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Effects of P deficiency on assimilation and transport of nitrate and phosphate in intact plants of castor bean (*Ricinus communis* L.)

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Abstract

An experimentally-based modelling technique was applied to describe quantitatively the uptake, translocation, storage, and assimilation of NO_3^- and $H_2PO_4^-$ over a 9 d period in mid-vegetative growth of sand-cultured castor bean (*Ricinus communis* L.) which was fed 12 mM NO_3^- and either 0.5 or a severely limiting 0.005 mM $H_2PO_4^-$. Model calculations were based on increments or losses of NO_3^- and reduced N or of $H_2PO_4^-$ and organic P in plant parts over the study period, on the concentrations of the above compounds in xylem and phloem sap, and on the previously determined flows of C and N in the same plants (Jeschke *et al.*, 1996).

Modelling allowed quantitative assessments of distribution of NO_3^- reduction and $H_2PO_4^-$ assimilation within the plant. In control plants 58% of total NO_3^- reduction occurred in leaf laminae, 40% in the root and 2% in stem and apical tissues. Averaged over all leaves more than half of the amino acids synthesized in laminae were exported via phloem, while the root provided 2.5-fold more amino acids than required for root growth. P deficiency led to severe inhibition of NO_3^- uptake and transport in xylem and even greater depression of NO_3^- reduction in the root but not in the shoot. Accentuated downward phloem translocation of amino acids favoured root growth and some cycling of N back to the shoot.

In control plants $H_2PO_4^-$ was the principal form of P transported in xylem with young laminae acting as major sinks. At the stem base retranslocation of P in the phloem amounted to 30% of xylem transport. $H_2PO_4^-$ assimilation was more evenly distributed than NO_3^- reduction with 54% occurring in leaf laminae, 6% in the apical bud, 19% in stem tissues, 20% in the root; young tissues were more active than mature ones. In P-deficient plants $H_2PO_4^-$ uptake was severely decreased to 1.8% of the control. Young laminae were the major sink for $H_2PO_4^-$. Considerable remobilization of P from older leaves led to substantial shoot to root translocation via phloem (50% of xylem transport). Young leaf laminae were major sites of $H_2PO_4^-$ assimilation (50%), followed by roots (26%) and the apical bud (10%). The remaining $H_2PO_4^-$ was assimilated in stem and mature leaf tissues. Old leaves exhibited 'negative' net assimilation of $H_2PO_4^-$, i.e. hydrolysis of organic P exceeded phosphorylation. In young laminae of low P plants, however, rates of $H_2PO_4^-$ assimilation per unit fresh weight were comparable to those of the controls.

Key words: *Ricinus communis* L., nitrate, nitrate reduction, phosphate, phosphate assimilation, partitioning, xylem, phloem, transport, P deficiency.

Introduction

In a previous paper using the modelling techniques of Pate *et al.* (1979*a*) and Jeschke and Pate (1991*a*) the

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Abbreviations: Since dissociation of phosphate varies with pH, the term PO_4 was used. Only when addressing phosphate as an anion or in the Figures was $H_2PO_4^-$ used.

marked effects of P deficiency on the uptake, flow and utilization of C, N and H_2O by whole castor bean plants were reported (Jeschke *et al.*, 1996). Budgets for N showed that not only was the uptake of NO_3^- substantially depressed, leading to very low N concentrations in plant tissues, but pronounced effects were also evident on the distribution of N. The latter included a strong depression in xylem transport and accentuated phloem transport of N towards the root. These findings of a major impact of P nutrition on the N economy of the whole plant are in good agreement with results published by other workers (Pilbeam *et al.*, 1993; Heuwinkel *et al.*, 1992; Schjorring, 1986; Rufty *et al.*, 1993).

Despite many studies on the P nutrition of higher plants, little is currently known concerning the forms (inorganic or organic) in which P moves or the rates at which it is transported from one region of a plant to another. Nevertheless, a relative increase in translocation of P from shoot to root is recorded as generally characteristic of plants subjected to low-P stress (Heuwinkel *et al.*, 1992; Fredeen *et al.*, 1989) and, as might be expected, this is associated with increased carbohydrate translocation to the root (Rufty *et al.*, 1993) and decreases in shoot : root dry matter ratio (Anghigoni and Barber, 1980; Fredeen *et al.*, 1989). For example, in a study of internal cycling of P flows in P-deficient plants of the tropical forage legume *Stylosanthes hamata* (Smith *et al.*, 1990), a rapid transfer of P was observed from shoots to roots and this was held responsible for maintaining root growth once shoot growth had ceased. Furthermore, under extreme deprivation P was remobilized from older parts of the root system as a source for continued growth of root meristems.

In an extension of the basic modelling technique (Pate *et al.*, 1979*a*) which was used in the earlier paper (Jeschke *et al.*, 1996), it has recently been possible to estimate separately the flows of NO_3^- and reduced N in a plant and thereby to gain a greater insight into the overall utilization of N, including nitrate assimilation, in individual organs (Jeschke and Pate, 1991*b*). In the present paper this method is applied to provide a detailed study of the N economy of P-sufficient (control) and of P-deficient plants of castor bean (*Ricinus communis* L.); the plants were the same as those already used in a companion study of the effects of P deficiency on C, N and H_2O partitioning between plant organs (Jeschke *et al.*, 1996). Moreover, the paper is specifically directed at calculating flows of phosphorus via phloem and xylem using *Ricinus communis* as a test plant because of ease of collection of xylem and phloem sap. As little previous information is available on perturbations in solute balances of translocation fluids in response to P deficiency, the chemical composition of phloem and xylem sap receives a detailed study. Special interest is given to amino acid and organic acid levels in phloem moving from shoot to root in view of earlier conclusions that such solutes might comprise signals to the root regulating NO_3^- uptake (Kirkby and Armstrong, 1980; Touraine *et al.*, 1994). The modelling exercise also allowed an assessment of the distribution of PO_4 assimilation, i.e. the phosphorylation of organic P compounds, within the individual organs of the plant.

Materials and methods

Culture of plants

Plants of castor bean (*Ricinus communis* L.) were grown in quartz sand culture in 5 dm^3 pots, 1 plant per pot, during May and June 1994 in a greenhouse and watered daily with an excess of a nutrient solution containing in mM: 4 KNO_3, 4 $Ca(NO_3)_2$, 1.5 $MgSO_4$, 0.5 NaH_2PO_4, and micronutrients as detailed in Jeschke *et al.* (1996). The plants were the same as those used in the above study of C, N and H_2O partitioning, and the basic details of nutrient and light regime and harvesting procedures were as described there. At 29 d after sowing (DAS) half of the pots were watered with a P-free nutrient solution (NaH_2PO_4 replaced by NaCl) and then from 41 DAS and throughout the study period from 44–53 DAS with a complete solution containing 5 μM $H_2PO_4^-$. The purpose of supplying a low, and still deficient, level of P was to facilitate a low continued uptake of P. Had no external P been supplied, net phosphate losses from the roots might have occurred, rendering the currently adopted modelling procedure difficult to apply. The other half of the plants continued to receive the complete solution containing 0.5 mM PO_4.

Plant development and harvests

The 9 d study period (44–53 DAS) coincided in the control plants with the final phase of expansion of leaf 4, rapid expansion of leaves 5 and 6, and early, exponential phases of leaf development of leaves 7 and 8. In the low-P plants expansion of leaves was markedly depressed and initiation of new leaves delayed to the extent that leaf 5 was expanding, leaf 6 was in its exponential phase and leaf 7 was just emerging (see Fig. 1 in Jeschke *et al.*, 1996). Lower leaves of P-deficient plants had senesced prematurely and some abscised during the study period. These fallen leaves were collected for analysis. A subset of seven of each of the control and low-P plants was harvested at the beginning and end of the study period and subdivided as detailed in the previous paper.

Collection and analysis of phloem and xylem sap

Phloem sap was obtained from shallow incisions into petioles and into the stem at various locations from the hypocotyl up to the youngest internodes. Ample bleeding occurred in the control plants, but it was slow and sporadic in low-P plants and thus not possible to collect sufficient amounts of sap from all sites of certain plants. Sap was collected on two occasions from seven plants of each of the control and low-P treatments. However, since phloem sap exudation was poor in low-P plants, it was not possible to collect it over the whole diurnal cycle; sap was therefore collected in the morning between 9 a.m. and 11 a.m., when the composition of phloem sap was close to the mean over their diurnal changes (Sharkey and Pate, 1976; Pate *et al.*, 1979*b*).

Xylem sap was collected in two ways: (a) by applying external pressure and (b) as root pressure exudate. (a) The moist quartz

sand substrate and the root system contained in a pressure vessel were pressurized (Passioura, 1980) and xylem sap was collected from incisions into the midrib of leaves as described in Jeschke and Pate (1991*a*). The pressures needed were similar in low-P (0.25–0.4 MPa) and high-P plants (0.25–0.45 MPa), depending on the conditions of transpiration. However, flow rates were lower in low-P plants. (b) Root pressure exudate (bleeding sap) was collected from detopped hypocotyl stumps. Sap samples were analysed for amino acids (Amino acid analyser LC 5001, Eppendorf/Biotronik Co., Maintal, Germany), for anions including $H_2PO_4^-$, NO_3^-, malate, and oxalate (Anionenchromatograph IC 1000, same manufacturer), for other organic acids by HPLC, and for cations by atomic absorption spectroscopy (FMD 3, Carl Zeiss, Oberkochen).

Determination of the incremental gains in total N, NO_3-N and reduced N and in total P, inorganic P and organically bound P in plant parts

Concentrations of total N in plant dry matter were measured in a CHN analyser (CHN-O-Rapid, Heräus, Hanau). Total P was analysed after digestion with nitric acid under pressure using an ICP spectrometer (JY 70 plus, ISA, Instrument S.A. division Jobin-Yvon, France). Anions including NO_3^- and $H_2PO_4^-$ were extracted with boiling water for 10 min and their concentrations were measured by anion chromatography, see above. Reduced N (soluble and insoluble) was assessed as the difference between total N and nitrate N, and in a similar fashion organic P (soluble and insoluble) was determined as the difference between total and inorganic P. The same procedure was applied to phloem sap to estimate the organic P fraction, while reduced N in phloem and xylem was obtained directly from the sum of amino acids.

Statistical treatment

Dry weight increments were obtained from seven replicates of each of both treatments at the first and second harvests. All further analyses were made using bulked material from two or three and sometimes up to seven individual samples of each organ. Where appropriate, data are presented as ± standard error (SE) of the mean. For the variation limits of increments see Pate *et al.* (1979*a*), and Jeschke *et al.* (1996).

Results

Phosphorus and nitrate concentrations in tissues

P concentrations in terms of dry matter in leaf laminae of P-sufficient plants decreased strongly with leaf age and tended to decrease from the first to the second harvest (Fig. 1). Concentrations changed similarly in the P-deficient plants, but, as expected were consistently lower than in the controls, particularly in old leaves. However, P concentrations in young leaf laminae of P-deficient plants remained at levels comparable to those normally regarded in shoots as sufficient, i.e. >0.15–0.2% (Marschner, 1995) or >1.5–2 mg g^{-1} DW, see the right hand scale.

Tissue N concentrations of the plants used in the present study have already been published (Jeschke *et al.*, 1996, Fig. 5) where it was shown that N concentrations in laminae decreased with leaf age in a similar manner to

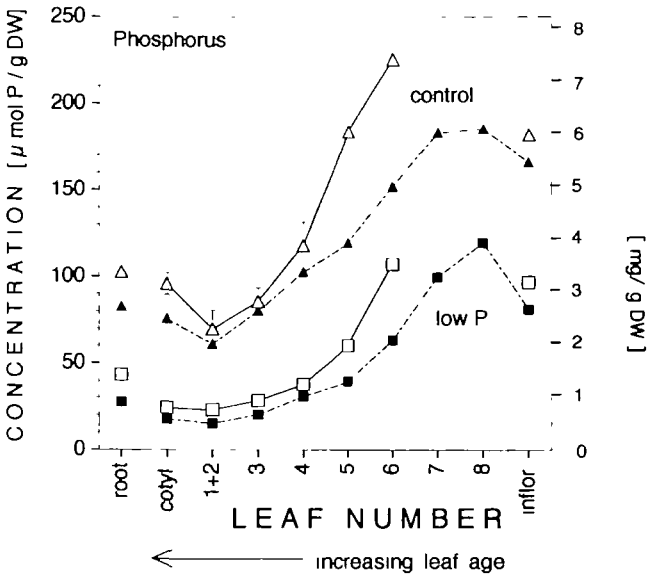

Fig. 1. Concentrations of P [μmol g^{-1} DW], or [mg g^{-1} DW] on right hand scale, in leaf laminae of different ages, root, and apical bud tissues of control (△, ▲) and P-deficient, low-P (□, ■) plants of sand-cultured castor bean (*Ricinus communis* L.). Control plants were fed 0.5 mM $H_2PO_4^-$ and P-deficient plants deprived of phosphate supply at 29 DAS and then supplied with 5 μM $H_2PO_4^-$ from 41 DAS onward. Plants were sampled at 44 DAS (open symbols) and at 53 DAS (closed symbols). Numbering of leaves starts at the primary leaves 1 and 2.

those of P. Moreover, as shown in that publication, concentrations of N like those of P were substantially diminished in P-deficient plants. Although the concentration of NO_3^- was relatively low in leaf laminae, it accumulated substantially in petioles and stem segments and increased in concentration with organ age (Fig. 2A). NO_3^- concentration increased in young petioles and the subtending stem segments during the study period, but there was some decrease in older ones (see insert in Fig. 2A). In P-deficient plants NO_3^- levels in almost all tissues were substantially lower (Fig. 2B), but changes with organ age and during the study period generally corresponded with those recorded for the control plants.

Phosphorus and phosphate contents and increments

Between 44 and 53 DAS the P content increased by 1830 μmol P $plant^{-1}$ in the controls, but by only 33 μmol P $plant^{-1}$ in the P-deficient plants. The distribution of these increments in P within the plant (Fig. 3B, D) was compared with that of the initial P content (Fig. 3A, C) to show absolute sink sizes and the changes relative to the first harvest. In high-P plants the highest P increments occurred in the expanding leaf 6 and in the exponentially growing leaf 7, which also showed the largest relative gain in P (cf. Fig. 1 in Jeschke *et al.*, 1996). Note that the total increment of 1830 μmol P in controls was larger than the initial content (1590 μmol). Older leaf laminae showed some senescence losses in P. Of the 1830 μmol P

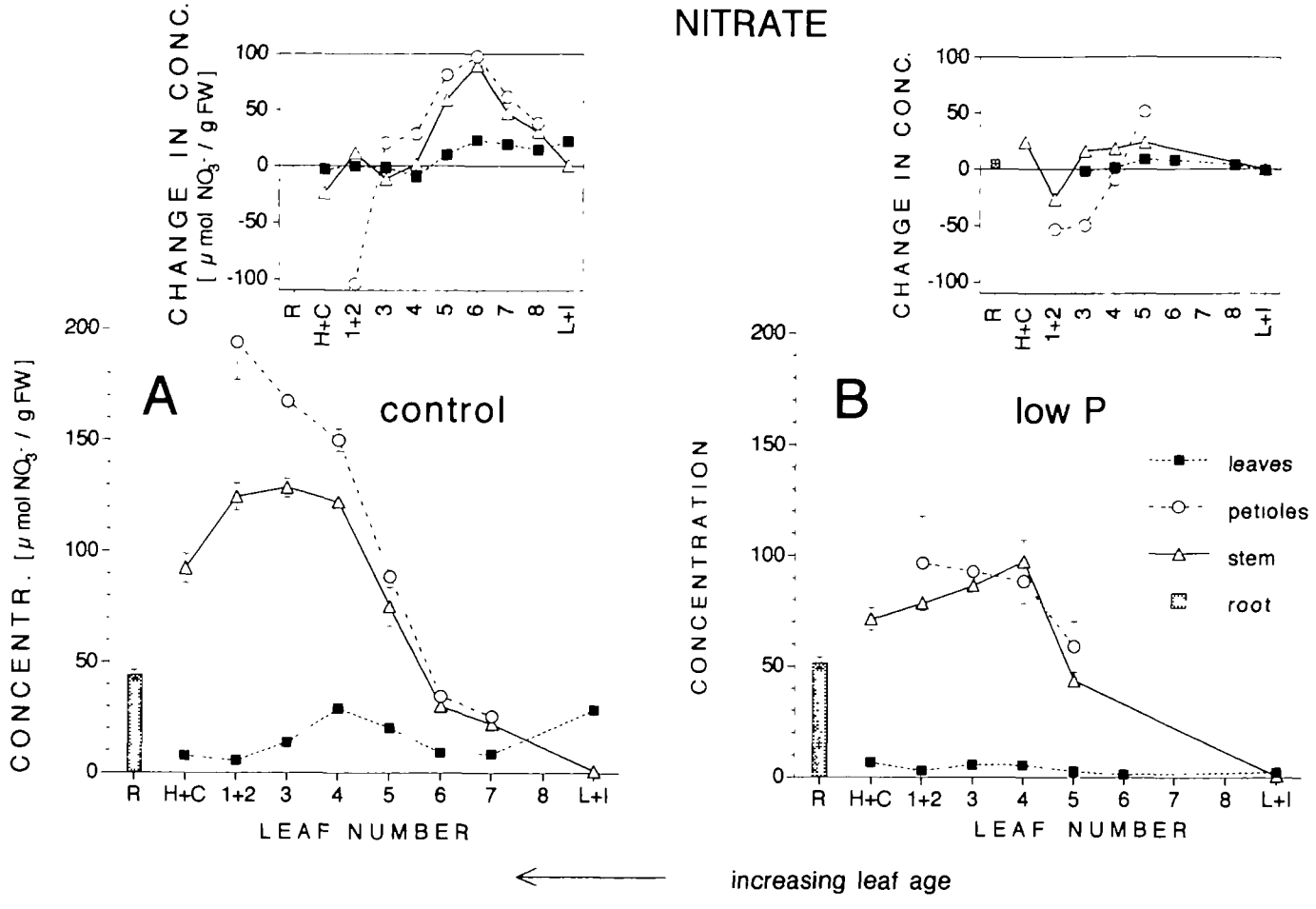

Fig. 2. Concentrations of NO_3^- [μmol g^{-1} FW] in leaf laminae (- - - ■ - - -), petioles (-·- ○ -·-), stem tissues (—△—) of different age and in the root (▣) of *Ricinus communis* L. control plants (A) and P-deficient plants (B) at 44 DAS (first harvest). Inserts: changes in nitrate concentration between 44 and 53 DAS.

taken up about 400 μmol were incorporated into the root (Fig. 3B), which contained about 20% of the total P.

In low-P plants initial P contents and increments between 44 and 53 DAS were much smaller than in the controls. Consistent with their retarded leaf development, the highest increments were shifted towards older leaves (Fig. 3C, D). P losses in senescing leaves were comparable to those in the P-sufficient plants. In contrast to the controls the sum of P increments (33 μmol) were much smaller than the P content at the first harvest (315 μmol). In the P-deficient plants the roots showed a slight loss of P, but nevertheless contained around 40% of the total P compared with 20% in the roots of control plants.

Although the inorganic phosphate (PO_4) contents and increments were distributed similarly to those of P, certain subtle differences were evident (Fig. 4): (a) PO_4 maxima were shifted towards older leaves as compared with total P, indicating that organic phosphate was preferentially incorporated into younger leaves; (b) senescence losses of PO_4 from older leaves were less pronounced than those of total P; (c) in P-deficient plants the losses of PO_4 from older leaves exceeded the gains in younger leaves; (d) particularly large PO_4 losses, exceeding those of total P, took place in the roots. In fact, in the whole plant there was a negative PO_4 balance of -19.4 μmol, compared with a gain in total P of 33 μmol and an initial PO_4 content of 315 μmol. This indicated that on balance some of the PO_4 initially contained in the plants had been assimilated into organic compounds.

Initial contents of and increments between 44 and 53 DAS in nitrate and reduced nitrogen

NO_3^- contents and increments in petioles and the stem by far exceeded those in the laminae in both control (Fig. 5A, B) and P-deficient plants (Fig. 5C, D). However, content and increments per plant were much lower in the low-P than in the control plants. Moreover, whereas NO_3^- increments (13.9 mmol $plant^{-1}$) exceeded the initial content of 10.2 in the controls, the opposite was true in P-deficient plants with increments of 1.2 compared with initial contents of 4.8 (all the same units).

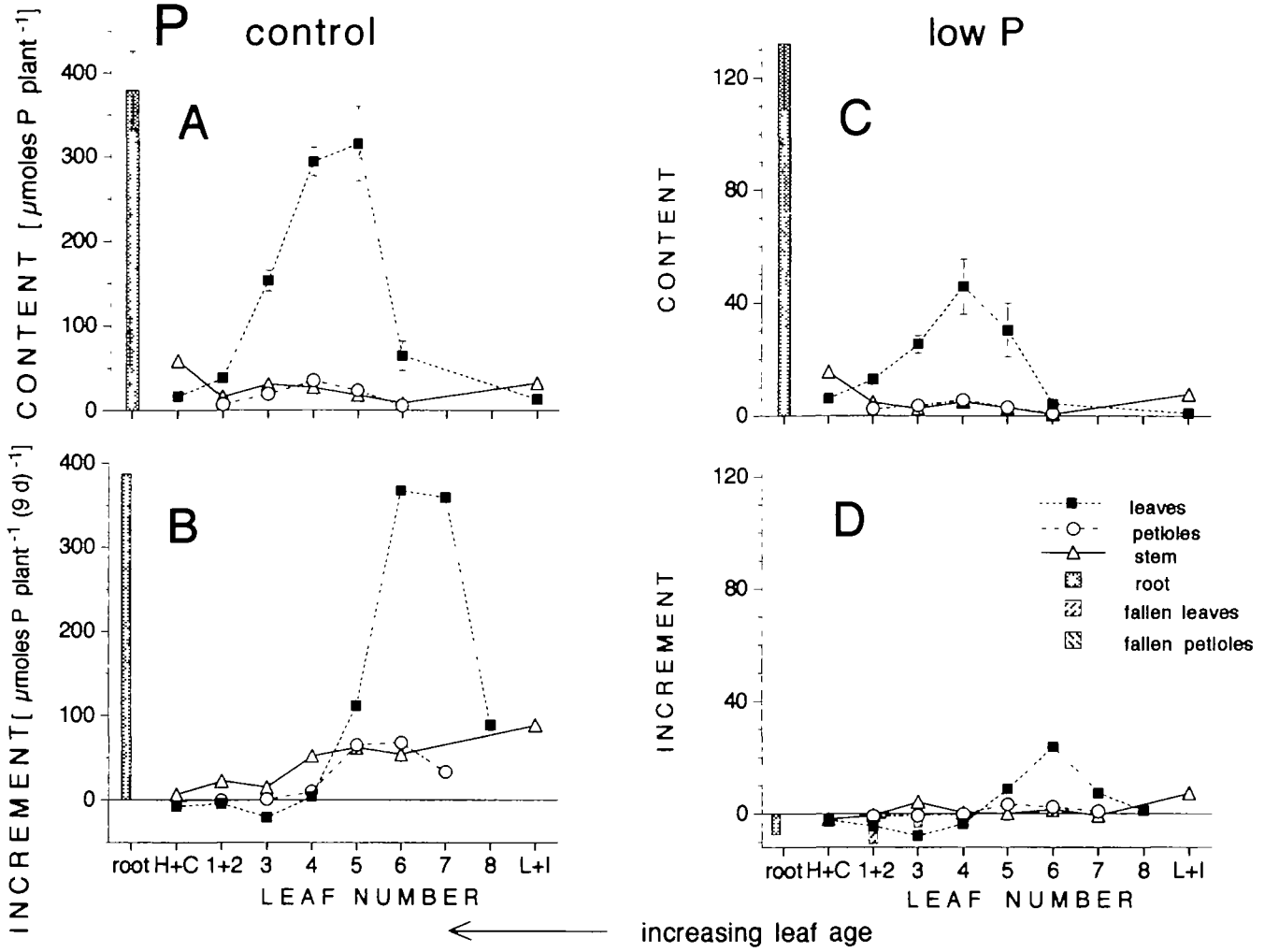

Fig. 3. Initial contents at 44 DAS (top, A, C) and increments or losses during the experimental period 44–53 DAS (bottom, B, D) of phosphorus in the root (□), leaf laminae (---■---), petioles (-·-○-·-) and stem segments (—△—) of sand-cultured *Ricinus communis*, grown in the presence of 0.5 mM $H_2PO_4^-$ (A, B) or under conditions of P deficiency (C, D), (see legend to Fig. 1) In graph D also the losses occurring due to abscission of laminae (▨) or petioles (▨) were also depicted. H + C = hypocotyl and cotyledons, L + I = lateral buds and inflorescence.

Reduced N, soluble and insoluble, showed totally different partitioning patterns from those for NO_3^-. Thus, laminae contained most of the reduced N and were the dominant sinks in both control and P-deficient plants (Fig. 6). The latter plants, however, showed much lower contents and increments than the controls (note the expanded scales in Fig. 6C, D). Moreover, increments in reduced N (5.7 mmol) were substantially lower than the initial content (14 mmol) in low-P plants, whilst both were of equal magnitude in the controls (35.3 and 36.7 mmol N).

Composition of phloem and xylem fluids in control and low P plants

Stem base phloem sap (Table 1) had high concentrations of sucrose and amino acids. Malate was the dominant organic and phosphate a prominent inorganic anion. Total P was 17% higher than PO_4, presumably due to the presence of organically bound P. K^+ and Mg^{2+} provided the principal balancing cationic charge to acidic amino acids which carried the bulk of the negative charge (Table 2). Glutamine was the principal amino acid in all phloem samples.

Low-P conditions diminished PO_4 by 75% and led to compensatory increases in glutamate, chloride and malate. The sum of amino acids was similar to the control, but the sum of amino acid N was slightly lower. In fact, there were some changes in the amino acid composition, among others principally a shift from glutamine to glutamate. This was clearly not due to hydrolysis during analysis, since the level of NH_3 was much smaller than that of glutamate. Sucrose was somewhat increased.

In the xylem sap of the control plants NO_3^- was the dominating anion followed by $H_2PO_4^-$, while organic

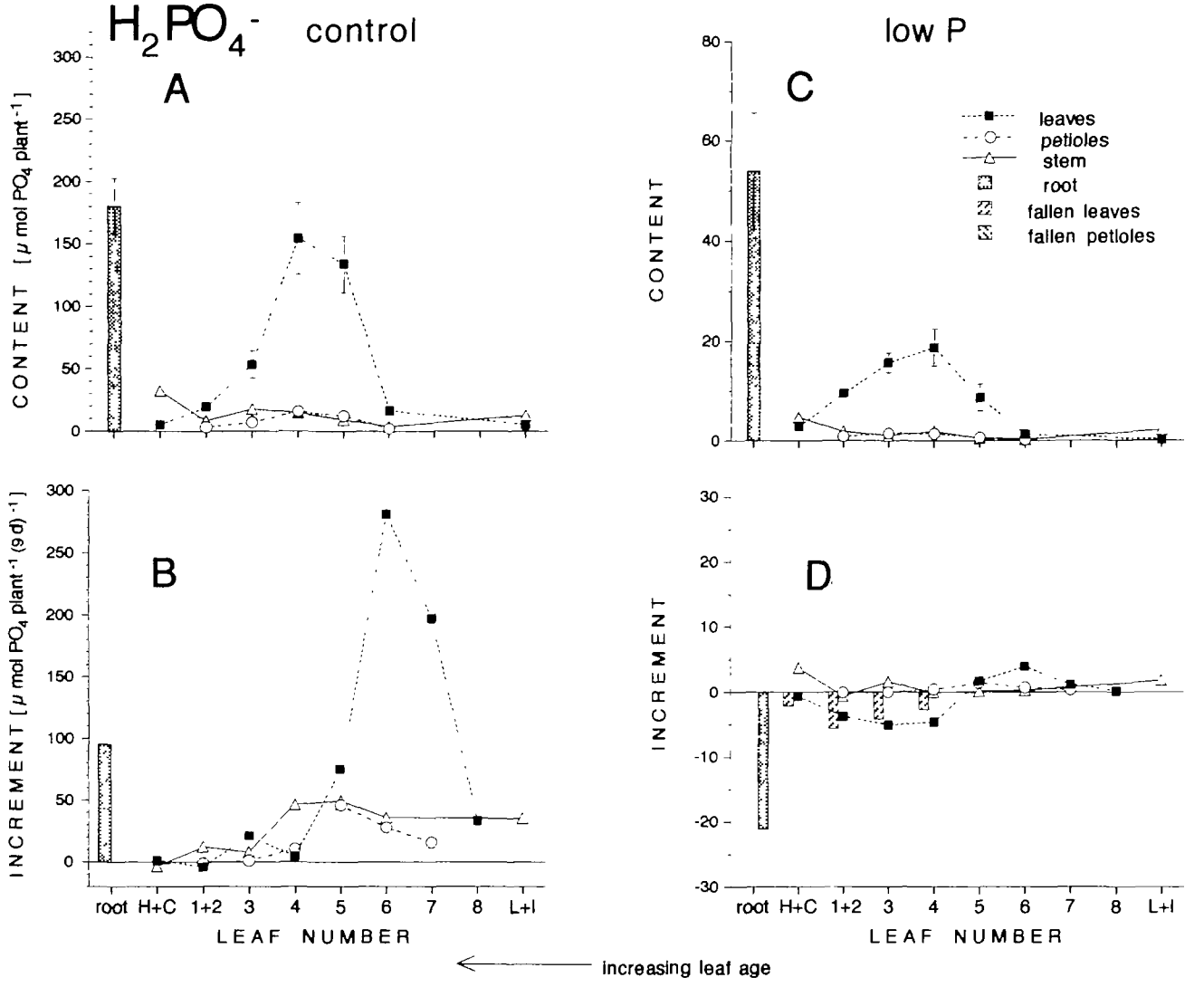

Fig. 4. Initial contents at 44 DAS (top, A, C) and increments or losses during the experimental period 44–53 DAS (bottom, B, D) of inorganic phosphate, $H_2PO_4^-$ in *Ricinus communis*, grown in the presence of 0.5 mM $H_2PO_4^-$ (control, A, B) or under conditions of P deficiency (control, C, D), (see legend to Fig. 1). Symbols and abbreviations as in Fig. 3.

anions were present at very low concentrations. K^+ was the major cation followed by Ca^{2+} and Mg^{2+}, but K^+ and Ca^{2+} had the same concentration in terms of milli-equivalents (Table 3).

Effects of low-P conditions on xylem sap were more severe than on phloem sap (see Table 3). $H_2PO_4^-$ was depressed by almost 90%, a decrease that was more than compensated for by increases in sap levels of Cl^- and SO_4^{2-}. The level of NO_3^- was more than halved by P deficiency, but organic acids, principally malate and citrate were substantially elevated. Apparently in response to lower NO_3^-, K^+ was diminished, but on balance an anion deficit was recorded for xylem sap of P-deficient plants. In the whole plant these decreases in concentration of, for example, nitrate, amino acids and phosphate were exacerbated, since the volume flow of xylem sap was substantially lowered in low-P plants (Table 3). As a consequence, the molar solute flows were much more depressed than suggested solely by lower concentrations. For example, the amino acid concentration was 1/6, the amino acid flow in the xylem, however, 1/66 of that in the control plants.

Separate modelling of the flows of reduced nitrogen and nitrate

Flows and partitioning of C, N and H_2O in the plants used here have been calculated (Jeschke *et al.*, 1996). As was shown in detail recently (Jeschke and Pate, 1991*b*), flows of nitrate and reduced N can be modelled separately in situations in which those of total N have been measured. For such a modelling procedure is required information on the ratios of reduced to total N in the phloem ($(N_{red}/N_t)_p$) and in the xylem ($(N_{red}/N_t)_x$) and the

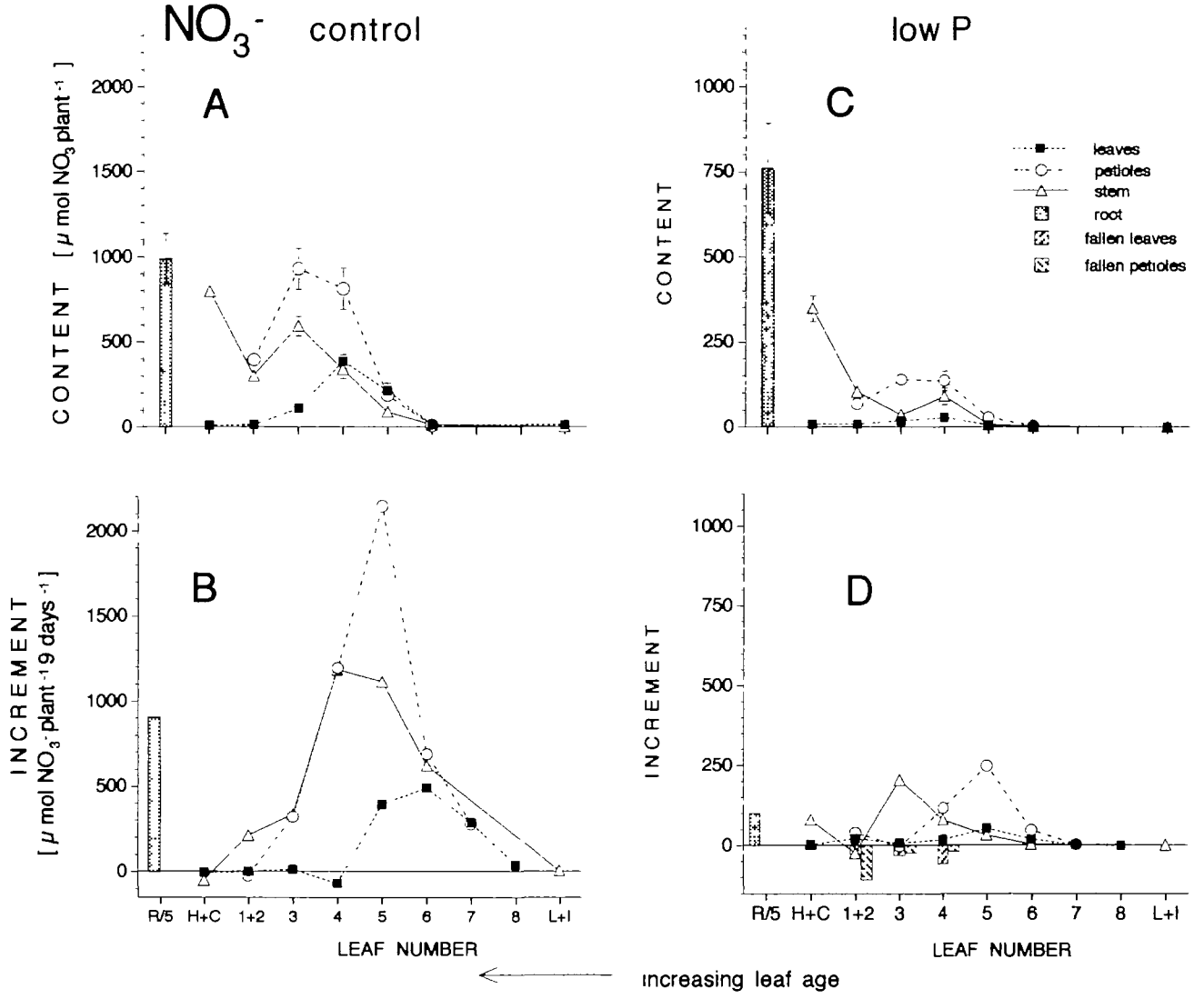

Fig. 5. Initial contents at 44 DAS (top, A, C) and increments or losses during the experimental period 44–53 DAS (bottom, B, D) of nitrate in *Ricinus communis*, grown in the presence of 12 mM NO_3^- and 0.5 mM $H_2PO_4^-$ (control, A, B) or under conditions of P deficiency, i.e. 12 mM NO_3^- and 0.005 mM $H_2PO_4^-$ (low-P, C, D). Symbols and abbreviations as in Fig. 3; the large contents and increments for the root are given as 1/5 of the data and are designated by R/5.

increments in NO_3^- and in reduced N in various organs. Besides yielding the flows of NO_3^- and of reduced N, the balance of exchanges and incorporation of reduced N also gives an estimate of the net nitrate reduction (*NR*) occurring in an organ

$$NR = \Delta_{N_{red}} - J_{N_{red},x} - J_{N_{red},p} \quad (1)$$

with $\Delta_{N_{red}}$, the increment in reduced N over the study period and $J_{N_{red},x}$ and $J_{N_{red},p}$, the net flows of reduced N (amino acids) in xylem and phloem; $J_{N_{red},p}$ would have a negative sign if a net phloem export occurred and hence add to the size of nitrate reduction..

Following the procedures outlined in Jeschke and Pate (1991*b*) detailed flow patterns showing flows of nitrate and reduced N to each individual leaf were calculated, but in Figs 7 and 8 these were condensed to bulk exchanges between root and stem and the bulk of the laminae. Corresponding information on partitioning of nitrate assimilation amongst differently-aged laminae and stem parts is contained in Fig. 9. The numbers in Figs 7 and 8 represent the flows and deposition of nitrate and reduced N and the reduction of nitrate in terms of mmoles N per plant over the study period.

According to Fig. 7 control (P-sufficient) plants exhibited the following features in respect of their economies of NO_3^- and reduced N:

(1) There was a virtually unidirectional translocation of NO_3^- in the xylem, which carried 61% of its N as nitrate and 39% in reduced form as amino acids.

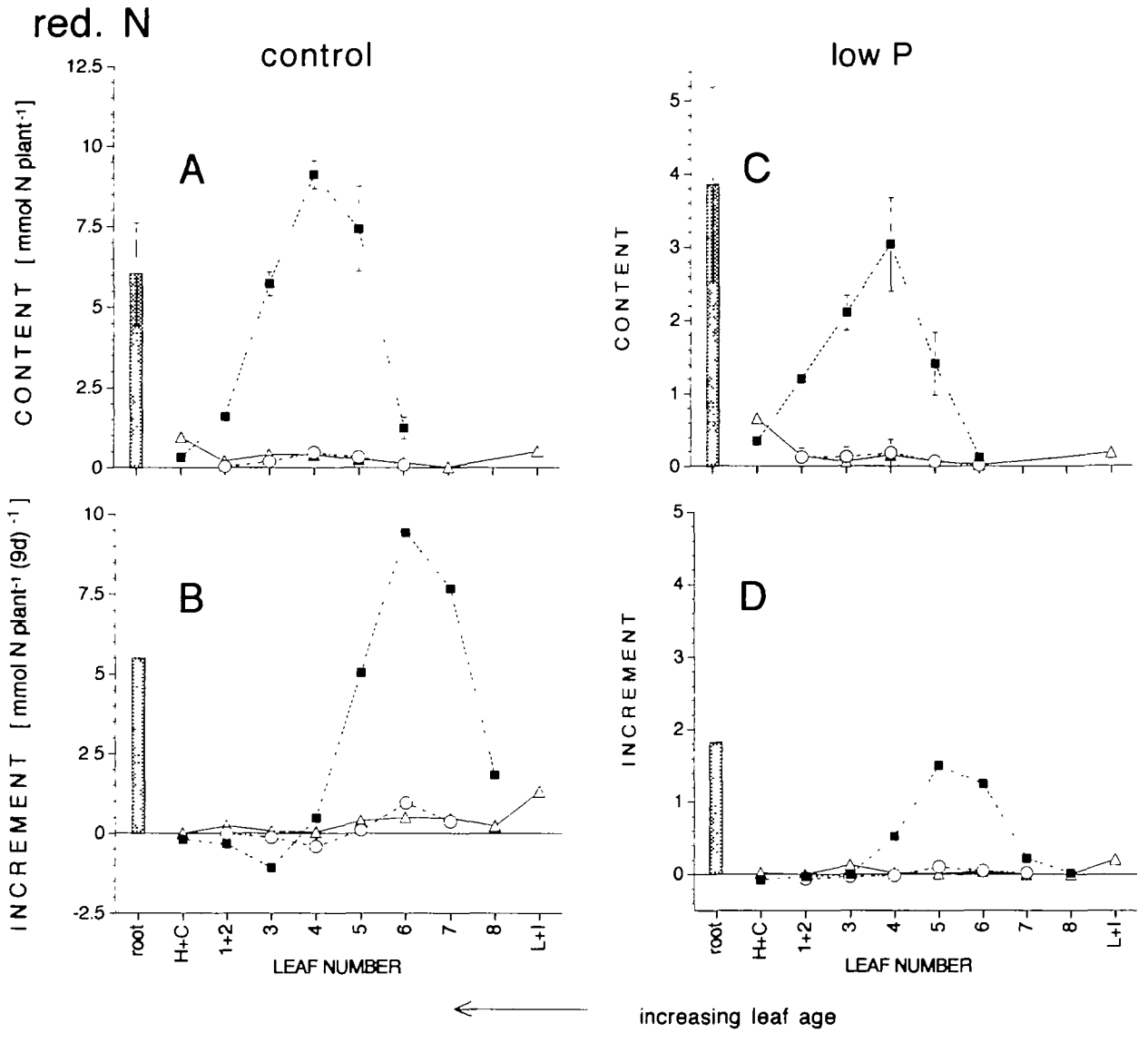

Fig. 6. Initial contents at 44 DAS (top, A, C) and increments or losses during the experimental period 44–53 DAS (bottom, B, D) of reduced nitrogen (soluble and insoluble) in *Ricinus communis*, grown in the presence of 0.5 mM $H_2PO_4^-$ (control, A, B) or under conditions of P deficiency (low-P, C, D); the NO_3^- concentration was 12 mM in both cases. Symbols and abbreviations as in Fig. 3.

(2) Xylem was a principal pathway also for reduced N, which originated primarily from amino acids synthesized in the root and from those recycled via phloem from the shoot. Amino acid transport in the xylem exceeded that in the phloem 1.8-fold.

(3) Laminae were the principal sinks for N and, as an overall average for all leaves, xylem-borne amino acids provided 60% of the reduced N required for the growth of leaf laminae.

(4) Within the laminae most of the xylem-imported NO_3^- was reduced. This reduction amounted to 58% of total nitrate reduction in the whole plant. However, more than half (54%) of the amino acids formed in the laminae were exported to meet the demand of phloem sinks—including the root and young, growing leaves. Laminae of leaves 6 to 8 were the major centres of nitrate reduction (Fig. 9).

(5) Roots contributed 40% of the total nitrate reduction in the plant, which is in good agreement with Van Beusichem *et al.* (1985) and thereby provided nominally 2.5-fold more amino acids than was required for its own growth. Reduction within stem segments was negligible compared with the demand of these parts for reduced N.

(6) 72% of the NO_3^- taken up during the study period was reduced and assimilated, and the remaining 28% stored mainly in stems and petioles.

The flow pattern for the P-deficient plants, as depicted in Fig. 8 using a greatly exaggerated scale, showed a severe

Table 1. *Mean composition of the phloem sap obtained from shallow incisions into the hypocotyl of P-sufficient control plants (fed 0.5 mM PO_4) and P-deficient low P plants (fed 0.005 mM PO_4) of castor bean* (Ricinus communis *L.*)

Compound	Concentration in stem base phloem sap	
	Controls (mM ± SEM)[a]	Low-P plants (mM ± SEM)[a]
Sucrose	370 ± 50	510 ± 60
Amino acids	108 ± 11	101 ± 15
amino acid N	164 ± 15	141 ± 19
Organic acids		
Malic	12.3 ± 2.8	16.5 ± 2
Shikimic	0.38 ± 0.15	0.68 ± 0.15
Citric	2.7 ± 1.2	1.6 ± 0.4
Fumaric	0.55 ± 0.34	0.33 ± 0.13
Succinic	n.d.[c]	1 4
Oxalic	2.5 ± 0 7	5.4 ± 0.7
Inorganic anions		
Chloride	5.5 ± 1	12.2 ± 1 5
Nitrate	2.4 ± 0.7	1.4 ± 0.2
Phosphate	7.3 ± 2	1 8 ± 0.3
Total P	8.4 ± 0 6	2.3 ± 0.1
Sulphate	3.7 ± 0.8	2.3 ± 0.4
Inorganic cations		
Potassium	94 ± 8	94 ± 3
Sodium	0.9 ± 0.1	0.4 ± 0.1
Magnesium	4.9 ± 0.8	7.9 ± 0.5
Calcium	1.5 ± 0.3	2 ± 0.25
Sum anions (mequ l^{-1})[b]	102	101.2
Sum cations (mequ l^{-1})[b]	107.7	114.2

[a]Standard error of the mean ($n = 4$, control; $n = 7$, low-P).
[b]The sum of anions and cations includes acidic and basic amino acids, respectively.
[c]Not detectable.

Table 2. *Mean amino acid composition of the phloem sap obtained from shallow incisions into the hypocotyl of P-sufficient control plants (fed 0.5 mM PO_4) and P-deficient low P plants (fed 0.005 mM PO_4) of castor bean (*Ricinus communis *L.*)

	Average of hypocotyl phloem sap			
	Controls		Low-P plants	
	mM ± SEM	% of amino acid N	mM ± SEM	% of amino acid N
ASP	13.1 ± 2.5	8.0%	6.3 ± 0.9	4.5%
THR	2.0 ± 0.3	1.2%	2.3 ± 0.3	1.6%
SER	6.2 ± 0.9	3.8%	6.6 ± 1.3	4.7%
GLU	22.6 ± 4.6	13.8%	33.9 ± 7.3	24.0%
GLN	54.1 ± 4.6	66 1%	35.9 ± 5.4	50.8%
PRO	0.48 ± 0.2	0.3%	0 55 ± 0.08	0.4%
GLY	1.3 ± 0.3	0.8%	3.0 ± 0.8	2.1%
ALA	1.5 ± 0.2	0.9%	3.3 ± 0.6	2.3%
VAL	1.4 ± 0.2	0.8%	1.8 ± 0 2	1.3%
CYS2	0.27 ± 0.04	0 3%	0.53 ± 0.05	0.8%
MET	0.17 ± 0.02	0.1%	0.27 ± 0.03	0.2%
ILE	0.44 ± 0.12	0 3%	0.71 ± 0.10	0.5%
LEU	0.26 ± 0.09	0.2%	0.37 ± 0.07	0.3%
PHE	0.47 ± 0 11	0.3%	0.71 ± 0.09	0.5%
β-ALA	0.09 ± 0.01	0.1%	0.09 ± 0.01	0 1%
GABA	1.2 ± 0.2	0.7%	2.0 ± 0 3	1 4%
NH3	1.1 ± 0.1	0.7%	0.81 ± 0.08	0.6%
ORN	0.24 ± 0.03	0.3%	0.47 ± 0.08	0.7%
LYS	0.32 ± 0.14	0.4%	0.45 ± 0.07	0.6%
HIS	0.35 ± 0.07	0.6%	0.43 ± 0.03	0.9%
ARG	0.12 ± 0 04	0.3%	0.43 ± 0.05	1.2%
CITR	0 08 ± 0.07	0.1%	0.35 ± 0.05	0.7%
Sum	107.7		101.3	

depression of transport of NO_3^- and amino acids (see the numbers in Fig. 8). Substantial alterations in N economy were evident in comparison with P-sufficient plants:

(1) Almost all of the restricted quantities of NO_3^- taken up during the study period were translocated in the xylem, which carried only 16% of its total N in the form of reduced N.
(2) In contrast to the control, phloem was the principal transport avenue for amino acids in P-deficient plants. Indeed, downward translocation in phloem from shoot to root exceeded upward xylem transport 2-fold, so that the phloem was the major source of reduced N for the relatively large growth increment made by the root.
(3) Translocation of reduced N in the xylem was only 6% of that in controls and supplied only 27% of the reduced N incorporated in the growing leaf laminae.
(4) NO_3^- assimilation proceeded to a proportionately greater extent in the shoot than was found in P-sufficient plants. The laminae contributed 82% to the total reduction, providing 73% of their reduced N. Nevertheless, as in P-sufficient plants nearly half the amino acids produced following nitrate reduction became available for phloem export.
(5) Nitrate reduction in the root represented only 12.7% of the total and was clearly only a minor provider of reduced N for root growth. Even though relatively small components, nitrate reduction outside leaves and roots was likely to have made substantial contributions to the reduced N utilized by stem and apical bud (Fig. 8).
(6) Of the small amounts of NO_3^- taken up, 83% was assimilated, a higher proportion than in the controls (72%).

The data for nitrate reduction per plant for nine days, as depicted in Figs 7–9, were related to mean organ fresh weights and estimates made of rates of nitrate reduction. Rates in leaves (Fig. 10A) were then found to be remarkably similar in low P and control plants and comparable to previously published rates in salt-treated *Ricinus* (Jeschke and Pate, 1991*b*). By contrast, nitrate reduction rates in roots were several fold lower in low-P plants (0.1 μmol g^{-1} h^{-1}) than in the controls (0.4 μmol g^{-1} h^{-1}) and the latter values in turn lower than in comparable salt-treated plants (0.9 μmol g^{-1} h^{-1}), Jeschke and Pate, 1991*b*). These rates of actually occurring nitrate reduction compare well with *in vivo* NRA in roots of *Ricinus* (Van Beusichem *et al.*, 1985) and in control tomato roots of 0.25 μmol g^{-1} h^{-1} (Cramer *et al.*,

Table 3. *Composition and volume flow of root pressure xylem exudates obtained from the hypocotyl stump of P-sufficient control plants (fed 0.5 mM PO_4) and P-deficient low P plants (fed 0.005 mM PO_4) of castor bean (*Ricinus communis *L.)*

Compound	Concentration in root pressure exudate	
	Controls (mM ± SEM)[a]	Low-P plants (mM ± SEM)[a]
Amino acids	9.2 ± 0.7	1 45 ± 0.2
Organic acids		
Malic	0.04 ± 0.01	0.82 ± 0.09
Shikimic	0.001	0.005
Citric	0.16	0.57
Fumaric	0.001	0.011
Inorganic anions		
Chloride	0.76 ± 0.03	1.4 ± 0.05
Nitrate	26.2 ± 1.3	11.7 ± 0.8
Phosphate	2.6 ± 0.24	0.33 ± 0 05
Sulphate	1.3 ± 0.1	2.5 ± 0.13
Inorganic cations		
Potassium	12.8 ± 0.6	5.6 ± 0.9
Sodium	0.14 ± 0.02	0 18 ± 0.02
Magnesium	3.4 ± 0.2	1.9 ± 0 1
Calcium	6.3 ± 0 4	8.2 ± 0.6
Sum anions[b]	32 8	21.4
Sum cations[b]	33.4	26.2
	$\mu l\ g^{-1}\ FW\ h^{-1}$	$\mu l\ g^{-1}\ FW\ h^{-1}$
Volume flow		
1st harvest	37.1 ± 2.3	5.1 ± 0.4
2nd harvest	39.6 ± 2.9	2 1 ± 0.3

[a]Standard error of the mean ($n = 58$, control; $n = 36$, low-P).
[b]The sum of anions and cations is given in mequ l^{-1} and includes acidic and basic amino acids, respectively

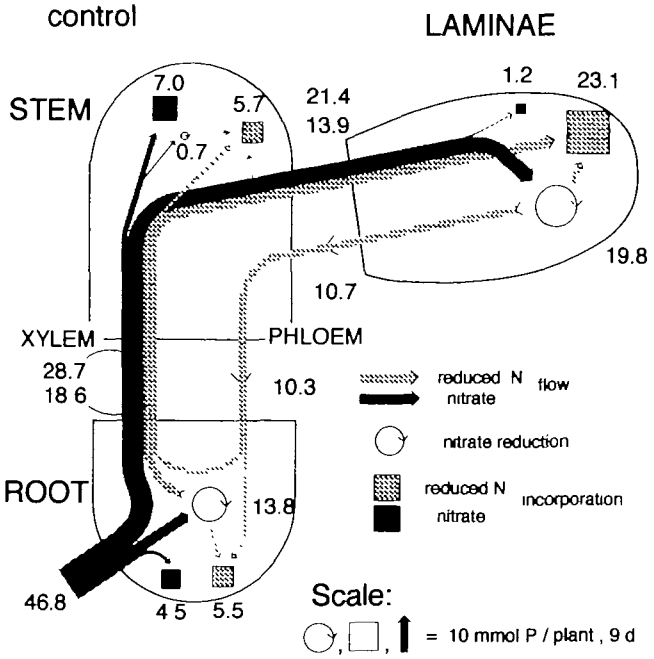

Fig. 7. Condensed model of the partitioning, flows and utilization of nitrate and reduced N in 44–53 d control plants of *Ricinus communis* L. fed 12 mM NO_3^- and 0.5 mM $H_2PO_4^-$. The economy of NO_3^- and reduced N is depicted in terms of exchanges between root and stem plus petioles including apical tissues and between stem axis and the bulk of all leaf laminae. Width of arrows (flows) and areas of squares (incorporation) and circles (nitrate reduction) are drawn in proportion to rates of these processes, see the scale. Numbers denote the flow rates in mmol N $plant^{-1}$ (9 d)$^{-1}$. Dotted arrows denote flow of NO_3^- in xylem, equally dotted squares storage of NO_3^-. Correspondingly cross-hatched arrows and squares denote flows and storage of reduced N The symbol ◯ refers to nitrate reduction.

1995), where rates were also increased in the presence of salt to 0.7 μmol $g^{-1}\ h^{-1}$.

Modelling of the flows of phosphorus

As a general principle it should be possible to model flows and partitioning of $H_2PO_4^-$ within a plant in a manner similar to that already accomplished for NO_3^- (Jeschke and Pate, 1991*b*), in this case deriving flows of $H_2PO_4^-$ from previously measured flows of C in the same plant. However, as for NO_3^- separate data need to be available for increments in inorganic (Pi) *and* organic P (P_{org}) and, in particular, information is needed on the molar ratios of Pi:C and of P_{org}:C in the phloem and xylem sap. The flows of both inorganic and organic P can then be estimated on the basis of the assumption that transport occurs by mass flow and hence in proportion to the molar ratios of the compounds present in the translocation fluids.

Increments of PO_4 (ΔPO_4) and of total P (ΔP) have been measured (Figs 3, 4) and their difference yielded those of organic phosphate (ΔP_{org})

$$\Delta P_{org} = \Delta P - \Delta PO_4 \qquad (2)$$

Similarly, concentrations of PO_4 and total P in the phloem sap have been determined (Table 1), and these data were used to calculate the molar ratios $(P:C)_p$ and $(PO_4:C)_p$ relative to C in the phloem. Using the carbon flows in the phloem (Jeschke *et al.*, 1996) those of P and PO_4 were obtained:

$$J_{P,p} = J_{C,p} \times (P:C)_p \qquad (3)$$

and

$$J_{PO_4,p} = J_{C,p} \times (PO_4:C)_p \qquad (4)$$

The net flow of organic P compounds in the phloem $J_{P_{org},p}$ was derived from the difference between (3) and (4).

$$J_{P_{org},p} = J_{P,p} - J_{PO_4,p} \qquad (5)$$

In the case of xylem sap, on the other hand, it has not been possible to detect organic P and it was therefore assumed that PO_4 was almost the sole P compound in the xylem and that

$$J_{p,x} \approx J_{Po_4,x} = J_{c,x} \times (PO_4:C)_x \qquad (6)$$

On this basis flow patterns of P have been calculated and the assimilation of PO_4 (P_{ass}) has been estimated as

$$P_{ass} = \Delta P_{org} - J_{P_{org},p} \qquad (7)$$

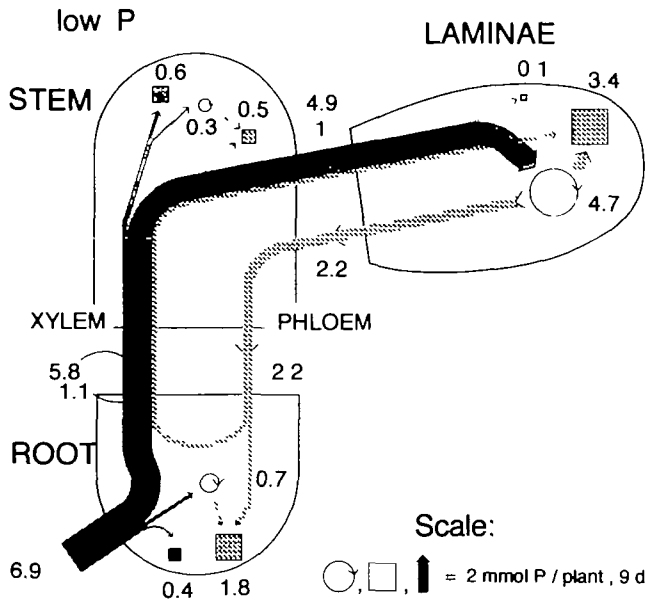

Fig. 8. Condensed model of the partitioning, flows and utilization of nitrate and reduced N during the study period 44–53 DAS in P-deficient plants of *Ricinus communis* L. fed 12 mM NO_3^- and 0.005 mM $H_2PO_4^-$. Other details as in Fig. 7, but note that the scale depicting flows and storage of NO_3^- and reduced N and NO_3^- reduction is approximately five times enlarged, see the scale. The symbol ↻ refers to nitrate reduction.

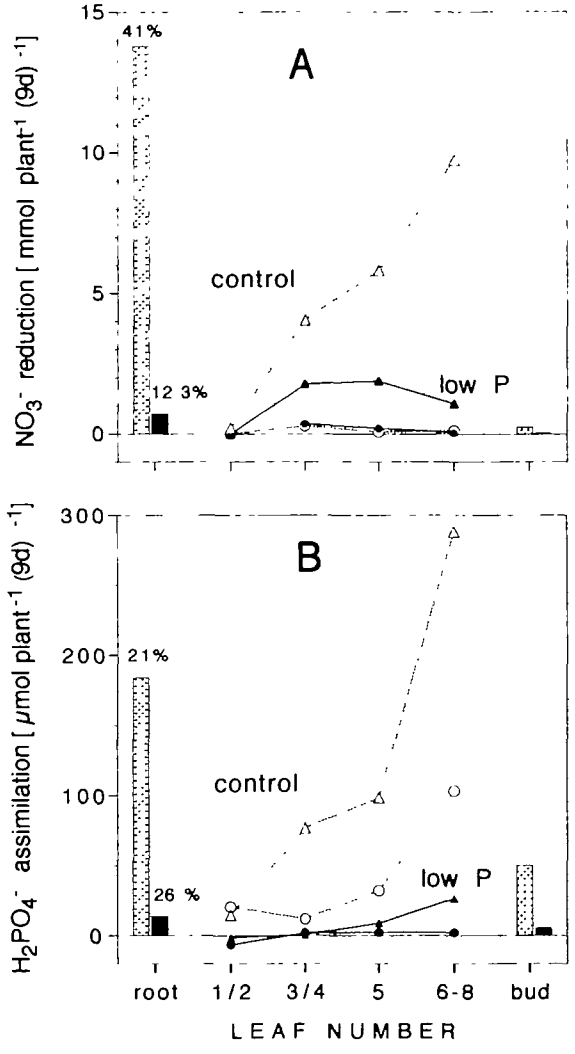

Fig. 9. Partitioning of (A) NO_3^- reduction [mmol N plant^{-1} (9 d)$^{-1}$] and (B) $H_2PO_4^-$ assimilation [µmol P plant^{-1} (9 d)$^{-1}$] between the individual organs of *Ricinus communis* plants during the study period 44–53 DAS. (---△---), (---○---), (▦): control plants fed 12 mM NO_3^- and 0.5 mM $H_2PO_4^-$; (—●—), (—▲—), (■): P-deficient plants fed 12 mM NO_3^- and 0.005 mM $H_2PO_4^-$. Triangles: leaf laminae, circles: stem segments + petioles. The rates of NO_3^- reduction have been obtained from detailed flow patterns of NO_3^- and reduced N corresponding to Figs 7 and 8. Similarly, rates of $H_2PO_4^-$ assimilation (phosphorylation) of organic P compounds have been obtained from detailed flow models of $H_2PO_4^-$ and of organic P corresponding to Figs 11 and 12 (not shown). The numbering of leaves and subtending stem segments started from the bottom with leaf 1 and 2 as the oldest, primary leaves. The numbers above the columns denote the percentage contribution of the root to total NO_3^- reduction or PO_4 assimilation.

where P_{ass} is defined as the sum of all phosphorylation processes leading to the formation of organic P compounds and $J_{P_{org,P}}$ is the net phloem flow of organic P into (positive sign) or out of (negative sign) the organ in question.

In Figures 11 and 12 detailed flow patterns of phosphorus have been condensed slightly to depict flows towards developmentally related organs. Leaves were grouped into four strata and leaves 3 and 4 combined because mobilization of P had commenced from both. The main features of P flows in P-sufficient control plants were:

(1) The principal translocation route for P was the xylem with the laminae and specifically those of the fast growing leaves 6 and 7 acting as the main sinks.
(2) The second major sink was the root, followed by stem segments, of which the youngest and growing ones had the greatest import capacity.
(3) While the uppermost leaves imported both from xylem and phloem (leaf 6 was close to transition from phloem import to export), substantial phloem retranslocation of P had started in leaf 5 and there was net P export, i.e. P mobilization from the lower leaves.
(4) Phloem retranslocation of P at the stem base amounted to 30% of xylem transport and the quantities retranslocated from shoot to root exceeded the requirements of the root by far. Some phosphate was accordingly recycled back to the shoot.

In comparison with the controls, the flow scheme for P in P-deficient plants (Fig. 12) highlighted the severe depression of P uptake and flows (see the numbers in Figs 11 and 12 and note the 10-fold amplification of the scale of arrow thicknesses depicting flows and of the areas

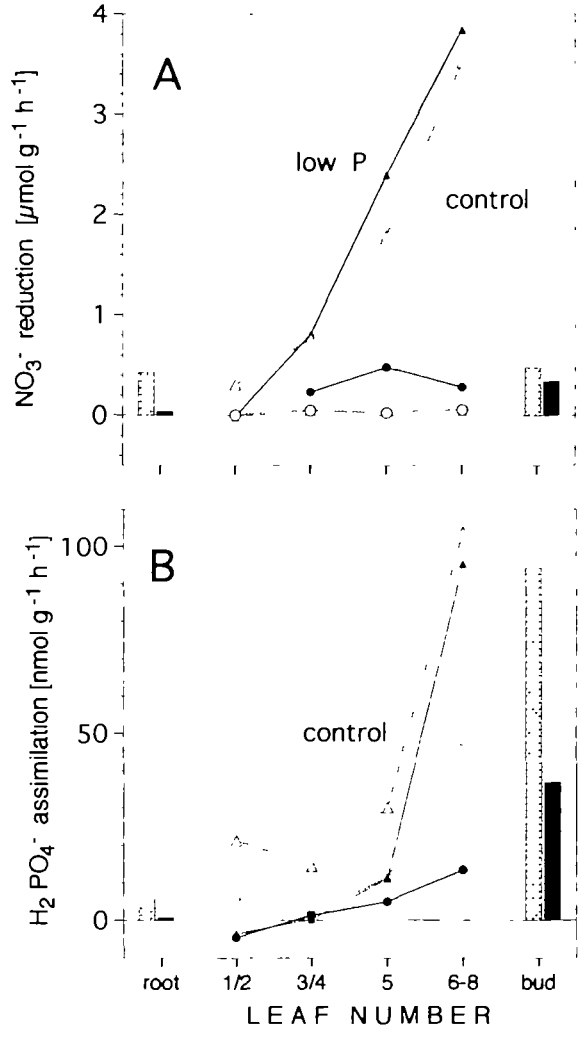

Fig. 10. Predicted rates of (A) NO_3^- reduction [µmol N g^{-1} FW h^{-1}] and (B) $H_2PO_4^-$ assimilation [nmol P g^{-1} FW h^{-1}] in root, stem segments plus petioles and leaf laminae of castor bean (*Ricinus communis* L.) for the interval 44–53 DAS. Estimates of NO_3^- reduction and PO_4 assimilation, respectively, are based on the values shown in Fig. 9 and the mean fresh weight of plant parts. Same symbols as in Fig. 9. (---△---), (---○---), (□): control plants fed 12 mM NO_3^- and 0.5 mM $H_2PO_4^-$; (—●—), (—▲—), (■) P-deficient plants fed 12 mM NO_3^- and 0.005 mM $H_2PO_4^-$. Triangles: leaf laminae, circles: stem segments + petioles. Numbering of leaves as in Fig. 9.

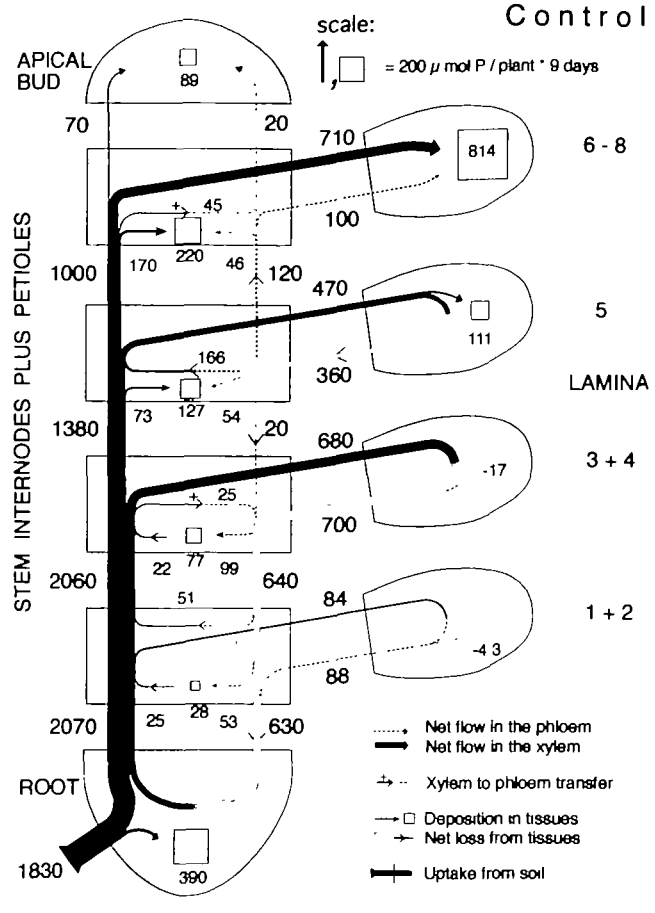

Fig. 11. Flow profiles for uptake, transport and utilization of total phosphorus in control plants of *Ricinus communis* L. over the study period 44–53 DAS. The plants were fed with 0.5 mM $H_2PO_4^-$ and 12 mM NO_3^-. The width of arrows (flows in xylem and phloem) and the area of squares (incorporation) are drawn in proportion to rates of flow of total P and of deposition of P into tissues (for the scale see the key at the top). The numbers indicate rates of flow and deposition in µmol P plant^{-1} (9 d)$^{-1}$. The numbers under the squares in each stem segment denote uptake from the phloem (right) or the xylem (left). For the sake of clarity flows into the young, growing leaves (6–8), into or out of the maturing but still expanding leaf 5, the mature leaves 3+4 and the oldest leaves (1+2) and the subtending stem segments plus petioles have been combined.

of squares denoting incorporation). The salient features for P-deficient plants were:

(1) The laminae of leaves 6 and 7 constituted the major sinks for P and they imported both from xylem and phloem.

(2) Although leaf 5 achieved the largest area increment during the study period (Fig. 1 in Jeschke *et al.*, 1996), it exhibited a low P increment and had commenced to export via the phloem to meet the demand of younger leaves. Its behaviour thus resembled that of the corresponding but developmentally more progressed leaf 5 of control plants.

(3) There was considerable remobilization of P from older leaves which in turn led to a substantial retranslocation of P from shoot to root. This phloem transport amounted to almost 50% of the P moving in the xylem stream exiting from roots.

(4) Since there was no net incorporation of P into the still actively growing P-deficient root, phloem-imported P was depicted in the models as being recycled to growing shoot parts via the xylem. Consequently, almost half of the xylem transport originated from this cycled P.

By separately modelling flows and partitioning of inorganic and organic P, comparable flow patterns as in Figs 11 and 12 have been obtained for these compounds (not shown). From these flows the distribution of the assimila-

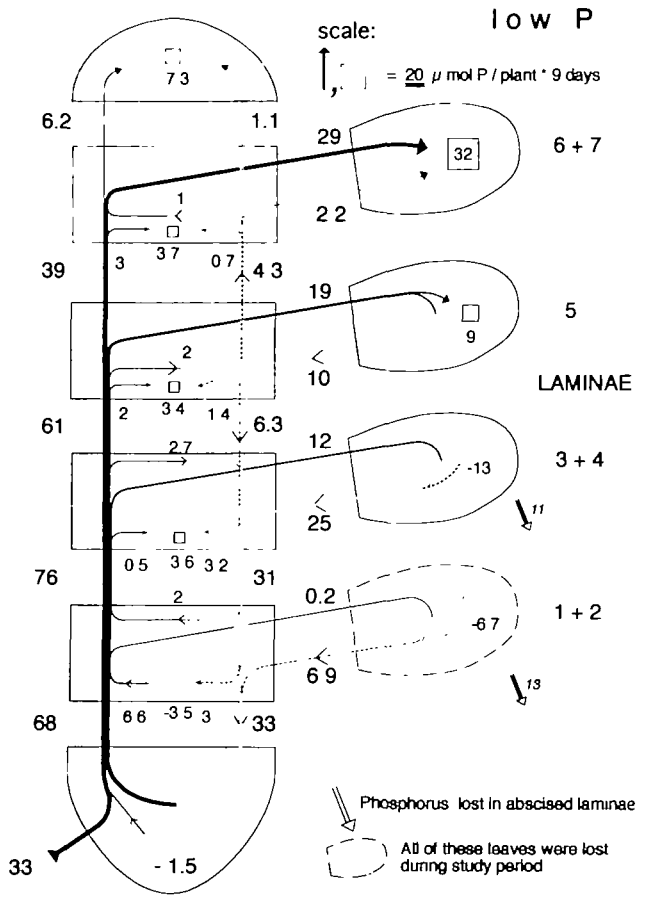

Fig. 12. Flow profiles for uptake, transport and utilization of total phosphorus in low-P plants of *Ricinus communis* L. over the study period 44–53 DAS. Phosphate had been withdrawn at 29 DAS and 5 μM $H_2PO_4^-$ was then given at 41 DAS. Details as in Fig. 11; note that the scale for arrows and squares is nearly 10-fold enlarged and, nevertheless, arrows (flows) and squares (P deposition) are smaller than in Fig. 11, highlighting the severe depression of P exchanges. In this model the young leaves (6, 7) were bulked in the calculations.

tion of PO_4 into organic compounds has been derived according to equation (7) (Fig. 9B). In control plants major sites of assimilation were leaf laminae, the root and apical tissues, followed by stem segments. In laminae and stem segments formation of organic P was primarily active in younger tissues. In general terms, P deficiency led to severe decreases in PO_4 assimilation and directed the major part of such assimilation into young laminae. Indeed, in lower leaves and stem parts 'negative' values for assimilation were recorded, i.e. hydrolysis of organic P compounds exceeded synthesis in net terms.

By relating PO_4 assimilation to the mean organ fresh weight, the rates of phosphorylation (PO_4 assimilation) in various organs have been obtained (Fig. 10B) showing by far the highest rates in young laminae and the terminal bud and notably low rates in root tissues. In P-deficient plants phosphorylation rates of young laminae were remarkably similar to the controls, but in other tissues rates of PO_4 assimilation were substantially decreased.

Discussion

Effects of P deficiency on the economy of nitrate and reduced nitrogen

P deficiency exercised very severe effects on the economy of nitrate and reduced N of *Ricinus communis*. These effects included (i) a restriction of nitrate uptake to 15% of the P-sufficient control, (ii) a depression of nitrate reduction in the root to 5% of the control, (iii) a shift of nitrate reduction from root to shoot, and (iv) an enormous relative increase in shoot:root cycling of reduced N equivalent to 200% of the N leaving the root in the xylem.

(i) Nitrate uptake rates on a root fresh weight basis were 1.45 μmol NO_3 g^{-1} FW h^{-1} in P-adequate control plants and 0.36 μmol NO_3 g^{-1} FW h^{-1} in P-deficient plants, i.e. an inhibition of 75%. This indicated that the even more reduced rates of uptake on a per plant basis (15% of the control) were due to lower uptake activity and to the small plant size. Low nitrate uptake rates under P deficiency were also observed by Schjorring (1986) and Pilbeam *et al.* (1993) and could well be related to a low sink demand in the slow-growing deficient plants. Demand-regulated N uptake has been suggested by a number of authors (Engels *et al.*, 1992; Muller and Touraine, 1992; Touraine *et al.*, 1994). This has been attributed to different shoot to root signals such as a negative feedback signal through the agency of phloem-borne amino acids (Muller and Touraine, 1992) or a promotive action of phloem-translocated organic acids. The first of these possibilities is clearly consistent with the results obtained here on phloem translocation in the low P plants where amino acid (reduced N) recycling from shoot to root was substantially increased in relative terms (Fig. 8) in comparison with the controls (Fig. 7). The alternative hypothesis of levels of phloem organic acids being instrumental in promoting NO_3^- uptake, on the other hand, assumes a stoichiometric process in which bicarbonate produced by decarboxylation of malate transported from shoot to root in the phloem is released to the rhizosphere in exchange for the uptake of NO_3^- (Touraine *et al.*, 1994). The present results appear to contradict such an effect in showing a 35% increase in malate concentration of phloem sap and, therefore, an expected promotion rather than the observed inhibition of NO_3^- uptake. This apparent contradiction may perhaps be explained by bearing in mind the altered organic acid metabolism and economy likely to occur in P-deficient plants especially in relation to enhanced organic acid exudation from roots (Dinkelacker *et al.*, 1989; Hoffland *et al.*, 1992). Such a release would mean that these organic acids would not be available for promoting NO_3^- uptake in the recycling

process as discussed above, since the charge from the organic acids would be dissipated. These findings of higher concentrations of malate, succinate and oxalate in the phloem of *Ricinus* would furthermore suggest that release of organic acids by the roots of P-deficient plants may involve organic acids originating in the shoot. This is in accordance with the observation of Hoffland *et al.* (1992) of higher PEP carboxylase activities in the leaves of P-starved rape plants, a species also showing enhanced root exudation of organic acids under deficiency. This shoot origin of the organic acids would thus provide an integrated response of the plants to a lack of P rather than simply a direct reaction of the root.

(ii), (iii) The very low proportional contribution of P-deficient *Ricinus* roots to nitrate reduction (Fig. 8) and the generally low rates in the roots (Fig. 10A) are somewhat difficult to explain. According to Andrews (1986) and Andrews *et al.* (1992) the high external NO_3^- concentration presently used (12 mM NO_3^-) and resulting high rates of uptake would be expected to favour reduction in the shoot. This is indeed true for the controls (Fig. 7). Low nitrate uptake as is found in the P-deficient plants would be expected similarly to lead to relatively high reduction in the root. This supposition clearly conflicts with our observations.

A possible explanation is that lower $H_2PO_4^-$ supply and hence restricted $H_2PO_4^-$ and NO_3^- uptake leads to an anion and an osmotic deficit in the xylem sap that, by forcing a release of NO_3^- into the xylem, leads to relatively higher allocation of NO_3^- to transport (Fig. 8) and a lack in NO_3^- taken up as the substrate for reduction in the roots. Indications for a close regulatory linkage between the strict need for cation–anion balance in xylem transport—prominently between K^+ and NO_3^-—and the size of nitrate reduction in the root (Rufty *et al.*, 1981) have been presented (Förster and Jeschke, 1993; Peuke *et al.*, 1996). On this basis a requirement for anionic charge in the xylem would depress the reduction of NO_3^- in the root as in the present case and a surplus of anionic charge as in the presence of NaCl (Peuke *et al.*, 1996) would have the reverse effect. Additionally, it cannot be excluded that the decreased PO_4 pool during phosphorus deprivation restricts the NAD(P)H needed for nitrate reduction.

(iv) Turning to the massive relative increase in retranslocation of reduced N from shoot to root (Fig. 8), it is no doubt related to favoured root growth in P-deficient plants (Fredeen *et al.*, 1989; Heuwinkel *et al.*, 1992).

The present flow schemes of NO_3^- and reduced N and the partitioning of NO_3^- reduction may be compared directly with the corresponding data obtained with salt-stressed *Ricinus* (Jeschke and Pate, 1991*b*), since plant age and the study period were identical in both experiments. In contrast to effects of P deficiency, in the presence of NaCl nitrate reduction was shifted towards the root, which then contributed 51% to total reduction compared with 40% in the present controls. This shift in response to salt agrees with other experiments with *Ricinus* (Peuke *et al.*, 1996) and with tomato (Cramer *et al.*, 1995). Similar to P deficiency, moderate salt stress severely inhibited nitrate uptake to 26% of the present controls, but the general patterns of the flows of reduced N and NO_3^- and their partitioning within the plant were not significantly altered in comparison with the present controls (cf. Fig. 7 in Jeschke and Pate, 1991*b*, with Fig. 7 here). Thus, under both conditions there was substantial retranslocation of reduced N, which amounted to 52% (salt) or 55% (control, Fig. 7) and the current nitrate reduction in the root exceeded the demand of the root nominally by 2.7-fold (salt) or 2.5-fold (control). By contrast the effects of P deficiency on the N economy already discussed were much more severe than those of salinity and appear to be more directed towards alleviation of low-P stress by improvement of root performance and maintenance of limited growth on the basis of remobilized P resources.

Flows and partitioning of P in P-sufficient and P-deficient Ricinus *plants*

For the first time, as far as we are aware, it has been possible to estimate flows of P in a whole plant. The major transport form for P is inorganic phosphate, but organic P compounds such as nucleotides (including ATP) have been found in phloem sap (Ziegler, 1975) and *Ricinus* phloem sap was found to contain 0.4–0.6 mM ATP or 1.2–1.8 mM organic P (Hall and Baker, 1972). These values are in fair agreement with the difference between total and inorganic P (1.1 mM, Table 1) recorded in the present study.

P is highly phloem-mobile (Ziegler, 1975) and was present at high levels in the sieve tube sap of the control plants of *Ricinus* (Table 1). Flow patterns derived from the data for these P-sufficient plants showed substantial retranslocation of P (about 30% of xylem transport) and were comparable with patterns of flow of other phloem-mobile ions such as K^+ and Mg^{2+} in lupin (Jeschke *et al.*, 1987) and in *Ricinus* (Jeschke and Pate, 1991*c*). As found earlier for N (Jeschke *et al.*, 1996), P was preferentially allocated to laminae and to younger organs (Fig. 11). In older leaves mobilization and net export of P was noted and retranslocated P greatly exceeded the current requirements of the root. Massive transfer of P from phloem to xylem accordingly took place in the root, to the extent that xylem transport of P to the shoot was substantially in excess of P uptake from the medium.

Separate modelling of the flows and partitioning of total P and of PO_4 in P-sufficient plants enabled us to estimate quantities of PO_4 that were in net terms assimilated into

organic compounds by various organs (Fig. 9B). Young laminae proved to be major sites of assimilation, followed by the root and young stem parts. Differences in PO_4 assimilation between laminae and the subtending petioles and stem parts were less marked than was encountered for nitrate assimilation (Fig. 9A, B), and this may be related to the close connection between nitrate reduction and photosynthesis. When relating PO_4 assimilation to fresh weights (Fig. 10B), rates varied greatly among organs and were especially high in young tissues of developing laminae and the apical bud and generally higher in laminae throughout the plant than in the corresponding stem parts and the root. The low rates of PO_4 assimilation per unit root fresh weight may reflect the inclusion of the large but metabolically less active main root.

Corresponding models of flows of P in P-deficient plants showed drastic curtailment of P uptake and translocation and also substantial alterations in the proportional involvement of phloem and xylem flows in the partitioning of P. Phloem retranslocation to the root was less restricted (to 5% of the control at the stem base) than uptake (to 1.8%), and remobilization of P from older leaves was relatively increased. These changes would together mitigate the effects of P deficiency on the plant. No *de novo* P incorporation into the root occurred despite greater relative growth in root than shoot in comparison with the controls (Jeschke *et al.*, 1996). Thus, incorporation of P into growing root tips must have been compensated for by proportionate mobilization from old root tissues as observed by Smith *et al.* (1990). As a consequence, all of the P imported into the root via the phloem was in net terms recycled to the shoot, and xylem transport thus was 2-fold higher than uptake from the medium. In the leaves, however, the process of remobilization did by no means amount to all of the P present, since it recovered only 18–34% of the P from senescing leaves, i.e. much less than reported for soybean (Lauer *et al.*, 1989). Substantial quantities of P were lost through precocious abscission of lower leaves. The characteristic brownish green colour of the necrotic leaves of P-deficient plants was consistent with tissue death preventing effective retrieval of nutrients.

In a study with the tropical forage legume *Stylosanthes hamata* Smith *et al.* (1990) showed rapid translocation of P into the root after withholding the external P supply. As can be seen from Fig. 11 the same would be expected to occur in *Ricinus* given the high potential for P retranslocation via the phloem in the P-sufficient plants. Were uptake to be prevented, retranslocation would continue and favour the import of P into the growing roots. The steady-state flows in the P-deficient plants in our experiment (Fig. 12), however, refer to plants 15 d after withdrawal of phosphate from the nutrient medium. At this stage they do not show any further net incorporation into the roots which already contained more than 40% of the total plant P (Fig. 3C) and twice as large a proportion as in the roots of the control plants.

Effects of P deficiency on xylem and phloem sap composition

The composition of xylem sap appeared to be much more affected by P deficiency than was that of phloem sap. Principal changes observed in root pressure xylem exudate were lowered $H_2PO_4^-$ levels (12% of the control) and, owing to specific restriction of nitrate uptake, drastically depressed amino acid (15% of the control) and somewhat lowered nitrate levels (45% of the control). Concentrations of the accompanying cations (K^+, Mg^{2+}) were decreased, apparently compensating for these changes, while levels of chloride, sulphate and, in particular, malate were increased. These P deficiency-induced changes appeared even more severe when solute flow rates are considered. Owing to the lower volume flow in low P plants (Table 3) flows of all solutes are then seen to be drastically depressed and compensations for the most severe decreases($H_2PO_4^-$, NO_3^-) are evident as smaller decreases only in flows of chloride and sulphate, while malate flow remained the same as in the controls (data not shown). The reason for the higher malate concentration (200-fold) in the xylem sap of the P-deficient plants probably relates to the marked depression of NO_3^- uptake associated with a net increase in cation over anion uptake and associated proton release (Heuwinkel *et al.*, 1992; Pilbeam *et al.*, 1993). This was indicated in the present experiments by daily falls in pH of the nutrient medium in this treatment, in contrast to the control plants in which comparative nutrient pH values increased.

The composition of phloem sap in control plants (Table 1) closely resembled published data for P-sufficient *Ricinus* (Hall and Baker, 1972; Van Beusichem *et al.*, 1988; Jeschke and Wolf, 1988). Changes in the phloem sap composition evident here for P-deficient plants can be attributed to two principal causes, firstly to the P deficit in tissues (Fig. 1), which led to a 75% decrease in $H_2PO_4^-$, and secondly to the inhibition of NO_3^- uptake, which was reflected in a measurable decrease in the amino N levels of the phloem sap (Table 1). As a consequence of lower $H_2PO_4^-$ levels, those of other anions including Cl^- and organic anions increased. Glutamine was diminished in favour of increases in glutamate as might be expected of incipient N deficiency where the amide-N of glutamine would be used for amino acid synthesis and the resulting glutamate loaded into the phloem. Interestingly, concentrations of malate, oxalate and shikimate were substantially increased in the phloem and succinate was found only in phloem sap of the P-deficient

plants. However, as indicated above, most of the changes in phloem sap were less severe than in the xylem, showing a high buffering capacity of the plant in terms of phloem loading. By maintaining relatively high levels of reduced N and even of $H_2PO_4^-$ in the phloem, the P-deficient plants remained capable of maintaining supply of these vital nutrients to sites of demand. The high sucrose levels in phloem of P-deficient plants may be considered indicative of a relatively low overall sink capacity in these slow-growing plants.

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