Nutrient absorption by pea plants during dinitrogen fixation. 1. Comparison with nitrate nutrition

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Summary

In experiments with pea plants, grown hydroponically for six weeks with nitrate as the only nitrogen source, an acidic nutrient uptake pattern was observed. When effectively nodulated plants were fixing dinitrogen, however, cation absorption exceeded anion absorption resulting in an alkaline ion uptake pattern.

Maintaining the ambient temperature at 13 °C allowed the comparison of the effects of nitrogen acquisition on nutrient absorption and proton or hydroxyl/ bicarbonate excretion by the roots, since plants both similar in dry matter yields and in nitrogen content were obtained.

Both for nitrate-supplied and dinitrogen-fixing plants the amount of excreted alkalinity or acidity, achieved by automatic titration, corresponded well with the respective excess absorption of nutritive anions or cations.

Some physiological and agronomic consequences of the alkaline nutrient uptake pattern and acidity generation by dinitrogen-fixing legumes are discussed.

Introduction

It is widely known that availability of nutrients for plants depends on many soil characteristics. One of the main factors affecting the solubility of nutrients such as phosphorus and micro-nutrients is the acidity of the soil. Deficiency of most nutrients can be induced by a high soil pH. Nutrient availability is generally favoured at a low soil pH, but sometimes solubility is enhanced to such an extent that some elements reach phytotoxic concentrations. Iron deficiency in soya bean crops grown on alkaline soils, generally referred to as lime-induced chlorosis, as well as aluminium toxicity in several crops grown on acidic soils, can be considered as world-wide problems in this respect.

An interesting property of a plant is its ability to change the pH of the rhizosphere during growth. Rhizosphere acidity or alkalinity generation is influenced by the operation of ion uptake processes. Vascular land plants generally take up unequal amounts of nutritive cations and anions when expressed in terms of charge equivalents. It has been found that the amount of excreted H^+ or OH^-/HCO_3^- by the roots is stoichiometrically equal to the respective excess cation or anion absorption (Breteler, 1973a; van Beusichem & van Loon, 1978; van Egmond & Aktas, 1977). Through this mechanism, electroneutrality of both plant and environment is maintained. As a consequence of rhizosphere acidity or alkalinity generation, plants are able to affect solubility of nutrients and hence their avialability and uptake. This phenomenon was clearly demonstrated by van Egmond & Aktas (1977), showing that Fe-efficient plant species and cultivars, grown hydroponically with nitrate as a nitrogen source, respond to iron stress by lowering the pH of the nutrient solution. In soils, this mechanism would enable the plant to mobilize precipitated iron compounds.

Because fertilizers can also change the pH of the soil, it is not easy to distinguish between the effects of the plant and the effect of the fertilizer on soil pH. Therefore, Pierre and co-workers gathered many data on the cation-anion balance of field-grown crops in order to separate the effects of crops and of fertilizers on soil acidity (Banwart & Pierre, 1975; Pierre & Banwart, 1973; Pierre et al., 1970).

In order to predict the extent of the H^+ or OH^-/HCO_3^- excretion process, it is necessary to have an insight in nutrient absorption characteristics of plants and in both external factors and internal processes, affecting the cation-anion uptake balance.

It is to be expected that the nutrient uptake pattern is affected by the ambient acidity. Up till now, this item has received little or no attention in literature. Data on the effects of the root medium pH on the cation-anion uptake balance and related H^+ excretion will be treated in a subsequent publication (van Beusichem, 1982). More information is available about the relations between nitrogen nutrition of plants and effects on the ambient pH. The uptake pattern of cations and anions and the resulting ionic balance in plants is influenced strongly by the nitrogen source, although differences exist between plant species. Because absorption of nitrogenous ions is substantial, ammonium nutrition results in an alkaline nutrient uptake pattern (net H^+ excretion by the roots), regardless of plant species. In contrast, nitrate nutrition generally results in an acidic nutrient uptake pattern (net OH^-/HCO_3^- excretion by the roots), but big differences exist between plant species with respect to the amounts of excreted alkalinity. Gramineae generally take up a large excess anions over cations. Some plant species, especially members of the Compositae, Polygonacea, Solanaceae, and Chenopodiaceae families, show a low acidic or even a neutral ion uptake pat-

tern when exposed to media in which nitrate is the only nitrogen source.

Many investigations have been carried out to study the effects of ammonium or nitrate nutrition on the uptake of different cations and anions, the ionic balance, organic acid and carbohydrate metabolism of different hydroponically grown plant species (Breteler, 1973a, 1973b; Chouteau, 1963; Clark, 1936; Dijkshoorn et al., 1968; Houba et al., 1971; Kirkby, 1968, 1969; Kirkby & Hughes, 1970). The number of publications concerning comparative studies of nitrogen sources, including urea, is very limited (van Beusichem & van Loon, 1978; van Beusichem & Neeteson, 1982; Kirkby & Mengel, 1967; Wallace & Ashcroft, 1956), may be partly because of experimental difficulties connected with hydrolysis of urea. When decomposition of urea in the nutrient solution is avoided, sugar-beet and maize plants show an alkaline nutrient uptake pattern, corresponding with acidification of the root medium (van Beusichem & Neeteson, 1981). Almost no information is available concerning nutrient uptake patterns of dinitrogen fixing plant species. Some authors have supposed that an alkaline uptake pattern occurs in soils, but this is based on incomplete plant analysis data (Andrew & Johnson, 1976; Israel & Jackson, 1978; Nyatsanga & Pierre, 1973). To the best of the author's knowledge, no quantitative data are available on the ionic balance of hydroponically grown dinitrogen-fixing plants. The present study deals with the effects of the nitrogen source (nitrate supply versus dinitrogen fixation) on dry matter production, nitrogen content, and ionic balance of young pea plants in relation to alkalinization or acidification of the nutrient solution.

Materials and methods

Seedling culture
Seeds of *Pisum sativum* L. cv. Rondo were surface-sterilized with 3 % hydrogen peroxide (v/v) for 30 minutes. The desinfected seeds were washed intensively with demineralized water and germinated on wetted filter paper at 20 °C in the dark.

After 10 days, selected seedlings were transferred to Mitscherlich pots (inner diameter 20 cm, height 22 cm) containing 6.5 litres of nutrient solution. The composition of the nutrient solution is given in Table 1 (pre-treatment). The pots were covered with perforated lids in which the seedlings were held in place by means of foam plastic (6 seedlings/pot). The pots were placed in a growth chamber maintained at 22 °C. The light intensity during the 14-h photoperiod was 13.5 J cm^{-2} h^{-1} while the relative air humidity varied between 70 and 75 %. The nutrient solutions were aerated continuously and the pH was adjusted daily at a value between 6.0 and 6.5.

Six days after transfer of the plants to the growth chamber, 10 ml of a dense suspension of *Rhizobium leguminosarum* were added to each of the pots. When

Table 1. Chemical composition (meq l^{-1}) of the nutrient solutions used in the experiments.

	K	Ca	Mg	H$_2$PO$_4$	Cl	NO$_3$	SO$_4$
Pre-treatment	2.5	2.5	2.5	2.5	2.5	—	2.5
Nitrate solution	2.5	4.0	2.5	2.5	—	4.0	2.5
Zero N solution	2.5	4.0	2.5	2.5	4.0	—	2.5

Trace elements in all solutions (mg l^{-1}): Fe 4.6; B 0.5; Mn 0.5; Zn 0.05; Cu 0.02; Mo 0.01.

in the subsequent experiment nitrate was used as a nitrogen source, the plants were inoculated with the strain P8, resulting in an ineffective symbiosis. The strain PF2 was used when the plants were committed to dinitrogen fixation; this strain accomplished an effective symbiosis.

Root nodules became visible in all pots about 4 days after inoculation. From this time onward the level of the nutrient solution was lowered daily by removing about 750 ml per pot. At the end of the pre-treatment period, 14 days after transfer to the growth chamber, about 10 cm of the nodulated root systems were above the solution level.

Plant growth

After the pre-treatment period, 40 plants were selected for each treatment and transferred to a 72-litre PVC box (surface 3000 cm^2, height 24 cm) containing 30 litres of a nutrient solution. The solution contained either nitrate as a nitrogen source or no combined nitrogen (Table 1). In the latter case the plants were solely dependent on symbiotic dinitrogen fixation as a nitrogen source. The solutions were circulated and consequently aerated by an electric pump with a capacity of about 15 litres per minute. The acidity of each nutrient solution was adjusted to pH 5.50 and kept constant by a pH-meter with pH-stat equipment (Radiometer T.T.T.2), operating an automatic burette (Radiometer A.B.U.12). The burette contained 0.1000 *M* NaOH (dinitrogen fixation) or 0.1000 *M* H$_2$SO$_4$ (nitrate nutrition). This technique was used successfully and described in detail by Breteler (1973a). The whole system was set up in a phytotron where the experimental conditions were: temperature 13 °C, photoperiod 16 h day^{-1}, light intensity 14.5 J cm^{-2} h^{-1}, and relative air humidity 70 %.

The plants were grown under constant climatic conditions for a period of 42 days. After 21 days the nutrient solutions were renewed and 20 plants of each treatment were harvested.

Only two treatments could be carried out at the same time. Each treatment combination was repeated two or three times. Repeats of experiments yielded data similar to those presented here.

Plant analysis

The plants were separated into shoots and roots prior to chemical analysis.

When the root system was nodulated the nodules were collected quantitatively. The roots were washed for 1 minute in 0.01 M HCl and then rinsed twice with demineralized water. Shoots, roots, and nodules were dried at 70 °C for a period of 24 hours. Subsequently, the dry weights were determined and the samples were ground for analyses.

Subsamples were analyzed for total nitrogen, potassium, sodium, calcium, magnesium, phosphate, chloride, nitrate, and sulphate. Total N, K, Na, Ca, Mg, and H_2PO_4 were determined after wet digestion of the samples in concentrated sulphuric acid and hydrogen peroxide (Lindner & Harley, 1942) in the presence of salicylic acid. In the diluted digests total nitrogen was measured colorimetrically by the indophenol-blue method (Novozamsky et al., 1974). K, Na, and Ca were determined by flame emission spectrometry, and Mg by atomic absorption spectrometry. H_2PO_4 was determined colorimetrically, using ammonium molybdate as a reagent, potassium antimonyl tartrate as a catalyst and ascorbic acid as a reductant. For the determination of Cl, NO_3, and SO_4 other subsamples were extracted with demineralized water (1:50, w/v). In the filtered extracts Cl was determined coulometrically with an Ag anode at constant current, NO_3 potentiometrically with a NO_3 selective electrode, and SO_4 turbidimetrically with $BaCl_2$ and Tween 80. For detailed description of the analyses see van Schouwenburg & Walinga (1979).

Results

Production of dry matter
In Fig. 1 the results are given of dry matter yields of shoots and roots of nitrate-supplied and dinitrogen-fixing pea plants. Both shoot and root production of the two treatments differed by less than 20 %, provided that the temperature in the phytotron was maintained at 13 °C throughout. Plants grown for six weeks produced four (nitrate nutrition) to five (dinitrogen fixation) times as much dry matter as plants grown for a three-week period. This may indicate that over the experimental period plants of both treatments were in their exponential phase of growth.

Inorganic chemical composition
Table 2 shows the results of the chemical plant analyses. The influence of the nitrogen acquisition is clearly reflected in the total inorganic cation content (C) in the shoots which was highest in nitrate-supplied plants at both harvests. In comparison with dinitrogen-fixing plants these plants had accumulated much more potassium. In the three-week-old plants also calcium contributed substantially to the higher cation content in nitrate-supplied plants. The cation content in the roots was about the same for both nitrogen sources. Substitution of chloride for
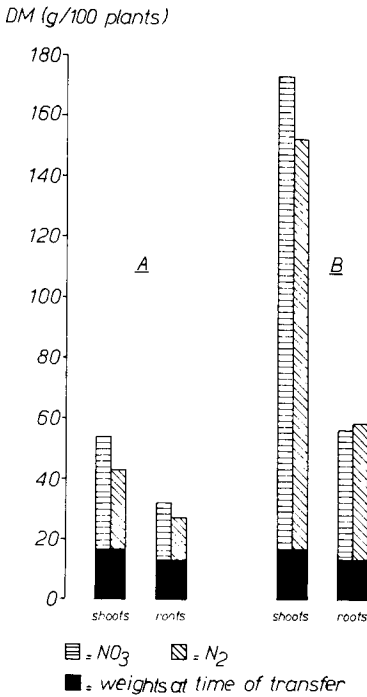
DM (g/100 plants)

Fig. 1. Dry matter yields of pea plants, 21 (A) and 42 (B) days after transfer to a nutrient solution with (NO₃) or without (N₂) combined nitrogen.

Table 2. Chemical composition (meq/kg DM) of shoots and roots of pea plants after 21 and 42 days growth on a nitrate-containing solution (NO₃) or on a nutrient solution without combined nitrogen (N₂).

	At transfer		21 days after transfer				42 days after transfer			
			NO₃		N₂		NO₃		N₂	
	shoots	roots	shoots	roots	shoots	roots	shoots	roots	shoots	roots
K	1049	1601	1245	1103	1000	1128	1282	905	1033	1016
Na	35	17	28	47	56	71	21	35	56	76
Ca	470	456	1054	488	784	396	766	490	693	431
Mg	385	259	466	298	411	192	374	166	307	172
Σ C	1939	2333	2793	1936	2251	1787	2443	1596	2089	1695
H₂PO₄	283	780	314	728	371	742	270	556	320	731
Cl	257	240	153	64	480	243	101	52	423	231
NO₃	0	0	232	192	0	0	153	145	0	0
SO₄	220	428	124	300	112	257	86	224	108	249
Σ A	760	1448	823	1284	963	1242	610	977	851	1211
Σ (C − A)	1179	885	1970	652	1288	545	1833	619	1238	484

nitrate in the nutrient solution and nitrate reduction in the roots and shoots of the plants resulted in a somewhat lower total inorganic anion content (A) in ni-trate-supplied plants than in dinitrogen-fixing plants. The anionic composition of the roots of dinitrogen-fixing plants did not change with age.

The difference between total cation and total anion content (C-A) is thought to be related about stoichiometrically to the amount of carboxylates (Houba et al., 1971). At both harvests the (C-A) value in the shoots of nitrate-supplied plants was 1.5 times as high as that in the shoots of dinitrogen-fixing plants (Table 2). The same picture was observed for the roots, although less pronounced. In alle cases much more carboxylates accumulated in the shoots than in the roots.

Nutrient absorption

The amounts of the different nutrients taken up during periods of 21 and 42 days are given in Table 3. These values are calculated from Table 2 and Fig. 1, taking into account the amounts absorbed during the pre-treatment period and using total nitrogen data (Table 5) for the calculation of nitrate absorption. Sulphate absorption was calculated as the sum of sulphate and organic sulphur, the latter being estimated as 5.4 % of the organic nitrogen amount (Dijkshoorn & van Wijk, 1967).

Differential uptake of cations and anions resulted in an alkaline nutrient up-take pattern (C_a-A_a) by dinitrogen-fixing plants and an acidic uptake pattern (A_a-C_a) by nitrate-supplied plants (Table 3). Nitrate absorption was partly re-

Table 3. Nutrient absorption (meq/100 plants) during 21 and 42 days growth on a nitrate-containing solution (NO_3) or on a nutrient solution without combined nitrogen (N_2). C_a, A_a = total cation and anion absorption, respectively. Figures in parenthesis represent total amounts of cations accumulated in the shoots.

	21 days after transfer		42 days after transfer	
	NO_3	N_2	NO_3	N_2
K	63	35	232	178
Na	1	3	4	12
Ca	58	30	145	116
Mg	24	13	63	47
C_a	146(118)	81(65)	444(390)	353(286)
H_2PO_4	24	21	62	76
Cl	1	19	11	70
NO_3	249	0	736	0
SO_4	19	13	55	56
A_a	293	53	864	202
$C_a - A_a$		28		151
$A_a - C_a$	147		420	

placed in dinitrogen-fixing plants by chloride uptake. However, in spite of the fact that chloride was completely substituted for nitrate in the nutrient solution of dinitrogen-fixing plants, chloride uptake of these plants could account only for about 8 % of the total nitrate absorption of nitrate-supplied plants. This indicates that chloride and nitrate are taken up by different mechanisms or, in case one mechanism is operative, that the affinity of the uptake system for both ions is different. Phosphate and sulphate absorption was not much affected by the form of nitrogen nutrition. Another way for compensating the amount of negative charge of nitrate is the suppression of cation uptake by dinitrogen-fixing plants. At both harvests nitrate-supplied plants had absorbed more of all cations (C_a) than dinitrogen-fixing plants. Between 80 and 88 % of the absorbed cations had been transported to the upper plant parts. Since nitrate absorption was only compensated for about 13 % by a lower potassium, calcium, and magnesium accumulation in the dinitrogen-fixing plants, this mechanism does not seem to play a dominant role. This implies that a mechanism through which pea plants, irrespective of the nitrogen source, aim at a constant internal pH only by way of changes in the absorption of non-nitrogenous ions, is not likely. The origin of these non-specific shifts in ion uptake is still unknown.
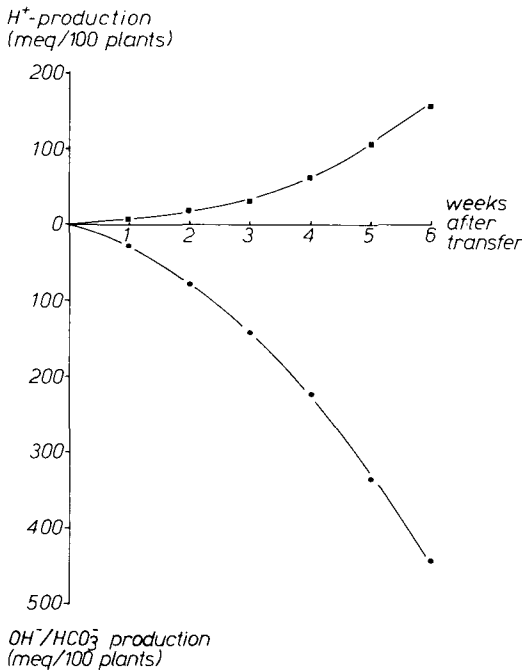

Fig. 2. Cumulative net hydroxyl/bicarbonate and proton production by pea plants grown on a nitrate-containing solution (●) or on a nutrient solution without combined nitrogen (■), respectively.

Net proton or hydroxyl/bicarbonate extrusion
Pea plants grown on a nitrate-containing nutrient solution extruded a consider-
able amount of alkalinity as a result of excess anion over cation absorption (Fig.
2). In contrast, when plants were fixing dinitrogen, the nutrient uptake pattern
was shifted, resulting in net proton excretion by the roots.

The curves presented in Fig. 2 are redrawn form the recorder sheets of the
pH-stat equipment. At both harvests the amounts of acid or base necessary to
keep the pH of the solutions at 5.50 corresponded well with the respective calcu-
lated values for excess anion or cation absorption (Table 4; Breteler, 1973a).

Nitrogen
In Table 5 the nitrogen contents in both shoots and roots of nitrate-supplied and
dinitrogen-fixing pea plants are compared. The nitrogen content in both organs
did not differ significantly for both treatments.

Because of a somewhat higher dry matter production in the nitrate treatment
(Fig. 1), the total amount of nitrate taken up by the plants over the 42 days was
about 13 % higher than the total amount of dinitrogen fixed during that period
(Table 6).

Table 4. Calculated and recorded acidic and alkaline nutrient uptake (meq/100 plants) by pea
plants after 21 and 42 days growth on a nitrate-containing solution (NO_3) or on a nutrient solution
without combined nitrogen (N_2).

	Days after transfer	
	21	42
NO_3		
A_a-C_a (calculated)	147	420
OH^- efflux (recorded)	141	444
N_2		
C_a-A_a (calculated)	28	151
H^+ efflux (recorded)	33	158

Table 5. Nitrogen content (mmol/kg DM) of shoots and roots of pea plants after 21 and 42 days
growth on a nitrate-containing solution (NO_3) or on a nutrient solution without combined nitrogen
(N_2). Initial nitrogen content: 1848 (shoots) and 1867 (roots) mmol/kg DM.

	21 days after transfer		42 days after transfer	
	shoots	roots	shoots	roots
NO_3	3927	2907	3668	3824
N_2	4051	3065	3593	2838

Table 6. Nitrate absorption (NO_3) and dinitrogen fixation (N_2) (mmol N / 100 plants) by pea plants during 21 and 42 days.

	Days after transfer	
	21	42
NO_3	249	736
N_2	198	653

Discussion

To obtain comparable results with respect to the influence of the nitrogen source on net proton or hydroxyl/bicarbonate extrusion by the roots it is desirable that the environmental conditions are chosen in such a way that both dry matter production and relative mass increment rate are in the same order of magnitude.

Maintaining the ambient temperature at 13 °C throughout, in combination with the prevailing lighting conditions (16 h day^{-1}; 14.5 J cm^{-2}h^{-1}), yielded morphologically comparable plants in both treatments. Over the experimental period no colour differences were observed, indicating that the dinitrogen-fixing plants did not suffer from possible limitations in nitrogen supply. Since dry matter production of nitrate-supplied plants was less than 20 % higher than that of dinitrogen-fixing plants the conclusion is justified that for comparative purposes temperature and illumination were 'in balance'. This conclusion is supported by data obtained from experiments at higher temperature (van Beusichem, 1982).

As is shown in Fig. 3, nutrient absorption characteristics, expressed as excess cation over anion absorption, and dry matter production of the dinitrogen-fixing plants were exponential over the 6-week period. On a relative scale, the dry matter production rate was somewhat lower than the alkaline uptake rate, a commonly observed phenomenon when dry matter production and nutrient absorption are compared. The same picture was observed in the nitrate treatment.

The nitrogen source had no significant influence on the total nitrogen content in the plants (Table 5). This implies that under the earlier described experimental conditions the dinitrogen-fixing process was not rate-limiting for optimal growth. It should be pointed out, however, that nutrient absorption and dinitrogen fixation were studied on plants which were already provided with an effectively nodulated root system. Differences between the treatments, associated with nodule initiation and development, were thus eliminated.

Nutrient absorption data and results from the pH-stat titrations clearly indicate that dinitrogen-fixing plants showed an alkaline nutrient uptake pattern (Fig. 2, Table 3). It is interesting to speculate on some physiological as well as agronomic aspects connected with this phenomenon.
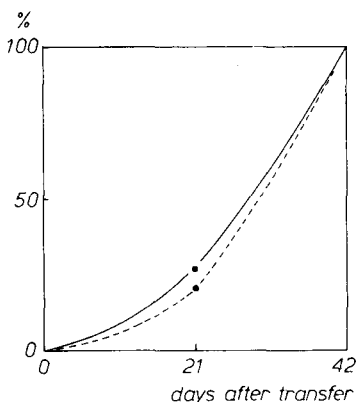
Fig. 3. Relative dry matter production (●) and net proton production (■) by dinitrogen-fixing pea plants.

In most cases, including this experiment, nitrate nutrition leads to an acidic nutrient uptake pattern by the roots, i.e. an excess anion over cation absorption. In apparent contrast, some authors reported that in nitrate-supplied plants inorganic cations in the xylem sap were almost completely balanced by inorganic anions. This phenomenon was found in a diversity of plant species, such as maize (Dijkshoorn, 1971), tomato (Wallace et al., 1971), dwarf bean (Breteler & Hänisch ten Cate, 1978), and castor oil plants (Kirkby & Armstrong, 1980). Equivalent xylem transport of inorganic cations and anions only occurs under conditions of adequate nitrate supply, so that nitrate deposition in the xylem is substantial. This implies that nitrate reductase activity must at least partly be located in the upper plant parts. Possible charge imbalances can be eliminated by a xylem-phloem recirculation of cations as proposed in the Dijkshoorn-Ben Zioni model (Dijkshoorn, 1958; Dijkshoorn et al., 1968; Ben Zioni et al., 1971; Kirkby, 1974). In our preliminary experiments, it was found that nitrate-supplied pea plants contained equal amounts of inorganic cations and inorganic anions in the bleeding sap (van Beusichem, to be published). This implies that the above-mentioned conditions are satisfied, although it is known that *Pisum* reduces substantial amounts of nitrate in the roots (Pate, 1973), indicating that the extent of cation recirculation in the pea plant is probably low.

To maintain electrically neutral longitudinal ion transport, in dinitrogen-fixing plants quite other processes must be operative than in nitrate-supplied plants. The excess of cationic over anionic nutrient uptake by dinitrogen-fixing plants (Table 3) and the small differences in total cation contents in the roots between both treatments (Table 2) suggest the necessity of organic anion synthesis in the roots and deposition of these compounds in the xylem. This could have an impact on the xylem loading rate of cations as a result of complexing ca-

pabilities of carboxylates and amino acids and their restricted radial movement in the root. The smaller cation accumulation in the upper parts of dinitrogen-fixing plants in comparison with nitrate-supplied plants (Table 3) is possibly a reflection of these processes. Careful examination of the organic chemical composition of bleeding saps of dinitrogen-fixing plants is necessary to get an insight into the dynamics of these uptake and transport phenomena.

Besides these physiological aspects, nutrient uptake patterns and acidity generation by dinitrogen-fixing legumes may have some agronomic significance. As a result of the proton extrusion pump operation, acidification of the rhizosphere can occur under field conditions. Soil rhizosphere acidity is influenced by operation of uptake processes. Variation in acidity of the rhizosphere through ammonium or nitrate nutrition has been shown to influence the solubility of soil phosphorus and thereby causing substantial modification in the amounts of phosphorus absorbed (Blair et al., 1971; Riley & Barber, 1971; Smiley, 1974; Soon & Miller, 1977).

The question arises which consequences rhizosphere acidity generation by dinitrogen-fixing legumes would have for the improvement of the efficiency of naturally occurring or added (rock) phosphates. An important difference between ammonium nutrition and dinitrogen fixation is the amount of acidity excreted by the roots per equivalent of nitrogen absorbed. For ammonium nutrition this value appears to vary between 1.10 and 1.25 for different plant species, such as sugar-beet (Breteler, 1973a), maize (van Beusichem & van Loon, 1978), and tomato (Kirkby & Mengel, 1967). In this experiment the amount of acidity excreted per unit nitrogen fixed was only 0.2 on an equivalence basis. When adequate nitrogen supply is sustained in leguminous plants, great deviations from this value are not likely to occur, so that no drastic effect of dinitrogen-fixing plants on acidification of the soil rhizosphere is to be expected.

In most of the tropical legumes, rhizosphere acidity generation appears to be lower than in temperate species (Andrew & Johnson, 1976). Moreover, in tropical and subtropical regions acidic ultisols and oxisols are predominant. An important aspect in this connection is the finding that the extent of acidity generation is highly pH-dependent (van Beusichem, 1982).

From these considerations the conclusion seems to be justified that it is necessary not to be too optimistic about the agronomic significance of the alkaline nutrient uptake pattern of legumes with respect to phosphate utilization. Probably positive effects are restricted to those cases in which legumes, provided with an intensive soil-exploring root system, are grown on non-acidic soils with very low pH-buffering and phospate-fixing capacities (Aguilar S. & van Diest, 1981). Data reported by Fried (1953), who found that the abilities to take up phosphorus, supplied as rock phosphate, were favoured by leguminous plants in comparison

with gramineous plants, should be considered as the result of a combination of the above-mentioned prerequisites.

Acknowledgements

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