A COMPARISON OF THE REACTION TO A LOCALIZED SUPPLY OF PHOSPHATE IN PLANTAGO MAJOR, PLANTAGO LANCEOLATA AND PLANTAGO MEDIA*

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SUMMARY

Plantago major ssp. *major*, *P. lanceolata* and *P. media* have been compared with respect to their ability to adapt themselves to a localized availability of phosphate. The growth and phosphorus content of plants of the three species were followed during 4–5 weeks in a divided-roots-experiment where at the start of the experiment about 25% of the root mass received a complete nutrient solution and the remaining roots a solution without phosphate.

The dry matter production of the treated plant is slightly depressed in *P. major* but unaffected in *P. lanceolata* and *P. media*. In all species the P-supplied roots of the treated plant show a stimulated relative growth rate and a stimulated rate of P-uptake.

These stimulations are smallest in *P. major* which also shows the lowest rate of P-uptake in control plants uniformly supplied with a complete nutrient solution. These facts point to the need for a more uniform supply of phosphate to the root system of *P. major* relative to *P. lanceolata* and *P. media*, correlating well with the nutrient-rich habitat of *P. major*.

The stimulated rate of uptake of phosphate after localization of the P-supply appears to be correlated with (a) lower P-concentration in the shoot, (b) lower P-concentration in the phloem and (c) lower concentration of inorganic phosphate in the root.

1. INTRODUCTION

Differences between habitats of different *Plantago* species may be correlated with variations of a number of factors like soil moisture content, soil oxygen level, the level of nutrients and soil compactness, the last factor influencing the other three (BLOM 1978). According to a number of authors, summarized in KUIPER & KUIPER (1979a and 1979b) the nutrient requirement of *P. major* (a), *P. lanceolata* (b) and *P. media* (c) is in a descending order a > b > c.

Part of the explanation for the greater requirement of nutrients, especially of *P. major*, could be a relative inability to use local enrichment of for instance phosphate which is known to be concentrated, in undisturbed profiles, in the upper layers of the soil.

The localized supply of nutrients to the root system of cultivated plants like maize (DE JAGER 1979) and barley (DREW & SAKER 1978) has been shown to give rise to two types of adaptation, viz. (1) stimulation of root growth in the enriched location and (2) stimulation of the rate of uptake.

In this paper a comparison is made of *Plantago major* ssp. *major*, *P. lanceolata* and *P. media* with respect to both types of adaptation.

2. MATERIALS AND METHODS

Seeds of *Plantago major* L. ssp. *major*, *P. lanceolata* L. and *P. media* L., obtained from Dr. C. W. P. M. Blom, Oostvoorne, were sown in sand. After five to six weeks seedlings were transferred to a complete nutrient solution for one week and then grown on a nutrient solution without P for another two or three weeks. The plants were then selected according to plant weight and their root system was divided into two parts, consisting of 20–25% and 75–80% of the root mass respectively. In *P. major* this meant a partitioning of several adventitious roots whereas in *P. lanceolata* and *P. media* a part of the primary root system (usually one thick lateral root) was selected to be supplied with the complete nutrient solution. By weighing the two parts of the root system preliminary to the experiments, it was established that the division of the root system was rather accurate with a deviation of only a few percent.

Two experimental set ups were used (DE JAGER 1979), (a) one to grow many plants together and to harvest frequently in order to determine fresh weight (roots were carefully blotted), dry weight (after 24 hours at 80 °C), and phosphate concentration, and (b) one to measure uptake of nutrients of individual plants during longer periods.

The nutrient solutions were aerated continuously and renewed regularly to prevent depletion. The pH varied between 6 and 7. The composition of the complete nutrient solution was as follows (mmol dm^{-3}): NO_3 5.95, H_2PO_4 0.5, SO_4 1.87, K 2.78, Ca 3.73, and Mg 1.87. Iron was added as FeEDTA. Added micronutrients were Cu, Zn, Mn, Mo and Bo. In the P-deficient solution H_2PO_4 was replaced by an equivalent amount of SO_4. The phosphate concentration of solution and of plant tissues (after drying, ashing and bringing the ash into solution with 3N HCl) was measured by colorimetry. Inorganic phosphate was determined according to DIJKSHOORN & LAMPE (1961).

Uptake of nutrients was measured as depletion of the nutrient solution, or calculated according to WILLIAMS (1948) using dry weight and nutrient concentration data.

Nitrate was measured with an ion-specific nitrate electrode, type Orion 97-02, and potassium by atomic absorption spectrophotometry.

During the experiments and the pre-experimental period *P. major* had to be treated with dodecyl (BASF) in order to prevent serious damage by powdery mildew. It was checked that this substance contained no phosphate.

3. RESULTS

3.1. Growth
Increase in dry weight is shown in *fig. 1* for whole plants (left) and roots (right) of *Plantago major* ssp. *major*, *P. lanceolata*, and *P. media*. In *P. major* the growth of
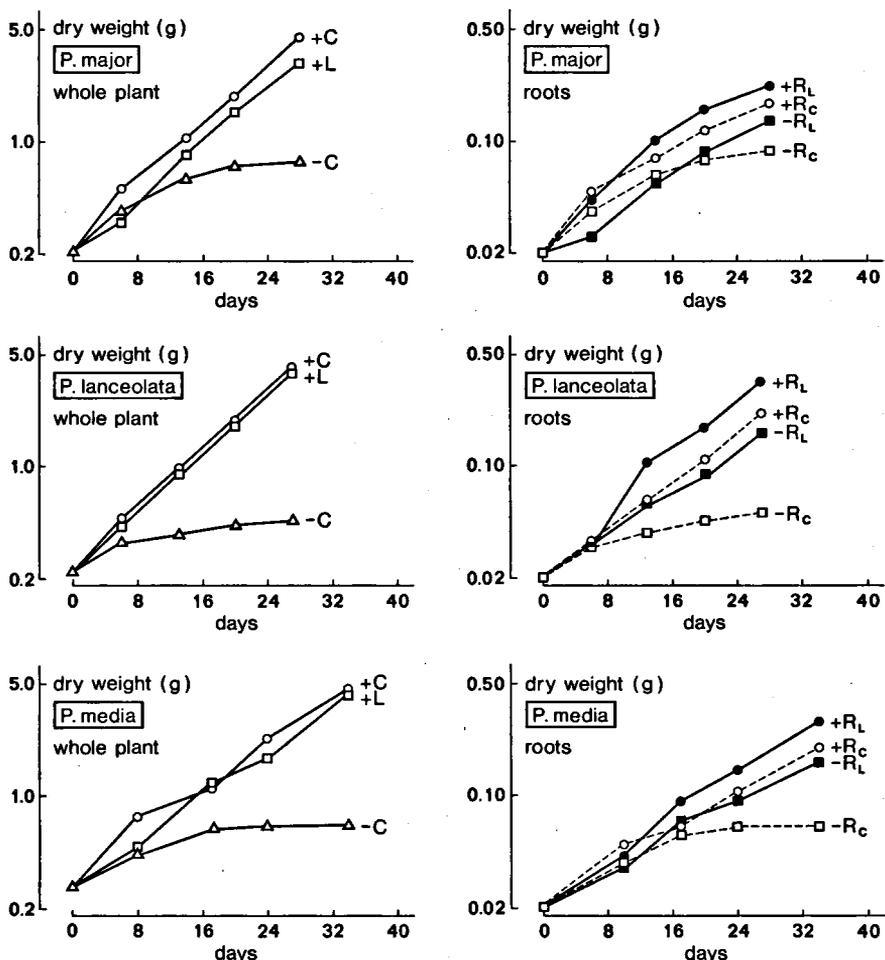
Fig. 1. Influence of a localized supply of phosphate to *Plantago major* (above), *P. lanceolata* (middle) and *P. media* on the increase in dry weight of the whole plant (left) and the roots (right). Control plants ($+C$ and $-C$) received a complete or a P-deficient nutrient solution at all roots ($+R_c$ and $-R_c$); treated plants ($+L$) received a complete solution at 20 to 25% of the root mass ($+R_c$) and a P-deficient solution at the remainder of the root system ($-R_1$). Growth conditions were, 16 hours light (HPI) at 70 to 80 W m^{-2}, 22°C and 55% RH; 8 hours dark at 17°C and 80% RH (n = 5).

In order to enable a direct comparison between the growth of different root parts the actual weight of the $-R_1$, $+R_c$ and $-R_c$ have been reduced to be equal to the $+R_1$ proportion at the start of the experiment. For *P. major* and *P. lanceolata* this meant reduction by a factor three ($-R_1$) or four ($+R_c$, $-R_c$) and for *P. media* by a factor four ($-R_1$) or five ($+R_c$, $-R_c$). The duration of the experiments was 28 days (p. major) 27 days (*P. lanceolata*) and 34 days (*P. media*).

the treated plant ($+L$) is somewhat decreased as compared with that of the $+$ control plant ($+C$), but here this is only an initial effect, the relative growth rate (RGR, the slope of the line) soon becoming similar for both the $+L$ and the $+C$.

In other experiments, however, there is mostly a reduction in RGR of the +L in *P. major*, also at a later stage. The two other species show no difference in RGR of the +L and the +C. Plants that received no phosphate (–C) show progressively inhibited growth. This is also apparent from *table 1*, where average RGR values of the +L and the +C are given for all species over the whole experimental period. Comparing the three species, + control plants of *P. major* and *P. lanceolata* show, under these conditions, identical RGR while those of P. media grow at a lower RGR.

Table 1. Average relative growth rate with standard deviation of the mean (n = 5) of *Plantago major*, *P. lanceolata* and *P. media* of the control plants (+C) and the treated plants (+L) at the end of 27 to 34 days lasting experiments. Further details are as under *fig. 1*.

	+C	+L
P. major	0.113 ± 0.011	0.097 ± 0.009
P. lanceolata	0.110 ± 0.007	0.106 ± 0.004
P. media	0.083 ± 0.010	0.082 ± 0.010

A comparison of the growth of the different types of roots, viz. supplied roots of control and treated plants (+ R_c and + R_1) and non-supplied roots of control and treated plants (– R_c and – R_1), reveals that in all three species root weights are ranked in the order + R_1 > + R_c > – R_1 > – R_c, at least in the second part of the experiment. Apparently the increase in weight of the + R_1 occurs at the expense of the – R_1, while total root weight remains unaffected.

Phosphate concentrations are shown in *fig. 2* for shoots (left) and roots (right). The low P-status of the plants at the start of the experiment is apparent from the rapid rise of the P-concentration in the + R_c, + R_1, + S_c and S_1.

Shoots of supplied control plants (+ S_c) always show higher P-concentrations then shoots of treated plants (+ S_1) while those of non-supplied plants (– S_1) show a steady decrease in concentration towards a lower limit of about 0.02 mmol g_{dry}^{-1}. The fact that this decrease is by a factor two, and the increase in dry weight (*fig. 1*) is by a factor three to four, points to the presence of some phosphate in the tapwater (3 μmol dm^{-3}). This fact, however, did not obscure any major effect in our experiments. P-supplied roots of the treated plant (+ R_1) show a slight but consistently higher P-concentration then roots of the + control plant (+ R_c). In other experiments, however, this was not always the case (see for example *table 4*) and at a lower P-concentration in the nutrient solution (100 μmol dm^{-3}) even the reverse effect was observed. Non-supplied roots of the treated plant (– R_1) show a rise in P-concentration, especially when compared to roots of -control plants (– R_c).

When corrections are applied for phosphate taken up from tapwater large differences remain, indicating internal supply of phosphate to these roots. Comparing the three *Plantago* species, *P. lanceolata* shows a higher P-concentration, expecially in the roots, than *P. major* and *P. media*, also when allowance is made for the higher dry weight to fresh weight ratio in *P. media*.
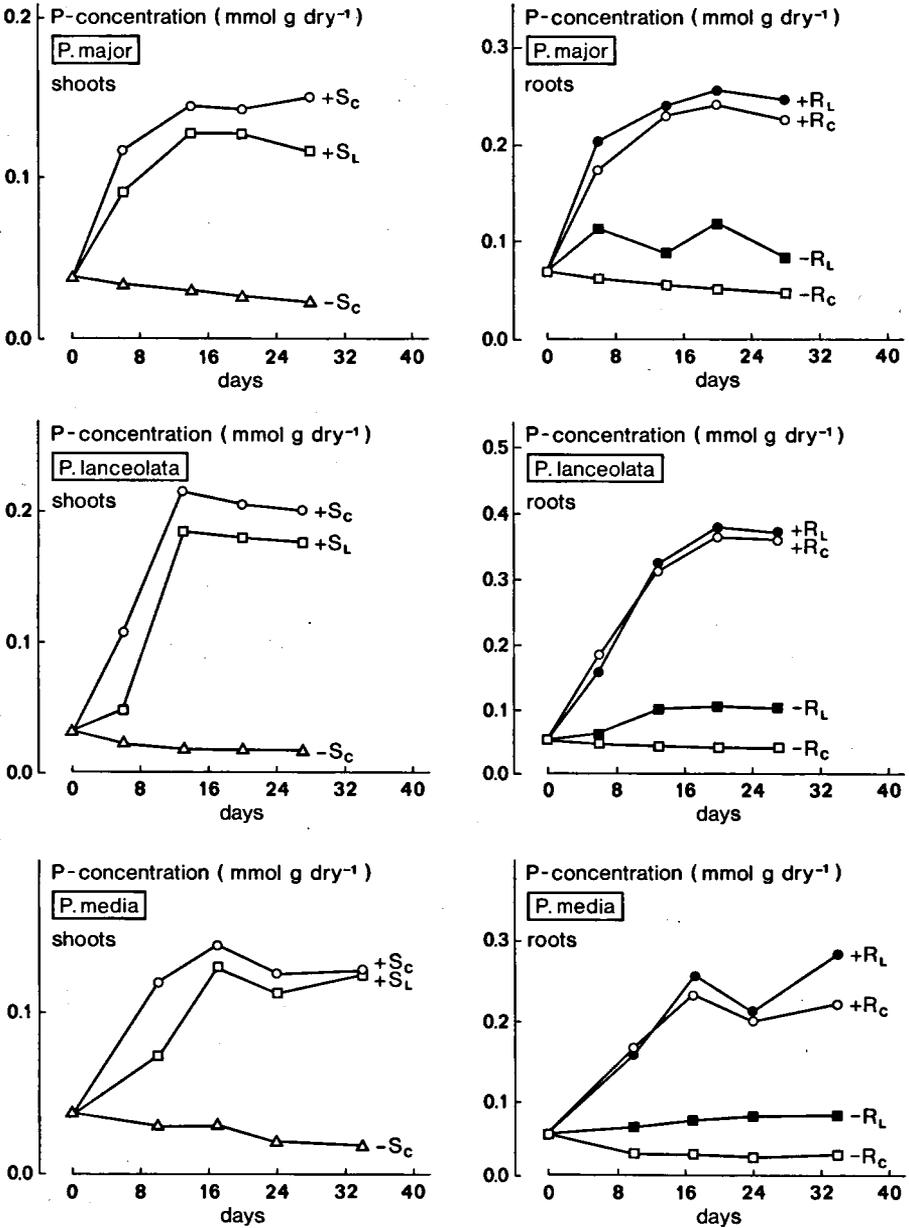
Fig. 2. Influence of a localized supply phosphate to *Plantago major* (above), *P. lanceolata* (middle) and *P. media* on phosphate concentrations in the shoots (left) and roots (right) of control plants and the treated plant.
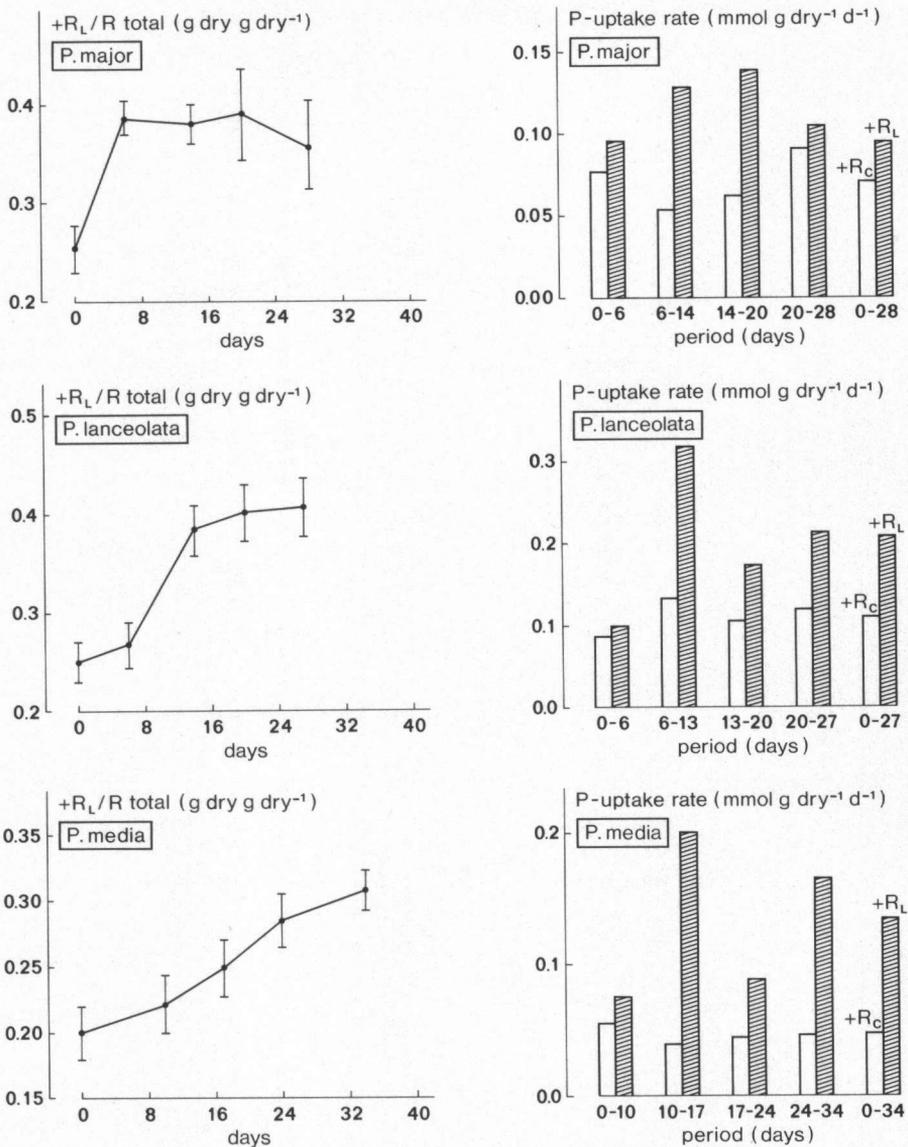For further details see under *fig. 1*.

Fig. 3. The stimulation of root growth (left) and rate of P-uptake (right) after localizing the supply of phosphate to *Plantago major* (above), *P. lanceolata* (middle) and *P. media*. On the left the weight of the supplied root ($+R_1$) is expressed as the fraction of the weight of the whole root system (mean of five replicates with standard deviation of the mean). On the right, calculated rate of P-uptake (mmol $g^{-}_{dry} d^{-1}$) is given for the periods between the harvests. The last bar represents the average rate of P-uptake over the whole period.

Further details are as under *fig. 1*.

Table 2. Shoot to root ratios (dry weight) and standard deviation of the mean (n = 5) of *P. major, P. lanceolata* and *P. media* in control plants (+C and –C) and treated plants (+L) at the end of 27 to 34 days lasting experiments. Further details as under *fig. 1.*

	+C	+L	–C
P. major	5.53 ± 0.35	3.96 ± 0.23	1.15 ± 0.05
P. lanceolata	3.92 ± 0.08	3.74 ± 0.17	1.31 ± 0.13
P. media	3.72 ± 0.35	3.57 ± 0.11	1.03 ± 0.18

The unimpaired RGR, the almost unaffected shoot to root ratio (*table 2*), and the rather high P-concentration in treated plants, especially in *P. lanceolata* and *P. media* point to the occurrence of compensational processes.

Fig. 3 shows, indeed, that the relative proportion of the $+R_1$ (left) increases and that the rate of uptake (right) is increased per unit weight, both increasing the total amount of phosphate taken up.

The compensational root growth in *P. major* appears to be different from that in the other species in that the relative $+R_1$ proportion shows a quick rise, then stabilizes and even decreases again after some time. This latter effect occurred in all experiments. In *P. lanceolata* and *P. media* on the other hand the relative $+R_1$ proportion keeps rising although in *P. lanceolata* a maximum seems to be and is indeed approached in other experiments; this maximum is always on a higher level than in *P. major*. The rate of P-uptake by the $+R_1$ seems to show some kind of an optimum, the maximum stimulation occurring at days 6–20 for *P. major*, 6–13 for *P. lanceolata* and 10 to 17 for *P. media*. The average stimulation (last bar of all right hand figures) appears to be greatest in *P. media* and least in *P. major*. The latter species also shows an intermediate rate of P-uptake in the control plant, lower than in *P. lanceolata* but higher then in *P. media*.

3.2. Regulation of P-uptake

The stimulation of the rate of P-uptake was investigated further in an experiment where the rate of uptake could be followed during several days on intact plants. *Table 3* shows some data on the simultaneous uptake of phosphate, nitrate and potassium by a specimen of *P. major* and one of *P. lanceolata*. Only at the end of the experiments rates of uptake per gram of roots could be calculated. From the left hand data it becomes clear that, as an average of several two-days periods, the $+R_1$ shows a higher uptake of phosphate relative to that of nitrate and potassium. The right hand data show that this is due to an increase in the rate of P-uptake and not to a decrease in the rate of NO_3 – and K-uptake.

In *P. major* the $+R_1$ and the $-R_1$ show normal rates of NO_3– and K-uptake; in *P. lanceolata* a slightly stimulated NO_3– and K-uptake in the $+R_1$ concurred with a slightly decreased uptake of these ions in the $-R_1$ to the effect that the total uptake of NO_3 and K by the treated plant was equal to that in the control plant.

Since the stimulation of the rate of P-uptake is a specific effect, the circulation

Table 3. The uptake of H_2PO_4 relative to that of NO_3 and that of K, by supplied roots of the control plant ($+R_c$) and the treated plant ($+R_1$) in *P. major* and *P. lanceolata* Data represent averages of 12 (*P. major*) or 6 (*P. lanceolata*) two-days uptake periods. Uptake rates of H_2PO_4, NO_3 and K (mmol $g^{-1}_{dry}d^{-1}$) are given at the end of the experiments, including NO_3- and K-uptake by non-supplied roots ($-R_1$) of the treated plant.
Further details as under *fig. 1*

Plantago major

	P/K	P/NO₃		P	K	NO₃
	mmol mmol⁻¹			mmol g^{-1}_{dry} d⁻¹		
+Rc	0.161	0.068		0.080	0.716	1.75
+R₁	0.215	0.096		0.105	0.751	1.74
−R₁	–	–		–	0.716	1.75

Plantago lanceolata

+Rc	0.176	0.063		0.151	1.58	3.53
+R₁	0.237	0.099		0.279	1.94	4.07
−R₁	–	–		–	1.13	2.92

Table 4. The relation between rate of P-uptake (V, mmol g^{-1}_{dry} d⁻¹), and P- concentration in the shoot ($[P]_s$), in the phloem ($[P]_{ph}$) and in the P-absorbing root ($[P]_r$) (mmol g^{-1}_{dry}) in *Plantago major* and *P. lanceolata*. To obtain different V-values, two different levels of $+R_1$ proportion at the start of the experiment were employed, i.e. 25% ($+L25$) and 10% ($+L10$). Further details as under *fig. 1*.

P. lanceolata					P. major			
	V	[P]ₛ	[P]ₚₕ	[P]ᵣ		V	[P]ₛ	[P]ₚₕ	[P]ᵣ
	mmol g^{-1}_{dry} d⁻¹	mmol g^{-1}_{dry}				mmol g^{-1}_{dry} d⁻¹	mmol g^{-1}_{dry}		
+C	0.11	0.15	–	0.26		0.07	0.12	–	0.19
+L25	0.20	0.13	0.04	0.26		0.11	0.10	0.03	0.20
+L10	0.26	0.12	0.02	0.26		0.18	0.09	0.02	0.21

of phosphate in the plant might be an important aspect of the regulation of the rate of P-uptake.

In *table 4* results are given of an experiment, comparable to those recorded in *figs. 1–3*, in which the rate of uptake was varied by modifying the relative $+R_1$ proportion at the start of the experiment. The P-concentration in the phloem was estimated roughly to be equal to the P-concentration in the $-R_1$ with corrections for the respiration (40% of the carbohydrate supply) and possible P-uptake from tapwater, assuming that half of that stays in the roots. The obtained values, 0.02 to 0.04 µmol g^{-1}_{dry} compare well with those measured in bleedingsap of *Yucca flaccida* i.e. 0.05 µmol g^{-1}_{dry} (TAMMES & VAN DIE 1964). A third correction, for the

Table 5. The concentration of inorganic phosphate in P-supplied roots of control plants ($+R_c$) and treated plants ($+R_1$) of *Plantago major* and *P. lanceolata*, as a function of time after localizing the supply of phosphate.
Further details as under *fig. 1*.

P. major			P. lanceolata	
	$+R_c$	$+R_1$	$+R_c$	$+R_1$
day		mmol P_i g_{dry}^{-1}		
6	0.19	0.21	0.21	0.24
13	0.23	0.19	0.31	0.23
18	0.22	0.21	0.23	0.20
25	0.22	0.20	0.23	0.21

amount of phosphate that is transported back to the shoot, cannot easily be applied but would result in an increase in the estimated P-concentrations in the phloem.

The rate of P-uptake appears to be negatively correlated with the P-concentration in the leaf (see also *fig. 2*) and the P-concentration in the phloem, but not (*P. major*) or not convincingly (*P. lanceolata*) with that in the root. The concentration in the root seems to be more dependent on the P-concentration in the nutrient solution.

In a separate experiment the concentration of inorganic phosphate was also measured. *Table 5* shows, that the concentration of P_i tends to be lower in the $+R_1$ than in the $+R_c$ while total-P-concentration is similar or higher in the $+R_1$. So, although differences are small, the rate of P-uptake seems also to be negatively correlated with the P_i-concentration in the root.

4. DISCUSSION

Qualitatively, the response of *P. major*, *P. lanceolata* and *P. media* to a localized supply of phosphate is similar in that these species all show a stimulation of root growth in the favoured area, at the cost of the other roots, and a stimulated rate of P-uptake per unit root weight. In this respect these dicotyledonous plants behave in a similar way as many cereals like oat (GOEDEWAAGEN 1942), barley (DREW & SAKER 1978) and maize (DE JAGER 1979).

Quantitatively, however, there are significant differences between *P. major* on the one hand and *P. lanceolata* and *P. media* on the other in that *P. major* shows lower levels of stimulation of root growth and of rate of P-uptake. At the same time, supplied control plants of *P. major* show lower rates of P-uptake compared to *P. lanceolata* which is reflected in the lower tissue-P-concentrations.

This indicates that *P. major* has less P-reserves than *P. lanceolata* when growing at the same rate. The lower P-concentration in the plant and the relative inability to benefit from local P-sources may be the cause of a necessity for a more uniform distribution of phosphate – both spatially and in time – in the environment of *P*.

major (KRUYNE, DE VRIES & MOOI 1967; BLOM 1978; KUIPER & KUIPER 1979a and 1979b) to maintain a RGR that is equal to that of *P. lanceolata*. A greater uniformity of P-availability could be a feature of disturbed habitats.

The lower shoot to root ratio in *P. lanceolata* and *P. media* as compared to *P. major* (*table 2*) is in accordance with results of KUIPER & KUIPER (1979b). They consider this fact to be an ecological advantage accounting for the lower requirement for nutrients in *P. lanceolata* and *P. media*. The equal RGR of *P. major* and *P. lanceolata* (*table 1*) and the lower shoot to root ratio in the latter species point to a higher net assimilation rate in *P. lanceolata*.

The fact that in the treated plants stimulation of the growth of supplied roots always concurred with a decrease of the growth of non-supplied roots strongly points to competition for assimilates, although dry-to-fresh weight ratios (not shown here), which may reflect concentration of soluble sugars (GROBBELAAR 1963), do not fully support this hypothesis.

One explanation for the new steady state that arises very quickly in *P. major*, but later on also in *P. lanceolata*, could be that the sink strength of the non-supplied roots is increased by internal supply of phosphate raising the P-concentration (*fig. 3*). Alternatively some factor in the supplied root may delimit the duration of the stimulation.

The lower rate of P-uptake (1) and the lower level of stimulation of P-uptake (2) when the supply is localized in *P. major* compared to *P. lanceolata* are not easy to explain. Both may be related to differences in a number of genetic factors (see LAUCHLI 1976 and EPSTEIN 1972, on intraspecific differences) like those governing the rate of uptake, the rate of secretion into the vacuole and into the xylem, and membrane leakiness. Ultimately, however, the rate of uptake is influenced since this is the process that brings nutrients into the plant. CRAM (1973) has shown that stimulation of the rate of NO_3-uptake in response to lowered NO_3-concentration in the whole cell comes about primarily as an increase of influx and not as a decrease of efflux. A decrease of the efflux would anyway fail to explain the stimulation of the rate of P-uptake by several factors as shown by CLARKSON et al. (1978) and BLASCO et al. (1976).

The stimulation of the rate of phosphate uptake in *P. major* and also in *P. lanceolata* appears to be P-specific (*table 3*). The slight stimulation of NO_3- and K-uptake in the supplied roots of *P. lanceolata* may be explained by the lower rate of uptake in the non-supplied roots, possibly as a consequence of P-deficiency (see also DREW & SAKER 1978). Thus a localized supply of P induces a kind of localized supply of NO_3 and K. The effect is, however, quantitatively unimportant. The specificity of the stimulation of P-uptake and the negative correlation between rate of uptake and phosphate concentration in the shoot and in the phloem (*table 4*), and the inorganic phosphate concentration in the root (*table 5*) support the hypothesis (DE JAGER 1979) that the regulation of the rate of P-uptake is exerted primarily by the concentration of the phosphate ion in the whole plant and consequently in the root.

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