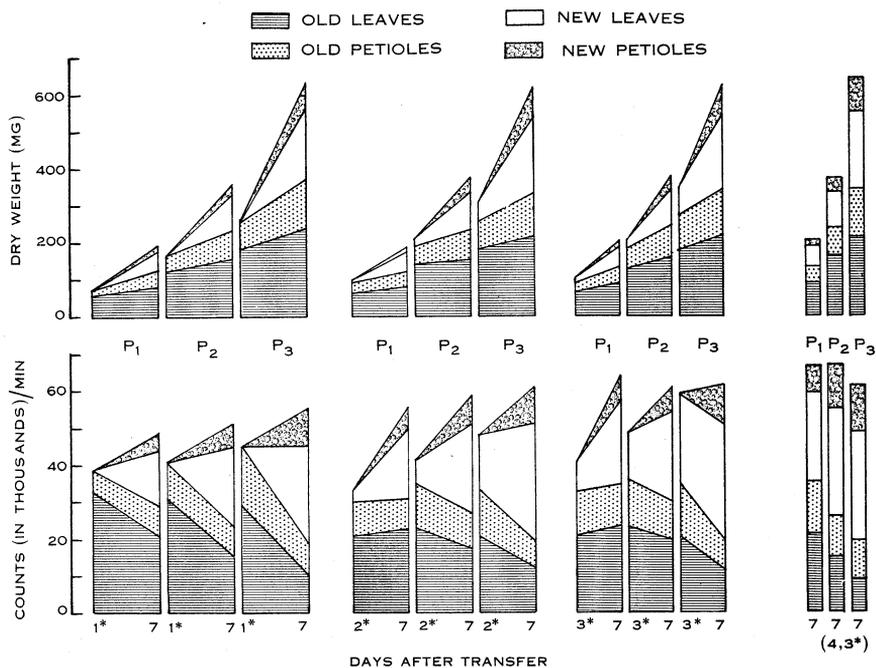


Fig. 3.—Changes in dry weight and distribution of ^{32}P during a period of 7 days after transfer of plants raised at three phosphorus levels to complete solutions. ^{32}P was present for 1, 2, or 3 days immediately after transfer or during the last 3 days. Plants were grown in tracer-free solutions for the remainder of the 7 days. These treatments are shown as follows (from left to right, respectively) 1*, 7; 2*, 7; 3*, 7; 4, 3* (expt. II), the asterisks referring to number of days in tracer solutions.

and day 7, or between day 3 and day 7, were considerably smaller than those for P_1 plants. At P_2 retranslocation of ^{32}P from older shoots during the 4 or 5 days in tracer-free solutions accounted for approximately one-third of the increase in ^{32}P of the new shoots during that period. At P_3 more than half of the increase in ^{32}P in new shoots was derived from old shoots.

The high demand for phosphorus in relatively old tissues of previously phosphorus-deficient plants, even during later stages of recovery, is indicated by the considerably greater amounts of ^{32}P translocated to old shoots of P_1 plants than to those of P_3 plants in the treatment where ^{32}P was present in the complete solutions during the last 3 days of the 7-day period.

IV. DISCUSSION

Williams (1955) reviewed the redistribution of mineral elements during development and emphasized the effect of competition for nutrients between different organs of the plant. He considered the rate of intake of nitrogen and phosphorus to be governed by the external supply of the element and by the demands set up by the various plant parts. Although the demand set up by each of the organs is usually met in part, if not entirely, by uptake from the medium, the rate of intake by the roots can be restricted to the extent that the nutrient is more readily available within the plant itself. This point was illustrated (Williams 1948) with results of an experiment with oat plants grown at different phosphorus levels. Deficient oat plants derived 30% of their inflorescence phosphorus from other plant parts, whereas plants grown with a high phosphorus supply derived no less than 93% of their inflorescence phosphorus from other plant parts. The external supply for the non-deficient plants was still plentiful, but a more accessible source was available in other plant parts.

The present experiments provided a clear illustration of the effects of the internal demand for phosphorus on its uptake by the plant and of the competitive stresses within the plant on the subsequent distribution patterns between organs. The dry weights on day 0 in experiment 1 were 46, 65, and 96 mg, while the leaf areas per plant were 4.5, 6.9, and 14.5 cm² at P₁, P₂, and P₃ respectively. To compare the nutrient uptake by plants of differing size, Williams (1948) suggested the use of a function which expresses the rate of nutrient uptake per unit root weight in unit time. This function allows for differences in root growth during the experimental period. The instantaneous intake of a nutrient element M is given by the equation

$$I_M = (1/R)(dM/dt),$$

in which R is the root dry weight at that instant. The mean value of I_M for a finite time interval ($t_2 - t_1$) may be calculated from the approximate formula:

$$I_M = \frac{\log_e R_2 - \log_e R_1}{t_2 - t_1} \times \frac{M_2 - M_1}{R_2 - R_1}.$$

The values for the first 3 days after transfer to complete solutions in experiment I were 2.73, 3.97, and 2.77 $\mu\text{g}/\text{mg}$ root weight/day at P₁, P₂, and P₃ respectively. The results of Figure 3 for experiment II also show clearly that the phosphorus intake by the shoots of previously deficient plants was, relatively speaking, considerably greater than that by the P₃ plants. In spite of a more than threefold difference in the weight of shoots, phosphorus in the shoots at the end of the first 24 hr after transfer differed by less than 20% between P₁ and P₃. Humphries (1951) grew barley plants at different levels of phosphorus nutrition and placed the excised roots in complete nutrient solutions. Plants raised at adequate levels showed a steady loss of phosphorus during the first 8 hr after transfer, while roots of deficient plants showed an increase in phosphorus content. Similar results were obtained for other elements and they also suggest a marked effect of the nutrient status of the plant on nutrient uptake.

The subsequent distribution of phosphorus between old and new shoots also depended on the phosphorus stress at transfer (Table 1). In previously deficient plants relatively large amounts of phosphorus were distributed to existing shoots during the first 3 days of recovery, while in non-deficient plants more phosphorus was distributed to new shoots than to old shoots. The distribution of assimilates in the same experiment (Bouma 1967) during the same period was quite the opposite. Of the dry weight increase of P_1 plants 11% was accounted for in old shoots and 48% in new shoots. For P_3 plants dry weight was equally distributed between old and new shoots (39 and 37% respectively). The differences in the dry weight distribution between the two groups of leaves were reflected in their contribution to the leaf area increases of the plant. Leaf expansion of existing leaves was smaller the greater the stress at transfer. The leaf area increase of P_1 plants was almost entirely attributable to the expansion of new leaf tissue (Bouma, unpublished data). It appears reasonable to suggest that the relatively large intake of phosphorus by older expanded leaves of previously deficient plants ensured the recovery of metabolic processes, including photosynthesis (Bouma 1967), essential for the production of assimilates required for new leaf tissue.

Part of the phosphorus in existing shoots was apparently available for redistribution to new leaves and petioles. This is in keeping with the known mobility of phosphorus (Biddulph 1941; Williams 1948; Bukovac and Wittwer 1957). However, the amounts and organs concerned in the redistribution were clearly determined by the competitive stresses within the plant and these in turn by the nutrient status of the plant and the availability of an external phosphorus supply. In the presence of an external supply (after transfer to complete solutions) little or no change occurred in absolute phosphorus of existing petioles, suggesting a low demand for phosphorus and a more or less passive role as conductors for phosphorus moving to the leaves. The leaves attached to these petioles showed a rapid initial accumulation of phosphorus followed by a retranslocation of phosphorus which was smaller the more severe the previous stress. In experiment II, retranslocation of radioactive phosphorus from existing leaves between day 1 and day 7 increased from 30% for P_1 to 58% for P_3 . In P_1 plants no radioactive phosphorus was translocated from existing leaves between day 2 and day 7, or between day 3 and day 7. By contrast, the importance of the roots as phosphorus sources for new leaves and petioles later during the recovery decreased with the severity of the previous stress. These changing source relationships for phosphorus at progressive stages of recovery from stresses of different intensity are clearly illustrated by the following comparison of radioactive phosphorus in new leaves derived from existing shoots and from roots during the periods in non-active solutions, expressed as a percentage of the total present in the shoots on day 7:

Treatment period (days)	1*—7			2*—7			3*—7		
Phosphorus pretreatment level	P_1	P_2	P_3	P_1	P_2	P_3	P_1	P_2	P_3
^{32}P from existing shoots in new leaves (%)	20	35	48	—	14	23	—	9	24
^{32}P from roots in new leaves (%)	21	19	18	40	29	21	33	22	4

* Day on which plants were transferred to tracer-free solution.

The apparent decrease in mobility of phosphorus taken up by existing leaves and petioles at lower pretreatment levels can be explained by assuming that the demand for phosphorus in the synthesis of less soluble organic compounds of phosphorus was greater the more severe the stress at transfer. Because the total intake of phosphorus by the shoots (Fig. 3) did not differ greatly between previous stress levels, the more mobile organic and inorganic phosphorus compounds available for later retranslocation would increase with the phosphorus status of the plant. The autoradiographs of Plate 1 support this suggestion. The old leaves of the P_1 plants were clearly distinguishable on day 7 by their considerably greater blackness than the leaves formed later. By contrast, radioactive phosphorus taken up by the P_3 plants had become evenly distributed between old and new leaves. That under extreme conditions even the less-available phosphorus sources in the leaves and petioles may be mobilized is apparent from experiment I after transfer of plants to solutions without phosphorus (Table 2). Considerable losses occurred at all pretreatment levels, which were relatively greater at P_3 than at P_1 .

Without an external phosphorus supply absolute phosphorus in the roots did not decrease at any of the phosphorus pretreatment levels (Table 2), suggesting that root phosphorus was a less-available source of phosphorus to meet the demands for new leaf growth than phosphorus in older leaves. Williams (1948) found that the roots of phosphorus-deficient oat plants retained relatively more of the absorbed phosphorus than non-deficient plants, so that relatively little was available for shoot growth. He suggested this as a possible explanation for the often observed increase in root weight ratios under conditions of phosphorus deficiency. He considered that the deficient roots, being nearest to the source of supply, would have first call on phosphorus entering the plant and so benefit at the expense of other plant parts. The present results suggest that phosphorus in the roots may be less mobile under conditions of starvation than phosphorus in other plant parts.

V. ACKNOWLEDGMENTS

The author is grateful to Dr. R. F. Williams for his interest in this work and for his criticism of the manuscript. Thanks are also due to Mr. E. J. Dowling for his assistance in much of this work.

The experiments with radioactive phosphorus were carried out at the Institute for Atomic Sciences in Agriculture, Wageningen, The Netherlands. The author expresses his gratitude to Dr. D. de Zeeuw, Director of the Institute, for his hospitality and the use of the facilities of the Institute. Special thanks are due to Messrs. E. Levi and H. Beek of the Institute for their advice, cooperation, and help.

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