ROCK-PHOSPHATE MOBILIZATION INDUCED BY THE ALKALINE UPTAKE PATTERN OF LEGUMES UTILIZING SYMBIOTICALLY FIXED NITROGEN

Promotor: dr.ir. A. van Diest, hoogleraar in de bodemvruchtbaarheid en de bemestingsleer

a. aguilar santelises

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Proefschrift
ter verkrijging van de graad van
doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op vrijdag 19 juni 1981
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen

PROPOSITIONS

1. The value of 0.1 to 0.2 H^+ excreted per N assimilated by plants utilizing symbiotically fixed nitrogen, as estimated by Raven and Smith, was arrived at without due consideration given to the ionic balance of nutrients absorbed by plants and, consequently, is too low.

   Raven, J.A. and F.A. Smith, 1976. Nitrogen assimilation and transport in vascular land plants in relation to intracellular pH regulation. *New Phytol 76:* 415-431.

2. Differences in the ionic uptake patterns among plant species form one of the most effective means of accounting for the differential "feeding power of plants for phosphates".

   Fried, M., 1953. The feeding power of plants for phosphates. *Soil Sci. Soc. Am. Proc. 17:* 357-359.
   Raij, B. van and A. van Diest, 1979. Utilization of phosphate from different sources by six plant species. *Plant Soil 51:* 577-589.

3. Absence of information on whether or not symbiotic nitrogen fixation was involved invalidates much of the work done to evaluate the feeding powers of legumes with regard to phosphate.

4. A research program on the ionic balance of many crop species, all grown on a range of soils differing in chemical characteristics, would yield a great deal of information needed to raise crop productivity and to promote a judicious use of fertilizers.

5. The presence of carboxylates in the rhizosphere of plant roots is often too easily interpreted as an indication of the excretion of organic acids by these roots.

6. Phosphorus toxicity in legumes occurs more frequently than is presently recognized.

7. When advocating the growing of leguminous crops in the Netherlands, agronomists should pay due attention to the ability of these crops to utilize P supplied in the form of relatively cheap rock phosphate.

8. In the curriculum of the specialization Soil Fertility of the M. Sc.-Course on Soil Science and Water Management (Agricultural University, Wageningen), more attention should be given to Plant Physiology and Soil Microbiology.

9. The Dutch word *gezellig* is not easily translated into other languages but its meaning becomes clear when one has the opportunity to live among the Dutch.

Proefschrift van A. Aguilar Santelises
Rock-phosphate mobilization induced by the alkaline uptake
pattern of legumes utilizing symbiotically fixed nitrogen.
Wageningen, 19 juni 1981.

WOORD VOORAF

Dit proefschrift is het resultaat van een 3-jarig gastmedewerkerschap bij de Vakgroep Bodemkunde en Bemestingsleer.

Met grote voldoening zal ik, zoals te doen gebruikelijk, beginnen met het uitspreken van dankbetuigingen aan degenen, die hebben bijgedragen tot het totstandkomen van dit werk.

Hooggeleerde Van Diest, U ben ik bijzonder veel dank verschuldigd. U was mijn leraar in het vak Soil Fertility van de M. Sc.- Course on Soil Science and Water Management en U begeleidde mijn werk verband houdende met de thesis van het hoofdvak van deze opleiding. Met enthousiasme heeft U mij ook gedurende de laatste drie jaren begeleid en geadviseerd zowel bij de uitvoering van het onderzoek als bij het schrijven van dit proefschrift. Ik beschouw U als een vriend en voorbeeld.

Vervolgens wil ik al degenen dankzeggen die mij met hun welgemeende commentaren en raadgevingen hebben bijgestaan.

- Dr. Lie van de Vakgroep Microbiologie voor de raadgevingen op het gebied van symbiotische stikstofbinding.
- Dr. Limonard van de Vakgroep Fytopathologie voor de adviezen in verband met het werken met mycorrhiza.
- Dr. Keltjens voor de hulp en het toezicht in het isotopenlaboratorium.
- Ir. Verdooren van de Vakgroep Wiskunde voor de waardevolle raadgevingen ten aanzien van de statistische aspecten van het onderzoek.
- Wim Menkveld en de heren Van Aggelen, Den Hartog en Jacobs van de "boerderij" voor de helpende hand geboden bij het gereedmaken en het verzorgen van de potproeven.
- De heer Houwers voor zijn hulp bij de acetyleen-reductietechniek en voor het veelvuldig verstrekken van het rhizobium inoculum.
- Rob van der Vossen voor het altijd weer beschikbaar stellen van het inoculum voor mycorrhiza.
- Ada Hoogendijk en haar medewerkers voor de chemische analyse van het gewasmateriaal.

- Arie van de Berg en Egbert Nab voor het analyseren van enkele grondmonsters.
- Piet Jansen voor de verzorging van het fotografische werk.
- De heer Matser en in het bijzonder Karen, Marian en Kitty voor de belangstelling die zij steeds getoond hebben zowel voor het werk als ook voor zaken in de persoonlijke sfeer.
- De doctoraalstudenten Bert de Boer, Lex Bouwman, Pieter Elzinga, Henk Haitsma, Tsjerk Hoekstra, Jan de Koning, Kees van Maaswaal, Ruud Paquay, Vincent Vinkenvleugel en Frank Wijnands voor het uitvoeren en verwerken van proeven die ten dele onderdeel zijn geworden van dit proefschrift. Ik heb jullie met veel genoegen begeleid en heb het bijzonder op prijs gesteld met jullie te mogen werken. Het is jammer dat een groot deel van de resultaten van jullie onderzoekingen niet in dit manuscript konden worden opgenomen door tijdgebrek.
- Voorts ben ik zeer erkentelijk voor de hulp van nog velen, medewerkers en personeel van de Vakgroep Bodemkunde en Bemestingsleer, die hier niet met name zijn genoemd.
- Jan Vermeer voor zijn daadwerkelijke hulp.
- Ina voor de verzorging van het typewerk en de tekeningen. Zonder jou zou de uitvoering van het onderzoek en het schrijven van dit proefschrift nauwelijks mogelijk zijn geweest.

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1 INTRODUCTION

The validity of the old concept that plant roots generally acidify their rhizosphere soil and thereby render soil nutrients more available for plant uptake, has been put to serious questioning in the past few years. Results of many investigations have demonstrated that, depending on the circumstances, plant roots may exert an increasing, a decreasing, or no effect on the pH of the soil in which their root systems develop. The form in which nitrogen enters the plant (NO_3^-, NH_4^+, or N_2) has been shown to exert an important influence on the type of effect to be expected. It has further been observed that pH changes in the rhizosphere strongly influence many processes important for the nutritional status and the health of a plant. In table 1, some examples of pertaining publications are listed.

Table 1 Publications on the effects of rhizosphere pH on various processes.

1.	P UPTAKE FROM SUPERPHOSPHATE BY VARIOUS PLANT SPECIES:	(Miller et al., 1970) (Riley and Barber, 1971)
2.	Fe UPTAKE BY SUNFLOWER:	(Venkat Raju et al., 1972)
	Fe UPTAKE BY SEVERAL PLANT SPECIES:	(van Egmond and Aktaş, 1977) (Aktaş and van Egmond, 1979)
3.	Al TOXICITY IN WHEAT AND BARLEY:	(Foy et al., 1965)
	Al TOXICITY IN LEGUMES:	(Helyar, 1978)
4.	NODULATION OF LEGUMES:	(Munns, 1978)
5.	CONTROL OF TAKE-ALL IN WHEAT:	(Smiley, 1979)
6.	SOIL pH	(Pierre and Banwart, 1973) (Nyatsanga and Pierre, 1973)

Under aerobic field conditions, plants absorb most of their nitrogen as nitrate. If this nitrate is formed in the soil as a result of nitrification of ammonium, its formation must lead to some soil acidification. In the traditional concept of plant nutrition, it was believed that the uptake of nutrients by plants gives rise to a further acidification of the soil. Presently, however, it is realized that the influence which a plant exerts on the pH of the soil, depends on the type of plant and on the form in which nitrogen is absorbed.

The large group of monocotyledonous plants appears to have a pH-raising effect on the soil, which effect is brought about by an uptake pattern in which, on an equivalence basis[*], more anionic than cationic nutrients are absorbed. How in such a situation the plant maintains electroneutrality inside its tissues will be explained in the next chapter.

In the group of dicotyledonous plants, more diversity is to be found. For a number of species, the situation is similar to that encountered with the monocotyledonous plants: they absorb more anionic than cationic nutrients. For others, however, the uptake of anions is practically equivalent to that of cations. The family of the Chenopodiaceae is representative of this group. As a result, these species are known to exert no effect on the pH of their root environment.

Among the dicotyledonous plants, a few species exist which, even in the case of NO_3^- nutrition, absorb more cationic than anionic nutrients. These plants then comply with the traditional concept that plants exert an acidifying effect on the root environment. The group contains few cultural plants, but it is not unlikely that a sizeable number of wild dicotyledonous species belongs to it.

The distinction between these groups with regard to their effects on the pH disappears when N is absorbed as NH_4^+. In such instances, the uptake of cationic nutrients always exceeds that of anionic nutrients, resulting in an acidifying effect. In acid soils, the mineralization of soil organic N leads to the formation of ammonium which will not or only slowly be converted into nitrate. Consequently, when in acid soils plants absorb their N mainly as NH_4^+, they thereby contribute to a further acidification of these soils.

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[*] Henceforth, when quantities of cations and anions absorbed or extruded by plants are compared, such comparisons are made on an equivalence basis, also when no specific mention is made of the use of equivalents as a means of comparison.

The experiments described in this dissertation are directed toward an elucidation of the reasons behind the common experience that some plants, like buckwheat and certain legumes, are "good feeders" on phosphorus supplied as rock phosphate, and that other plants, like wheat, in this respect can be characterized as "poor feeders" (e.g. Dean and Fried, 1953). With regard to buckwheat and wheat, it must be emphasized that the former crop absorbs more cationic than anionic nutrients, even when N is absorbed as NO_3^- (van Raij and van Diest, 1979), thereby acidifying its root environment, whereas the latter crop absorbs more anionic than cationic nutrients and thus tends to have a pH-increasing effect on its root environment. It is obvious that the acidity generated by buckwheat promotes the solubilization of alkaline rock phosphates, and that the pH-raising effect of wheat impedes such a solubilization.

As dicotyledonous plants, the legumes in general tend to absorb more anionic than cationic nutrients. However, these legumes distinguish themselves from other plants in that their symbiosis with Rhizobium enables them to keep on growing and absorbing nutrients also when the soil fails to supply them with any nitrate or ammonium. In such a situation, they can be expected to absorb many more cationic than anionic nutrients and thus to cause an acidification of the soils in which their root systems develop. Nyatsanga and Pierre (1973) showed this to be the case for nodulated alfalfa, and Israel and Jackson (1978) for nodulated soybean.

The question then arises whether such an acidification will be large enough to exert a significant influence on the solubilization of alkaline rock phosphates applied as fertilizer in finely ground form. If such an influence is present, it would mean that for the production of legumes not only large investments in expensive nitrogen fertilizers can be omitted, but that also expenditures for P fertilizers can be confined to those needed for purchasing the relatively cheap rock phosphates.

2 LITERATURE

2.1 IONIC UPTAKE BY PLANTS AND ITS INFLUENCE ON THE pH OF THE RHIZOSPHERE

2.1.1 Introduction

The quantities of essential nutrients that are taken up by plants from the soil and their relationships with the various factors affecting plant growth have for many years been the subject matter of much research conducted by scientists of various disciplines.

Already in 1804, de Saussure found that the ash content of plants varied with the plant species, its age, the plant part and the soil on which the plant grew, and Liebig in 1852 advanced some ideas on the possibility to establish fertilizer recommendations on the basis of the amounts of nutrients extracted by crops from the soil. Hall (1905) elaborated on the concept of a "normal nutrient content" which in his opinion, would serve as a guide to the fertilization of field crops. However, it still took many years before the interrelationships between the different nutrients and particularly their possible significance in relation to yield and quality of crops began to receive due attention (e.g. Hoagland and Martin, 1923; Lundegårdh, 1935), mainly because of the complexity of the soil-plant relationships.

Of great importance was the work published by van Itallie (1938, 1948) on the mutual replacement of cations in plants grown on soils of different composition. He was the first scientist to study the equilibrium between the nutrients taken up by plants from the soil in relation to the quantities of the same nutrients and their equilibrium in the soil. In 1950 Bear reported that "the number of me of K + Ca + Mg + Na per unit weight of the top portions of alfalfa plants that had been grown under uniform environmental conditions tended to be a constant" and that "the me sums of the N, P, S, Cl and Si anions tended to be equally as constant as those of the cations, even though the me of the individual ions varied greatly" and an equation "to express the overall cation-anion equivalent relationship of plants at any given pH value" was presented (Bear, 1950):

$$\frac{K + Ca + Mg + Na}{N + P + S + Cl + Si} = \text{constant.}$$

Although the validity of this relationship has been questioned since (e.g. Dijkshoorn, 1958; Kirkby, 1969), the pioneering work of the above-mentioned workers was followed by a large number of investigations into the ionic balance of plants and its relationship with plant growth.

2.1.2 Processes and terminology

In order to emphasize some of the most important aspects of ionic uptake of nutrients and its influence on the pH of the rhizosphere, reference is made to the scheme proposed by Israel and Jackson (1978) to illustrate the interrelationships between some processes involved in inorganic ion accumulation by higher plants and the export of nutrients from their root systems (Fig. 1).

In studies of the ionic balance in plants, the total amount of cations taken up by a given plant is estimated as the sum of K^+, Ca^{2+}, Mg^{2+} and Na^+ and sometimes NH_4^+ (in the case of NH_4-fed plants). Other cations that may enter the plant tissues such as some of the so-called minor elements do not contribute significantly to the pool of ionic species with positive charge inside plants. With the exception of plants taking up all or a portion of their N in the form of NH_4^+, the sum of cations present within the plant (C) is equal to C_a, being the sum of nutritive cations absorbed, because the cations are not subjected to metabolic transformations in the plant.

The net quantity of nutritive anions entering the roots (A_a) comprises the ions NO_3^-, SO_4^{2-}, $H_2PO_4^-$, Cl^-. Silica is absorbed by many plants to a considerable extent but due to its highly polymerized state, its contribution to the ionic balance seems negligible (Dijkshoorn, 1958, 1962). Since a large proportion of NO_3^- and SO_4^{2-} is incorporated into organic compounds, A_a has a much higher numerical value than has A, being the sum of inorganic anions inside the plant.

According to Israel and Jackson (Fig. 1), the primary step in the overall ion uptake process is an electrogenic H^+ extrusion ① to the ambient medium. The existence of such a mechanism is widely accepted and, at present, demands the attention of many investigators (Pitman, 1970; Raven and Smith, 1974, 1976a, 1976b; Marrè, 1979; Spanswick et al., 1980). Cations enter the

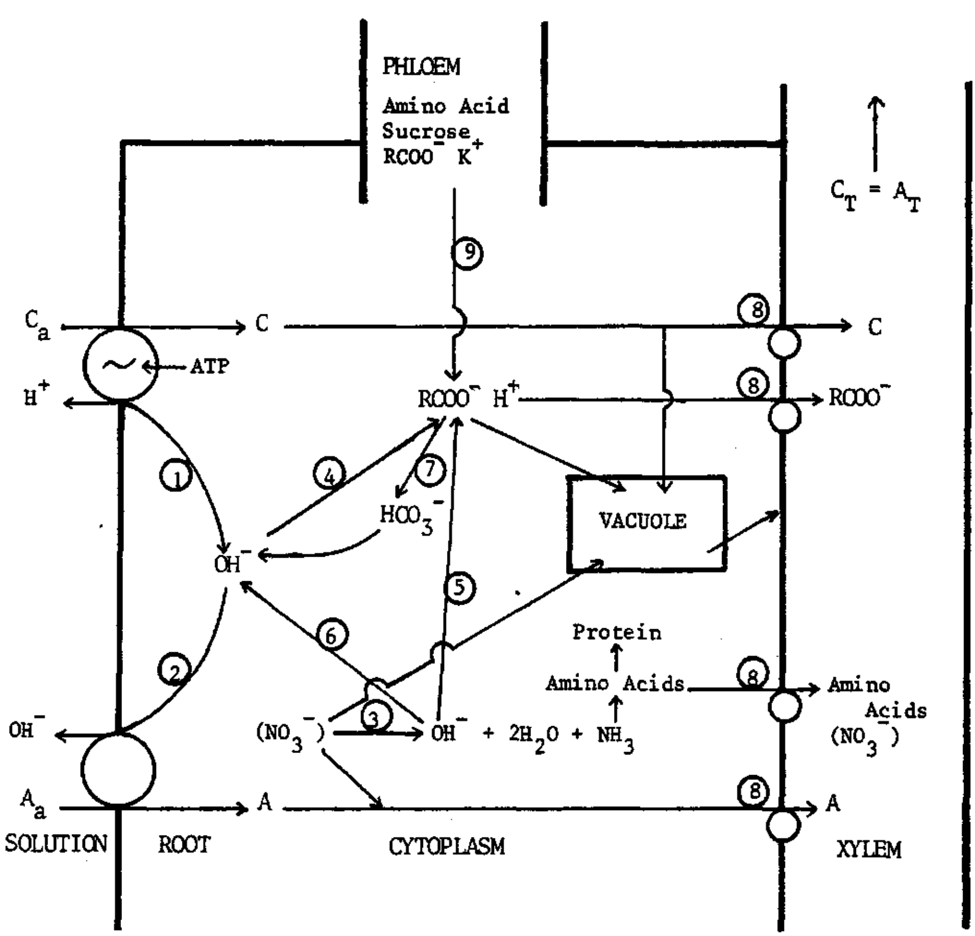


Fig. 1 Scheme of Israel and Jackson (1978) on processes involved in the uptake of ions by root systems of higher plants and their extrusion from these *root* systems. The large circles denote transport mechanisms for cations and anions between the soil solution and the cytoplasm and the small circles denote the export of products to the xylem. C_a and A_a stand for total absorption of inorganic cations and anions from the soil solution, C and A for inorganic cations and anions in the plant and C_T and A_T for total cations and anions in the xylem.

tissue in response to the resulting electrical potential gradient or possibly as an integral part of the pump operation. As a consequence of the H^+ extrusion process cytoplasmic OH^- is generated which may serve as counter-ion for the inward transport of ambient anions ② . Another source of cytoplasmic OH^- is the reduction of NO_3^- by the combined action of the enzymes nitrate reductase and nitrite reductase producing ammonia which is then incorporated into organic compounds ③ . A simplified representation of the last mentioned reactions is:

$$NO_3^- + 8(H) \rightarrow (NH_3) + 2H_2O + OH^-.$$

The process of NO_3^- reduction can occur in the roots, in the shoots or in both. Pate (1971, 1973), by means of analyses performed on bleeding sap collected from a range of herbaceous plants showed that the ability to reduce nitrate in the roots increases in the order of Xanthium, Stellaria Trifolium, Perilla, Avena, Zea, Impatiens, Heliantus, Hordeum, Phaseolus, Vicia, Pisum, Raphanus and Lupinus. Xanthium transports all its nitrate to the shoots while on the other hand Raphanus and Lupinus reduce practically all nitrate in the roots.

Whether nitrate reduction takes place in the root system or in the above-ground parts of plants has important implications for step ② , that is the disposal of OH^- to the external medium. OH^- originating in roots will be easily extruded, but OH^- from shoots will first have to be transported all through the plant toward the roots before it can be extruded. An explanatory mechanism has been proposed to account for this phenomenon and it will be discussed in the following section.

Ammonium present in plant tissues as a consequence of NO_3^- reduction or of the direct uptake of ammonium from the external medium is incorporated into organic compounds by the combined activities of glutamine synthetase and glutamate synthetase (Beevers, 1976):

$$NH_4^+ \rightleftarrows NH_3 + H^+$$

$$NH_3 + \text{glutamate} + \text{ATP} \rightarrow \text{glutamine} + \text{ADP} + P_i$$

$$\text{Glutamine} + \alpha\text{-ketoglutarate} + \text{NADPH}^+(\text{NADH}^+) \rightarrow 2\ \text{glutamate} + \text{NADP(NAD)}.$$

The process in which a part of the sulphate taken up by the plants is reduced is another source of OH^-. This source of cytoplasmic OH^- is quantitatively of less importance than is the NO_3^- reduction reaction. On an equivalence basis, the ratio of reduced SO_4^{2-} to reduced NO_3^- is about 0.054 : 1 (Dijkshoorn and van Wijk, 1967):

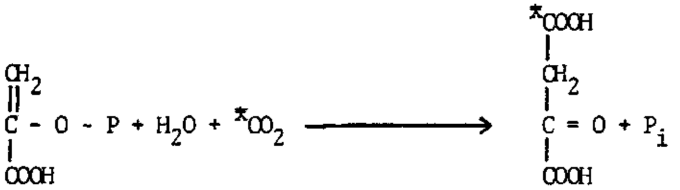
$$SO_4^{2-} + 8(H) \rightarrow (H_2S) + 2H_2O + 2OH^-$$

In the reduction reactions of SO_4^{2-} and NO_3^-, the symbols (NH_3) and (H_2S) stand for the many organic compounds in which N and S are present.

The OH^- released both during the operation of the H^+ extrusion pump and during the assimilation of NO_3^- and SO_4^{2-} favors the solvation of CO_2:

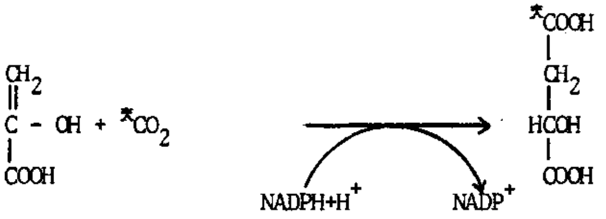
$$CO_2 + OH^- \rightarrow HCO_3^-$$

thus leading to an increase in the bicarbonate concentration in the cytoplasm. It has become well established (Bedri et al., 1960; Chouteau, 1963) that an increase in the bicarbonate concentration stimulates the synthesis of organic anions. Hence, it can be postulated that the negative charges of the nitrates and sulphates are transferred by way of OH^- and HCO_3^- to organic anions ④ , ⑤ . The synthesis of organic acids in plant tissues can be represented by:



$$CH_2{=}C(-O-P)-COOH + H_2O + {}^*CO_2 \longrightarrow {}^*COOH-CH_2-C(=O)-COOH + P_i$$

phosphoenolpyruvate oxalacetate



$$CH_2{=}C(-OH)-COOH + {}^*CO_2 \xrightarrow{NADPH+H^+ \;\rightarrow\; NADP^+} {}^*COOH-CH_2-HCOH-COOH$$

enolpyruvate malate

Carboxylates, inorganic cations and inorganic anions together with amino acids are deposited in the xylem ⑧ to participate in plant metabolism.

2.1.3 Nitrogen assimilation and ionic balance in plants

Among many others, the processes illustrated in Figure 1 take place in all plants and some of them have been known since long by botanists and plant physiologists (e.g. Pfeffer, 1881; Wehmer, 1891). However, their direct application to plant nutrition is comparatively new (van Tuil, 1965). The extent to which each one of these processes can take place depends on many factors inherent in the plant species, in the soil and in general in the environment in which the plant grows. However, two fundamental principles exist which limit the extent of these processes and partially govern their balance. These principles also provide the two sound tenets from which the study of the above-mentioned processes as well as their interrelationships can be approached.

At first, the electroneutrality of the plant as a whole must be maintained, which implies that all anion equivalents in the plant (or in the growth medium) are always balanced by stoichiometric amounts of cation equivalents, and *vice versa*. The fact that living cells have a negative potential does not constitute a serious violation of this assumption because at a difference in electrical potential of about 100-200 mV the surplus of anions in the cell is so small that it can hardly be measured by chemical methods (Mengel, 1974). Thus, the net quantities of cations and anions that plants absorb must be equivalent. However, the differential requirements of plants for nutrients often dictate patterns of non-equivalent uptake of cationic and anionic nutrients. In turn, these differential requirements arise from variations in the extent of internal metabolic processes and they manifest themselves in variations in the activities of uptake- and extrusion mechanisms operative in plant roots.

Electroneutrality is thus maintained by means of ion absorption from and ion extrusion to the rooting medium. In this context, next to the nutritive ions present in the soil, the ions H_3O^+, OH^- and HCO_3^- are also of importance and their supply is potentially unlimited as they are in equilibrium with water and with atmospheric CO_2:

$$2H_2O \rightleftarrows H_3O^+ + OH^-$$

$$CO_2 + 2H_2O \rightleftarrows H_3O^+ + HCO_3^-.$$

The second important governing agent is the internal pH of the plants, which must be maintained within narrow limits in the neighborhood of neutral reaction. Even though large acidity differences may be found in different parts of the cells, the overall reaction of plants remains always fairly neutral. This is a condition that requires the simultaneous participation of several pH-regulating mechanisms because the pH stresses originating from the metabolic reactions are so large that they would lead to enormous pH changes in the cytoplasm (Raven and Smith, 1973, 1974, 1976a).

Smith and Raven (1979) have discussed the pH-regulation mechanisms in plant cells. At first, mention should be made of some buffers present in the cell cytoplasm such as bicarbonate ($pK_a \approx 6.38$), phosphatic compounds ($pK_a \approx 6.8 - 7.0$), and others. These authors calculated that the cytoplasmic buffer capacity in the range of pH 6.0 - 8.0 is at most 20 mmol H^+ 1^{-1}(pH unit)$^{-1}$ and they suggested that this is effective in countering pH changes of 0.2 - 0.3 units for only a few minutes.

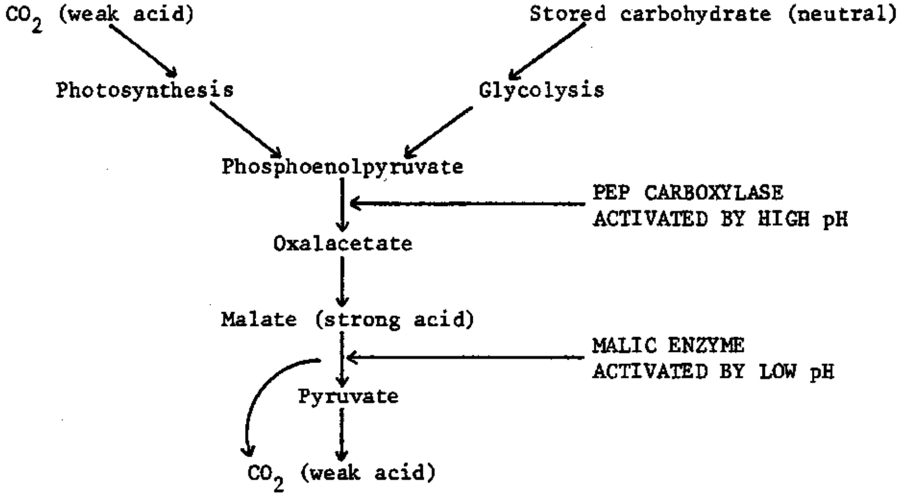


Fig. 2 The biochemical pH-stat (after Davies, 1973a, 1973b).

Another mechanism of pH-regulation is the biochemical pH stat proposed by Davies (1973a, 1973b) which is illustrated in Fig. 2. According to this scheme, excess OH^- production (or H^+ consumption) increases the cytoplasmic pH sufficiently to increase the activity of the phosphoenolpyruvate (PEP) carboxylase, thus leading to the formation and dissociation of a strong acid (malic acid). Conversely, H^+ production (or OH^- consumption) lowers the cytoplasmic pH inhibiting malate formation and activating malic enzyme so that pyruvate, CO_2 and OH^- are formed. This mechanism permits the control of H^+ production to only a limited extent since the malate ion needed to neutralize the H^+ requires the previous synthesis of malic acid accompanied by H^+-cation exchange. In contrast, carboxylation is of widespread importance in countering OH^- production by cells.

In spite of the existence of the above-mentioned mechanisms for pH-regulation, in a large number of plant species the transport of H^+ and/or OH^- through cell membranes toward the soil solution represents the only possible mechanism for pH-regulation, with the other mechanisms (except carboxylation) being helpful only in countering small pH stresses. In relation to ionic uptake, the most important reason for the generation of acid or base in plant tissues is associated with the form in which N is assimilated by plants. Figure 3 shows a scheme by Raven and Smith (1976a) in which the quantities of H^+ or OH^- produced per unit of N (NO_3^-, NH_4^+ or $\frac{1}{2}$ N_2) assimilated are given.

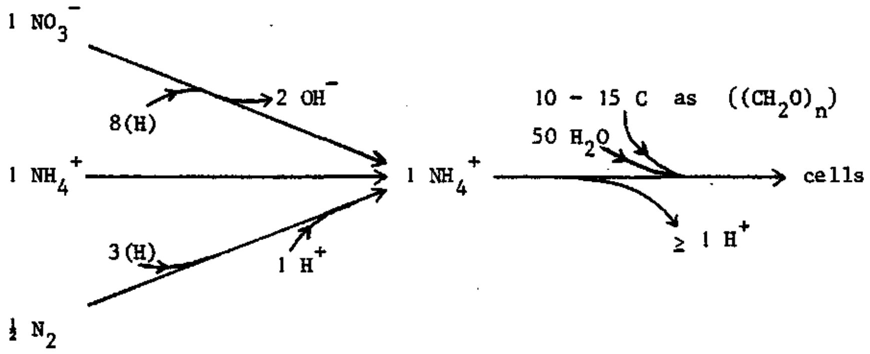


Fig. 3 Schematic illustration of the quantity of H^+ (or OH^-) produced by plant cells per N assimilated. After Raven and Smith (1976a).

2.1.3.1. Ammonium assimilation

Under aerobic conditions, plants growing on soils absorb most of their N in the form of NO_3^- (Walker, 1960). However, sometimes plants develop under circumstances in which nitrogen is not absorbed in the nitrate but in the ammonium form. The best known example is rice growing under flooded conditions (e.g. Koop and van Diest, 1971; Yoneyama, 1977).

There is good evidence that the major site of ammonium assimilation in land plants is in the roots and that N is transported through the xylem to the shoots as a mixture of amino acids and their amides (Yoneyama and Kumazawa, 1974). If there is to be no excess production of H^+ during subsequent metabolic transformations involved in the production of other materials in the shoot from photosynthetic products and the translocated N compounds, these compounds must carry a net negative charge balanced by inorganic cations other than H^+. This then requires the extrusion of H^+ ions to the rooting medium and the assimilation of other nutritive cations instead. This reasoning is supported by experimental evidence showing that 1.1 - 1.25 H^+ is extruded per NH_4^+ absorbed (Becking, 1956; Breteler, 1973). A more complex mechanism arises when due consideration is given to the fact that some ammonium is transported to the shoots and that the nature of the N-compounds found in xylem sap may be basic or acidic. This phenomenon would cause a net production of H^+ or OH^- in the shoots which has to be neutralized by the biochemical pH stat (carboxylation or decarboxylation) possibly associated with vacuolar storage of excess acid or base and transport of malic acid to the roots.

On the other hand, when NH_4^+ assimilation is considered in relation with the required electroneutrality it becomes clear that NH_4-fed plants will absorb more nutritive cations (NH_4^+, Na^+, K^+, Ca^{2+},Mg^{2+}) than nutritive anions. The difference will be accounted for by the extrusion of H^+:

$$C_a - A_a = H^+ \text{ extrusion}$$

or

$$(NH_4^+, Na^+, K^+, Ca^{2+}, Mg^{2+}) - (H_2PO_4^-, SO_4^{2-}, Cl^-) = H^+ \text{ extrusion.}$$

These relationships have proved to be useful when H^+ extrusion from NH_4-fed plants was estimated from the ionic composition of the plants (Breteler, 1973). In NH_4-fed plants, owing to the assimilation of NH_4^+ accompanied by

H^+ extrusion, C is much smaller than C_a, whereas A does not differ much from A_a. The quantities of organic anions are small because of a lack of HCO_3^- needed for carboxylation.

2.1.3.2. Nitrate assimilation

A more normal situation is found when plants absorb their nitrogen in the form of NO_3^-. In this case they take up more nutritive anions A_a (NO_3^-, $H_2PO_4^-$, SO_4^{2-}, Cl^-) than nutritive cations C_a (Na^+, K^+, Ca^{2+}, Mg^{2+}). The pH-regulation mechanisms must cope with a large production of OH^- associated with the assimilation of NO_3^-. Such a regulation may occur mainly through the formation of carboxylates which remain associated with cations (other than H^+) in the cells, and through the extrusion of OH^- to the soil solution. There is evidence that plants reducing most of their NO_3^- in the roots tend to export the excess OH^- to the external solution and thus produce relatively few organic acids whereas plants that reduce most of their NO_3^- in the shoots tend to accumulate carboxylates as soluble or insoluble salts (e.g. calcium oxalate).

The relationship between organic anion content (carboxylates) and the organic nitrogen contents of the shoots of NO_3-fed plants has been studied in a number of species. Organic anions in quantities equivalent to about 50% of the organic N are found in Lolium and Zea (Dijkshoorn, 1962, 1971), 60 - 80% in Hordeum and Sorghum (Watanabe et al., 1971), up to 90% in Nicotiana (Vickery et al., 1940), Lycopersicon (Kirkby and Mengel, 1967) and Beta (Houba et al., 1971; Breteler, 1973), 100% in Atriplex (Osmond, 1967) and more than 100% in Fagopyrum and Gossypium (Bornkamm, 1969; Watanabe et al., 1971). These values, therefore, reflect the amounts of OH^- neutralized through the formation of carboxylates, the remainder being excreted to the rooting medium.

The scheme of figure 1 includes the possibility that carboxylates (malate) generated in the leaves may be transported to the roots via the phloem ⑨ . Decarboxylation of these carboxylates in the roots gives rise to OH^- which in turn may serve to sustain anion uptake in excess of cation uptake (Dijkshoorn et al., 1968; Ben-Zioni et al., 1970, 1971; Lips et al., 1971) ⑦.

From the above, it is clear that most NO_3-fed plants neutralize the OH^- ions originating from NO_3-assimilation partly by means of an accumulation of carboxylates in their tissues and partly by transport of OH^- ions to the rhizosphere. From the available data, Raven and Smith (1976a) conclude that the OH^- production in NO_3-fed plants can be 0.05-0.9 equivalents per NO_3^- assimilated. However, this range does not take into consideration the data for Fagopyrum and Gossypium according to which the quantities of carboxylate equivalents accumulated are larger than those of organic N present in the plant. This is confirmed by the results of Pierre et al. (1970) who found a carboxylate/organic N ratio for buckwheat larger than 1 and an acidification of the soil on which the plants grew. Similar results had already been obtained by de Wit et al. (1963) working with nutrient solutions.

Approaching the ionic balance of NO_3-fed plants from the standpoint that electroneutrality is required, we arrive at the following relationships:

a) carboxylates = C - A ≈ organic-N

$C_a \approx A_a$

for species, such as Lycopersicon, Beta, Atriplex which accumulate carboxylates in amounts almost equivalent to those of reduced NO_3^-.

b) carboxylates = C - A < organic-N

$C_a \approx A_a - OH^-$ extrusion

for plants, such as Lolium and Zea, whose carboxylate accumulation falls short of the quantity of nitrate reduced.

c) carboxylates = C - A > organic-N

$C_a \approx A_a + H^+$ extrusion

for plants, such as Fagopyrum and Gossypium, that tend to accumulate carboxylates in quantities exceeding those of reduced NO_3^-.

2.1.3.3. Symbiotic nitrogen fixation

There are very few reports in the literature concerning studies on aspects of pH-regulation and ionic balance in plants depending on N_2 fixation. It is well known, however, that by the action of nitrogenase N_2 is reduced to ammonium:

$$N_2 + 12ATP + 6e^- + 8H^+ \rightarrow 2NH_4^+ + 12ADP + 12P_i$$

and that ammonium is immediately transformed into organic-N compounds, such as amides of dicarboxylic acids (Pate and Wallace, 1964; Pate et al., 1969; Gunning et al., 1974). Recent studies have indicated that the N products exported from the root nodule are species-specific (Pate, 1980). Ureides (allantoin and allantoic acid) are the main compounds in certain tropical legumes and asparagine and glutamine are the translocated solutes in many temperate legumes.

In their review on nitrogen assimilation in terrestrial vascular plants in relation to intracellular pH-regulation, Raven and Smith, pointing at the similarities between the xylem transport of products of symbiotic N_2 fixation and the xylem transport of products of ammonium and nitrate assimilation in the root system, advanced the idea that the pH stress involved in N_2 fixation amounts to about 0.1 - 0.2 H^+ per N assimilated (Fig. 3). However, no experimental evidence supports this statement.

Indirect evidence exists that vascular plants depending for their N on symbiotic N_2 fixation, acidify their growth medium (Bond, 1950; Bond and Mackintosh, 1975; Ahmed and Evans, 1960).

However, studies related to ionic balance in legumes and its effect on the pH of the rhizosphere are almost nonexistent. In this realm Nyatsanga and Pierre (1973) and Israel and Jackson (1978) have detected an alkaline uptake pattern in legumes, i.e. more nutritive cations taken up than nutritive anions. The difference will have to be balanced by the extrusion of acidity to the root environment

$$C_a - A_a = H^+ \text{ extrusion.}$$

From this difference, Nyatsanga and Pierre (1973) deduced that 0.8 me of acid per gram total dry plant weight was released to the soil and this acidity was sufficient to lower the pH of the loamy soil in which the plants grew, by 1 unit. By means of the same method of calculation, Israel and Jackson (1978) found that nodulated soybean plants, growing in a nitrogen-free medium, released 1.08 me of acid per gram total dry plant weight. Recent work by van Beusichem (1981) with peas grown hydroponically confirms an alkaline uptake pattern in nodulated peas and shows an excellent agreement between the calculated H^+ production ($C_a - A_a$) and the production recorded in the culture solution by means of a pH stat.

2.1.4 Rhizosphere pH

Any study of the rhizosphere is faced with a great deal of technical difficulties. It is, therefore, not surprising that progress in our knowledge on physical, chemical and biological processes in the rhizosphere has been slow.

Hiltner (1904) was the first to use the term rhizosphere for the zone of soil in which the microflora is influenced by the presence of a root system. In later years, many workers have advanced various other definitions of the term rhizosphere and have proposed subdivisions of the zone influenced by plant roots (Rovira and Davey, 1974). For instance, Riley and Barber (1969, 1970, 1971) distinguished between the following segments: rhizosphere soil, being soil that adheres loosely to the roots after removal of the bulk soil, rhizocylinder, consisting of roots and strongly adhering soil, and rhizoplane soil, being the soil in the rhizocylinder. In practice, the definition of the term rhizosphere is dependent upon the experimental technique employed to study it.

With regard to research on the pH of the rhizosphere, various methods have been used. Foy et al. (1965) measured the pH in aqueous suspensions of roots plus adhering soil. Goss and Gould (1967) determined the pH of soil sampled from the root zone of densely planted turf grasses, and Riley and Barber (1969) collected soil peds located near the roots. Papavizas and Davey (1961) constructed a device for removing concentric cylinders of soil from the zone around Lupinus roots. Boero and Thien (1979) devised a method to confine the entire root system of a plant to a small volume of soil thus creating a "macrorhizosphere"in which five concentric zones could be distinguished. In a more elaborate technique, Farr et al. (1969) and Bagshaw et al. (1972) made use of a cryostatic microtome and a pH microelectrode to determine pH profiles of the rhizosphere consisting of values acquired from soil samples taken at 0.5-mm intervals between 0.5 a 6.0 mm from the root surface.

The results obtained by the above-mentioned workers made it clear that the old concept that plant roots always acidify their root zones as a consequence of CO_2 evolution (e.g. Jenny and Overstreet, 1939) had to be abolished. Although this concept had found wide acceptance over many decades, already in 1928 Metzger reported that he had found higher concentrations of HCO_3^- in soil samples from near the roots than in those taken at a distance from the roots. He, however, attributed the HCO_3^- accumulation to CO_2 respiration. On the basis of data from Cunningham (1964) and Parker (1924), Nye and Tinker (1977) advanced the thought that HCO_3^- excreted by roots of many plants has a pH-raising effect in many cases large enough to overcome the acidification caused by CO_2 evolution from the roots.

These results of pH measurements in the rhizosphere appear to agree rather well with the concepts of ionic balance in plants as discussed in the previous sections. For example, Riley and Barber (1969) showed with NO_3-fed soybean plants that a situation of excess anion uptake and ensuing HCO_3^- release can lead to a considerable pH increase not only in nutrient solutions but also in the rhizosphere of plants grown on soil. Later on, the same authors (Riley and Barber, 1971) made it clear that under conditions of NH_4^+nutrition, on an equivalence basis, soybean absorbs more cationic than anionic nutrients thus giving rise to a pH decrease in the rhizosphere soil. Smiley (1974) found differences in rhizosphere pH of up to 2.2 units for NH_4-vs. NO_3-fed wheat plants grown on different soils. The same author also reported that all 8 plant species included in his study considerably reduced their rhizosphere pH when grown with NH_4^+ as N source, but that the same plant species, when supplied with NO_3-N, brought about very different increases in rhizosphere pH. He concluded that the principal cause of this differential behavior was the assimilation of NO_3^- taking place in either the roots or the shoots.

All reports mentioned in this section deal with NO_3- or NH_4-fed plants. No information is to be found in the literature on pH measurements made in the rhizospheres of legumes symbiotically associated with Rhizobium. Owing to the experimental technique used, the investigations conducted by Nyatsanga and Pierre (1973) can be regarded as coming closest to this subject. These authors found a decrease in pH of 1 unit in soil in which nodulated alfalfa plants had grown and they related this finding to the alkaline uptake pattern as verified by means of chemical analysis of the plant material obtained.

2.2 PHOSPHORUS UPTAKE BY PLANTS

2.2.1 Introduction

In this section, attention will be given mainly to published information on the utilization of rock-phosphate P by different plant species as affected directly by the plant itself, or indirectly through chemical or biological processes taking place in the root-soil interface, resulting from the presence of a root system. As early as 1898, Merrill reported that the effect of rock phosphates on the growth of plants varied among plant species. This finding, together with the observations of practical agriculturists led to the concept of the so-called "feeding power of a plant species", implying that plants differ in their abilities to utilize native phosphate, or rock phosphates applied to soil as fertilizers. Reports concerning this concept were published by Truog (1916, 1922), Bauer (1921), Davies et al. (1923) and Thomas (1930).

In their review on soil-plant relationships in connection with the phosphorus nutrition of plants, Dean and Fried (1953) emphasized that, in general, the most efficient users of rock phosphate are lupines, buckwheat, sweet clover, mustard, swiss chard, vetch and to a lesser extent alfalfa, pea, rape and cabbage. The same authors mention cotton, cowpea, bur clover, rice, oats, barley, millet, rye and wheat as crops considered to be poor users of rock phosphate. Further research conducted with ^{45}Ca- and ^{32}P-labeled materials confirmed the evidence that plant species differ in their ability to utilize phosphate sources and that the pertaining differences among species are not due only to differences in size and extensiveness of root systems but also to specific species-source interactions. Additionally, Fried (1953) acknowledged that his experimental results did not reveal the nature of these interactions and he pointed to some possible explanations previously proposed by other researchers, such as a theory advanced by Truog (1916) who emphasized the relation between the calcium uptake by plants and the absorption of phosphate from basic calcium phosphates, and a theory suggesting the possible involvement of soil microorganisms (Gerretsen, 1948). Murdock and Seay (1955) confirmed that clover is a better feeder on rock-phosphate P than is wheat. They included in their P sources a mixture of rock phosphate and superphosphate and showed that a small quantity of superphosphate P added to a relatively large amount of rock-phosphate P increased the uptake of rock-phosphate P but that superphosphate P, when applied in a quantity equal to that of rock-phosphate P did not enhance the utilization of rock-phosphate P.

In more recent investigations, Ozanne et al. (1969) and Keay et al. (1970) found marked differences in the response of eight crop species to phosphate applied to a soil which was severely deficient in phosphate. They compared the rates of phosphate absorption by these species grown on soil with those obtained by Asher and Loneragan (1967) who determined rates of phosphate absorption from fast-flowing nutrient-culture solutions. Five species were common to both investigations: cape-weed (*Arctotheca calendula*), erodium (*Erodium botrys*), silver grass (*Vulpia myuros*), subterranean clover (*Trifolium subterraneum*) and West Australian blue lupine (*Lupinus digitalis*). It was observed that the rates of P absorption from soil were lower than those from solutions for all species, except lupine. In discussing their results, Keay et al. (1970) made mention of several physiological and anatomical characteristics, such as the distribution of phosphate between roots and tops, translocation of phosphate within the plant, surface area of root systems, without being able to attribute the observed differences to any single factor. They admitted that other factors, such as differences in root exudates or in microbial rhizosphere populations could also play a role in accounting for these distinct behavioral differences among species with respect to utilization of P-sources. On the basis of the results obtained in the above-mentioned investigation, it can be assumed that plants exert an influence on the phosphate present in the vicinity of their root systems. Also, from the many literature reviews that have been published on the direct use of rock phosphates as fertilizers, it is clear that soil scientists assume that crops differ in their abilities to utilize rock-phosphate P and native P present in low-phosphate soils. However, from the same reviews it becomes evident that the exact nature of this specific difference is one of the less understood plant characteristics governing uptake of P from rock phosphates (e.g. Cooke, 1956; Khasawneh and Doll, 1978).

Some of the theories which have been proposed to account for the differences in feeding power of plants with respect to phosphate will be discussed in the next sub-section. In general, the possibility that plant species show large variations in the ratio in which fertilizer-P and soil-P are utilized can probably be disregarded as several investigators, working with soils to which monocalcium phosphate labeled with ^{32}P had been added, have observed that the specific activity of the phosphorus in their species tended to be alike (Fried, 1953; Nye and Foster, 1958; Keay et al., 1970; Marais et al., 1970).

2.2.2 Theories on the differential P uptake by plant species

Black (1968) distinguishes 3 theories to account for the observed differences among plant species in ability to absorb phosphates: a. the *root-character theory*, b. the *phosphorus-requirement theory* and c. the *ionic-equilibrium theory*.

ad a. The *root-character theory* is based on differences among species with regard to phosphorus-absorption characteristics and extensiveness of root systems. The work of Noggle and Fried (1960) provides an example related to the P absorption characteristics of roots. In short-term nutrient-culture experiments they determined the uptake of ^{32}P-labeled orthophosphate by detached roots of millet, barley and alfalfa seedlings. Large differences in the P uptake per gram of roots were detected, but no relationship was found between the rates of P uptake and the surface areas of the root systems. Apparently, real differences existed in root-surface characteristics of the three plant species.

On the other hand, it appears self-evident that the extensiveness of the root system will affect the response of the plant to the supply of phosphorus. However, the results of experimental tests conducted to explain species differences on this basis are difficult to interpret, as root characteristics vary from one root section to another (Rovira and Bowen, 1970) and root surfaces are difficult to measure accurately (Pearson, 1974). Some workers (Khasawneh and Copeland, 1973; Newman and Andrews, 1973) emphasize the importance of root elongation as a factor in phosphorus nutrition, but each of these investigations was conducted with one test crop only, which makes it difficult to attach a general meaning to the results obtained.

ad b. The *phosphorus-requirement theory* is based on observed differences in the rate with which various crop species absorb phosphorus. For instance, in a review of work on the mechanisms of phosphate absorption by cells and plants, Jennings (1963) suggested that the distinct metabolic reactions leading to the incorporation of P into organic compounds could influence the rate of the P absorption process. Hence, the differences that species exhibit in their rate of metabolic reactions involving P could explain the different rates of phosphate absorption, independently of differences in the absorption mechanism itself.

According to Black (1968), the *phosphorus-requirement theory* is an expression of the common observation that slow-growing species low in phosphorus content are better adapted to grow under conditions of phosphorus deficiency than are fast-growing species high in content of phosphorus. For instance, Rorison (1969) determined the dry weights of several plant species grown on solution cultures varying in phosphate concentrations and observed that *Deschampsia flexuosa*, a slow-growing plant, could yield satisfactorily at a phosphate level too low for an optimum growth rate of the other species. Rorison attributed the ability of Deschampsia to grow at very low phosphate levels to its low relative growth rate and low phosphate demand.

In an attempt to account for the differential response of crop species to rock-phosphate applications, Khasawneh and Doll (1978) emphasized the importance of differences in relative growth rates and their effects on the quantities of both Ca and P required for normal growth. In accordance with this statement, to maintain a normal growth rate fast-growing plants are known to show a high rate of phosphate uptake. If a plant species has an extensive root system, the P demand per unit length of root will be lower than for a species having a root system of limited extensiveness. Again, for a given level of P-intake per unit root length, the concentration of P in solution needs to be above a certain critical value which is determined by the kinetics of P uptake for the species involved. If a rock phosphate cannot maintain such a concentration in solution, the rate of P absorption per unit of root will be insufficient and the relative growth rate will decline accordingly. Additionally, with regard to extensiveness of roots mention must be made of the fact that different species and sometimes different varieties within a species have different root systems and that the development of a root system is affected by many factors such as compactness of the soil, drainage, soil acidity, etc.

ad c. In their coverage of "the use of phosphate rock for direct application to soils", Khasawneh and Doll (1978) did not mention any direct effect roots may have on the solubility of soil and fertilizer phosphates. Nevertheless, much research work has been conducted on this subject. Black (1968) includes it in the so-called *ionic-equilibrium theory*. Evidence that plants actively influence the external ionic environment that modifies phosphorus solubility in soil is to be found in the classical experiments demonstrating the etching of polished marble in contact with roots, presumably as a result of the action of hydronium ions originating from the carbonic acid exuded by the roots. Further evidence was provided by Johnston and Olsen (1972) who observed a much larger phosphate uptake when roots of squash, barley and wheat were in direct contact with Virginia fluor-apatite than when they were confined to the supernatant liquid. These authors concluded that pH changes in the liquid phase of the growth medium, CO_2 excretion and exudation of chelates (except for squash) were not responsible for the observed increase in P uptake, but that the efficient feeders (squash and soybean) were most effective in removing calcium from the immediate environment of the solid rock phosphate with a concomitant shift in the mass-action equilibrium of the calcium phosphate compound. In other words, a decrease in Ca concentration (increase in pCa) will be accompanied by an increase in phosphate concentration (decrease in pPO_4). This is in accordance with Truog's original ideas on variations in "feeding power" (1916, 1922). However, Johnston and Olsen (1972) admitted that the shift in Ca concentration would be insufficient to account for a significant solubilization of the phosphate unless the mass-action effects were confined to the immediate environment of the rock. In previous work by Leggett et al. (1965) with detached barley roots and by Wild (1964) with plants grown on nutrient solutions, no effect of variations in Ca concentration upon the uptake of phosphorus was found.

A modification of Truog's theory is to be found in the work of Drake et al. (1951) and Drake and Steckel (1955) who emphasized the relationship between the differences in ability of plant species to utilize soil nutrients and the cation-exchange capacity of the roots. They postulated that calcium is inactivated on cation-exchange sites of the roots and that, hence, the availability of phosphorus present in phosphate rock should be greater for plants having roots with a high CEC-value. For example, Fox and Kacar (1964) observed that, when grown on a calcareous soil, sainfoin (*Onobrychis viciaefolia*) with a CEC_r-value of 77 me/100 g could make better use of applied phosphate than could wheat with a CEC_r-value of 9 me/100 g. However, most of the evidence supporting this theory was derived from CEC determinations performed on dead roots whose exchange capacities are known to differ from those obtained with living roots. Furthermore, as Fried and Broeshart (1967) pointed out, there appears to be little ground for an assumption that CEC-values of roots of a certain plant species bear a direct relationship with ion-uptake characteristics of that species.

Another way in which a plant may influence the availability of soil and fertilizer phosphates is through a secretion of organic materials by its roots (e.g. Kepert et al., 1979). Such secretions, if taking place, would directly modify the chemistry of the root environment, besides having an effect on the size and the activity of the microbial population utilizing the secreted materials as substrate. Yet, there is little certainty about the amounts of exuded substances, their composition or their effects. Rovira and Davey (1974) reviewed the literature on this matter and indicated that the amounts, the nature and the proportions of compounds in root exudates differ among plant species. Such a statement could be interpreted as lending support to the assumption that root exudates exert an important influence on the degree of availability of soil and fertilizer phosphates to a certain plant species, but in reality there appears to be little work published to support such a view. From published data, Nye and Tinker (1977) have attempted to arrive at some quantitative conclusions on the effect of exudates upon the uptake of nutrients but they did not succeed, mainly because of lack of published evidence:

The presence of an enriched microbial population in the rhizosphere is rather well documented (Rovira and Davey, 1974) and it has been demonstrated that many of those micro-organisms which are abundantly present in the rhizosphere produce organic compounds which could be active in the dissolution of rock phosphates (Dalton et al. 1952). There is, however, no evidence of a specific relationship between certain plants and certain micro-organisms generally present in rhizospheres (Tinker and Sanders, 1975).

Next to free-living bacteria and fungi in the rhizosphere, mention must be made of fungi associated with plants in the formation of a mycorrhiza. It is well known that the host plant and the fungus live together in an intimately balanced relationship and that generally the association is beneficial to both organisms (Gerdemann, 1974). An improved rock phosphate utilization by the infected plant has often been observed (Mosse et al., 1976; Powell and Daniel, 1978; Islam et al., 1980) and several authors have reported interactions with phosphate-solubilizing bacteria (Azcon et al., 1976; Bagyaraj and Menge, 1978) and in the case of legumes with Rhizobium bacteria (Bagyaraj et al., 1979; Barea et al.,1980). However, mycorrhizal plants take up more phosphorus only from the labile pool of soil phosphate and have no extra solubilizing effect on fertilizer phosphates (e.g. Owusu-Bennoah and Wild, 1980). Furthermore, no specificity has been proved in these associations (Kruckelmann, 1975; Mosse, 1975; Tinker, 1975) and no relationship could be ascertained between mycorrhizal infections and the differential ability of plants to take up phosphates.

2.2.3 The influence of nitrogen on P uptake

In general terms, nitrogen added to a soil is believed to enhance the uptake of phosphorus by plants. This phenomenon has been known for a few decades. In 1942, Scarseth et al. observed it when nitrogen fertilizer was applied as a side dressing to maize. In 1944, Breon et al. noted that P was taken up more rapidly by tomato plants supplied with urea-N than by those that had received NO_3-N. Presumably, the urea was largely hydrolyzed to NH_4^+, and the effect observed may have to be attributed to NH_4^+ ions. Lorenz and Johnson (1953) reported that the yield of potatoes that had received NO_3-N and phosphate fertilizer was equal to that of plants that had received $(NH_4)_2SO_4$ only. Volk (1944) and Jones (1948) reported that N fertilizers increased the availability of P supplied as rock phosphate to plants.

After having reviewed a large number of publications on this subject, Grunes (1959) concluded that indeed NH_4-N promotes P absorption more than does NO_3-N. In a discussion of this effect, Miller (1974) summarized the factors which have been shown to play a role. These factors are presented in the scheme of Fig. 4.

In subsection 2.2.2, mention was made of some processes and factors which exert influences on the uptake of P by plants, and some of these play a role in accounting for the differential feeding power of species and varieties with respect to rock phosphates.Several of these factors are to be found back in Miller's scheme (1974), but in this scheme relatively little attention is paid to the influence of rhizosphere pH changes, induced by unequal uptake of cationic and anionic nutrients, on the availability of P, as was demonstrated by various experimenters (Riley and Barber, 1971; Smiley, 1974; Mc Lachlan, 1976; Soon and Miller, 1977a). As was mentioned in section 2.1, the form in which N is utilized (NO_3^-, NH_4^+ or N_2) has a bearing on this pH change and consequently on P availability. Therefore, in the next subsections attention will be paid to the influences exerted by different forms of nitrogen on the uptake of phosphorus.

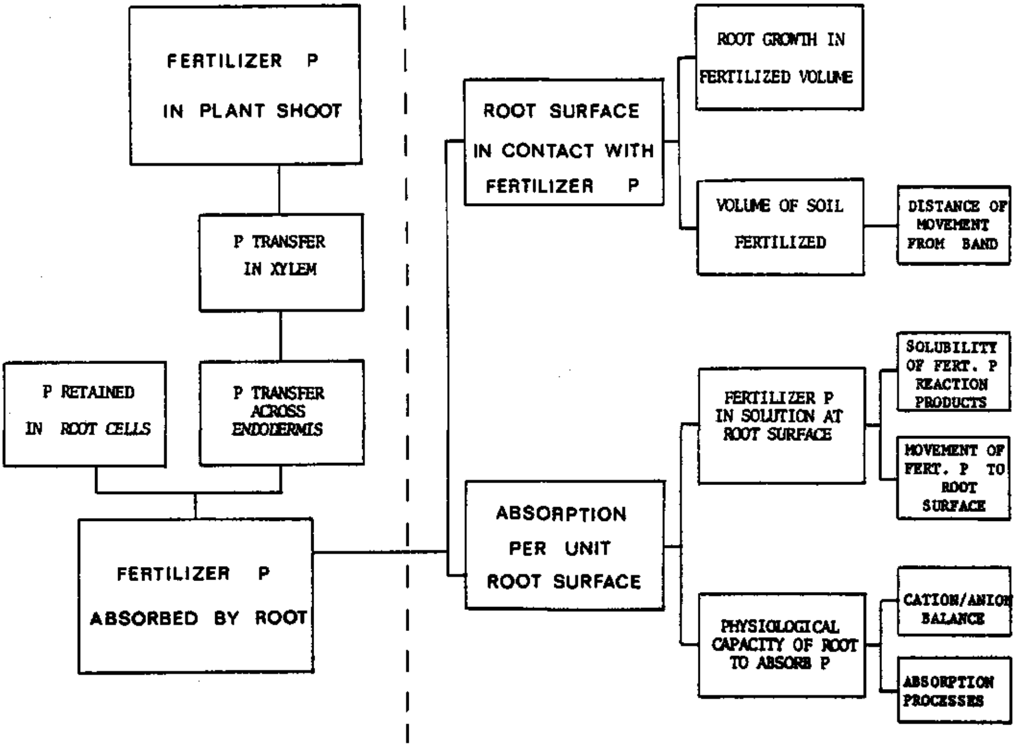


Fig. 4 Processes and factors involved in the effect of N on absorption of phosphate (after Miller, 1974).

2.2.3.1. Nitrate and ammonium absorption

Arnon (1939), working with barley plants grown on nutrient solutions, acknowledged the importance of the ionic form in which nitrogen is absorbed by plants "in view of the very large amounts of this element used by the plant and the rapid rate of absorption of both ammonium and nitrate ions". He detected higher concentrations of phosphate in NH_4- than in NO_3-fed plants and stated that a rapidly absorbable cation such as ammonium would favor the absorption of anions such as $H_2PO_4^-$. Many other authors found higher P concentrations in plants growing on ammonium solutions than in those growing on nitrate solutions. An example is Blair et al. (1970) who attributed the phenomenon to an NH_4-induced stimulation of the anion uptake. They also observed an increase in absorption of sulphate ions and a decrease in solution culture pH. In fact, several decades ago (Pirschle, 1931; Trelease and

Trelease, 1935) it was already known that a pH decrease results when plants absorb more nutritive cations than anions, and in recent years this relationship was firmly substantiated (e.g. Breteler, 1973; see also section 2.1).

When this phenomenon is studied in soil, many more variables are involved than is the case with the use of nutrient solutions, but nevertheless in recent soil studies considerable progress was made. When investigating the environment of soybean roots, Riley and Barber (1969) observed an accumulation of HCO_3^- and an increase in pH. The magnitudes of the HCO_3^- accumulation and the pH increase were related to the level of NO_3^- in the soil solution. This finding was attributed to the excess uptake of anions over cations by the soybean plants, inducing a release of HCO_3^- ions which serves to maintain electroneutrality both inside and outside the plant. The pH near the roots was found to have risen about 1 unit, and the HCO_3^- accumulation was as large as 35 me/100 g dry roots. In a later publication (1971) the same authors made it clear that under conditions of NH_4^+ nutrition, soybean absorbs more cationic than anionic nutrients, leading to a pH decrease in the rhizosphere soil. In this experiment, it was observed that the difference in pH between the rhizocylinders of NH_4- and NO_3-fed plants was as large as 1.9 pH units, when the initial soil pH was 5.2 and as small as 0.2 units, when prior to N application the pH was 7.8. Furthermore, the NH_4-fed soybean plants absorbed more P and had a higher P content than the NO_3-fed plants. These findings led the authors to conclude that the higher uptake of P from soil that contained NH_4^+ was due mainly to the pH-lowering effect on the rhizosphere soil, resulting from an uptake pattern in which soybean plants absorbed more cationic than anionic nutrients.

Other authors, however, have postulated that an increased rate of P uptake is not to be ascribed to shifts in the balance of cation- and anion uptake caused by NH_4^+ nutrition, but more to physiological processes taking place inside the plant. Thien and McFee (1970) placed two-week old maize plants for 24 hours in nutrient solutions containing either NH_4Cl or $NaNO_3$. Next, the plants were transferred to solutions containing ^{32}P-labeled phosphate together with either one of the two N forms. After 6 hours, the quantities of P absorbed from the two nutrient solutions were found to be equal. On the basis of these results the authors argued that an NH_4-induced increase in the quantity of P taken up from a soil is likely to be a reflection of generally better growth conditions associated with lower leaching losses of N applied as NH_4^+ than when applied as NO_3^-.

A different approach is based on the results obtained by Hagen and Hopkins (1955), who postulated that $H_2PO_4^-$ and HPO_4^{2-} are absorbed over different carrier sites and that the $H_2PO_4^-$ form is absorbed several times faster than is the HPO_4^{2-} form. With this in mind, Miller et al. (1970) related the increased absorption of fertilizer P from a neutral soil by NH_4-fertilized maize plants to an increase in the $H_2PO_4^-/HPO_4^{2-}$ ratio in the soil solution brought about by the acidifying effect of an alkaline uptake pattern. However, Blair et al. (1971), after having tested this postulate on several soils, concluded that the NH_4-induced increase in P uptake from soils was independent of soil pH. Since one of the soils had a pH value of 5.5, at which level practically all inorganic soil solution-P is present in the $H_2PO_4^-$ form these authors felt justified in stating that in this soil a shift in $H_2PO_4^-/HPO_4^{2-}$ ratio could not have been involved in causing an increase in P uptake as observed when NH_4 was used as N-source.

In a recent, more elaborate study, Soon and Miller (1977a, 1977b) succeeded in reconciling the two viewpoints on the cause of NH_4-induced increases in P uptake. They grew maize seedlings on 3 soils having pH levels of 7.1, 5.5 and 4.3. The soils received monocalcium phosphate and furthermore either $(NH_4)_2SO_4$ or $Ca(NO_3)_2$. It was noted that, in comparison with that of the bulk soil solution, the pH of the rhizosphere solution decreased as a result of NH_4^+ absorption and increased slightly as a result of NO_3^- absorption. The authors verified the relationship between lowered rhizocylinder pH and increased P concentration in the rhizocylinder solution, resulting in a rise in P uptake, but they also detected an increase in P absorption in both the NO_3- and NH_4-fed plants in comparison with plants that had not received any nitrogen. They ascribed these latter increases to an enhanced physiologically-controlled capacity to absorb P and to the development of a more extensive root system, as was reported earlier by others (Duncan and Ohlrogge, 1958, 1959; Drew, 1975).

2.2.3.2. Symbiotic nitrogen fixation

The special ability of legumes to utilize rock phosphates has been known since the end of the last century (subsection 2.2.1). Comparisons among leguminous species in this respect have shown that some species are more effective than others. For instance, Bryan and Andrew (1971) grew seven pasture legumes on a lateritic podzol and found that *Lotononis bainesii*, *Stylosanthes guianensis* (Schofield stylo), *Centrosema pubescens* (centro), *Indigofera spicata* and *Medicago sativa* (alfalfa) responded more favorably to Nauru rock phosphate than did *Desmodium uncinatum* and *Medicago lathyroides*. In a field experiment, Andrew (1973) observed that Morocco rock phosphate was very well utilized by *Lotononis bainesii*, but not by *Medicago atropurpureum*.

Why some plant species are more efficient than others in utilizing soil and fertilizer phosphorus is a question that continues to attract the attention of many researchers. Some of them point to variations in extensiveness of root systems, others stress the specific demands for Ca and P, the differences in length of the growth period, or the occurrences of mycorrhizal infections (Terman, 1971; Hundal and Sekhon, 1976; Andrew and Jones, 1978, and others cited in subsection 2.2.2). Some researchers, however, have commented that the ability of legumes to utilize rock phosphates might be related to the ability of these plants to acidify their rhizosphere (van Diest, 1975; Israel and Jackson, 1978).

Leguminous plants, when making use of symbiotically fixed nitrogen, display an alkaline uptake pattern which induces them to excrete H^+ ions to the external root environment. The extent of such H^+ excretions has recently been measured for some leguminous species (Nyatsanga and Pierre, 1973; Israel and Jackson, 1978; van Beusichem, 1981). Some authors have speculated on the possible consequences of acidity generated by nodulated legumes. Nyatsanga and Pierre (1973) mentioned its influence on soil management and soil development, Nambiar (1976) stressed its effects on the solubilities of relatively immobile trace elements, while Munns (1978) and Israel and Jackson (1978) spoke of its influence on the nodulation process. These last authors expressed the thought that the magnitude of the H-extrusion may determine the quantity of lime to be applied for optimal nodulation. Yet, very few experimental data are to be found on this issue.

As the aims of the present study are to confirm that legumes acidify their rhizosphere and to investigate whether such an acidification is sufficient to affect the availability of rock-phosphate P, it is of importance to pay due attention to possible constraints to these aims. Some of these constraints are biological and environmental factors, which will be discussed in the next section.

2.3 FACTORS LIMITING SYMBIOTIC N FIXATION AND DISSOLUTION OF ROCK PHOSPHATES

In connection with sharp increases in prices of nitrogenous fertilizers, ample attention has recently been given to factors affecting the efficiency of symbiotic nitrogen fixation (Bergensen, 1973; Lie, 1974; Vincent, 1974; Quispel, 1974; Dart et al., 1976; Hardy and Havelka, 1976; Pate, 1976; Munns, 1977).

A similar situation exists for rock phosphates, which have received renewed attention as a result of increases in prices of manufactured phosphate fertilizers, like superphosphate and triple superphosphate (Terman, 1971; Barnes and Kamprath, 1975; Khasawneh and Doll, 1978; McClellan and Gremillion, 1980).

2.3.1 Symbiotic nitrogen fixation

Nodulation and nitrogen fixation in the nodules depend on the collective action of a number of different biological factors such as (a) the presence in the rooting medium of cells of an efficient Rhizobium strain, (b) the increase in number of Rhizobium cells in the rhizosphere, (c) the degree of infection of the roots by the bacteria, (d) the growth, and (e) the longevity of the nodules, and (f) the activity of the Rhizobium bacteroids (Mulder et al., 1969).

In turn, these biological factors are influenced by variations in both endogenous and exogenous conditions. As an example of the former ones, pronounced differences in nodulation and nitrogen fixation among associations of a particular Rhizobium strain with various varieties of a certain species have been observed (Mulder et al., 1969). Furthermore, large differences have been found in the physiological responses of leguminous species to infection by different strains of Rhizobium (Bethlenfalvay et al., 1978b). The association of a particular species with different bacterial strains can yield large variations in response, ranging from the formation of highly efficient nodules in which ample nitrogen is fixed for the growth of the symbionts, to a total absence of nodulation (ineffective Rhizobium strains) or to the formation of non-efficient nodules being more a burden than an asset to the host plant.

As for the exogenous conditions, it is known that the symbiosis between host plant and the bacterium is easily disturbed by environmental stress, even when plant growth depending on combined nitrogen is apparently unaffected

(Lie, 1974). The optimum temperature for nodule formation is often similar to that for nodule development and subsequent nitrogen fixation, and both are inhibited by high and low temperatures. In the case of the tropical legumes, establishment of a symbiosis requires a well defined range of temperatures.For instance, Dart and Mercer (1965) reported that cowpea nodulated poorly and fixed little nitrogen at daytime/nighttime temperatures of $21^{o}C/15^{o}C$ or of $36^{o}C/31^{o}C$. In well nodulated cowpea plants, however, ample nitrogen fixation took place at $40^{o}C$ (Dart and Day, 1971). These same authors reported that effectively nodulated species of Vicia, Medicago and Trifolium reduced considerable amounts of $N_2(C_2H_2)$ at $2\text{-}5^{o}C$ and those of soybean and cowpea at $10^{o}C$. For *Medicago sativa*, nodules were most active at $35^{o}C$ and for two species of Vicia and Trifolium each, at $20^{o}\text{-}30^{o}C$.

Since for their growth and functioning root nodules depend upon photosynthates, all factors affecting photosynthesis, such as light, pCO_2, and temperature, will also exert influences on N_2 fixation (Hardy and Havelka, 1976). Within certain limits, a direct relationship can be found between light intensity, nodulation and nitrogen fixation (Lie, 1974). The effect of light is clearly illustrated in a publication of Sloger et al. (1975) who measured seasonal and diurnal variations in $N_2(C_2H_2)$ fixing activities in soybean plants. The average specific activity ($N_2(C_2H_2)$ fixing activity per gram fresh weight of nodules per hour) for all sampling times was highest in the 14.00-16.00 h period and was significantly correlated with cumulative solar-radiation values for each date, but not with cumulative solar radiation for the day prior to the sampling date.

Soil moisture is another important factor governing the efficiency of nitrogen fixation. Fred et al. (1932) found that for alfalfa and soybean the optimum soil moisture content lies at about 60-75% of water-holding capacity. It is also known, however, that a thin layer of moisture around the nodules reduces $N_2(C_2H_2)$ reduction (Schwinghamer et al., 1970).

It is well known that N_2 fixation is influenced by soil pH in the sense that pH affects survival and growth of Rhizobium in the soil, the infection of the roots and the formation of the nodules, the efficiency of the N_2-reduction process, and the nutrition and general well-being of the host plant. Rhizobium species are known to differ in their tolerance of soil acidity, e.g. the slow-growing species such as *Rhizobium japonicum* and *Rhizobium lupini* are more acid-tolerant than are fast-growing species like *Rhizobium meliloti*. In general, the pH range of 6-7 is considered to be optimal. In experiments with sub-irrigated sand cultures of 5 tropical and 6 temperate legumes, Andrew (1976) found that tropical legumes became well nodulated in the range of pH 4-6, but that amongst the temperate species none nodulated well at pH 4.

It has been stated that, once nodulated, legumes become self-sufficient with respect to N and grow optimally, irrespective of substrate-pH (Loneragan and Dowling, 1958), but Andrew (1976) showed that nodulated plants, grown at pH levels below the optimum one for nodulation, grew less well and accumulated less N than did plants grown at the pH level optimal for nodulation. Spurway (1941) mentioned optimum pH levels for a number of legumes: alfalfa, pH 6.2; red clover, pH 6.0; sweet clover, pH 6.5; soybean, pH 6.0; cowpea, pH 5.0; lespedeza, pH 4.5.

Another important factor, related to soil pH, is calcium, which element is known for its importance in the nodulation process (Lowther and Loneragan, 1970; Munns, 1970). Soil acidity is normally associated with low levels of Ca availability. In neutral soils, however, the concentration of Ca in the root-soil interface can be so high that it leads to accumulation at the root surface, as for instance has been observed with subterranean clover (Barber and Ozanne, 1970) and with soybean (Riley and Barber, 1969) supplied with NO_3-N. On the other hand, it can be expected that nodulated legumes growing in acid soils in the absence of NO_3-N should develop a micro-environment, with high acidity and low Ca concentration, which is certainly not conducive to a normal development of root nodules. In this connection, there is reason to attach a causal meaning to the finding that legumes which nodulate well in acid soils, e.g. cowpea, peanut and soybean, are low-Ca plants, whereas acid-sensitive species, such as alfalfa and white clover, are high-Ca plants (Munns, 1978).

Soil acidity also induces harmful concentrations of Al and Mn in soil solution (e.g. Morris and Pierre, 1949; Munns, 1965; Helyar, 1978), and furthermore a reduction in availability of Mo which is known to be essential to the process of N_2 fixation. Parker and Harris (1967) conducted field experiments with nodulating and non-nodulating soybean plants in soils with a pH range of 4.5-5.6, in which soybean responded favorably to added Mo. Their results indicated that in these soils enough Mo was available to allow a normal functioning of nitrate reductase in reducing absorbed NO_3^-, but that insufficient Mo was available to accommodate the proper functioning of nitrogenase in the N_2-fixing process.

In acid soils, the availability of phosphate is usually low. Andrew (1978) discussed the interrelationship between pH, Ca, P and Al in connection with the nutrition of legumes. He stated that "low pH reduces the availability of soil Ca and also reduces Ca uptake by the root, increased Al further restricts the uptake of Ca and roots become Ca-deficient, interactions of P and Al occur in or on the roots, nodulation is impaired or absent, and the plant becomes acutely N-deficient".

On soils well supplied with P, a depressing effect of high or moderately high levels of P on plant growth has been mentioned by several investigators (Shive, 1918; Rossiter, 1952; Dunphy et al., 1968). This problem appears to be particularly serious for legumes relying on symbiotic nitrogen fixation. For instance Rossiter (1952) indicated that, when no N-fertilizer was applied, the growth of clover was depressed by a high P level, whereas in the presence of applied N, the plants responded favorably to the same P level. The efficacy of nitrate-nitrogen in overcoming P toxicity in soybean was reported by Hamner (1940).

The presence of combined nitrogen at moderate or high levels hampers both the formation of nodules and the activity of nitrogen-fixing bacteria (e.g. Allos and Bartholomew, 1959; Houwaard, 1979). However, symbiotic nitrogen fixation appears to be stimulated by low levels of combined N (Oghoghorie and Pate, 1971; Harper, 1974; Bethlenfalvay et al., 1978a). Small amounts of fertilizer nitrogen applied at the start of the vegetative growth period were shown to result in higher quantities of atmospheric nitrogen fixed due to increased growth of the nodules (e.g. Pate and Dart, 1961).

2.3.2 Dissolution of rock phosphates

The phosphatic raw materials of interest to agronomists for possible use as fertilizer as such in ground form or as basic materials for manufactured fertilizers, are minerals grouped under the general heading of phosphate rock. Most of the economically important phosphate rocks are Ca phosphates of sedimentary origin and belong to the broad category of apatite minerals. In spite of their structural similarity, the chemical and mineralogical compositions of these apatites usually differ significantly from that of fluorapatite which is commonly assumed to be the phosphatic component of phosphate rock (c.f. McClellan and Gremillion, 1980). Natural phosphates are commonly accompanied by accessory minerals which are mostly silica, alkalineearth carbonates, layer silicates, Fe- and Al-oxides and -hydroxides and evaporites (chlorides and sulphates).

The mineralogical composition of phosphate rocks can be derived from isomorphous substitutions in the basic fluorapatite structure. Ca^{2+} is partially replaced by Mg^{2+} and Na^+, $PO_4{}^{3-}$ by $CO_3{}^{2-}$, and F^- by OH^-. The generalized empiric formula:

$$(Ca_{10-a-b}\, Na_a\, Mg_b)(PO_4)_{6-x}(CO_3)_x\, F_{2+0.4x}$$

represents the natural sedimentary apatites, in which x is the number of moles of $CO_3{}^{2-}$ per mole apatite, $a = 1.327x/(6-x)$ and $b = 0.515x/(6-x)$. When $x = 0$, the formula reduces to that of fluorapatite:

$$Ca_{10}(PO_4)_6F_2.$$

Plant responses to direct application of ground phosphate rock vary from almost equal to responses to superphosphate to no response at all. The effectiveness of rock phosphate, being the common name for ground phosphate rock, depends on such variables as soil type, soil pH, soil redox potential, time of reaction, particle size and nature of the phosphate rock. An often used criterion for the usefulness of a rock phosphate is its solubility in a 2% citric acid solution (e.g. Caro and Hill, 1956).

In regard to the particle size, it is known that in both acid and alkaline soils, plant response to rock phosphate increases with decreasing particle size (increase in surface area per unit of P) (Terman, 1971). However, it is also agreed that usually yields obtained with extremely fine grindings are not higher than those obtained with commercially ground material passing a 100-mesh sieve (Khasawneh and Doll, 1978).

The rate of dissolution of rock phosphates in soil depends on the rate of dissociation of the ions Ca^{2+}, $PO_4{}^{3-}$ and F^- from the apatite surfaces. The $PO_4{}^{3-}$ released is rapidly protonated to $H_2PO_4{}^-$ or $HPO_4{}^{2-}$. Soil acidity has always been considered the most important factor enhancing the solubilization of rock phosphates. Next to soil acidity, Ca- and P concentrations in the soil solution also play important roles. To illustrate this, in Figure 5 the solubility isotherms of fluorapatite in equilibrium with fluorite (CaF_2), according to:

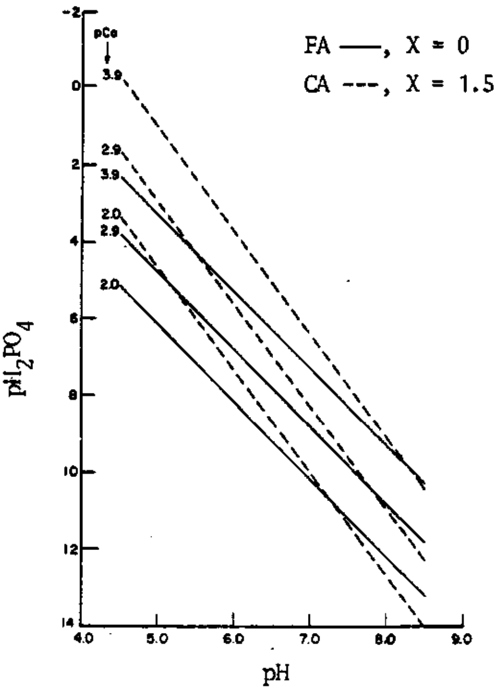


Fig. 5 Solubility isotherms of fluorapatite (FA) and a highly substituted carbonate apatite (CA) in equilibrium with fluorite as related to pH and pCa (after Khasawneh and Doll, 1978).

$$pH_2PO_4 = 2pH - 1.5pCa - 0.82$$

are presented. This equation was calculated with the use of a value of 122.2 for the pK value of fluorapatite (Chien and Black, 1976) for three values of Ca activity namely 1.25×10^{-4}M, 1.25×10^{-3}M and 1.0×10^{-2}M. The same figure shows the solubility isotherms of a substituted carbonate apatite ($x = 1.5$). In this case, the solubility product of the apatite was estimated from the following relationship (Chien and Black, 1976):

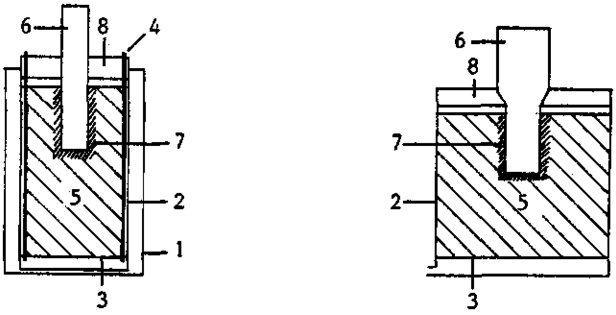
$$pK_{CA} = 122.2 - 6.3x.$$

Figure 5 presents information on pH_2PO_4 as a function of pH and pCa. It must be borne in mind, however, that pH_2PO_4 does not represent total P in solution, since other ionic forms of P might be present whose activities in turn depend on pH and activities of other ions. The actual dissolution of rock phosphate in soils has been measured only by indirect methods, such as extractions of P with acids and bases and with exchange resins (e.g. Chien and Hammond, 1978).

3 MATERIALS AND METHODS

A series of pot experiments was conducted between August 1978 and January 1981 under greenhouse conditions. Some experimental features are common to almost all experiments and these will be described first in this chapter. More information pertaining to each experiment will be given in chapter 4.

Pot technique - The experiments were conducted according to a technique described by Schuffelen et al. (1952) which guarantees good growing conditions with only small differences between replicate pots. Figure 6 shows drawings of the types of pots used. The small cylinders were filled with 2.7 kg of soil or sand and the large cylinders with 6 kg of sand.



(a) small cylinders (b) large cylinders

Fig. 6 Pots used in the Department of Soil Science and Plant Nutrition.
1: wooden box (16.5x16x24 cm); 2a: plastic cylinder (d = 12.5 cm, h = 25 cm, total contents = 2.7 l.); 2b: large enameled cylinder (d = 20 cm, h = 22 cm, total contents = 6.9 l.); 3: sieve resting on a triangular standard allowing an air space (h = 2 cm) in the bottom of the pot; ventilation by means of two plastic tubes (4); 5: soil; 6a: watering hopper (h = 17 cm, d = 3 cm); 6b: watering hopper (h = 17 cm, d (top) = 6 cm, d (bottom) = 3,7 cm); 7: quartz sand; 8: layer of ground gravel of pure quartz.

Moisture supply - Water was supplied twice daily through the watering hoppers (Fig. 6) and the pots were weighed every morning in order to add enough water to restore a moisture level at 60% of the water-holding capacity. The weights of the plants were periodically estimated and added to the total weights of the pots.

Placement of the pots - The pots were placed on stationary greenhouse tables, on carts which could be wheeled into an open-air cage, or on an endless conveyor belt. To prevent environmental differences due to unequal exposure, after each weighing the pots were moved up two places on the table or the carts. When experiments were conducted in the fall or winter, the temperature in the greenhouse was set at 22°C daytime/18°C nighttime, and artificial lighting was provided.

Fertilizers - Before the pots were filled, fertilizers were mixed thoroughly with the sand or the soil to be transferred to the pots. In most of the experiments, two forms of nitrogen nutrition were included, namely NO_3^- nutrition by means of addition of $Ca(NO_3)_2$, and N_2 nutrition by means of symbiotic nitrogen fixation. In both cases a quantity of 5 me NO_3-N per pot (10 me in experiment 1) was added to the sand or the soil. Phosphate fertilizer was applied as triple superphosphate (18.8% P), as KH_2PO_4, or as rock phosphate in the form of either Morocco rock phosphate, with 13.7% total P and 5.5% P soluble in 2% citric acid, or Mali rock phosphate, with 13.3% total P and 3.4% soluble in 2% citric acid. The quantities of N, P and Ca applied will be mentioned in the description of each individual experiment.

K and Mg were applied in a fixed ratio as sulphates in the form of the commercial fertilizer sulpomag (patentkali), containing 28% K_2O and 8% MgO, at a rate of 0.9g/kg soil or sand. Fe was supplied as Fe-citrate (0.7mg salt per kg soil or sand) instead of as Fe-EDTA which has been shown to inhibit the nodulation of legumes (Lie and Brotonegoro, 1969). Sufficient quantities of the other micronutrients were added in the form of a multi-element solution, including cobalt.

Growth media - Two different growth media were used, namely fine quartz sand or a soil. The soil is a sandy loam found in a quarry covered by a few meters of sand, near Lunteren, Netherlands. The soil was selected for its low contents of phosphorus and nitrogen. Some characteristics of it are listed in Table 2. The data presented are averages of results obtained on several samples, representing different loads collected at the same location over a number of years.

Table 2 Characteristics of the Lunteren soil before treatment with lime

granular composition	: fraction > 50 μ = 70% 50 - 16 μ = 5% 16 - 2 μ = 10% < 2 μ = 15%
cation exchange capacity	: 6.0 me/100g soil
base saturation	: 2.4 me Ca/100g soil 2.2 me Mg/100g soil 0.2 me K/100g soil
pH (KCl)	: 4.4
pH (H_2O)	: 5.8
C-total	: 0.03%
N-total	: 0.01%
P-available (P-Al)	: 0.4 mg P/100 g soil
P-total (mostly Al-P and Fe-P)	: 14 mg P/100 g soil
P-maximum adsorption	: 0.5 mg P/g soil
water-holding capacity	: 250 ml/kg soil

Before the soil was used in the pot experiment, its pH was adjusted to values of around pH(H_2O) 6.0. To this aim, the soil was incubated for several weeks with $Ca(OH)_2$ at 40% of water-holding capacity. Shortly before use, the soil was dried and passed through a 5-mm sieve. The actual soil-pH value at the start of each experiment will be given in the separate descriptions of the experiments.

Crops and Rhizobium strains - Alfalfa (*Medicago sativa*) var. DuPuits, soybean (*Glycine max*) var. Portage, and peanut (*Arachis hypogaea*) var. Matjan were used as test plants. Prior to planting, the seeds were surface-sterilized by rinsing them for 30 minutes in a 3% H_2O_2 solution to which a drop of detergent (Teepol) had been added. The seeds germinated in a germination tray containing pure quartz sand, and the seedlings were transplanted into the soil or sand at a rate of 10 plants of alfalfa or 3 plants of soybean per 2.7-1 pot or 5 plants of peanut per 6-1 pot.

Plants that were meant to utilize symbiotically fixed N were inoculated with Rhizobium strains by applying 5 drops of a liquid culture of the bacteria to the soil or sand close to each plant. For soybean, a mixed culture of the effective *Rhizobium japonicum* strains CC2053, KA107 and SM was used, for alfalfa the *Rhizobium meliloti* strain K24 and for peanut *Rhizobium* spp. strain DMG432. For alfalfa and soybean, the strains were obtained from the Department of Microbiology, Agricultural University, Wageningen, and the strain for peanut was made available by the Department of Microbiology, University of Guelph, Canada.

Analytical techniques for soil analysis - Chemical determinations on soil and sand constituents were performed according to methods described by Houba et al. (1979). Determinations of pH were carried out in the supernatant liquid of a 1:2.5 sand/soil:solution ratio. The liquid used was either demineralized water or 1 N KCl; the shaking time was 2 hours. A titration curve of the soil was obtained by shaking soil samples with 1 N KOH solution in the same soil:solution ratio for periods of 12 hours.

A phosphate-adsorption isotherm was obtained by shaking suspensions of 5.0-g samples of soil in 100-ml volumes of a series of phosphorus solutions in 0.1 M $CaCl_2$ for 72 hours at 24°C. P was determined in the supernatant liquid obtained by means of centrifugation. The value for maximum P adsorption in Table 2 was obtained from the isotherm constructed with the use of the Langmuir equation. Phosphate extractable with ammonium lactate-acetic acid, pH 3.75 (P-Al) and phosphate extractable in water were used as indices of plant-available phosphate.

In some sand-culture experiments, at the end of the growth periods rhizosphere sand and bulk sand were separated. To achieve this, at harvest the plant roots were carefully removed from the sand. The complete root systems were exposed to a mild stream of warm air for periods of 10-15 minutes, during which period the sand adhering to the roots dried out, so that it could be easily shaken from the roots and subjected to separate analyses.

Acetylene-reduction technique - At harvest time, the plants were usually separated into shoots and roots. For determinations of nitrogenase activity, portions of root systems were transferred to 2-liter erlenmeyer flasks containing a gas mixture consisting for 10% of acetylene gas. After 30 minutes the quantities of ethylene gas produced were measured with a Becker 417 gas chromatograph equipped with a flame-ionization detector and a stainless steel column filled with Poropak R, at 80°C. The ethylene content was calibrated with a standard gas mixture of 100 ppm C_2H_4 in N_2. The acetylene-reduction technique and the method to calculate the quantities of C_2H_2 reduced from the peaks obtained from the recorder coupled to the gas chromatograph is described by Hardy et al. (1968) and by Dart et al. (1972).

Analytical techniques for plant analysis - Roots were thoroughly washed with tap water, submerged for 2 minutes in a 0.1 N HCl solution and rinsed with demineralized water. The plant material was then dried for 48 hours at $70^{\circ}C$, weighed and ground. Subsamples of dried plant material were wet-ashed in H_2SO_4 and H_2O_2 in the presence of salicylic acid. In the digest, Na, K and Ca were determined flame-photometrically and H_2PO_4 and total N by colorimetric methods. Other subsamples were extracted with water (0.5 g of plant material and 50 ml water, shaking time 2 hours). In the extract NO_3^- (nitrate electrode), Cl^- (chlor-o-counter) and SO_4^{2-} (colorimeter) were determined. All methods were described in detail by Slangen and Hoogendijk (1971).

Methods of calculation - The method for calculating acid and alkaline excretion by plants has been described by Breteler (1973) and by van Egmond and Aktaş (1977)(Chapter 2). The total number of equivalents of cationic nutrients absorbed by the plants (C_a) is calculated directly from the analytical values of the Na, K, Mg and Ca determinations as described above. For the NO_3-fed plants, the number of equivalents of anionic nutrients absorbed (A_a) is calculated from the values of total N, total S, H_2PO_4 and Cl. Total S is calculated from the sum of SO_4-S analytically determined and organic S estimated at 0.054 times the equivalents of organic N (Dijkshoorn and van Wijk, 1967). For plants making use of symbiotically fixed N, only the 10 or 5 me of NO_3-N added as starter fertilizer are entered into the calculation of anionic-nutrient uptake.

The contents of nutrients in the soybean and peanut seeds were taken into account for a determination of C_a and A_a. The quantities of nutrients in the alfalfa seeds were considered to be too small to warrant inclusion in the calculations.

4 RESULTS AND DISCUSSION

4.1 EXPERIMENT 1

The main purpose of this experiment was to verify that the manner in which legumes obtain their nitrogen (either as NO_3^- or through symbiotic fixation) can exert an influence on the pH of the medium in which the root systems of these legumes develop. The test crop was soybean, which was grown on pots filled with sand as described in chapter 3. Each pot contained 3 soybean plants. In one-half of the pots, the plants were inoculated with the proper Rhizobium strains, whereas in the remaining pots inoculation was omitted, so that here the plants were supposed to depend entirely on the 8.8 me N supplied as $Ca(NO_3)_2$ to all pots, including the inoculated ones, as a starter fertilizer. In this experiment, the only P source used was triple superphosphate. The quantity applied was 155 mg P per pot.

The experiment was started on September 21, 1978. After 4 weeks, of the inoculated pots three were harvested, and weekly harvests were carried out in the subsequent 8 weeks (with 1 week omitted) so that eventually data could be obtained on plants and sand over a period of 9 weeks. Harvests of non-inoculated plants were carried out less frequently, as indicated in Table 3. Next to complete chemical analyses performed on plant materials (mixtures of tops and roots), determinations of pH and Ca-, Mg- and K contents of rhizosphere- and non-rhizosphere sand were made.

In Fig. 7, information is given on the dry-matter production per pot. It can be seen that over an initial 60-day period, little difference in dry-matter production of inoculated and non-inoculated plants was observed. Subsequently, yields started to diverge mainly as a result of N-shortage in non-inoculated plants, so that at the end of the growth period the yields of inoculated plants were significantly higher.

The results of the chemical analyses of plant material are presented in Table 3. It can be seen that indeed the N-contents of non-inoculated plants started to decline appreciably after harvest IV and further that in the inoculated plants N_2 fixation apparently remained insignificant until after

Table 3 Chemical composition of soybean plants in different stages of growth on sand, with and without the availability of symbiotically fixed N_2.

Content of nutrients, me/kg dry matter	N_2 fixation	Harvest periods							
		I	II	III	IV	V	VI	VII	VIII
Total N	+	2996	2506	1781	1354	1188	1637	2038	2433
	-		2501		1156		695		748
Na	+	8	10	8	8	12	5	6	5
	-		8		8		7		11
K	+	1071	989	755	615	486	509	521	570
	-		1004		541		533		626
Ca	+	670	564	446	370	478	342	346	346
	-		532		386		358		387
Mg	+	449	405	298	249	275	255	253	249
	-		397		270		280		329
H_2PO_4	+	521	417	317	238	190	192	196	203
	-		420		240		206		241
Cl	+	27	20	11	6	7	10	5	9
	-		21		6		1		8
NO_3	+	138	73	23	13	0	4	4	5
	-		62		12		4		3
SO_4	+	160	123	122	145	141	147	138	134
	-		105		145		229		354
ΣC	+	2198	1968	1507	1242	1251	1111	1126	1170
	-		1941		1205		1178		1353
ΣA	+	846	633	473	402	338	353	343	351
	-		608		403		440		606
ΣC-ΣA	+	1352	1335	1034	840	913	758	783	819
	-		1333		802		738		747

harvest V. It is further noteworthy that at the end of the growth period the contents of all nutrients, except nitrogen, were higher in non-inoculated than in inoculated plants, but that for both treatments similar decreases in (C-A) values with time were observed.

On the root material of harvest II, the first nodules were to be seen in a quantity of about 10 small nodules per pot. This number had increased

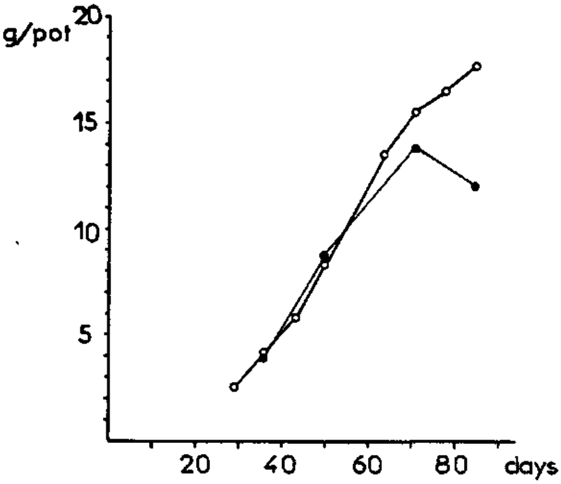


Fig. 7 Yield of soybean (g dry matter per pot) in different stages of growth on sand with (-○-) and without (-●-) the availability of symbiotically fixed N_2.

to approximately 120 well developed, pink nodules per pot at the time of harvest VIII. Spontaneous infection of non-inoculated plants was insignificant to non-existent, as can be judged from the information supplied in Table 4. Here,the quantities of N contained in the seeds must also be taken into account. For 3 seeds, this quantity is approximately 2 me N. In a later experiment, it was observed that this seed-N is used incompletely by the developing plant.

At harvests I and II, it was observed that small necrotic spots had developed on the young leaves. Later on, the spots coalesced to form completely necrotic leaves. New leaves appearing after harvest III were not affected any more. It was later learned that these symptoms are indicative of P-toxicity. The H_2PO_4 content of 521 me per kg dry matter in plants of harvest I, as listed in Table 3, corresponds with a P content of 1.6% which indeed is unduly high.

The data of Table 3 can be used to calculate for each treatment and each harvest date the total quantities of cationic and anionic nutrients absorbed by the plants. For all nutrients, except nitrogen, the pertinent data can be calculated directly from those listed in Table 3. For nitrogen it was assumed that 90% of the NO_3-N originally supplied as $Ca(NO_3)_2$ was absorbed by the plants. This assumption is based on the finding that in the control pots

Table 4 Total N in plant material and NO_3-N in bulk sand in various stages of growth of inoculated and non-inoculated soybean plants.

Harvest	Total N in plants, me/pot*		NO_3-N in sand, me/pot	
	inoculation	no inoculation	inoculation	no inoculation
I	7.5		2.7	
II	10.5	9.6	0.2	0.2
III	10.4		0.2	
IV	11.2	10.1	0.1	0.1
V	16.0		0.1	
VI	25.3	9.7	0.0	0.0
VII	33.7		0.0	
VIII	43.1	9.0	0.0	0.0

*original application: 8.8 me NO_3-N per pot

Table 5 Sums of cationic and anionic nutrients absorbed by soybean plants grown on sand with and without the availability of symbiotically fixed N_2.

Harvest	$\Sigma A_a - \Sigma C_a$, me per pot (acidic uptake pattern)		$\Sigma C_a - \Sigma A_a$, me per pot (alkaline uptake pattern)	
	inoculation	no inoculation	inoculation	no inoculation
I	4.2			
II	2.4	3.0		
III	2.2			
IV	1.3	1.2		
V			4.0	
VI			3.5	2.1
VII			4.7	
VIII			6.3	0.8

without plants, some nitrogen applied as NO_3^- could not be recovered as such, possibly as a result of some microbial immobilization taking place. For those cases in which more than 7.9 me total N was recovered in the plant material, it was assumed that any excess N was not absorbed as NO_3^-, but was obtained from sources such as seed-N and symbiotically fixed N.

From the calculated sums of cationic and anionic nutrients and their differences, as presented in Table 5, it can be seen in which stages of development the soybean plants showed acidic or alkaline uptake patterns.

A comparison of the data in Table 5 with those in Table 3 reveals that for inoculated plants in the period of rapidly increasing N_2 fixation an initially acidic uptake pattern changed into an alkaline uptake pattern. However, also for the non-inoculated plants a conversion of an acidic into an alkaline uptake pattern is to be seen. Here, after having exhausted the external N-source, the plants tried to sustain a growth process by redistribution of their nitrogen. In this period, all other nutrients were still absorbed, but many more cationic than anionic ones, thus leading to an alkaline uptake pattern.

In Fig. 8, information is supplied on the pH value found in the rhizo-

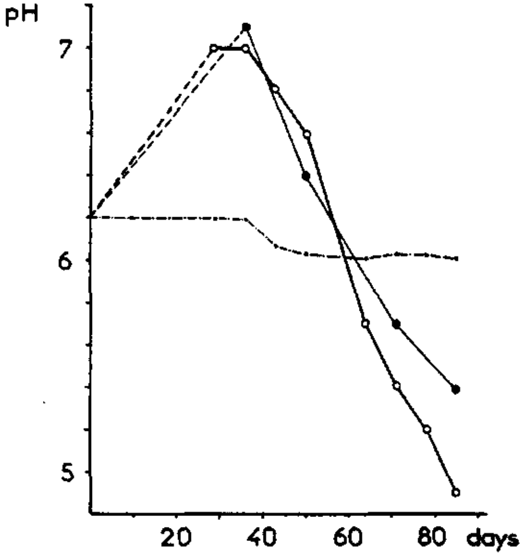


Fig. 8 pH(H_2O) values measured at various growth stages of inoculated (—○—) and non-inoculated (—●—) soybean plants in the rhizosphere sand. The reference curve (-·-·-) represents pH(H_2O) values measured on samples of sand from pots without plants.

sphere sand of plants harvested at the various stages. An initial rise in pH caused by the acidic uptake pattern was followed by a rapid decline. Here it must be noticed that the turning point of a pH increase changing into a pH decrease does not coincide with the point at which an acidic uptake pattern turns into an alkaline uptake pattern. A ready explanation for this lack of synchronism is not available.

It can be seen from the graph (Fig. 8) that the pH decrease extends to lower values for the inoculated than for the non-inoculated plants. This difference was to be expected, on account of the much higher values for alkaline uptake in the inoculated than in the non-inoculated plants (harvests VI and VIII, Table 5). Lack of nitrogen causes a gradual decline in growth rate and rate of nutrient uptake in non-inoculated plants, whereas in inoculated plants the availability of symbiotically fixed N_2 enables these plants to sustain a normal rate of growth and rate of nutrient absorption.

A comparison of pH values in rhizosphere and non-rhizosphere sand (Fig. 9) discloses that only in an early stage of growth a difference can be observed. The data obtained for harvest II were significantly different from each other, with the higher value found for the rhizosphere sand as was to be

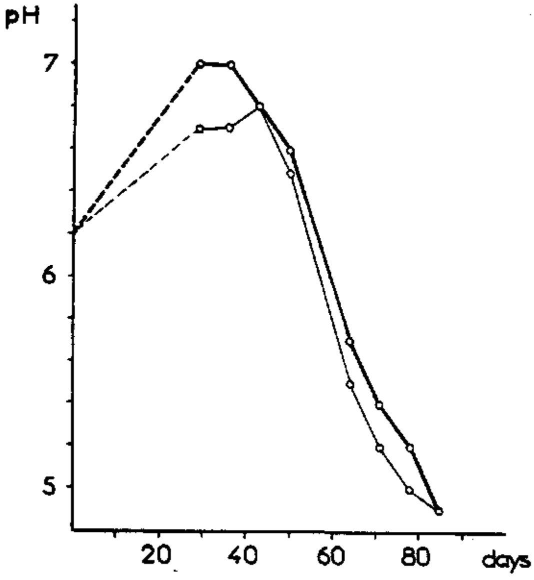


Fig. 9 pH(H_2O) values measured at various growth stages of inoculated soybean plants in rhizosphere (━○━) and non-rhizosphere (–○–) sand.

expected. A statistical analysis of data obtained at later harvests indicated that no differences were present. A likely explanation for such an absence of difference is that at a certain stage the root systems of 3 plants in a relatively small pot become so dense that hardly any sand remains which is not or has not been in intimate contact with a root. It must also be taken into account here that in an unbuffered medium like sand any influence that a root may exert on the chemical composition of its environment will let itself be noticed over a relatively long distance. Because of these considerations, in subsequent experiments no distinction was made any more between rhizosphere and non-rhizosphere sand or -soil.

The rhizosphere and non-rhizosphere sand obtained after each harvest was analyzed for Ca, Mg and K. The results of these analyses are shown in Table 6.

The values of Ca in the rhizosphere sand being always higher than those in the non-rhizosphere sand can be considered to reflect a situation in which the quantities of Ca transferred to the roots as a result of mass flow exceed the quantities absorbed by the roots. Backdiffusion of accumulated calcium

Table 6 Ca, Mg and K concentrations in the rhizosphere and non-rhizosphere sand at various stages of growth of inoculated and non-inoculated soybean plants.

Harvest	cationic concentrations, me per 100 g dry sand											
	Ca				Mg				K			
	inocul.		non-inocul.		inocul.		non-inocul.		inocul.		non-inocul.	
	-R[*]	R	-R	R	-R	R	-R	R	-R	R	-R	R
I	0.65	1.14			0.35	0.19			0.34	0.35		
II	0.69	1.17	0.69	0.86	0.38	0.24	0.36	0.23	0.32	0.42	0.31	0.38
III	0.79	2.00			0.38	0.29			0.30	0.56		
IV	0.69	1.17	0.69	0.86	0.33	0.15	0.35	0.13	0.20	0.38	0.24	0.39
V	0.65	1.14			0.27	0.14			0.16	0.38		
VI	0.63	0.74	0.59	0.62	0.27	0.11	0.11	0.13	0.17	0.26	0.14	0.29
VII	0.50	1.00			0.25	0.10			0.14	0.28		
VIII	0.62	1.13	0.69	0.85	0.19	0.09	0.20	0.07	0.12	0.23	0.16	0.26
Control (no plants)	0.72				0.35				0.35			

[*] -R = non-rhizosphere sand; R = rhizosphere sand

apparently does not proceed rapidly enough to eliminate the concentration gradient. These results on Ca are in agreement with those obtained by other investigators, but this is not the case with the findings on Mg and K. For Mg, the data of Table 6 show that the concentrations are lower inside than outside the rhizosphere. This finding could indicate that the demand of the plant exceeds the supply through mass flow, so that a concentration gradient is created which gives rise to a situation in which most of the Mg is transferred to the absorbing roots by means of diffusion instead of mass flow. Usually, such a situation exists for K instead of for Mg, but it can be seen that in the present experiment K accumulated in the rhizosphere, which suggests that the rate of K supply to the roots by means of mass flow exceeded the rate of absorption of K by the roots. The absence of any adsorbens in the sand will have contributed to a situation in which the K concentration in the aqueous phase was considerably higher than usually encountered in soils.

The observations made on movement and accumulation of these cations in the vicinity of the soybean roots are not sufficient to give a full account of pH changes found to have taken place in the sand. If this would have been the objective, a complete balance sheet of nutritive cations and -anions, together with H_3O^+, HCO_3^- and/or OH^- ions absorbed or extruded by the roots, should have been drawn up. Such an objective, however, was beyond the scope of the present investigation.

The main objective of experiment 1 was to investigate whether an alkaline uptake pattern of soybean utilizing symbiotically fixed N_2 would generate enough acidity to warrant an expectation that under such conditions rock phosphate could become a useful source of P for the soybean plant.

4.2 EXPERIMENT 2

In this experiment, use was made again of soybean as a test crop, grown on 2.5-l pots filled with sand as described in chapter 3. The experiment lasted from August 29, 1979 to November 20, 1979. To the sand of each pot a quantity of 370 mg P was added as either triple superphosphate, Morocco rock phosphate or Mali rock phosphate. In comparison with the previous experiment, the quantity of starter NO_3-N was reduced to 5 me per pot, supplied as $Ca(NO_3)_2$. To inoculated plants no further quantities of combined nitrogen were applied, whereas non-inoculated plants received weekly applications of

$Ca(NO_3)_2$ up to a total of 90 me (1250 mg) N per pot. The pots with inoculated plants received 50 me Ca in the form of $CaSO_4$ as a basal dressing. In a factorial experiment, with three forms of phosphate* and two forms of N-nutrition (either as NO_3-N or by means of symbiotic N_2 fixation), the number of treatments amounted to six. In each treatment, 21 replicate pots were present. The plant material of each time 3 pots with inoculated plants was harvested 14, 21, 28, 35, 49, 64 and 83 days after sowing (harvests I - VII). Non-inoculated plants were harvested less frequently, as shown in Fig. 10.

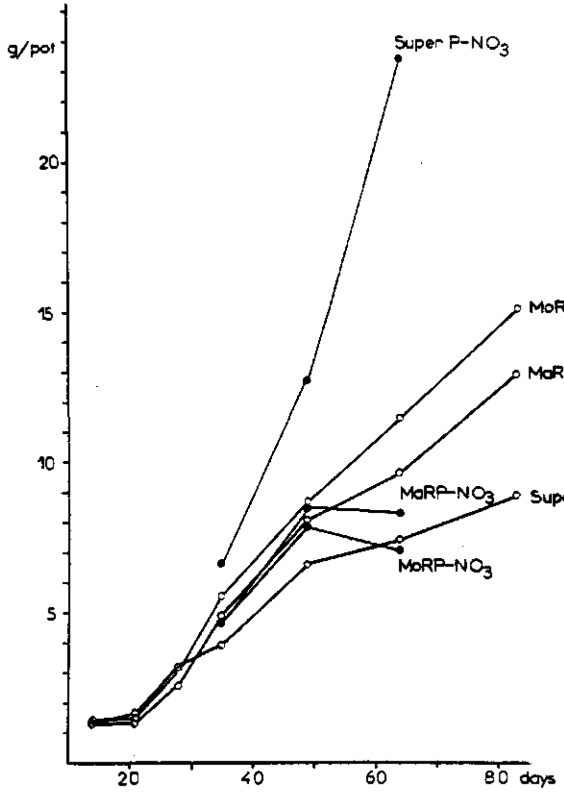


Fig. 10 Dry-matter yield of soybean grown on sand with varying sources of N and P, and harvested at different stages of growth (g dry matter per pot).

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*Henceforth indicated as superP (= P applied as triple superphosphate), MaRP (= P applied as Mali rock phosphate) and MoRP (= P applied as Morocco rock phosphate).

After each harvest, the pH(H_2O)- and pH(KCl) values of the sand were determined. The plant material was analyzed for the usual nutrients involved in calculations of cation-anion uptake balance sheets. Symbiotic N_2 fixation was measured by means of acetylene reduction in nodulated root material.

Some characteristics of the plants harvested at the various dates are listed below:

Harvest I (14 days)

- no nodules present

Harvest II (21 days)

- second trifoliate leaf begins to emerge
- no nodules present

Harvest III (28 days)

- third and fourth trifoliate leaves begin to emerge
- plants utilizing superphosphate-P show the first symptoms of P toxicity
- some small nodules are present

Harvest IV (35 days)

- plants of NO_3^- treatments are dark green and begin to lose their lower leaves
- first flowers appear

Harvest V (49 days)

- inoculated plants utilizing superphosphate-P are lighter in color than those utilizing rock phosphate-P

Harvest VI (64 days)

- inoculated plants utilizing superphosphate-P have already lost many leaves
- inoculated plants utilizing rock phosphate-P have lost 2 or 3 leaves per plant
- all plants bear some pods
- NO_3-fed plants utilizing superphosphate-P have 7-9 trifoliate leaves and several lateral branches
- NO_3-fed plants utilizing Morocco rock phosphate-P have lost almost all leaves.

The dry-matter yields of the plants harvested at the various dates are presented graphically in Fig. 10. Attention will first be paid to the data pertaining to plants utilizing superphosphate-P. Very large differences were obtained between yields of NO_3-fed plants and those of plants utilizing fixed N_2. The latter ones were strongly affected by P toxicity which caused severe reductions in nodule development and symbiotic N_2 fixation, as will be shown later. In spite of initial symptoms of P toxicity, the NO_3-fed plants produced the highest yields in the experiment.

Regarding the rock phosphate treatments, it can be seen that after an initial lag period, the plants utilizing symbiotically fixed N_2 were able to maintain a fairly steady growth rate over a period of approximately 80 days, with yields obtained with Morocco rock phosphate being somewhat higher than those obtained with Mali rock phosphate. During the first 7 weeks, the NO_3-fed plants maintained a growth rate not much lower than that of the plants utilizing fixed N_2. After the 7th week, the NO_3-fed plants deteriorated rapidly. All through the experiment, symptoms of P deficiency were quite apparent in these plants. To a lesser extent, however, this was also the case in the plants utilizing fixed N_2.

Table 7 supplies information on the chemical composition of the plants harvested at various growth stages. The phosphate contents of the plants utilizing fixed N_2 and supplied with superphosphate were found to be extremely high. It is likely that an absence of NO_3^- as anion competing with the absorption of $H_2PO_4^-$ induces an excessively high rate of phosphate uptake. To a much lesser extent, this also applies to sulphate uptake.

With increasing age of the plants, the phosphate contents of NO_3-fed plants utilizing rock phosphate-P fell farther behind those of plants using fixed N_2. The pH-values of the sand to be shown in Fig. 11 will make it clear that in the latter part of the experiment, conditions became increasingly unfavorable for dissolution of rock phosphates.

A comparison of N contents of plants utilizing fixed N_2 for the various harvests discloses that apparently toward the end of the experiment conditions for symbiotic N_2 fixation became increasingly favorable, which induced better availability of rock phosphate-P reflected in rising phosphate contents of the plant material.

Changes in pH-values of the sand during the growth period are pictured in Fig. 11. It can again be seen that after slight initial increases during the period in which starter-NO_3 was still available, the pH-values in sand serving as growth medium for plants depending on fixed N_2, showed a considerable decline. This decline was least pronounced in the superphosphate treatment owing to the fact that P toxicity obstructed proper nodulation which in turn resulted in poor growth and, hence, in a relatively small acidifying effect of the plants on the sand. The acidifying effect of plants utilizing Mali rock phosphate-P was largest, even though the yields of these

Table 7 Chemical composition of soybean plants at three stages of growth on sand, with three forms of P supplied, and with or without the availability of symbiotically fixed N_2.

Harvest period and treatment	Yield of d.m., g/pot	Contents of nutrients, me/kg dry matter									
		N	Na	K	Ca	Mg	H_2PO_4	Cl	NO_3	SO_4	ΣC−ΣA
Harvest IV											
SuperP-N_2	3.94	1540	13	936	582	388	611	11	0	192	1105
MaRP-N_2	4.90	1470	8	592	226	326	47	15	3	152	935
MoRP-N_2	5.56	1262	12	580	222	298	55	11	0	120	926
SuperP-NO_3	6.66	2726	16	844	588	374	303	17	62	90	1350
MaRP-NO_3	4.54	1959	13	555	224	326	37	22	99	114	846
MoRP-NO_3	4.61	2081	13	538	220	304	34	18	85	102	836
Harvest V											
SuperP-N_2	6.60	1602	2	788	400	354	522	17	6	184	815
MaRP-N_2	8.08	1361	4	441	276	276	43	24	4	120	806
MoRP-N_2	8.70	1671	9	477	319	291	66	28	4	106	892
SuperP-NO_3	12.78	2683	2	696	658	388	219	26	61	74	1364
MaRP-NO_3	8.50	2122	3	492	595	346	27	30	72	166	1141
MoRP-NO_3	7.86	2013	1	502	354	341	27	31	89	156	895
Harvest VI											
SuperP-N_2	7.43	2399	12	843	380	358	466	16	6	168	937
MaRP-N_2	9.63	2526	13	593	322	306	66	31	1	74	1062
MoRP-N_2	11.48	3189	13	606	377	334	106	37	0	74	1113
SuperP-NO_3	23.39	1953	12	442	580	360	130	21	24	54	1165
MaRP-NO_3	8.32	2493	15	608	959	513	24	48	221	227	1575
MoRP-NO_3	7.07	2739	22	694	1186	644	22	62	378	228	1856
Portage seed	0.56	5184	3	517	60	192	254	7	10	18	

plants lagged somewhat behind those obtained with Morocco rock phosphate-P.

Among the NO_3- fed plants, the pH-raising effect was largest for the plants utilizing superphosphate-P. As was shown in Fig. 11, plant growth in this treatment was more vigorous than in any other treatment. The pH values

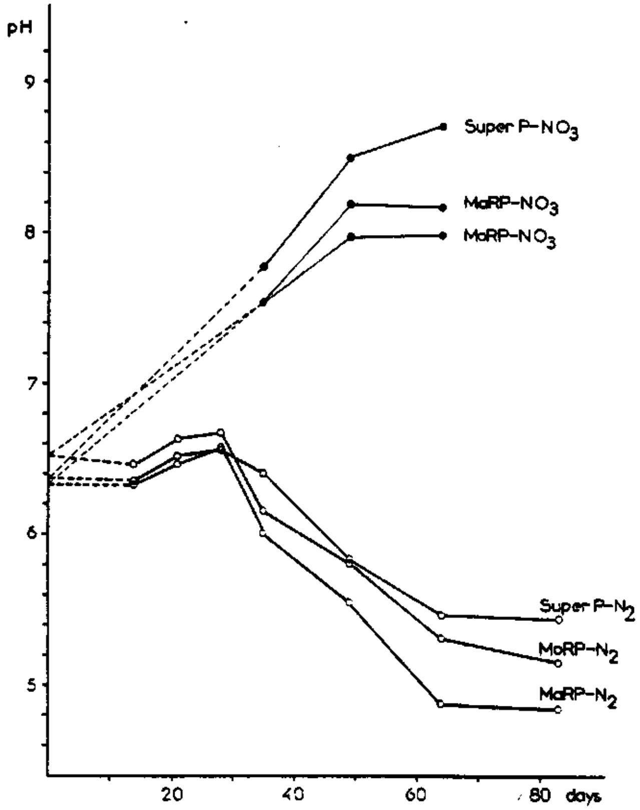

Fig. 11 pH(H_2O) values determined in samples of bulk sand as affected by the type of N-nutrition of soybean.

of approximately 8, found for the sand on which plants grew that depended on rock phosphate-P, is considered to be high enough to form a serious obstacle to the solubilization of rock phosphates. The extremely low level of availability of phosphates in these treatments is borne out by the data presented in Table 8.

The information on acidity and alkalinity generated, as appearing in this table, was obtained from the yield data presented in Fig. 10 and the nutrient contents of Table 7. It can be noticed that the availability of phosphate in the two apatitic sources is strongly dependent on the manner in which the soybean plants acquired their nitrogen. Since an alkaline uptake pattern generates an acid production in the growth medium, and an acidic

Table 8 The effect of variation in mode of N nutrition on the influence exerted by 64-day old soybean plants upon the pH of the sand, leading to variations in P availability and yield of dry matter.

Treatment	N_2 fixed, me/pot	NO_3-N absorbed, me/pot	Acidity generated, me/g d.m.	Alkalinity generated, me/g d.m.	pH(H_2O) of sand	Yield of P, mg/pot	Yield of d.m., mg/pot
SuperP-N_2	13		0.5		5.4	107	7.4
MaRP-N_2	20		0.7		4.9	20	9.6
MoRP-N_2	33		0.7		5.3	38	11.5
SuperP-NO_3		44		0.8	8.7	94	23.4
MaRP-NO_3		19		0.6	8.2	6	8.3
MoRP-NO_3		17		0.4	8.0	5	7.1

uptake pattern generates an alkali production, the numerical consequences of these uptake patterns were presented in the table under the headings "acidity and alkalinity generated". The values of acidity generated by soybean plants utilizing rock phosphate-P are not much lower than the value of 0.8 me/g reported for alfalfa grown in soil (Nyatsanga and Pierre, 1973), and of 1.08 me/g reported for 65-day old soybean plants grown on a N-free medium (Israel and Jackson, 1978).

Each time that plants were harvested, root nodules, if present, were collected separately, counted, weighed, and analyzed for their $N_2(C_2H_2)$ reducing ability. The information on nodule weights, nodule numbers and $N_2(C_2H_2)$-fixing activities is presented in Fig. 12. All three values were found to be highest for the plants that had received Morocco rock phosphate. The values for Mali rock phosphate were lower, possibly as a result of the lower solubility of this rock phosphate. The low values obtained with the use of superphosphate are likely to have been caused by P toxicity.

It is evident from the results of this trial that the acidity generated by the alkaline uptake pattern resulting from N_2 fixation is sufficient to solubilize alkaline rock phosphates in an unbuffered medium like sand. The question remains whether any such effect will also be achieved when soybean plants are grown on a soil. This was the object of investigation in the next experiment.

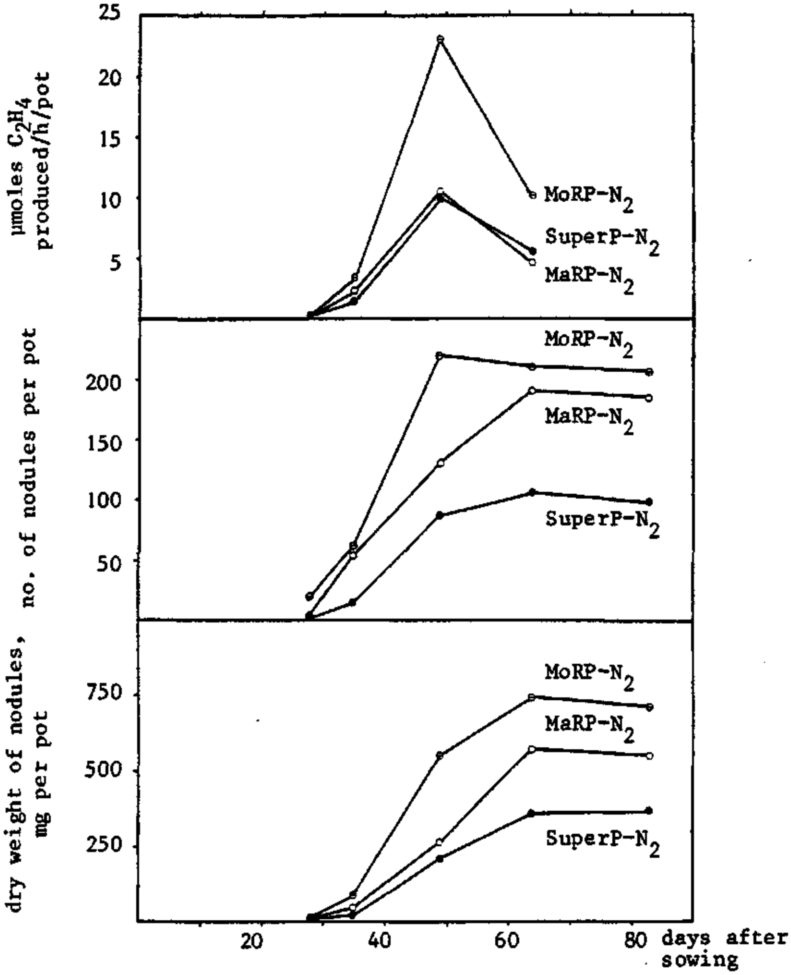

Fig. 12 Nodule weight, number of nodules and $N_2(C_2H_2)$-fixing activity of soybean plants grown on sand, as affected by variation in type of phosphate applied.

4.3 EXPERIMENT 3

The experimental conditions for this experiment were largely similar to those of the previous one, except that the Lunteren subsoil was used as a growth medium. Because of the P toxicity encountered in the previous experiments superphosphate-P was now applied in two quantities, namely 185 mg P (½SuperP treatments) and 370 mg P (SuperP treatments) per pot. The experiment lasted from June 21 to August 7, 1979.

Since the results obtained were not so promising as the ones of the previous experiments, the information on the present experiment will be limited to that included in Table 9.

It can be seen that at the end of the experiment the pH-values in the pots with plants depending on N_2 fixation were lower than those in pots with NO_3-fed plants. The higher yields obtained with superphosphate also resulted in lower pH-values. Apparently, with the readily available superphosphate-P, symbiotic N_2 fixation proceeded more efficiently than when rock phosphate was applied, resulting in lower pH-values. However, the P content of 0.11% P in dry matter of plants growing with the lower quantity of superphosphate applied, indicates that this quantity was too low to maintain a proper availability of P to the plants. In this experiment, no symptoms of P toxicity have been observed.

From the results, it can be reasoned that possibly in this soil with a low level of available P and a relatively high P-fixing capacity, the starter-P needed to initiate an N_2 fixation mechanism is lacking, also when P is applied in rock phosphate form. It might, therefore, be necessary to add a

Table 9 The effect of variation in mode of N nutrition on the growth and P nutrition of soybean plants grown on a sandy loam treated with different forms of phosphate fertilizer.

Treatment	Total N in plants, mg/pot	pH(H_2O) at harvest	Yield of dry matter, mg/pot	Yield of P, mg/pot	% P in dry matter
ZeroP-N_2	144	5.7	9.9	6.7	0.07
½SuperP-N_2	460	5.2	23.8	27.3	0.11
SuperP-N_2	646	5.3	24.7	57.3	0.23
MaRP-N_2	165	5.8	10.0	7.1	0.07
MoRP-N_2	221	5.8	13.3	11.1	0.08
ZeroP-NO_3	199	6.1	5.7	3.3	0.06
½SuperP-NO_3	792	6.2	30.1	26.1	0.09
SuperP-NO_3	801	6.0	38.3	62.9	0.16
MaRP-NO_3	250	6.2	7.0	4.3	0.06
MoRP-NO_3	298	6.4	8.2	5.1	0.06

little superphosphate-P as a starter fertilizer to create conditions under which N_2 fixation can bring about an acidifying effect through which rock phosphate-P can be solubilized. The validity of this assumption was investigated in experiment 6.

The NO_3-fed plants were found to accumulate even less P from soil- and rock phosphate sources than was the case with plants utilizing fixed N_2. The higher pH-values resulting from an acidic uptake pattern apparently exerted an unfavorable influence on the availability of these P sources. Even the plants that had received superphosphate showed P contents that were subnormal.

4.4 EXPERIMENT 4

It became clear from the previous experiments that under the environmental conditions prevailing in these experiments, rock phosphate-P might become available to a short-season crop like soybean only when the medium on which the crop developes has a low phosphate-sorption capacity. Among the tropical legumes, peanut (*Arachis hypogaea*) is often grown on sandy soils, and for that reason it was investigated whether this crop can utilize rock phosphate-P when grown on sand.

This experiment was conducted in a greenhouse of the Department of Tropical Crop Husbandry, which has better facilities to control climatic conditions than have the other greenhouses in which experiments were carried out. The daytime/nighttime temperatures were 28°C/20°C, and the relative humidity was kept at 80%. The experiment lasted from March 14 to April 29, 1980.* The 6-liter pots used contained 6 kg sand on which 5 plants were grown.

The Rhizobium strain used was tested in a preliminary experiment in the Department of Microbiology together with several other strains, all applied to pots on which seedlings of the peanut variety "Matjan" were grown. The strain selected for the present experiment had been found to be the most effective one.

Because of P toxicity encountered in previous experiments with soybean grown on sand, in the present experiment the quantities of P applied were reduced to 400 mg P per 6 kg sand ("SuperP" treatment). In an additional treatment, only 200 mg P per 6 kg sand ("½SuperP" treatment) was applied. The

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*Thanks are due to H.J. Haitsma, who was in charge of this experiment.

relatively large quantity of N contained in peanut seed was considered sufficient to support the early growth of the seedlings. Consequently, no starter-NO_3 was applied to the pots with inoculated seeds. To the pots with NO_3-fed plants, a total of 1600 mg N per pot was applied in periodic dressings.

Of the analytical data obtained, only the most pertinent ones are listed in Table 10.

The results of this experiment were influenced greatly by poor N_2 fixation, caused in the first place by poor nodulation, and in the second place by P toxicity, even there where the superphosphate application had been halved. The data show that in the "superP-N_2" treatments N_2-fixation capacity was low to non-existent, consequently growth was very poor and the influence of the plants on the pH of the sand was erratic. In the "rock phosphate-N_2" treatments, growth was somewhat better, but still seriously inhibited by N shortage. Here, poor nodulation and poor N_2 fixation were not caused by lack of available P, as can be seen from the comparatively high P content and yield-of-P values. For both forms of rock phosphate, these values for yield of P were significantly higher than those obtained with NO_3-fed plants, in spite of the much higher dry-matter yields in the NO_3-treatments.

Table 10 The effect of variation in mode of N nutrition on some characteristics of peanut grown on sand treated with various forms of phosphate fertilizer.

Treatment	Yield of N, mg/pot	N_2-fixation capacity, μM C_2H_4/h/pot	% N in d.m.	pH(H_2O) at harvest	Yield of d.m., g/pot	Yield of P, mg/pot	% of P in d.m.	$\Sigma C_a - \Sigma A_a$, me/bg
ZeroP-N_2	150	2.8	1.3	4.7	11.8	10.6	0.09	690
SuperP-N_2	104	0.0	2.3	6.1	4.5	91.4	2.03	1030
½SuperP-N_2	118	0.2	1.5	5.1	7.9	100.8	1.28	690
MaRP-N_2	195	8.5	1.3	5.0	15.5	69.7	0.45	1100
MoRP-N_2	246	9.9	1.6	5.5	15.3	85.3	0.56	940
ZeroP-NO_3	388		2.4	5.4	15.9	11.8	0.07	370
SuperP-NO_3	819		1.4	5.2	58.5	255.4	0.44	-140
½SuperP-NO_3	737		1.1	4.9	68.9	196.3	0.28	140
MaRP-NO_3	702		2.1	5.2	33.1	29.7	0.09	-190
MoRP-NO_3	805		1.7	5.7	46.8	62.3	0.13	100

Much better yields were obtained in the "superP-NO_3" treatments. Obviously, in these treatments, neither nitrogen nor phosphorus was limiting growth. It can be deduced from the data presented that excessive uptake of P in legumes is harmful mainly in that it inhibits proper nodulation and N_2 fixation. The plants in the "½superP-NO_3" treatment absorbed 92% of the phosphate applied which even for a sand-culture experiment is an excessively high value. Even more remarkable is the P-recovery value of 45% in the "½superP-N_2" treatment, considering the fact that all this P had accumulated in only 7.9 g of dry matter. There is reason to postulate again that in cases of low availability of anionic nutrients, due to absence of NO_3^-, seed legumes display a remarkable ability to absorb phosphate, even to the extent that it leads to self-destruction.

It is interesting to note that plants having NO_3^- as sole N source displayed an uptake pattern which was close to neutral ($\Sigma C_a - \Sigma A_a$ values in Table 10 not deviating widely from the zero-value). Consequently, a pH-rise, as experienced with NO_3-fed soybean plants, was not observed for NO_3-fed peanut plants.

It was unfortunate that in this experiment N_2 fixation proceeded so poorly that it prevented a proper testing of the ability of peanut to utilize rock phosphate-P. However, it was again evident that the availability of this rock phosphate-P was better for plants depending on fixed N_2 than for NO_3-fed plants.

4.5 EXPERIMENT 5

In comparison with soybean, alfalfa has the advantages of possessing a denser root system and of being a perennial crop that can exert its influences on the soil over a much longer period. In the experiment to be discussed, alfalfa was therefore grown on 2.5-l pots, filled with the Lunteren sandy loam subsoil, to examine whether this crop can exert a longer-lasting acidifying effect strong enough to overcome the capacity of this soil to buffer pH changes.

The experiment was initiated on June 21, 1979 and lasted for 210 days. In this period, 5 cuttings were collected. Each time after a cutting, the plants were allowed to regrow until in flower. At the final harvest, the root systems were used for measurements of nitrogenase activity and were also processed for chemical analysis.

The usual phosphate treatments were applied in quantities of 370 mg P per pot. In each phosphate treatment, the 8 plants in one-half of the pots were inoculated with the *Rhizobium meliloti* strain K24. Each of these pots also received 5 me NO_3-N in $Ca(NO_3)_2$ form as a starter fertilizer. The remaining pots with NO_3-fed plants received periodic applications of $Ca(NO_3)_2$ up to a total of 1750 mg N per pot.

The values on production of dry matter over the 210-day period are presented in Fig. 13. The results shown in this figure can best be viewed in combination with other information supplied in the Tables 11 and 12 and in Fig. 14. In the former table, the analytical data on plant material of 3 harvests are presented. As mentioned before, in the final harvest also root material was included. With the use of the basic data on plant material of each harvest separately, total quantities of cationic and anionic nutrients absorbed could be calculated. These data, in turn, were used for a calculation of acidity or alkalinity generated per unit quantity of plant material,

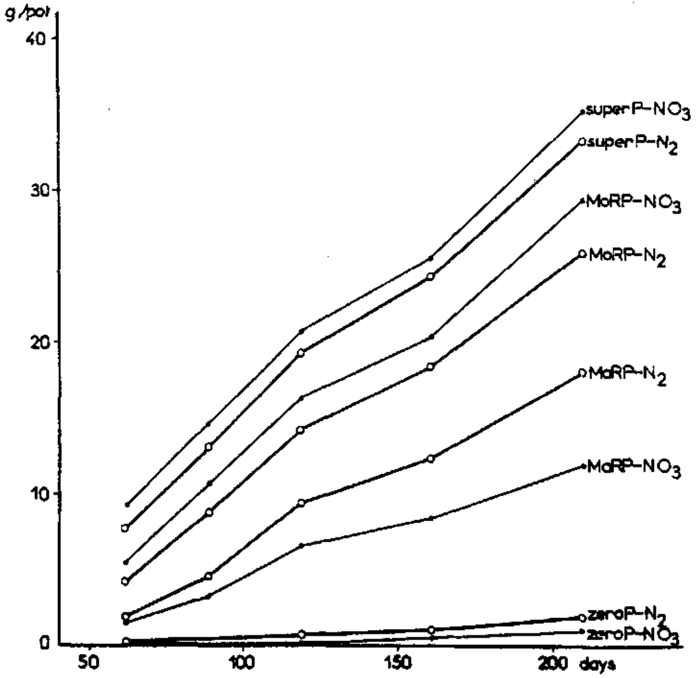

Fig. 13 Cumulative yields of dry matter of alfalfa grown on a sandy loam in a pot experiment.

Table 11 Chemical composition of alfalfa plants grown on a sandy loam with three forms of phosphate fertilizer applied, with and without the availability of symbiotically fixed N_2.

Harvest no. and treatment	Yield of d.m., g/pot	Contents of nutrients, me/kg dry matter									
		N	Na	K	Ca	Mg	H_2PO_4	Cl	NO_3	SO_4	ΣC-ΣA
Harvest III											
ZeroP-N_2	0.7	1914	0	916	1260	694	41	56	8	118	2647
SuperP-N_2	4.8	2331	2	674	467	346	96	52	2	44	1295
MaRP-N_2	4.8	2011	7	846	817	568	52	75	1	77	2033
MoRP-N_2	5.4	2177	0	733	704	505	57	71	0	65	1749
ZeroP-NO_3	0.0										
SuperP-NO_3	6.4	2489	9	583	814	313	83	47	120	41	1428
MaRP-NO_3	3.2	2574	9	691	1007	381	50	69	217	38	1714
MoRP-NO_3	6.0	2207	7	660	1018	391	53	58	116	43	1806
Harvest IV											
ZeroP-N_2	0.3	1984	9	680	1540	1180	34	71	0	348	2956
SuperP-N_2	5.0	2643	4	554	713	551	103	56	0	68	1595
MaRP-N_2	2.9	2287	8	702	1238	871	65	104	0	190	2460
MoRP-N_2	4.1	2512	2	676	896	673	75	84	0	95	1993
ZeroP-NO_3	0.2	3153	45	494	1724	1290	24	132	634	144	2619
SuperP-NO_3	4.8	2478	7	594	1100	430	93	52	77	96	1813
MaRP-NO_3	1.8	2985	6	528	1649	596	56	89	336	103	2195
MoRP-NO_3	4.1	2556	0	642	975	650	65	86	115	104	1897
Harvest V											
shoots											
ZeroP-N_2	0.9	1898	20	760	1210	1202	33	94	26	454	2585
SuperP-N_2	8.6	2076	16	599	705	555	77	242	1	135	1420
MaRP-N_2	5.7	1833	7	724	1033	1011	55	240	0	207	2273
MoRP-N_2	7.4	1971	25	642	906	681	58	201	0	136	1859
ZeroP-NO_3	0.6	3153	45	494	1724	1290	24	132	634	144	2619
SuperP-NO_3	9.5	2083	14	562	1102	397	65	139	111	144	1616
MaRP-NO_3	3.7	2394	10	545	1625	635	37	129	324	93	2232
MoRP-NO_3	8.8	2050	15	598	1515	553	45	128	121	105	2282
roots											
ZeroP-N_2	2.7	1607	14	358	130	494	21	62	3	102	808
SuperP-N_2	11.4	2210	64	311	121	273	120	116	0	69	464
MaRP-N_2	8.9	1745	21	381	127	335	64	116	0	88	596
MoRP-N_2	13.9	2052	38	490	135	321	82	115	0	59	728
ZeroP-NO_3	1.6	2710	2	455	210	566	17	43	159	64	950
SuperP-NO_3	14.9	1985	36	546	398	337	84	73	51	102	1007
MaRP-NO_3	6.6	2488	1	544	231	411	25	41	182	52	887
MoRP-NO_3	15.2	2005	7	653	270	411	41	70	49	73	1108

and these values together with other pertinent data are presented in Table 12.

The yield data on the superphosphate treatments, considered in combination with the N contents listed in Table 12, show that under conditions of

Table 12 The effect of acidity and alkalinity generated by alfalfa plants utilizing different N sources, on the pH of the soil and on the availability of rock phosphates.

Treatment	Yield of d.m., g/pot	µmol C_2H_4/h/pot	Acidity generated, me/g d.m.	Alkalinity generated, me/g d.m.	pH(H_2O) of soil	Org. N in plants, mg/pot	Yield of P, mg/pot
ZeroP-N_2	4.7	0.5	0.5		6.2	98	4
SuperP-N_2	44.5	12.2	1.0		5.1	1190	138
MaRP-N_2	26.9	4.5	1.4		5.3	728	49
MoRP-N_2	39.9	9.1	1.2		5.3	1218	85
ZeroP-NO_3	2.5	0.0		1.1	6.3	84	1
SuperP-NO_3	49.9	0.0		0.8	7.5	1484	122
MaRP-NO_3	18.8	0.0		0.8	7.3	616	23
MoRP-NO_3	44.4	0.0		0.5	7.4	1316	90

high phosphate availability symbiotically fixed N could support a growth rate not falling far behind that obtained with an ample supply of NO_3-N. A comparable picture arises from the data concerning Morocco rock phosphate. Here, the availability of the phosphate was lower, but not so low that growth of the NO_3-fed plants was seriously hampered by P deficiency, in spite of the rise in soil pH caused by the acidic uptake patterns of these plants.

For the plants utilizing P from the Mali rock phosphate source, the picture is different. Here, the rise in soil pH induced by the acidic uptake pattern of the NO_3-fed plants lowered the solubility of the rock phosphate-P to such an extent that plant growth was hampered primarily by P deficiency. The plants making use of fixed N_2 were able to lower the soil pH enough to mobilize a portion of the Mali rock phosphate so that values on yield of dry matter and yield of P were definitely higher than those of the NO_3-fed plants (Figs. 13 and 14). In spite of the comparatively low quantity of N_2 fixed in the "MaRP-N_2" treatment, the reduction in soil pH for this treatment was equal to that encountered in the "MoRP-N_2" treatment, possibly as a result of the higher quantity of acidity generated per unit dry matter produced in the former treatment.

Although the dry-matter yields in the "Zero-P" treatments were very low, the differences between the two treatments serve to show that symbiotic N_2 fixation could also induce the mobilization of some of the sparsely present soil phosphate. The pH-values of the soil in these two treatments hardly

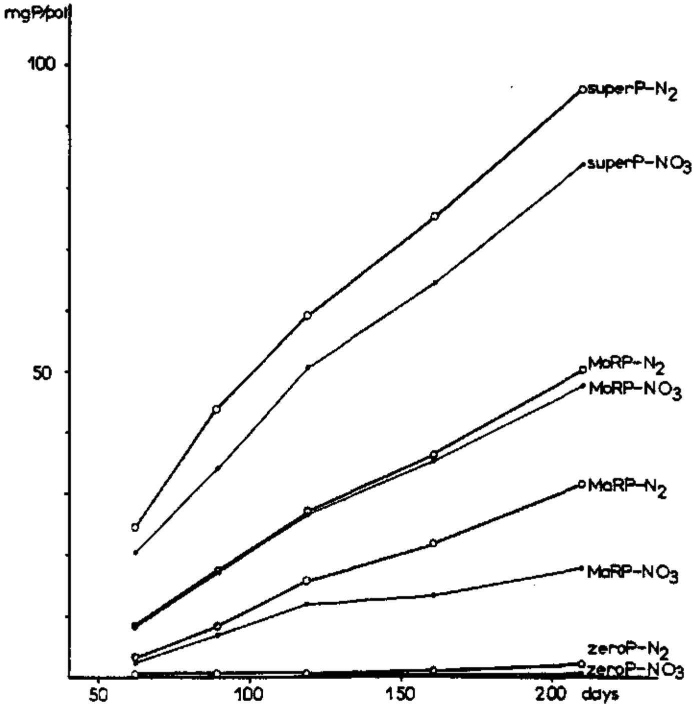

Fig. 14 Cumulative yields of P in alfalfa grown on a sandy loam in a pot experiment.

differ, but it is not unlikely that in these two cases of very poor root growth, measurements of the pH of the bulk soil were not representative of pH-values in the rhizosphere.

It can be observed that the acidity generated by alfalfa plants making use of fixed N_2 and supplied with fertilizer P was responsible for a pH decline in the soil of somewhat more than 1 pH unit (pH of fallow soil: 6.4), whereas a pH increase of approximately 1 pH unit resulted from the alkalinity generated by the NO_3-fed plants. These findings clearly indicate that imbalances in the uptake of cationic and anionic nutrients by plants can cause considerable pH changes not only in unbuffered media like nutrient solutions and sand, but also in soils.

4.6 EXPERIMENT 6

The chain of events and circumstances required to eventually bring about a solubilization of rock phosphate is thought to be as follows: a. initially present plant-available phosphate enables leguminous seedlings to develop and to form nodules in which symbiotic N_2 fixation can take place; b. such an N_2 fixation allows the host plant to grow also when little or no NO_3-N is available; c. the absence of NO_3^- induces an uptake pattern in which the quantity of cationic nutrients absorbed exceeds that of anionic nutrients absorbed; d. this alkaline uptake pattern is responsible for a certain degree of acidification of the medium on which the legume grows; e. the acidity generated promotes the solubilization of alkaline rock phosphates; f. the phosphate made available enables the legume to keep on growing also when the source of initially available phosphate has been exhausted.

Earlier, mention was made of the possibility that the absence of initially available phosphate might prevent this chain of reactions from being set into motion. In other words, a priming effect of a limited quantity of plant-available phosphate may be needed to enable leguminous plants to create conditions under which sparsely soluble rock phosphate can be mobilized.

The objective of the present experiment, therefore, was to examine the effect of a small quantity of superphosphate added to sand or soil as a starter fertilizer upon the extent to which rock phosphates were utilized by soybean plants. For this purpose, in one-half of the pots of this experiment only rock phosphate was applied to sand or soil, whereas in the other half of the pots, application of rock phosphate was combined with that of a ^{32}P-labeled quantity of easily soluble P in the form of KH_2PO_4. To avoid complications arising from isotopic exchange, the two sources of phosphate were kept apart as much as possible. To achieve this, quantities of 370 mg P in rock phosphate form were mixed with 2-kg quantities of moist sand or soil which were placed in the pots first. This material was covered with a 200-g layer of moist sand or soil to which no P was added. Above this layer, a perforated plastic tube with a 3-cm diameter, used for daily additions of water to the pots, was placed in the remaining quantity of 500 g of dry sand or soil added to complete the filling of the pots.

At the start of the experiment, the labeled quantity of P as KH_2PO_4 (40 mg P to sand and 100 mg P to soil) dissolved in water, was added by pouring the solution into the plastic tube. The KH_2PO_4 was expected to be retained mainly by the dry sand or soil surrounding the plastic tube. The first roots of the germinating soybean plants were expected to mainly utilize this labeled P source which was meant to enable these plants to build up an N_2-fixation apparatus needed to induce acidification of the growth medium and solubilization of rock phosphate.

All pots received 5 me NO_3-N in $Ca(NO_3)_2$ form as a starter fertilizer. To the pots with NO_3-fed plants, periodic dressings of $Ca(NO_3)_2$ brought the total N applied to 70 me per pot. The other pots received 50 me Ca as $CaSO_4$.

The sowing date was June 3, 1980. Plants were harvested 14, 28, 42 and 70 days after sowing. The above-ground material was analyzed for total N, total P and P absorbed from the labeled source.

The disadvantage of low solar irradiation was particularly noticeable in this experiment. Safety regulations ruled out the possibility of aerating the greenhouse during periods of bright sunshine. The lack of air conditioning made it necessary to make use of a device with which screens along the roof of the greenhouse were automatically drawn as soon as radiation exceeded the level of 300 Wm^{-2}. As a result, the plants were somewhat etiolated.

The most pertinent information on the effect of the presence of a starter-P fertilizer is presented in Table 13. Here, mention is made of fresh weight produced and of quantities of nutrients accumulated in a period of

Table 13 Quantities of fresh material produced and N and P accumulated over a 56-day period by soybean plants grown on sand or soil and utilizing either one of two sources of N and two sources of P.

Treatment	Fresh weight produced, g/pot		Nitrogen accumulated, mg/pot		P absorbed from starter KH_2PO_4, mg/pot		P absorbed from rock phosphate, mg/pot	
	sand	soil	sand	soil	sand	soil	sand	soil
MaRP-N_2	47	15	232	80			24.3	1.5
MaRP-H_2PO_4-N_2	47	49	400	360	27.9	19.4	30.4	2.5
MaRP-NO_3	24	12	205	150			3.2	0.6
MaRP-H_2PO_4-NO_3	102	70	642	491	32.4	22.1	8.3	3.2
MoRP-N_2	56	28	304	151			30.9	6.1
MoRP-H_2PO_4-N_2	57	48	447	411	26.1	19.6	43.9	6.1
MoRP-NO_3	43	15	326	171			6.4	1.5
MoRP-H_2PO_4-NO_3	107	76	622	559	30.6	24.3	12.3	2.6

56 days. These quantities are arrived at by subtracting the values obtained with 14-day old plants from those obtained with 70-day old plants. It was verified that during the first 2 weeks of growth, the plants developed mainly on nutrients present in the seed and on the 5 me NO_3-N applied. Hence, data listed in Table 13 pertain to quantities of nutrients absorbed from the growth medium or quantities of N_2 fixed and to quantities of fresh material produced by utilizing these nutrients.

The results obtained with the plants that had not received any starter-P fertilizer confirmed the information supplied by Experiment 2: on sand, the plants utilizing fixed N_2 grew better than the NO_3-fed plants on account of more rock phosphate-P being mobilized as a result of acidification of the growth medium; on soil, the beneficial influence of N_2 fixation on rock phosphate-P mobilization was noticeable for the Morocco rock phosphate only.

The data on P absorbed by the soybean plants from the starter KH_2PO_4 fertilizer show that high percentages of the 40-mg P quantities added to the sand were indeed absorbed by the plants. However, from the soil to which 100-mg P quantities had been added as KH_2PO_4, the plants absorbed much lower percentages of added starter P, which again attests to the high P-fixation capacity of the Lunteren subsoil used.

Plants growing on sand and depending on fixed N_2 were found to utilize significantly more P from the Mali and Morocco rock phosphate in the presence than in the absence of starter KH_2PO_4 (30.4 mg P vs. 24.3 mg P and 43.9 mg P vs. 30.9 mg P, respectively).

Examination of comparable data for NO_3-fed plants reveals that also for these plants addition of starter KH_2PO_4 improved the utilization of rock phosphate-P. In these cases, however, the much better growth of the plants that had received starter KH_2PO_4 is likely to have improved contact between roots and rock phosphate to the extent that the rock phosphate sources became more accessible.

When the plants were grown on soil, no improvement in utilization of rock phosphate-P was obtained from adding starter KH_2PO_4. Again, fixation of P by soil constituents is thought to have been responsible for this finding.

The results obtained in this experiment show that indeed the presence of a small quantity of starter P can be helpful in initiating processes by which sparsely soluble rock phosphates can be mobilized. In the present experiment, the positive effect of the starter P was confined to cases in which plants were grown on sand. It is not unlikely, however, that under more favorable light conditions, the positive effect will manifest itself also in cases of plants grown on soil.

4.7 EXPERIMENT 7

This experiment[*] deals with the influences that vesicular-arbuscular mycorrhiza (VAM) can exert on the utilization by legumes of soil- and fertilizer phosphate needed to build up an N_2-fixing apparatus. In a sense, here again it appears justified to contemplate the possible presence of a priming effect, this time exerted by VAM: when through the action of VAM a legume can utilize some phosphorus needed to set into motion an N_2-fixation process, the soil acidification indirectly resulting from this N_2 fixation may induce a further mobilization of soil- and/or fertilizer phosphate needed to sustain legume growth and N_2 fixation over a longer period. The reasoning is that possibly the role of the starter-P fertilizer, as discussed in section 4.6, can be taken over by VAM.

Furthermore, it is of interest to know whether the solubilization of rock phosphate-P, as induced by N_2 fixation, can also be obtained when VAM and legume operate as symbionts, but this time with an ample quantity of NO_3-N available to sustain legume growth.

Finally, it can be reasoned that possibly the combined actions of nodulated legume and VAM can lead to a more complete solubilization of rock phosphate-P than is obtained with the single actions of VAM and legume separately.

In this experiment, alfalfa, var. DuPuits was used as test crop, grown on the sandy loam from Lunteren in 2.5-l pots (10 plants per pot). The experiment was initiated on August 1, 1980 and lasted for 222 days. In this period 5 cuttings were taken. At the time of the final harvest, the roots were also removed from the columns of soil.

The PxN treatments were identical to those of Experiment 5: zero-P, superP, MaRP and MoRP, combined with non-inoculated and inoculated alfalfa plants. In each of the resulting treatment combinations, a further differentiation was made in pots that had or had not received VAM. Hence, a total of 16 treatments resulted, with each treatment represented by 3 replicate pots.

The VAM inoculum was obtained from the Department of Phytopathology. The

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[*] The cooperation of L.E.C.R. Paquay, who was in charge of this experiment, is gratefully acknowledged.

species used was *Glomus mosseae*, present in tomato roots of which a quantity of 1.5 g was mixed with the soil of each pot belonging to a VAM treatment.

At the start of the experiment, all pots received 5 me NO_3-N as starter fertilizer in the form of $Ca(NO_3)_2$. Periodically, additional $Ca(NO_3)_2$ was added to pots with NO_3-fed plants up to a total of 140 me NO_3-N. To compensate for the quantity of Ca thus applied, the remaining pots received 100 me Ca as $CaSO_4$, all applied as a basal dressing. The plants of these pots were inoculated with *Rhizobium meliloti*, as described before. Whenever phosphate fertilizer was applied, the quantity of P per pot was 370 mg.

The most pertinent data obtained in this experiment are presented in Table 14. All yield data appearing in this table apply to measurements conducted on samples taken from pooled plant material of 5 harvests, including the roots harvested after the final cutting. A discussion of these data can best take place in association with the information supplied in the graphs of Fig. 15. The yield data of the final harvest shown in these graphs pertain to above-ground material only.

From the data of Table 14 and Fig. 15 it can be deduced that lack of available soil P prevented Rhizobium from inducing an alkaline uptake pattern through which enough acidity could be generated to mobilize the sparsely present soil P. It is evident that, when NO_3-N was applied, VAM succeeded in mobilizing a sizeable quantity of soil P. From the much lower yield of the RM treatment, it can be concluded that the ability of VAM to mobilize soil P was insufficient to meet the requirement of both the host plant and Rhizobium. Judging from the combination of low P content of plant material and low dry-matter yield, P deficiency was most severe in the "NO_3 -only" plants (control treatment). Due to poor growth of the alfalfa plants depending on fixed N_2, the acidifying effect exerted by these plants on the soil was relatively small.

The picture presented in Fig. 15B differs strongly from that of the previous one. It can be observed that the various yield levels were determined primarily by the degree of availability of nitrogen. Such, of course, is to be expected when use is made of superphosphate as P source. In spite of the absence of a P shortage, symbiotic N_2 fixation did not proceed rapidly enough to allow the plants depending on fixed N_2 to maintain a growth rate similar to that of the NO_3-fed plants. Poor light conditions may be assumed to have been responsible for the difference, although it must be noted that such an assumption is not supported by lower N contents of the plants depending on fixed N_2. In other words, there are no signs that, as a result of N shortage, the plants

Table 14 Growth and chemical composition of alfalfa plants, as influenced by variations in form of P fertilizer applied and in manner of N nutrition, with and without the presence of VA mycorrhiza.

Treatment	Yield of d.m., g/pot	Yield of N, mg/pot	Yield of P, mg/pot	% N in d.m.	% P in d.m.	Final pH(H_2O) in soil	$\Sigma C_a-\Sigma A_a$, me/g d.m.
ZeroP-N_2-VAM	9.1	427	20	4.7	0.22	6.2	0.93
ZeroP-N_2	4.6	238	10	5.2	0.22	6.2	0.92
ZeroP-NO_3-VAM	18.9	786	42	4.2	0.22	7.2	-1.25
ZeroP-NO_3	3.1	190	5	6.1	0.16	- *	-1.22
SuperP-N_2-VAM	32.9	1334	185	4.1	0.56	5.1	0.76
SuperP-N_2	30.8	1258	166	4.1	0.53	4.9	0.88
SuperP-NO_3-VAM	43.4	1658	200	3.8	0.47	7.2	-1.20
SuperP-NO_3	43.3	1704	204	3.9	0.47	-	-1.29
MaRP-N_2-VAM	32.9	1245	205	3.8	0.62	5.1	1.09
MaRP-N_2	23.1	976	124	4.2	0.54	4.8	1.20
MaRP-NO_3-VAM	30.6	1312	138	4.3	0.45	7.4	-1.28
MaRP-NO_3	21.6	1034	78	4.8	0.36	-	-1.16
MoRP-N_2-VAM	30.0	1238	121	4.1	0.40	5.2	1.06
MoRP-N_2	30.3	1227	129	4.0	0.43	5.1	1.14
MoRP-NO_3-VAM	32.7	1314	93	4.0	0.28	6.9	-1.12
MoRP-NO_3	33.5	1414	102	4.2	0.30	-	-1.05

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*The soil of the NO_3-N treatments without VAM was erroneously discarded before pH measurements had been made. It is to be expected that the pH values of the soil in these treatments did not differ much from those in the NO_3-N treatments with VAM, as evidenced by the similarities of the pertaining ($\Sigma C_a-\Sigma A_a$) values.

started to redistribute the N already accumulated in order to maintain growth at the expense of N concentration. There are numerous indications in the literature of the occurrence of such dilution phenomena. The absence of it in this case also led to higher P contents than obtained in the NO_3-fed plants.

The situation arising from the use of Mali rock phosphate is different again (Fig. 15C). Here, the influence of VAM was very dominant: when it was present, the yields were significantly higher, irrespective of the form in

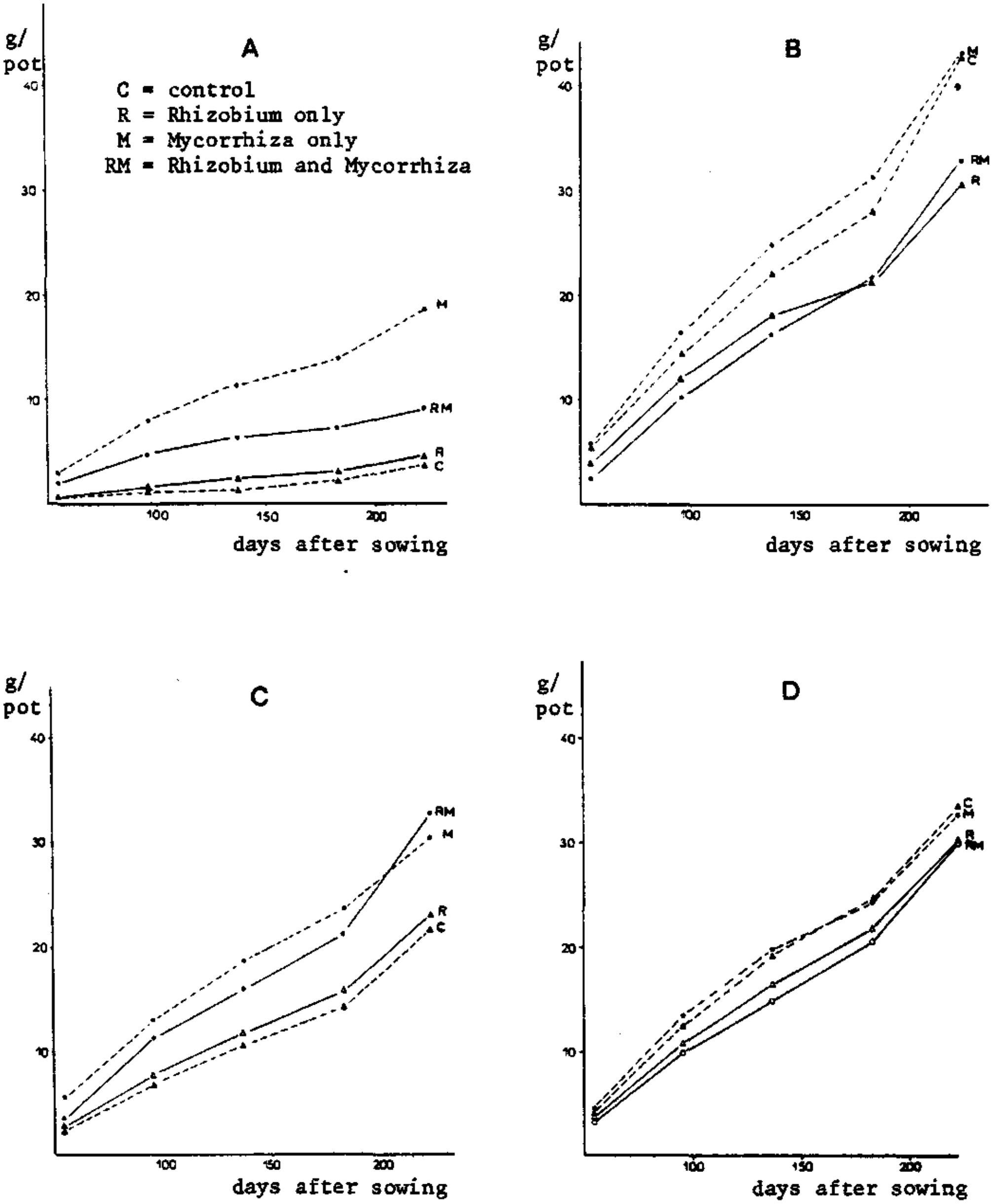

Fig. 15 Cumulative yields of dry matter of alfalfa grown on a sandy loam, without phosphate applied (A), with triple superphosphate (B), with Mali rock phosphate (C), and with Morocco rock phosphate (D), as affected by the activities of Rhizobium and VA mycorrhiza.

which N was obtained by the plants. In spite of the sharp pH decline observed in the "MaRP-N_2" treatment, the mobilization of rock phosphate-P was insufficient to satisfy the requirements of both alfalfa plant and Rhizobium. Consequently, in spite of a higher value for yield of P in this treatment in comparison with the "MaRP-NO_3" treatment, inadequate N_2 fixation prevented the yield of dry matter in the former treatment from rising above that of the latter treatment.

The picture changed when VAM was present: in spite of the pH rise encountered for the pots with NO_3-fed plants, VAM apparently succeeded in mobilizing enough P to allow these plants to grow better than those of the "MaRP-NO_3" treatment. Apparently, the increase in P-absorbing surface area, brought about by VAM, was more than enough to overcome the disadvantage of a pH rise brought about by the acidic uptake pattern of the NO_3-fed plants. As for the plants depending on N_2 fixation, it can be seen that the combined effects of Rhizobium and VAM on the availability of the rock phosphate-P was sufficient to allow the plants in the "MaRP-N_2-VAM" treatment to grow as well as those in the "MaRP-NO_3-VAM" treatment.

In fact, when the growth of the plants in the "MaRP-N_2-VAM" treatment between the third and the fourth harvest is compared with that of the plants in the "superP-N_2-VAM" treatment (Fig. 15B), it can be seen that there is no difference. This means that in the latter part of the growth period, which was the early spring of 1981 when light conditions became appreciably more favorable than in the preceding winter period, alfalfa plants living in symbiosis with both Rhizobium and VAM could make use just as well of Mali rock phosphate as they could of superphosphate.

The dry-matter yields in the treatments with Morocco rock phosphate were all alike (Fig. 15D). The yields of the plants depending on N_2 fixation were similar to those obtained with superphosphate, which indicated that under the conditions prevailing in this experiment the acidifying effect arising from N_2 fixation was sufficient to mobilize enough Morocco rock phosphate to meet to requirements of both host plant and Rhizobium. Any further contribution of VAM in this respect was not reflected in higher dry-matter yields, probably because N_2 fixation and not P availability was the factor primarily limiting yield.

There is no ready explanation to be given for the observation that for NO_3-fed plants the presence of VAM did not raise the availability of rock phosphate-P, contrary to what was found with the use of Mali rock phosphate.

Returning to the considerations advanced in the early part of this section, it can be remarked that indeed in the case of Mali rock phosphate, the P-solubilizing effect, as arising from N_2 fixation, could also be obtained with the presence of VAM in pots with NO_3-fed plants. Furthermore, for both the "ZeroP" and "MaRP" treatments, it could be observed that the combined effects of N_2 fixation and VAM activity led to a higher degree of P mobilization than was obtained with either one of the two effects separately.

4.8 EXPERIMENT 8

The last experiment to be discussed here will be one in which it is verified that gramineous plants largely lack an ability to utilize rock phosphate-P as was shown to be present in legumes depending on symbiotically fixed N_2. For this purpose, maize was grown (3 plants per pot) on the 2.5-1 pots filled with the Lunteren subsoil. The experiment lasted from June 21 to July 23, 1979.

The quantity of P added per pot was 370 mg. The forms were again triple superphosphate, Mali rock phosphate or Morocco rock phosphate. In an additional treatment, the quantity of superphosphate-P added was reduced to 185 mg P per pot. Nitrogen was added in the form of $Ca(NO_3)_2$ in periodic dressings up to a total of 600 mg N per pot.

The most relevant results are presented in Table 15. It can be seen that both the yields of dry matter and of P obtained with the use of the rock phosphates were practically similar to those of the "zero-P" treatment. Only with superphosphate a reasonable growth could be achieved, although the low P con-

Table 15 The response of maize to three forms of phosphate fertilizers used in a pot experiment.

Treatment	Yield of dry matter, g/pot	pH(H_2O) of soil at harvest	Yield of P, mg/pot	% P in dry matter
ZeroP	14.5	6.2	9.9	0.07
½SuperP	40.4	6.1	37.5	0.09
SuperP	42.5	6.0	69.8	0.16
MaRP	15.1	6.2	10.7	0.07
MoRP	16.1	6.2	12.0	0.07

tent of the plant material in the "½Super" treatment would probably have resulted in poor ear formation, if the plants had been allowed to develop into a generative stage. This was prevented on account of the fact that at the time of harvest the plants in the other treatments were on the verge of dying from lack of P. The short duration of the growth period was likely to be also responsible for a lack of any influence of the plants on the pH of the soil.

The results of this experiment make it quite clear that without an acidifying effect, such as brought about by legumes when utilizing symbiotically fixed N_2, the availability of the two rock phosphates used in these experiments is quite poor.

5 CONCLUSIONS

Gradually, the traditional concept of plant roots acidifying their environment is losing ground to a more diversified view on the effects plants have on the pH of the soil on which they grow. There is a growing recognition of the fact that gramineous plants are responsible for a rise in pH of the soils in which their root systems develop. Such a pH-raising effect, however, is usually not sufficient to neutralize the acidification brought about by the nitrification of ammonium applied in fertilizer form and/or arising from the mineralization of soil organic nitrogen.

Soil acidification resulting from nitrification of ammonium is avoided when soil pH is low enough to inhibit the activities of nitrifying bacteria. Under such conditions, ammonium is the dominant form of plant-available nitrogen and, hence, gramineous plants, absorbing the majority of their N as NH_4^+, will indeed conform to the traditional concept of plants acidifying their soils. However, soil pH values at which such a situation prevails, are certainly too low for optimum growth of cereals like wheat and barley, and in many cases the same applies to maize.

Consequently, the conclusion will be that through their acidic uptake pattern cereals will bring about only a partial neutralization of the acidity arising from the nitrification of ammonium applied as fertilizer. If, however, fertilizer-N is applied in nitrate form, e.g. as $Ca(NO_3)_2$, it can be expected that the pH-raising effect induced by the acidic uptake pattern of cereals will be more than enough to overcome the slightly acidic effect exerted by the $Ca(NO_3)_2$, provided that a major portion of the NO_3 is absorbed by the crop and thus is prevented from being lost due to leaching.

In the realm of dicotyledonous plants, the picture is more diversified. Here, we find plants that in the case of nitrate nutrition show a neutral uptake pattern resulting in an absence of any influence exerted on the pH. Among the leguminous plants, peanut was found to belong in this category (Experiment 4). Other legumes, such as soybean and alfalfa, were found to show an acidic uptake pattern (Experiments 2 and 5),which puts these plants in the same category as in which the cereals are to be placed.

As was said earlier, the category of cultural plants exhibiting an alkaline uptake pattern and thus acidifying their soils even when N is absorbed as NO_3^-, is thought to be small. The findings of the experiments presented in this dissertation have confirmed earlier reports on the acidifying action of legumes once they have become dependent on fixed N_2. Although, in essence, this characteristic is to be distinguished from that of e.g. buckwheat, which crop acidifies its soil even when N is absorbed as NO_3^-, the practical consequences are identical: these plants share an ability to acidify their soils.

Phosphate is known to be a nutrient whose displacement in soil is usually counted in distances of a few millimeters. It is therefore considered important for plants to develop root systems that are sufficiently dense and widespread to enable them to build up an intimate contact with phosphate present in the soil. Nevertheless, the quantity of phosphate potentially available to a crop may not exceed the quantity of phosphate which once or more frequently during a growing season is located within that portion of a soil which is penetrated by root hairs at that moment actively involved in the absorption of nutrients. If, next to establishing contact between soil phosphate and plant, the absorbing roots can also affect the soil pH in such a way that the solubilization of soil- and fertilizer phosphate is improved, it is to be expected that the availability of these phosphates is enhanced. Such an enhancement is to be expected when roots of leguminous plants absorb more cationic than anionic nutrients from a soil containing most of its P in the form of calcium phosphates.

In some respects, the conditions under which the experiments discussed in this dissertation were conducted, were made as favorable as possible for a mobilization of rock phosphates added. When sand was used as growth medium, it was expected that any effect exerted by the plants on the pH of the sand would not be obscured by a high pH-buffering capacity of the medium. When the sandy loam subsoil was used, it was chosen for its combination of relatively low pH-buffering capacity and relatively high P-sorption capacity, but by previous liming the pH was adjusted to a level considered favorable for the growth of most legumes, yet not as high as to create a situation in which any effect on the pH would be neutralized by the solubilization of free calcium carbonate present. Under these experimental conditions, it was found that a relatively short-season, annual crop like soybean succeeded in mobilizing rock phosphate-P when grown on sand, but not when grown on soil, and that a perennial crop like alfalfa succeeded in mobilizing rock phosphate-P even when grown on soil.

One other factor which was optimalized in the present experiments was the contact between plant and soil. It is not to be expected that under field conditions this contact will be as intimate as encountered in a pot experiment. Nevertheless, under field conditions the contact between plant and phosphate fertilizer can be improved by placing the phosphate in a band. It is not customary to practice band placement in cases of rock phosphate application, but in view of the present finding that the mobilization of rock phosphate-P is likely to be enhanced by an intensification of the contact between plant and phosphate, it is certainly worthwhile to investigate in future field experiments whether the usefulness of rock phosphates to legumes is improved by placing the phosphates in bands close to the plant rows.

One condition which in the present experiments was certainly not ideal for optimal rock phosphate-P utilization was light intensity. For optimal utilization of the benefits originating from the acidifying effects of legumes with respect to rock phosphate mobilization, conditions for symbiotic N fixation should be as favorable as possible. In this regard, one of the main requirements of the symbiotic N-fixation process is an adequate availability of photosynthates which can be ascertained by proper lighting conditions. The experiments described in chapter 4 were conducted in the years 1978, 1979 and 1980 under greenhouse conditions. During the summers of all three years, light intensity in the Netherlands was below normal. It is therefore certain that in none of the experiments the ability of legumes to mobilize rock phosphates has been put to full use. Experimentation under field conditions in countries with higher average solar radiation than encountered in the Netherlands might yield a more realistic picture of the ability of legumes to mobilize rock phosphates.

Next to suboptimal light intensity, a lack of a small quantity of immediately available soil phosphate may have prevented a normal development of the Rhizobium population. The results obtained with alfalfa indicate that the combination of low solar radiation and low soil phosphate availability formed no obstacle to the creation of conditions under which rock phosphate could be mobilized. Aside from the advantage that alfalfa is a slow starter which does not have a great immediate need of P, its dense root system supplies the additional advantage that this crop might have been able to utilize the sparsely present native soil P and might have competed with the P-fixing soil constituents for P released by the added rock phosphates. The finding that alfalfa was capable of utilizing Morocco rock phosphate, even under conditions of pH increase resulting from NO_3^--N nutrition (Experiment 5) attests to the effectiveness of its root system. Only when the presence of a pH rise exerted its negative effect on the solubility of an already highly insoluble P form like Mali rock phosphate, did alfalfa severely suffer from P deficiency. It was for this rock phosphate that the benefit of soil acidification initiated by symbiotic N_2 fixation became clearly evident (Figs. 13 and 14).

It is, however, to be expected that faster growing crops, such as soybean and peanut, having less dense root systems than alfalfa has, may be more seriously hampered by low soil phosphate availability in building up a symbiotic N_2-fixation apparatus. In Experiment 6, designed to examine the possible usefulness of providing a small quantity of easily available starter-P fertilizer to initiate an N_2-fixation process expected to be eventually leading to a mobilization of rock phosphate-P, the disadvantage of low irradiation was particularly troublesome. Nevertheless, the results obtained in this experiment showed that, when sand was used as growth medium, the presence of some easily available P in the period of nodule growth promoted the subsequent utilization of P added in rock-phosphate form (Table 13).

In practical terms, this finding could be extrapolated to indicate that the usefulness of rock phosphate added to a neutral soil, low in available P, on which a leguminous crop is to be grown, can possibly be enhanced by mixing a small quantity of superphosphate with a larger quantity of rock phosphate before applying the phosphate to the soil. It can, however, also be reasoned that the priming effect exerted by superphosphate-P might not be needed when mycorrhiza is capable of supplying the young seedlings with a quantity of soil P necessary to initiate nodulation and ensuing N_2 fixation.

The results obtained in Experiment 7 have made it clear once more how useful mycorrhiza can be in mobilizing soil phosphate (Fig. 15A). Nevertheless, from a soil containing nothing but a small quantity of sparsely available soil P, the amount of P mobilized by mycorrhiza proved to be insufficient to meet the requirements of both host plant and Rhizobium. However, when the relatively insoluble Mali rock phosphate was used (Fig. 15C), it was evident that the activities of the mycorrhiza enabled the host plant to support a Rhizobium population that not only helped the host plant to acquire nitrogen, but also allowed it to generate a quantity of acidity needed to mobilize a portion of the added rock phosphate. When more soluble forms of phosphate were used, such as Morocco rock phosphate and triple superphosphate, it was observed (Fig. 15B and D) that no particular benefit was obtained from the presence of mycorrhiza, but that at the same time the host plant did not seem to be negatively affected by the presence of mycorrhiza, as sometimes is found.

Interesting as these results obtained with mycorrhiza may be, it must nevertheless be realized that they are as yet inadequate to allow a full evaluation to be made of the importance of mycorrhiza in aiding a leguminous host plant to utilize both fixed N_2 and insoluble rock phosphate-P. The relevant experiment included in the present dissertation should only be seen as a first step toward a more thorough investigation of the possible contribution to be made by vesicular-arbuscular mycorrhiza in enabling legumes to mobilize sparsely soluble rock phosphates.

It may, however, be useful to warn at this place against unwarranted expectations about the value of mycorrhiza in making sparsely soluble soil phosphates available to agricultural crops. It must be realized that in many cases mycorrhiza can only help to deprive already impoverished soils of their last remaining resources of phosphate, and that mycorrhiza can never serve as a lasting substitute of phosphates needed, either in fertilizer form or in the form of organic materials, to restore the fertility of a soil depleted by the removal of P in the form of agricultural produce.

It is to be hoped that the overall results of the experiments discussed in this dissertation will serve to stimulate interest in the use of sparsely soluble apatitic rock phosphates for the growth of legumes, especially in developing countries. In a number of these countries, phosphate-rock materials have been discovered. Many of these materials do not lend themselves to beneficiation needed to manufacture superphosphates. Instead, in finely ground form such materials may prove to be useful as P sources for leguminous crops. If they are found to be functional in this respect, they may play a keyrole in the growing of legumes which then can be produced without any need for large investments to be made for the purchase of expensive nitrogenous and phosphatic fertilizers, which often have to be imported from other countries.

6 SUMMARY

The ability of plants to exert a certain degree of selection when absorbing nutrients often has as a consequence that on an equivalence basis unequal quantities of cationic and anionic nutrients are absorbed. To maintain electroneutrality inside and outside its tissues, the plant absorbs or extrudes ions not considered as nutritive ones. As a result of such regulatory actions of the plant, the pH in the root environment can be affected which in turn can exert an influence on the solubility and availability of nutrients.

In this dissertation, attention is paid to the implications of utilization of symbiotically fixed N_2 by legumes for the usefulness of rock phosphates as phosphatic fertilizers. The chain of thoughts is, as follows: 1. originally present soil phosphate and soil nitrate enable legumes to form nodules in which symbiotic N_2 fixation can take place which for its further growth will make the host plant independent of any form of combined N (NH_4^+ and/or NO_3^-); 2. when the legume has exhausted the initial NO_3^- supply, but can continue to grow because of the availability of symbiotically fixed N_2, it will absorb more cationic than anionic nutrients; 3. on account of this cationic uptake pattern, the growth medium in the vicinity of absorbing roots will acidify; 4. as a result of this acidification alkaline rock phosphates, when added as fertilizer, might be partially solubilized; 5. because of this solubilization induced by N_2 fixation, rock phosphates might be more useful P sources for legumes than for other crops such as cereals, and they might also be more useful for legumes utilizing symbiotically fixed N_2 than for the same legumes under conditions of an ample supply of NO_3-N.

In a series of experiments, it was investigated whether this chain of thoughts could be verified and, if so, which limiting factors could manifest themselves and how these might be eliminated. These experiments were conducted in greenhouses with the use of two growth media, namely pure quartz sand and a sandy loam low in P which was located at a depth of a few meters in a sand quarry near Lunteren. The experimental crops used were soybean, alfalfa, peanut, and maize. The phosphate fertilizers employed were triple superphosphate and alkaline rock phosphates from Mali and Morocco.

In experiment 1, use was made of sand as growth medium, soybean as test crop and superphosphate as P fertilizer. It could be shown that, as long as the crop could make use of NO_3-N, soybean exerted a pH-raising effect on the sand. Shortly after the stage at which the NO_3-N was exhausted and the plants transferred to symbiotically fixed N_2 as N source, a decline in pH could be observed. In the first weeks of growth, the pH-values of rhizosphere- and non-rhizosphere sand were found to differ. Probably as a result of intensification of the root system, later on these differences disappeared.

In experiment 2, an evaluation was made of the ability of soybean, grown on sand, to make use of P supplied in the form of the two rock phosphates. With an ample supply of NO_3-N, the plants appeared to be unable to utilize these P sources, but this was different for plants utilizing symbiotically fixed N_2, on account of the fact that these latter plants succeeded in acidifying their root environment. With the use of superphosphate, the growth of plants utilizing fixed N_2 was very poor, which could be ascribed to P toxicity. To compensate for the absence of NO_3^- as major anionic nutrient, these plants appeared to have absorbed excessive quantities of phosphate. In the case of NO_3-fed plants, P uptake was less extreme.

Experiment 3 was comparable to experiment 2, except that the sandy loam was used as growth medium. It appeared that in this case the acidifying effect of the plants utilizing fixed N_2 was insufficient to solubilize the rock phosphates used. The short duration of the experiment, the P-fixing capacity and the pH-buffering capacity of the soil, and a shortage of available soil phosphate were factors likely to be responsible for the negative results obtained in this experiment.

Since peanut is often grown on light-textured soils having low pH-buffering capacities, this crop was chosen as test crop in experiment 4 to examine its ability to utilize P applied in the 3 different fertilizer forms to sand. Even when the superphosphate quantity applied was reduced to one-half of that applied to soybean in a previous experiment, P toxicity was still noticeable. For unknown reasons, symbiosis between host plant and Rhizobium strain was insufficiently effective to bring about an acidification of the sand. As a result, solubilization of rock phosphates did not take place. It was furthermore observed that peanut belongs to the group of plants exhibiting a neutral uptake pattern (equivalent quantities of cationic and anionic nutrients absorbed) when N is absorbed as NO_3^-.

In experiment 5, alfalfa was grown on the sandy loam. With this perennial crop, the acidification induced by plants utilizing fixed N_2 appeared large enough to enable alfalfa to make use of P applied in rock phosphate form. In the case of NO_3^- nutrition, the soil pH became high enough to render the sparsely soluble Mali rock phosphate largely unavailable to alfalfa. It could be concluded that the influence exerted by perennial legumes on the soil pH can be large enough to overcome a buffering capacity the soil may have.

The possibility exists that in extremely poor soils, lack of available P prevents the development of nodules so that symbiotic N_2 fixation cannot take place. In such cases, application of a small quantity of soluble P fertilizer may be sufficient to put into motion the chain of reactions eventually resulting in solubilization of rock phosphate that was also applied. In experiment 6, a small quantity of ^{32}P-labeled KH_2PO_4 was added to sand or sandy loam which was placed in pots on top of a larger quantity of sand or soil to which either one of the two rock phosphates was added. With the use of sand as growth medium, it was found that with the aid of the small quantity of KH_2PO_4 applied, soybean could indeed make better use of the rock phosphates. With the soil, such a priming effect of the starter-KH_2PO_4 was not observed. More so than in other experiments, low light intensity constituted an obstacle to a normal development of the N_2-fixing mechanism, thus reducing the ability of the root system of soybean to acidify its root environment.

It is known that vesicular-arbuscular mycorrhiza (VAM) can make a contribution to the P nutrition of plants growing on P-depleted soil or on soil to which sparsely soluble rock phosphate was applied. In experiment 7, it was examined whether the joint actions of Rhizobium and VAM in mobilizing soil P and P added as rock phosphate would be more effective than the single actions of each of the micro-organisms. The experimental crop was again alfalfa grown on the sandy loam. It was found that for the control treatment (no fertilizer-P added) and for the Mali rock phosphate treatment the quantities of P absorbed by the plants were indeed largest when both Rhizobium and VAM were active. In the superphosphate- and Morocco rock phosphate treatments, VAM did not make a contribution to the P nutrition of the plants, which can be seen as an indication that the availability of these fertilizers was high enough to meet the requirements of the alfalfa plants without any aid rendered by VAM.

In experiment 8, it was shown that maize, as an example of a crop that in the case of NO_3^- nutrition raises the pH of its root environment, lacks the ability to utilize P applied in rock phosphate form. In this respect, the behavior of maize is similar to that of leguminous crops, like soybean and alfalfa, when these crops are amply supplied with NO_3-N and do not utilize symbiotically fixed N_2.

The conclusion to be drawn from the results obtained is that alkaline rock phosphates are relatively useful as P sources to crops which are capable of acidifying their root environment. Legumes possess this ability, provided that they utilize symbiotically fixed N_2. In such cases, they withdraw from the soil more cationic than anionic nutrients, thereby exerting an acidifying effect on their root environment.

Such an ability to solubilize rock phosphates is, therefore, dependent on the extent to which N_2 fixation can take place. Such a fixation can be hampered by low light intensities, but also by lack of a small quantity of readily available phosphate needed by the young plants to build up an N_2-fixation mechanism. Once such a mechanism is operative, it can indirectly make a contribution to the mobilization of alkaline rock phosphates when these have been applied to the soil. From the results obtained it appeared that the initiation of a chain of reactions eventually leading to the solubilization of rock phosphate can be brought about by adding a small quantity of superphosphate or by establishing a symbiosis of legume and VA mycorrhiza.

The results obtained provide opportunities for those countries which possess alkaline rock phosphates that do not lend themselves to being used as basic materials for the manufacturing of superphosphates. Application of these rock phosphates creates a possibility to grow legumes with only a slight investment in the form of rock phosphate needed to supply the plants with two highly important and expensive nutrients, namely nitrogen and phosphate.

ACKNOWLEDGMENTS

I wish to express my sincere gratitude to the authorities and personnel of the Agricultural University of Wageningen for having made their facilities available to me to conduct this research work.

I am greatly indebted to Prof. Dr. Ir. A. van Diest, my promotor.

Furthermore my thanks are addressed to the Mexican Organization CONACYT (Consejo Nacional de Ciencia y Tecnología) which granted me a fellowship for the period of my stay in Wageningen.

SAMENVATTING

Het vermogen van planten om bij de opname van voedingselementen een zekere mate van selectie toe te passen heeft vaak mede tot gevolg, dat op equivalentbasis ongelijke hoeveelheden voedingskat- en -anionen worden opgenomen. Ter handhaving van de electroneutraliteit binnen en buiten de plant neemt de plant ionen op of scheidt de plant ionen uit, die niet gerekend worden tot de voedingsionen. Als gevolg van deze regulerende werking van de plant kan de pH van de wortelomgeving beïnvloed worden, hetgeen weer gevolgen kan hebben voor de oplosbaarheid en beschikbaarheid van voedingselementen.

In deze dissertatie wordt aandacht besteed aan de consequenties van het benutten door leguminosen van symbiotisch gebonden luchtstikstof voor de bruikbaarheid van ruwe fosfaten als fosfaatmeststoffen. De gedachtengang is hierbij als volgt: 1. oorspronkelijk aanwezig bodemfosfaat en -nitraat stelt leguminosen in staat om wortelknolletjes te vormen, waarin luchtstikstofbinding gaat plaatsvinden die de waardplant voor haar verdere groei onafhankelijk maakt van het al dan niet beschikbaar zijn van bodemstikstof (NH_4^+ en/of NO_3^-); 2. als de leguminoos geen NO_3^- meer opneemt, maar wel kan doorgroeien vanwege de beschikbaarheid van symbiotisch gebonden luchtstikstof, zal op equivalentbasis de plant meer voedingskationen dan voedingsanionen opnemen; 3. vanwege dit z.g. alkalische opnamepatroon zal het groeimedium in de omgeving van de absorberende wortel verzuren; 4. door deze verzuring zouden in het groeimedium aanwezige alkalische ruwe fosfaten die als P meststof zijn toegediend gedeeltelijk in oplossing kunnen komen; 5. als gevolg van deze door N_2 binding geïnduceerde mobilisering van ruwe fosfaten zouden deze fosfaten voor leguminosen bruikbaarder kunnen zijn dan voor andere gewassen, zoals granen, en ook bruikbaarder voor leguminosen die symbiotisch gebonden luchtstikstof benutten dan voor dezelfde leguminosen, wanneer deze een grote hoeveelheid nitraatstikstof tot hun beschikking hebben.

In een serie proeven werd onderzocht of deze gedachtengang geverifieerd kon worden en, zo ja, welke limiterende factoren zich hierbij zouden kunnen manifesteren en hoe deze uit de weg geruimd zouden kunnen worden. Deze proeven werden uitgevoerd in kassen met gebruikmaking van twee groeimedia, n.l. zuiver kwartszand en een P-arme zandige leemgrond aangetroffen op enkele meters diepte in een zandafgraving bij Lunteren. De gebruikte proefgewassen waren sojaboon, lucerne, aardnoot en mais. Als fosfaatmeststoffen werden gebruikt dubbelsuperfosfaat en alkalische ruwe fosfaten uit Mali en Marokko.

In proef 1 werd gebruik gemaakt van zand als groeimedium, sojaboon als proefgewas en superfosfaat als P meststof. Er werd aangetoond dat, zolang het gewas kon beschikken over NO_3-N, sojaboon de pH van het zand verhoogde. Kort na het moment waarop het gewas overschakelde van NO_3-N op symbiotisch gebonden N, kon een pH daling van het zand geconstateerd worden. In de eerste weken na kieming bleken de pH-waarden van zand binnen en buiten de rhizosfeer te verschillen. Later was waarschijnlijk de beworteling zo dicht geworden, dat van verschil geen sprake meer was. De pH waarden van zand binnen en buiten de rhizosfeer werden dan ook vrijwel gelijk.

In proef 2 werden met gebruikmaking van zand en sojaboon de twee ruwe fosfaten getoetst op hun vermogen om fosfaat te leveren. Het bleek dat bij NO_3 voeding de planten niet in staat waren om gebruik te maken van P in de ruwe fosfaten, terwijl dit bij afhankelijkheid van symbiotisch gebonden N en de daarmee gepaard gaande verzuring wel het geval was. Bij gebruik van superfosfaat ontwikkelden de planten die gebonden N benutten, zich erg slecht vanwege het optreden van P vergiftiging. Ter compensatie van het wegvallen van NO_3^- als belangrijkste voedingsanion bleken deze planten zeer grote hoeveelheden fosfaat op te nemen. Bij de met NO_3^- gevoede planten was de P opname niet abnormaal hoog.

In proef 3 werd de vorige proef herhaald maar nu met gebruikmaking van de leemgrond. De verzurende invloed van de planten die gebonden N benutten bleek nu te gering om de ruwe fosfaten te kunnen mobiliseren. De korte groeiduur van het gewas, het P-fixerend vermogen en het pH-bufferend vermogen van de grond, en een gebrek aan beschikbaar bodemfosfaat waren waarschijnlijk verantwoordelijk voor de negatieve resultaten in deze proef verkregen.

Omdat aardnoot vaak op lichte gronden met een gering pH-bufferend vermogen wordt verbouwd, werd in proef 4 dit gewas geteeld op zand met gebruikmaking van de drie fosfaten. Zelfs bij een halvering van de eerder gebruikte superfosfaatgift werd nog P vergiftiging geconstateerd. Om onduidelijke redenen bleek de symbiose tussen waardplant en Rhizobiumstam in deze proef niet voldoende effectief geweest te zijn om een verzuring van het zand te bewerkstelligen. Als gevolg daarvan trad geen ontsluiting van de ruwe fosfaten op. Er werd verder geconstateerd dat aardnoot tot de groep van planten behoort die bij NO_3^- voeding een neutraal opnamepatroon (gelijke opname van equivalenten kationen en anionen) vertonen.

In proef 5 werd lucerne verbouwd op de zandige leemgrond. Bij dit meerjarige gewas bleek de verzuring, teweeggebracht door planten die symbiotisch gebonden N benutten, groot genoeg te zijn om de lucerne in staat te stellen gebruik te maken van P aangeboden in de vorm van ruwe fosfaten. Bij NO_3^- voeding steeg de bodem pH zodanig, dat P in het zeer slecht oplosbare Mali-fosfaat grotendeels onbeschikbaar bleef voor de lucerne. Er kon geconcludeerd worden, dat bij leguminosen met een relatief lange groeiduur de invloed van het gewas op de pH groot genoeg was om het bufferend vermogen van de grond te overwinnen.

De mogelijkheid is aanwezig, dat bij de teelt van leguminosen op extreem P-arme gronden de stikstofbinding achterwege blijft als gevolg van onvermogen van de waardplant om wortelknolletjes te vormen vanwege P gebrek. Een kleine hoeveelheid van een goed oplosbare fosfaatmeststof zou dan eventueel voldoende kunnen zijn om de keten van reacties op gang te brengen die uiteindelijk moet leiden tot ontsluiting van een eveneens toegediende ruwe fosfaat.

In proef 6 werd een kleine hoeveelheid met ^{32}P gemerkte KH_2PO_4 toegevoegd aan zand of leemgrond die in de potten als afdekking diende van zand of grond waaraan één van de ruwe fosfaten was toegediend. Bij gebruik van zand als groeimedium werd geconstateerd, dat sojaboon met behulp van de kleine hoeveelheid KH_2PO_4 inderdaad in staat was P in ruw fosfaat beter te benutten. Bij gebruik van de leemgrond werd geen effect van de starter-KH_2PO_4 geconstateerd. Meer nog dan bij de andere proeven bleken lage lichtintensiteiten een belemmering te vormen voor de normale ontwikkeling van het stikstofbindingsproces, waardoor uiteindelijk het vermogen van leguminosen om hun wortelomgeving te verzuren niet volledig werd verwezenlijkt.

Het is bekend, dat vesiculaire-arbusculaire mycorrhiza (VAM) een bijdrage kan leveren aan de fosfaatvoeding van planten die zich ontwikkelen op P-arme gronden of op gronden waaraan slecht oplosbaar ruwfosfaat is toegediend. In proef 7 werd onderzocht of de gezamenlijke werking van Rhizobium en VAM effectiever is dan de afzonderlijke werkingen van elk der beide micro-organismen bij het mobiliseren van bodemfosfaat en P toegediend in ruwfosfaat-vorm. Het proefgewas was wederom lucerne geteeld op de zandige leemgrond. Er werd gevonden, dat bij de controle potten (geen meststof-P toegediend) en bij de potten met Mali ruwfosfaat de hoeveelheden door het gewas opgenomen fosfaat inderdaad het grootst waren bij de aanwezigheid van zowel Rhizobium als VAM.

Bij gebruik van superfosfaat en Marokko ruwfosfaat bleek de VAM geen bijdrage te leveren aan de P opname door planten, die in deze gevallen ook zonder VAM in voldoende mate gebruik konden maken van deze beter beschikbare vormen van meststoffosfaat.

In de laatste proef (proef 8) werd aangetoond, dat mais, als voorbeeld van een gewas dat met NO_3^-voeding een pH verhogende invloed op zijn wortelomgeving uitoefent, niet in staat is om P aangeboden in de vorm van ruwfosfaat te benutten. In dit opzicht kwam het gedrag van mais overeen met dat van de gewassen sojaboon en lucerne, wanneer deze konden beschikken over een ruim aanbod van NO_3-N en niet gebruik maakten van symbiotisch gebonden N.

De conclusie die getrokken kan worden uit de verkregen resultaten is, dat alkalische ruwe fosfaten relatief bruikbaar zijn als P bron voor gewassen die een vermogen bezitten om de omgeving van hun wortels te verzuren. Dit vermogen is aanwezig bij leguminosen, althans wanneer deze gebruik maken van symbiotisch gebonden N. In dat geval nemen zij namelijk uit de bodem meer voedingskationen dan voedingsanionen op en oefenen daarmee een verzurende invloed uit op de wortelomgeving.

Dit vermogen om ruwe fosfaten in oplossing te brengen is derhalve afhankelijk van de mate waarin luchtstikstofbinding kan plaatsvinden. Luchtstikstofbinding kan belemmerd worden door lage lichtintensiteiten, maar ook door gebrek aan een kleine hoeveelheid beschikbaar fosfaat die de jonge planten nodig hebben om een luchtstikstofbindingsmechanisme op te bouwen. Als dit mechanisme eenmaal in werking is, kan het indirect een bijdrage gaan leveren aan de ontsluiting van alkalische ruwe fosfaten wanneer deze zijn toegediend. Uit de verkregen resultaten is gebleken, dat het op gang brengen van de keten van processen waarmee uiteindelijk ruw fosfaat wordt gemobiliseerd, bewerkstelligd kan worden door het toedienen van kleine hoeveelheden superfosfaat of door het tot stand brengen van een symbiose tussen leguminoos en VA mycorrhiza.

De verkregen resultaten openen perspectieven voor die landen, welke beschikken over alkalisch ruwe fosfaten die zich er niet toe lenen om als grondstof voor de superfosfaatfabricage gebruikt te worden. Toepassing van deze ruwe fosfaten bij de teelt van leguminosen schept de mogelijkheid om met geringe investeringen een gewas te verbouwen dat kan beschikken over twee zeer belangrijke en dure nutrienten, namelijk stikstof en fosfaat.

RESUMEN

Las plantas pueden modificar el pH del suelo que está directamente en contacto con sus raices y así ejercer cierta influencia sobre la disolución de algunos productos químicos presentes en esa zona de contacto. Esto se debe a que, hasta cierto grado, las plantas absorben selectivamente los iones nutritivos presentes en la solución del suelo lo cual trae como consecuencia que las cantidades de aniones y cationes nutritivos absorbidos, en muchos casos, no sean equivalentes. Para mantener la electroneutralidad tanto dentro como fuera de la planta, ésta absorbe o libera otros iones que no están considerados entre los iones nutritivos tales como son: H_3O^+, OH^- y HCO_3^-.

Cuando las leguminosas, simbióticamente asociadas con bacterias del género Rhizobium, hacen uso del nitrógeno atmosférico, se produce una acidificación del medio ambiente de sus raices. En esta disertación se presta atención a la relación entre dicha acidificación y la utilización por parte de la planta de roca fosfórica finamente pulverizada, aplicada al suelo como fuente de fósforo. La cadena de ideas relacionadas con este estudio es: 1. utilizando pequeñas cantidades de fosfatos y nitratos originalmente presentes en el suelo, las plantas forman los nódulos en donde la fijación simbiótica de nitrógeno toma lugar permitiendo a la planta continuar su crecimiento sin depender de nitrógeno en forma de NH_4^+ o NO_3^-; 2. al crecer a base de la fijación simbiótica de nitrógeno, la leguminosa absorbe más cationes (Ca^{2+}, Mg^{2+}, K^+, Na^+) que aniones ($H_2PO_4^-$, Cl^-, SO_4^{2-}) nutritivos; 3. a consecuencia de tal patrón de absorción iónica, se acidifica el suelo en contacto con las raices; 4. como resultado de la acidificación se facilitaría la disolución de roca fosfórica alcalina aplicada como fertilizante; 5. como resultado de la disolución de la roca fosfórica, ésta podría ser más útil como fuente de fósforo para las leguminosas que para otros cultivos como son los cereales, asi mismo, sería más útil para las leguminosas dependientes de la fijación simbiótica de nitrógeno que para aquellas desarrollándose en condiciones bajo las cuales puedan disponer de suficiente nitrógeno en forma de NO_3^-.

Mediante una serie de experimentos de invernadero, se investigó si estas ideas podrían ser confirmadas y de ser así, cuáles serían los factores limi-

tantes y cómo podrían ser eliminados. Los experimentos fueron conducidos usando arena pura de cuarzo o un subsuelo localizado a una profundidad de varios metros, cubierto por una capa de arena, cerca de Lunteren, Holanda. Las plantas de ensayo fueron frijol soya, alfalfa, cacahuate y maíz. Los fertilizantes fosfóricos utilizados fueron superfosfato triple y dos rocas fosfóricas alcalinas de Marruecos y Mali.

En el experimento 1 se usó arena como medio de crecimiento, soya como cultivo de prueba y superfosfato simple como fertilizante fosfórico. Se demostró que mientras las plantas utilizaron nitrógeno en forma de nitrato, el pH de la arena subió. Cuando la pequeña cantidad de nitrato aplicada al inicio del experimento fue consumida por las plantas y éstas empezaron a depender de nitrógeno atmosférico fijado simbióticamente, se observó que el pH descendió. Se tomaron muestras de la arena adherida a las raices y muestras representativas de toda la arena contenida en las macetas. Durante las primeras semanas, el pH de los dos tipos de muestras fue diferente pero en las subsecuentes semanas la diferencia desapareció.

En el experimento 2, con plantas de frijol soya cultivadas en arena, se evaluó la capacidad de este cultivo para utilizar las dos rocas fosfóricas como fuente de fósforo. Cuando las plantas recibieron una cantidad amplia de nitrato, no fueron capaces de utilizar la roca fosfórica. En cambio, las plantas dependientes de N_2 fijado simbióticamente, si lo hicieron debido a que acidificaron el medio ambiente de sus raices. Las plantas inoculadas que recibieron superfosfato triple crecieron muy poco debido a que sufrieron toxicidad de fósforo. Esto se debe a que para compensar la ausencia de iones NO_3^-, estas plantas absorbieron cantidades excesivas de fosfato. En el caso de la soya provista de NO_3^-, la absorción de P fue menos extrema.

El experimento 3 fue similar al 2 excepto en que el suelo fue usado como medio de crecimiento. En este caso se observó que la acidificación producida por las raices de las plantas dependientes de la fijación de nitrógeno atmosférico, fue insuficiente para disolver la roca fosfórica. Los factores probablemente responsables de ésto fueron la corta duración del experimento, la capacidad de fijación de fósforo del suelo y su capacidad reguladora de pH.

En el experimento 4 se usaron plantas de cacahuate ya que a menudo se cultivan en suelos ligeros cuya capacidad reguladora del pH es muy reducida. Se usó arena como medio de cultivo. La dósis de fósforo aplicada en forma de superfosfato triple fue reducida considerablemente con respecto al experimento 2, sin embargo, síntomas de toxicidad de fósforo fueron observados nuevamente en las plantas dependiendo de la fijación de nitrógeno atmosférico. A pesar de que en pruebas preliminares se comprobó la efectividad de la asociación simbiótica utilizada, en el transcurso del experimento no se redujo suficiente nitrógeno para un buen crecimiento de las plantas y no se produjo acidificación de la arena. Se observó además que el cultivo utilizado pertenece al grupo de plantas que presentan un patrón neutro de absorción iónica, lo que significa que cuando N es absorbido en forma de NO_3^-, la planta asimila cantidades equivalentes de cationes y aniones nutritivos.

En el experimento 5 se cultivó alfalfa en el suelo de Lunteren. Con este cultivo perenne, la acidificación inducida por las plantas utilizando N_2 atmosférico fue suficiente para permitir que la alfalfa utilizara la roca fosfórica aplicada como fuente de fósforo. En el caso de las plantas provistas de NO_3^-, el pH del suelo subió lo cual ocasionó que las plantas no pudieran absorber el fósforo presente en la roca fosfórica de Mali. En este experimento se llegó a la conclusión de que las leguminosas perennes pueden ejercer una influencia sobre el pH del suelo suficientemente grande para vencer su capacidad reguladora del pH.

En suelos extremadamente pobres, la falta de fósforo disponible para las plantas puede evitar el desarrollo de los nódulos de tal manera que la fijación simbiótica de nitrógeno no pueda llevarse a cabo. En tal caso, la aplicación de una pequeña cantidad de fósforo en una forma soluble puede ser suficiente para iniciar la cadena de procesos que pueden inducir la disolución de la roca fosfórica también aplicada. En el experimento 6, la roca fosfórica finamente pulverizada fue mezclada con 2 kg de suelo o de arena de cuarzo y la mezcla resultante fue colocada en el fondo de las macetas. Sobre ella, 0.7 kg de arena o suelo fueron colocadas y una pequeña cantidad de KH_2PO_4 marcada con ^{32}P fue adicionada a la superficie. En la parte del experimento conducida con arena se encontró que con la ayuda de la pequeña cantidad de KH_2PO_4, las plantas de frijol soya si utilizaron mejor la roca fosfórica. Con el suelo no se observó ningún efecto causado por el KH_2PO_4 en cuanto a disolución de la roca fosfórica. En este experimento se observó una amplia proliferación de nódulos en las raices de las plantas pero no se alcanzó una optima fijación de nitrógeno a causa de la baja intensidad de luz prevaleciente, reduciendo así la capacidad de las raices para acidificar el suelo.

La asociación con hongos endomicorrícicos puede contribuir a la nutrición fosfórica de las plantas cultivadas en suelos muy pobres en fósforo. En el experimento 7 se investigó si la acción combinada de las bacterias del género Rhizobium y de los hongos endomicorrícicos de la especie *Glomus mosseae* es superior a su acción independiente. Se encontró que en los tratamientos donde no se aplicó ninguna fuente de fósforo y en aquellos en que se usó la roca fosfórica de Mali, las cantidades de P tomadas por las plantas si fueron mayores cuando ambos, bacterias y hongos estuvieron presentes. En los casos en que se usó la roca fosfórica de Marruecos o el superfosfato triple se encontró que la asociación simbiótica con los hongos micorrícicos no contribuyó a la nutrición fosfórica de las plantas lo cual puede tomarse como una indicación de que,en sí, la disponibilidad de estos fertilizantes fue suficientemente alta para satisfacer los requerimientos de las plantas.

En el experimento 8, se usó maíz como ejemplo de un cultivo que al recibir nitrógeno en forma de NO_3^-, eleva el pH del suelo adyacente a las raices y carece, por lo tanto, de la habilidad de poder utilizar fósforo en la forma de roca fosfórica. En este sentido, el comportamiento de las plantas de maíz es similar al de las plantas de alfalfa y de frijol soya, cuando éstas reciben una amplia cantidad de nitrógeno en forma de NO_3^- y no utilizan nitrógeno fijado simbióticamente.

La conclusión que se puede derivar de los resultados obtenidos es que las rocas fosfóricas usadas como fertilizantes son relativamente útiles como fuentes de fósforo para aquellos cultivos capaces de acidificar el suelo en contacto con sus raices. Las leguminosas poseen esta capacidad cuando fijan nitrógeno atmosférico al estar simbióticamente asociadas con bacterias del género Rhizobium, en cuyo caso absorben más cationes que aniones nutritivos y acidifican el medio ambiente de sus raices. Esta capacidad de disolver roca fosfórica, depende por lo tanto de una eficiente fijación de nitrógeno atmosférico. Este proceso puede ser obstaculizado por muchos factores entre los cuales está una baja intensidad de luz y la ausencia de fósforo en las primas etapas de crecimiento vegetal. Los resultados obtenidos indican que la iniciación de los procesos que resultan en la disolución de roca fosfórica puede ser estimulada con la adición de una pequeña cantidad de superfosfato o mediante la asociación de las plantas con hongos micorricicos.

Los resultados obtenidos ofrecen la posibilidad de usar directamente fosfatos naturales que no se prestan para la fabricación de superfosfatos. La aplicación de dichos fosfatos crea la posibilidad de cultivar leguminosas mediante una pequeña inversión en la forma de la roca fosfórica necesaria para proveer el fósforo necesario para el crecimiento vegetal y para la fijación simbiótica de nitrógeno atmosférico.

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CURRICULUM VITAE

Andrés Aguilar Santelises werd op 31 augustus 1946 in Mexico-Stad geboren. Hij studeerde af in 1970 aan de Escuela Nacional de Ciencias Biológicas, Mexico. In 1973 ontving hij de graad *Master of Science* na het volgen van de M. Sc.- Course on Soil Science and Water Management te Wageningen, met als hoofdvak bodemvruchtbaarheid en als bijvak grond- en gewasanalyse.

Hij trad in maart 1974 in dienst bij de afdeling Bodemkunde van de Universidad Agrícola Chapingo, Estado de México. Vanaf maart 1978 tot heden was hij werkzaam als gastmedewerker bij de vakgroep Bodemkunde en Bemestingsleer van de Landbouwhogeschool te Wageningen.