MOBILIZATION OF ROCK PHOSPHATE BY RAPE (*BRASSICA NAPUS* L.)

Promotor: dr G.R. Findenegg, hoogleraar in de Bodemvruchtbaarheid en Plantevoeding

Co-promotor: dr ir P.A. Leffelaar, universitair docent bij de vakgroep Theoretische produktie-ecologie

Ellis Hoffland

Mobilization of rock phosphate by rape (*Brassica napus* L.)

Proefschrift
ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen
op gezag van de rector magnificus
dr H.C. van der Plas
in het openbaar te verdedigen
op vrijdag 7 juni 1991
des namiddags te vier uur in de aula
van de Landbouwuniversiteit te Wageningen

Abstract

Hoffland, E., 1991. Mobilization of rock phosphate by rape (*Brassica napus* L.). Ph. D. thesis, Wageningen Agricultural University, Wageningen, The Netherlands. 93 pages.

Rape (*Brassica napus*) is known as an effective user of sparingly soluble rock phosphates. The research reported in this thesis aimed to establish the cause of this phenomenon.

With the help of an agar plate technique it was established that phosphate-deficient rape plants grown with nitrate as nitrogen source acidify their rhizosphere. The acidification is restricted to a root zone of about 1.5 cm behind the root tip, and is not related to nutrient uptake. Enzymatic analyses revealed that more malic and citric acid is exuded in the acidified part of the rhizosphere than in the alkalinized part and that the concentrations of these acids in the exuding root segments are higher. It is concluded that acidification of the rhizosphere by exudation of organic acids might enable rape to mobilize rock phosphate.

The concentration of citric acid in the shoots of phosphate-deficient rape plants is also higher. The results of experiments in which the shoots of rape plants were exposed to labelled carbon dioxide indicated that the exuded acids originate from the shoot.

To calculate the effect of organic acid exudation on phosphate uptake from rock phosphate a simulation model was used. The first version model describes the uptake of a growth-limiting, dissolved nutrient from soil by a growing root system. The uptake of the nutrient depends on the rate of nutrient supply towards the roots by mass flow and diffusion. Allowance is made for both time-dependent root density and inter-root competition. Each root is assigned a finite cylindrical soil volume delivering nutrients. This soil volume per unit root length declines with increasing root density.

Simulated and experimental results agreed well when uptake of nitrate or (dissolved) phosphate from a quartz sand/nutrient solution mixture was described at growth-limiting supply (a).

An experimentally determined relation was used to describe the effect of decreasing pH on the solubility of Mali rock phosphate (b). Parameters on exudation were measured in rape plants grown without phosphate. The values of these parameters were assumed to describe the potential of rape plants to mobilize rock phosphate. It appeared that simulated phosphate uptake was greater than observed uptake (c). From (a), (b) and (c) it is concluded that measured rates of organic acid exudation are more than sufficient to explain the relatively large uptake of phosphate from rock phosphate by rape.

Additional index words: rhizosphere acidification, root exudation, malate, citrate, pH gradient, zero-sink, cation-anion balance, $^{14}CO_2$, root hair, CSMP, split pot experiments, Ca uptake

Stellingen

1. Het vermogen van kruisbloemigen om slecht oplosbare ruwfosfaten te benutten als fosfaatbron berust op door fosfaatgebrek geïnduceerde uitscheiding van organische zuren.

   Van den Boogaard H A M G 1989 Exudatie van organische zuren als reaktie op P gebrek. Doktoraalverslag, Vakgroep Bodemkunde en Plantevoeding, Landbouwuniversiteit Wageningen.
   Dit proefschrift.

2. Voor het verkrijgen van meer inzicht in de samenhang tussen rhizosfeerprocessen vormen simulatiemodellen een onmisbaar instrument.

   Dit proefschrift.

3. Er zijn redenen om te twijfelen aan de juistheid van de konklusie van Venkat Raju *et al.* (1972) dat ijzergebrekkige planten geen organische zuren uitscheiden.

   Venkat Raju K, Marschner H en Römheld V 1972 Effect of iron nutritional status on iron uptake, substrate pH and production and release of organic acids and riboflavin by sunflower plants. Z. Pflanzenernaehr. Bodenkd. 132, 178-191.

4. Resultaten van experimenteel onderzoek dienen op korte termijn een eind te maken aan spekulaties van modelbouwers over de rol van wortelharen bij de opname van nutriënten.

   Brewster J L, Bhat K K S en Nye P H 1976 The possibility of predicting solute uptake and plant growth response from independently measured soil and plant characteristics. V. The growth and phosphorus uptake of rape in soil at a range of phosphorus concentrations and a comparison of results with the predictions of a simulation model. Plant Soil 4, 295-328.
   Itoh S en Barber S A 1983 Phosphorus uptake by six plant species as related to root hairs. Agron. J. 75, 457-461.

5. Uit de waarneming dat bakteriën *in vitro* ruwfosfaat oplossen, kan niets worden gekonkludeerd omtrent hun bijdrage *in vivo* aan de fosfaatbeschikbaarheid voor planten gekweekt met ruwfosfaat als fosfaatbron.

6. Het is niet uitgesloten dat bij inokulatie van anjerstengels met *Fusarium* in kombinatie met bakterisatie van de wortels met siderofoor-producerende pseudomonaden kompetitie om ijzer tussen schimmel en bakterie een rol speelt bij de onderdrukking van Fusarium-verwelkingsziekte door

deze bakteriën.

Van Peer R 1990 Induced resistence and phytoalexin accumulation in biological control of Fusarium wilt of carnation grown on rockwool by *Pseudomonas* sp. strain WCS417r. *In* Microbial Interactions and Plant Responses in Soilless Cultures. pp 109-122. Proefschrift, Rijksuniversiteit Utrecht / Phytopathol. In druk.

7. De eis dat om toegang tot de promotie te krijgen een promovendus ten minste zes niet op het proefschrift betrekking hebbende stellingen op wetenschappelijk gebied moet toevoegen, is onredelijk.

Anonymus 1990 Promotiereglement Landbouwuniversiteit. Landbouwuniversiteit Wageningen.

8. Aangezien er voor een groot aantal AIO's aan de Landbouwuniversiteit nog geen passend onderwijsaanbod is, is het AIO-stelsel ten minste vijf jaar te vroeg ingevoerd.

9. Als een onderzoeker louter op eigen gezag zegt dat zijn onderzoek maatschappelijk relevant is, zegt dat iets over de onderzoeker, maar niets over zijn onderzoek.

10. Boeren hebben beter dan de Dienst Landbouwvoorlichting begrepen dat milieubelang boerenbelang is.

11. Zonder *duende* geen flamenco.

12. De aktie "Help de Russen de winter door" had beter kunnen heten: "Help ons geweten de kerstdagen door".

13. De toekomst is vrouwelijk.

Stellingen, behorende bij het proefschrift "Mobilization of rock phosphate by rape (*Brassica napus* L.)". Ellis Hoffland, Wageningen, 7 juni 1991.

Aan mijn ouders

'To know you are ignorant is the beginning of wisdom'

Marion Bradley, *The Mists of Avalon*

Voorwoord

Het feit dat dit proefschrift slechts één schrijfster heeft, doet zeer ten onrechte vermoeden dat het werk van één persoon betreft. Niets is minder waar. Dit voorwoord wil ik daarom graag gebruiken om een aantal van de mensen die hebben meegewerkt te noemen en te bedanken.

In de allereerste plaats wil ik Jaap Nelemans vermelden. Hij heeft met nietaflatende inzet en barstensvol goede ideeën een groot deel van het werk uitgevoerd dat in dit proefschrift wordt beschreven. Als hij er niet was geweest, was dit boekje heel wat dunner geworden. Ik ben hem dankbaar voor de prettige, stimulerende en kameraadschappelijke samenwerking.

Günter Findenegg wil ik bedanken voor zijn begeleiding. Zijn enorme belangstelling voor ons werk, vooral in de eerste jaren, was meer dan een AIO zich kan wensen. Peter Leffelaar is een onmisbare steun geweest bij het ontwikkelen van simulatiemodellen. Hoe hij het klaar speelde weet ik niet, maar elke keer als ik bij hem vandaan kwam, dacht ik weer dat het allemaal tòch gewoon moest kunnen. Zijn kritische en nauwgezette kommentaar op manuscripten heeft een groot deel van dit proefschrift aanzienlijk verbeterd.

Alle medewerkers/sters van de vakgroep Bodemkunde & Plantevoeding wil ik bedanken voor hun bijdrage aan hetzij de uitvoering van het werk, hetzij de gezelligheid van mijn verblijf in Wageningen, hetzij beiden. In het bijzonder wil ik bedanken Erik Heij, die ondanks een overvolle agenda toch nog altijd een gaatje wist te vinden om wat monsters te analyseren, en de mensen van de Boerderij, met name Karel van Gaalen en Peter Pellen, die ontelbare koolzaadplantjes door hun handen hebben zien gaan.

Henny Bloemhof, Riki van den Boogaard en Chekole Degeffa hebben in het kader van een afstudeervak aan dit onderzoek meegewerkt, en hebben een stimulerende invloed gehad. Henny heeft de basis gelegd voor de hoofdstukken 5 t/m 7, waarvoor ik haar erg dankbaar ben.

Hans Overbeek van het CABO stelde zijn laboratorium aan ons beschikbaar. Dr Lie van de vakgroep Microbiologie wordt bedankt voor de prettige samenwerking. De tekeningen zijn verzorgd door de mensen van de tekenkamer van het Biotechnion, de foto's door Piet Jansen. Mw Joy Burrough-Boenisch korrigeerde het Engels van de hoofdstukken 1, 7 en 8. Leen Muurmans ontwierp de omslag.

Het LEB-fonds heeft de drukkosten van dit proefschrift gesubsidieerd.

Aan het thuisfront wil ik Wim Dijkman bedanken voor de morele en logistieke ondersteuning, en voor zijn stimuli, vooral 's morgens erg vroeg.

Wageningen, februari 1991

Ellis

The research reported in this thesis was done at the Department of Soil Science and Plant Nutrition, Wageningen Agricultural University, P.O.Box 8005, 6700 EC Wageningen, The Netherlands.

Contents

Chapter 1

General Introduction

General introduction

In this chapter the main characteristics of rock phosphates are described. Factors affecting the fertilizer value of rock phosphates are discussed. To outline the scientific framework of the work reported in this thesis, emphasis is given to the plant factors affecting phosphate uptake from rock phosphates. The final section presents the aim of the research and an outline of this thesis.

Use and composition of rock phosphate

Phosphate fertilizers have always been of great interest to agricultural workers. The first phosphate fertilizers used were ground bones from battlefields and catacombs. These sources supplied only a fraction of the needs and therefore the discovery, in the first half of the 18th century, of phosphate deposits that could be used as fertilizer was of great importance. The first commercial mining started in 1847, in Suffolk, Great Britain. The mineral was finely ground and it was called rock phosphate, phosphate rock, raw phosphate or phosphorite. Very soon it became clear that rock phosphate was not as good a source of phosphorus as bonemeal, because of its poor availability to plants (Burlison, 1916). In 1843 it was discovered that a more available source of phosphorus could be produced by treating rock phosphate with sulphuric acid, resulting in a product called superphosphate (Gray, 1944). Now, most of the rock phosphate mined is used as a raw material in the production of superphosphate. Nevertheless, rock phosphate is also used for direct application to the soil. Although no statistics are available for many countries, the total registered use of rock phosphate as fertilizer was about 0.9 million metric tons of P_2O_5 in 1986 (Anonymous, 1987), which was about 4% of the total world consumption of phosphate fertilizers. Of these 0.9 million tons, 83% was used in the USSR and 15% in developing countries. The consumption in China, which was estimated to be about 0.4 million metric tons of P_2O_5 in 1973 (Khasawneh and Doll, 1978) is not included in these statistics.

Rock phosphate is especially important as phosphate fertilizer in developing countries. These countries are in great need of phosphate fertilizer to increase crop yield because most soils are phosphate-deficient and very phosphate-retentive too. However, severe financial constraints often restrict the import of artificial fertilizers. Hence, indigenous rock phosphate is used. Deposits are found in many developing countries and processing is simple and cheap. In developed countries rock phosphate is used in organic farming. Usually it is composted, mixed with manure. Rock phosphate has the advantage that its heavy metal content can be less than that of superphosphates. Another reason for using rock phosphate is that it takes less energy per mole phosphorus to process than to process superphosphates, and that it is

cheaper.

Rock phosphate is a collective noun for ground sedimentary rocks with a high phosphorus content (15-40% P_2O_5). The sedimentary rocks originate from organic material, deposited on the sea floor and may be interbedded with a variety of other rocks, usually limestones and shales (Collings, 1955). Apatite is the main constituent of rock phosphate. The basic form of apatite is fluorapatite ($Ca_{10}(PO_4)_6F_2$), the calcium of which can be replaced by sodium and/or magnesium whereas the phosphate can be replaced by carbonate. The general formula for apatite is

$$Ca_{10-a-b}Na_aMg_b(PO_4)_{6-x}(CO_3)_xF_{2+y} \quad \text{with} \quad x/(6-x) \leq 0.33$$

(Khasawneh and Doll, 1978). Besides apatite, rock phosphate can contain a great variety of impurities, like silica, silicates, carbonates and/or oxides of iron and aluminium, which have a profound influence on its chemical behaviour. The precise composition depends on the geological and geographical origin.

Several factors affect the fertilizer value of rock phosphate. Since plants absorb phosphorus in the form of phosphate, most factors concern the solubilization of rock phosphate. They can be subdivided into soil factors, rock phosphate factors and plant factors. Plant factors will be discussed in detail below. Soil and rock phosphate factors are reviewed by Khasawneh and Doll (1978), but are mentioned here, for the sake of completeness.

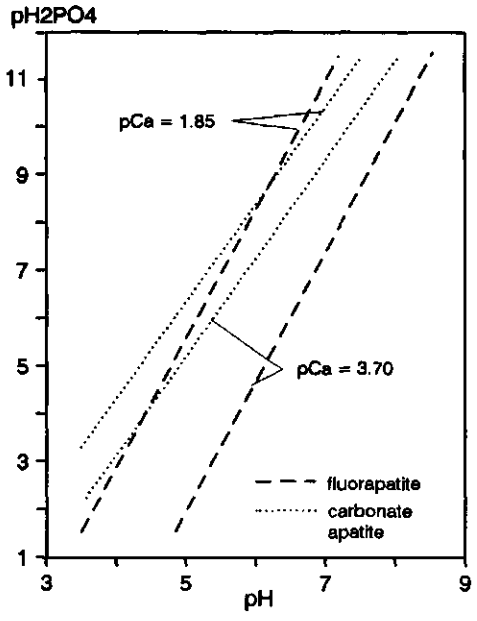

Figure 1. Relation between pH and pH_2PO_4 for fluorapatite and the maximally substituted carbonate apatite ($[Ca_{9.4}Na_{0.4}Mg_{0.2}(PO_4)_{4.5}(CO_3)_{1.5}F_{2.6}]^{0.5+}$) for high and low pCa (derived from Johnston and Olsen (1972) and Kirk and Nye (1986a)).

The two most important soil factors are the pH and pCa of the soil solution, regardless of the precise composition of the rock phosphate applied (see Fig. 1). At low pH and high pCa more phosphate will be available for plant uptake. It is generally known that the use of rock phosphate is more effective on acid soils than on neutral or alkaline soils. The presence of bacteria that dissolve phosphate minerals can also be considered a soil factor. Although the existence of such microorganisms is generally accepted, their effect on the availability of rock phosphates for plant uptake is a conflicting issue. Some report a stimulating effect (Bajpai and Rao, 1971;

Gaur and Ostwal, 1972; Lyval and Berthelin, 1989), others report no effect (Azcon *et al.*, 1976; Laheurte and Berthelin, 1988) of inoculation of plant roots with phosphate solubilizing microorganisms on phosphate uptake.

Both chemical and physical rock phosphate factors can be distinguished. The chemical composition of rock phosphate affects its solubility (Fig. 1) and thus its value as fertilizer. The size and distribution of rock phosphate particles in the soil determine the dissolution rate, as described by Kirk and Nye (1986b).

Since all these factors interact, it is impossible to state anything in general on the fertilizer value of rock phosphate. The interaction of this set of factors may result in a rock phosphate being nearly as effective as superphosphate or in its being nearly inert.

Plant factors promoting phosphate uptake from rock phosphates

As long ago as 1895 Merill established that plant species differ in their ability to grow on rock phosphate. He demonstrated that plants of the cruciferae family (turnip and rutabagas; both *Brassica napus*) grow relatively well on Florida rock phosphate. However, he gave no data on phosphate uptake. He distinguished between species that were able to grow well on rock phosphate ("strong feeders" or "strong feeding power") and species that showed poor growth on rock phosphate ("weak feeding power"). Bauer (1921) mentioned that legumes and buckwheat were strong feeders too.

Recent experiments done in our laboratory on phosphate uptake have confirmed that plants differ in their ability to absorb phosphate from rock phosphate (Fig. 2). Most of the species tested did not take up any phosphate from Mali rock phosphate. Most crucifers appeared to be able to absorb relatively large amounts of phosphate from rock phosphate. Since all experimental conditions were the same for all species, differences in phosphate uptake must have originated from plant factors.

Several plant factors known to affect phosphate uptake will be discussed below. Whenever possible, particular attention will be paid to phosphate uptake from rock phosphate.

Root morphology

The phosphate concentration in the soil solution is usually low in comparison with concentrations of other nutrients and with plant demand. Hence, phosphate uptake by mass flow is relatively small, and phosphate uptake by diffusion is a quantitatively important process. This means that an increase in the root surface can

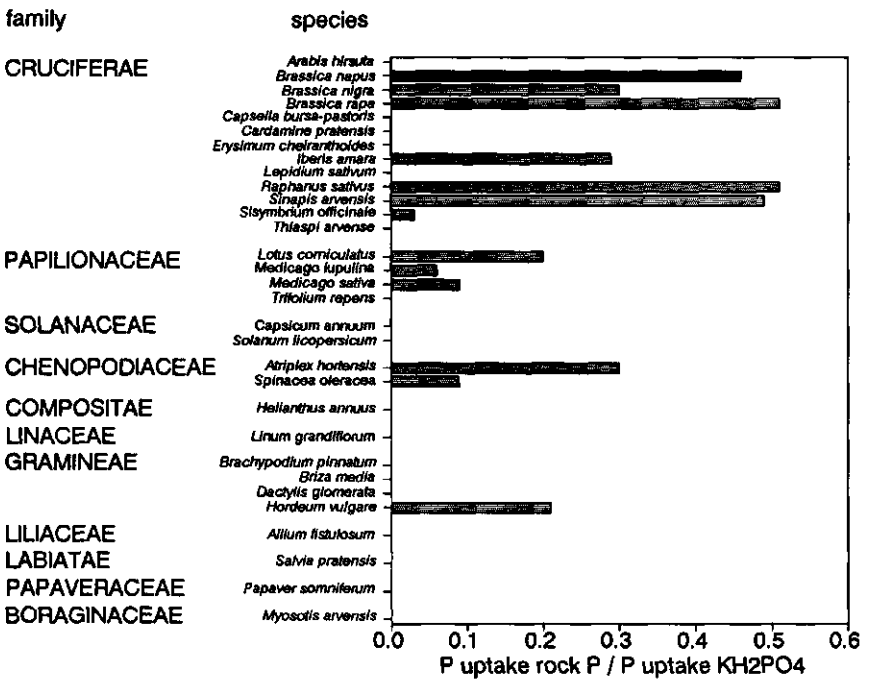

Figure 2. Ability of plant species to absorb phosphate from rock phosphate as phosphate source. Phosphate uptake from rock phosphate is given as a fraction of phosphate uptake from dissolved KH_2PO_4. Methods are described in Chapter 2 (*'Comparison of rock phosphate mobilization'*).

result in more phosphate uptake. Root hairs play an important role in this. They can contribute to phosphate uptake considerably. Although no information is available on the role of root hairs in phosphate uptake from rock phosphates, it is likely that they are as important as they have been shown to be in studies on the poor availability of phosphate.

Bhat and Nye (1973) concluded from model calculations that the phosphate gradient around roots of rape (*Brassica napus*) can only be explained by intense root hair activity. From simulation work by Itoh and Barber (1983) it can be estimated that about 70% of total phosphate uptake by Russian thistle (*Salsola kali*) and about 50% of total phosphate uptake by tomato (*Lycopersicon esculentum*) must be attributed to root hair activity. The quantitative contribution of root hairs to phosphate uptake appeared to depend on their length and density.

Phosphorus deficiency can induce considerable changes in root morphology. Foehse and Jungk (1983) found that the length and density of root hairs of plants grown on nutrient solution increase when the phosphorus content of the root is low. Schenk and Barber (1979) found that genotypes of maize (*Zea mays*) that were more

efficient in phosphate uptake than others reduce the root radius as a reaction to phosphate stress and thus increase the root surface per unit of root weight. A distinct reaction to phosphorus deficiency is shown by white lupin (*Lupinus albus*; Gardner *et al.*, 1981) and by many genera of the family Proteaceae (Jeffrey, 1967). These plants develop proteoid roots: "dense bottle-brush-like clusters of rootlets of limited growth covered in a dense mat of root hairs" (Gardner *et al.*, 1981). Besides providing an increased surface for absorption of phosphate, these proteoid roots have, in the case of white lupin, more functions in enhancing phosphate uptake (see below).

Vesicular-arbuscular mycorrhizae

Evidence has accumulated that vesicular-arbuscular mycorrhizae (VAM) can improve plant growth by increasing phosphate uptake. Also, when rock phosphate is used as a phosphate source, it has been found that inoculating plant roots with VAM fungi stimulates phosphate uptake greatly (Murdoch *et al.*, 1967; Jackson *et al.*, 1972; Powell and Daniel, 1978). Usually VAM are considered to increase the surface area through which phosphate can be absorbed. The uptake efficiency is great because the VAM hyphae can penetrate the soil well beyond the zone of phosphate depletion surrounding the root plus root hairs (Sanders and Tinker, 1971).

The mycorrhizal symbiosis is controlled by the phosphorus content of the host plant: high phosphorus content inhibits VAM infection. Ratnayake *et al.* (1978) and Graham *et al.* (1981) proposed that low phosphorus content causes increased root membrane permeability, which enhances the exudation of sugars and amino acids. The levels of these metabolites in the rhizosphere should be adequate to sustain the germination and growth of mycorrhizal fungi.

pH of rhizosphere

The pH of the rhizosphere is often modified by plant roots, *e.g.* by nutrient uptake and exudation of organic acids. Since pH mainly determines the solubility of rock phosphate (Fig. 1) this modification is very relevant in relation to the use of rock phosphate as a source of phosphate.

Plant roots extrude protons when cation uptake exceeds anion uptake. Hydroxyl- or bicarbonate-ions are extruded when anion uptake exceeds cation uptake. The ratio of cation uptake to anion uptake and thus the rhizosphere pH largely depend on whether the nitrogen is absorbed as ammonium, nitrate or symbiotically fixed nitrogen. Usually the rhizosphere is acidified when ammonium or symbiotically fixed nitrogen is the main nitrogen source and alkalinized when nitrate is the main nitrogen source.

Already at the beginning of this century Prianischnikov (cited by Truog, 1916) found that using ammonium nitrate instead of sodium nitrate as a source of nitrogen greatly increased the availability of rock phosphate to plants with "weak feeding powers". Bekele *et al.* (1983) demonstrated that soil pH was lower and phosphate uptake from different types of rock phosphate two to four times greater when Rhodes grass (*Chloris gayana*) and ryegrass (*Lolium perenne*) were grown with ammonium instead of nitrate as nitrogen source.

Aguilar and Van Diest (1981) demonstrated the induction of increased phosphate uptake from rock phosphate by symbiotic fixation of nitrogen for soy bean (*Glycine max*) and alfalfa (*Medicago sativa*) and Bekele *et al.* (1983) did so for field bean (*Vicia faba*). A two- to tenfold increase of phosphate uptake from rock phosphate was reported when symbiotically fixed nitrogen was used instead of nitrate, depending on the species, the type of rock phosphate and the soil type used.

The exudation of organic acids also increases the solubility of tricalcium phosphate, mainly by its effect on pH (Johnston, 1959). Lipton *et al.* (1987) established that roots of alfalfa (*Medicago sativa*) exude citric, malic and succinic acid and that the exudation of citric and succinic acid almost doubles when the supply of phosphate is limited. They proposed that this increased exudation is a mechanism by which phosphorus-stressed plants enhance the availability of phosphate in the rhizosphere. Dinkelaker *et al.* (1989) presumed that proteoid roots of white lupin (*Lupinus albus*) secrete citric acid which causes a strong pH decrease in the rhizosphere (Gardner *et al.*, 1981) and may promote solubilization of rock phosphate. Lupin was recognized very early as a "strong feeder" on rock phosphate (Prianischnikov; cited by Truog, 1916).

pCa of rhizosphere

Some plants can reduce the calcium concentration in the rhizosphere by high calcium uptake, which increases the solubility of rock phosphate (see Fig. 1). Truog (1916) already tried to relate "high feeding power" to high calcium uptake. McLachlan (1976) and Bekele *et al.* (1983) concluded that high calcium uptake is the cause of the capacity of buckwheat (*Fagopyrum esculentum*) to mobilize rock phosphate. Increased calcium uptake can also lead to the rhizosphere being less alkalinized or acidified as a result of excessive cation uptake. It is difficult to distinguish between these two effects.

Some plant species are able to excrete organic substances that chelate calcium. This also reduces the concentration of free calcium in the rhizosphere. White lupin (*Lupinus albus*) excretes large amounts of citrate (Gardner *et al.*, 1983) that can chelate calcium (Johnston, 1959). At high calcium concentrations in the soil solution, precipitation of calcium citrate can occur (Dinkelaker *et al.*, 1989). It is not

known whether this process is quantitatively important in the release of phosphate from rock phosphate.

Aim of the research and outline of this thesis

The aim of the research reported in this thesis was to establish the cause of the great capacity of rape (*Brassica napus*) to mobilize rock phosphate. As reported above, crucifers were recognized as "strong feeders" on rock phosphate early (see also Fig. 2). The work presented here was done to find the processes responsible for relatively high phosphate uptake from rock phosphate and to calculate whether these processes could fully explain the relatively high phosphate uptake from rock phosphates by rape.

In Chapter 2 existing theories on rape's capacity to mobilize rock phosphate are evaluated. In Chapter 3 a new theory is presented, indicating exudation of organic acids as the reason for rape's efficient use of rock phosphate. The physiological reactions to phosphorus deficiency that might lead to this exudation are discussed in Chapter 4.

A simulation model was developed in order to find the quantitative impact of organic acid exudation by rape on phosphate uptake from rock phosphate. The basic part of this model is presented and evaluated in Chapter 5. In Chapter 6 the effect of the activity of roots of rape to solubilize rock phosphate is estimated using model calculations. The effect of organic acid exudation on phosphate uptake from rock phosphate is calculated in Chapter 7.

The thesis concludes with an evaluation of the significance of the results presented and with a discussion of the prospects for future research (Chapter 8).

References

Aguilar S A and Van Diest A 1981 Rock-phosphate mobilization induced by the alkaline uptake pattern of legumes utilizing symbiotically fixed nitrogen. Plant Soil 61, 27-42.

Anonymous 1987 1986 FAO Fertilizer Yearbook. Food and Agriculture Organization of the United Nations, Rome, Italy.

Azcon R, Barea J M and Hayman D S 1976 Utilization of rock phosphate in alkaline soils by plants inoculated with mycorrhizal fungi and phosphate-solublilizing bacteria. Soil Biol. Biochem. 8, 135-138.

Bajpai P D and Rao S W V B 1971 Phosphate solubilising bacteria. III. Soil inoculation with phosphorus solubilising bacteria. Soil Sci. Plant Nutr. 17, 46-53.

Bauer F C 1921 The relation of organic matter and the feeding power of plants to the utilization of rock phosphate. Soil Sci. 22, 21-41.

Bekele T, Cino B J, Ehlert P A I, Van der Maas A A and Van Diest A 1983 An evaluation of plant-

borne factors promoting the solubilization of alkaline rock phosphates. Plant Soil 75, 361-378.
Bhat K K S and Nye P H 1973 Diffusion of phosphate to plant roots in soil. I. Quantitative autoradiography of the depletion zone. Plant Soil 38, 161-175.
Burlison W L 1916 Availability of mineral phosphates for plant nutrition. J. Agr. Res. 6(13), 485-514.
Collings G H 1955 Commercial Fertilizers. McGraw-Hill Book Company, Inc., New York *etc.*, USA.
Dinkelaker B, Römheld V and Marschner H 1989 Citric acid excretion and precipitation of calcium citrate in the rhizosphere of white lupin (*Lupinus albus* L.). Plant Cell Environ. 12, 285-292.
Foehse D and Jungk A 1983 Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants. Plant Soil 74, 359-368.
Gardner W K, Parberry D G and Barber D A 1981 Proteoid root morphology and function in *Lupinus albus*. Plant Soil 60, 143-147.
Gardner W K, Barber D A and Parberry D G 1983 The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. Plant Soil 70, 107-124.
Gaur A C and Ostwal K P 1972 Influence of phosphate dissolving bacilli on yield & phosphate uptake of wheat crop. Indian J. Exp. Biol. 10, 393-394.
Graham J H, Leonard R T and Menge J A 1981 Membrane-mediated decrease in root exudation responsible for phosphorus inhibition of vesicular-arbuscular mycorrhiza formation. Plant Physiol. 68, 548-552.
Gray A N 1944 Phosphates and Superphosphate. Heron & Co., Ltd., London, Great Britain.
Itoh S and Barber S A 1983 Phosphorus uptake by six plant species as related to root hairs. Agron. J. 75, 457-461.
Jackson N E, Franklin R E and Miller R H 1972 Effects of vesicular-arbuscular mycorrhizae on growth and phosphorus content of three agronomic crops. Soil Sci. Soc. Am. Proc. 36, 64-67.
Jeffrey D W 1967 Phosphate nutrition of Australian heath plants. I. The importance of proteoid roots in *Banksia* (Proteaceae). Aust. J. Bot. 15, 403-411.
Johnston H W 1959 The solubilization of "insoluble" phosphate. 4-The reaction between organic acids and tricalcium phosphate. New Zeal. J. Sci. 2, 109-120.
Johnston W B and Olsen R A 1972 Dissolution of fluorapatite by plant roots. Soil Sci. 114, 29-36.
Khasawneh F E and Doll E C 1978 The use of phosphate rock for direct application to soils. Adv. Agron. 30, 159-206.
Kirk G J D and Nye P H 1986a A simple model for predicting the rates of dissolution of sparingly soluble calcium phosphates in soil. I. The basic model. J. Soil Sci. 37, 529-540.
Kirk G J D and Nye P H 1986b A simple model for predicting the rates of dissolution of sparingly soluble calcium phosphates in soil. II. Applications of the model. J. Soil Sci. 37, 541-554.
Laheurte F and Berthelin J 1988 Effect of a phosphate solubilizing bacteria on maize growth and root exudation over four levels of labile phosphorus. Plant Soil 105, 11-17.
Leyval C and Berthelin J 1989 Interactions between *Laccaria laccata*, *Agrobacterium radiobacter* and beech roots: Influence on P, K, Mg, and Fe mobilization from minerals and plant growth. Plant Soil 117, 103-110.
Lipton D S, Blanchar R W and Blevins D G 1987 Citrate, malate and succinate concentration in exudates from P-sufficient and P-stressed *Medicago sativa* L. seedlings. Plant Physiol. 85, 315-317.
McLachlan K D 1976 Comparative phosphorus responses in plants to a range of available phosphorus situations. Aust. J. Agric. Res. 27, 323-341.
Merill L H 1895 Investigation on the foraging powers of some agricultural plants for phosphoric acid. Ann. Rep. Maine State Coll., Agr. Expt. Sta., 10-14.
Murdoch C L, Jackobs J A and Gerdemann J W 1967 Utilization of phosphorus sources of different availability by mycorrhizal and non-mycorrhizal maize. Plant Soil 27, 329-334.
Powell C LL and Daniel J 1978 Mycorrhizal fungi stimulate uptake of soluble and insoluble phosphate fertilizer from a phosphate-deficient soil. New Phytol. 80, 351-358.
Ratnayake M, Leonard R T and Menge J A 1978 Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation. New Phytol. 81, 543-552.

Sanders F E and Tinker P B 1971 Mechanism of absorption of phosphate from soil by *Endogone* micorrhizas. Nature 233, 278-279.

Schenk M K and Barber S A 1979 Root characteristics of corn genotypes as related to P uptake. Agron. J. 71, 921-924.

Truog E 1916 The utilization of phosphates by agricultural crops, including a new theory regarding the feeding power of plants. Agr. Exp. Sta. Univ. Wisconsin, Res. Bull. 41.

Chapter 2

Solubilization of Rock Phosphate by Rape

I. Evaluation of the role of the nutrient uptake pattern

Plant and Soil 113, 155-160 (1989)

with: Günter R. Findenegg and
Jacob A. Nelemans

Solubilization of rock phosphate by rape
I. Evaluation of the role of the nutrient uptake pattern

Key words: *Brassica napus*, Ca uptake, cation-anion balance, rock phosphate, split pot experiments

Abstract

Rape and sunflower were compared with respect to their rock phosphate mobilizing capacities, cation-anion balance and uptake of Ca and NO_3 at P-starvation. Rape was able to mobilize P from rock phosphate, whereas sunflower was not.

When grown on a complete nutrient solution with NO_3 as the only nitrogen source, both species took up more nutrient anions than cations. Withholding phosphate from the nutrient solution did not change the uptake pattern of rape, but sunflower took up more nutrient cations than anions at P-starvation, due to a strong decline in NO_3 uptake. With both species, Ca uptake was not affected by phosphate in the nutrient solution.

In split pot experiments, with rock phosphate supplied spatially separated from other nutrients, rape was still able to mobilize rock phosphate. A high Ca concentration had no effect on this capacity.

The results indicated that in our experiments rock phosphate mobilization by rape was not associated with an excess of cation over anion uptake and neither with a high Ca uptake rate.

Introduction

Rape (*Brassica napus*) is known as an effective, nonmycorrhizal (Gerdemann, 1968) user of rock phosphates. Several suggestions to explain this phenomenon have been made. Brewster *et al.* (1976a) demonstrated that rape plants have fine roots and abundant root hairs. The root hairs proved to increase in length and number at decreasing P supply (Foehse and Jungk, 1983). A relatively large soil compartment can thus be exploited. However, it was demonstrated by simulation that P uptake by rape from soils poor in P was higher than expected on the basis of P movement to the enlarged root surface (Brewster *et al.*, 1976b).

Modification of the P-solubility by root-borne acidification of the rhizosphere has been put forward as another mechanism leading to enhanced P uptake from sparsely soluble P sources. Distinct effects of the cation-anion uptake pattern of rape plants on rock phosphate mobilization were demonstrated (Bekele *et al.*, 1983). With NH_4 as nitrogen source, more cations than anions were taken up and in order to maintain an electrical charge balance, a net H-ion extrusion from the roots occurred. NH_4 nutrition therefore resulted in increased P uptake from rock phosphate compared with NO_3 nutrition, where the rhizosphere was alkalinized. However, it is still an open question whether differences in uptake pattern of species growing with NO_3 as the only nitrogen source, can explain the differences in rock phosphate mobilization among those species. In this respect changes in uptake pattern induced by nutrient stress have to be considered.

Changes in uptake pattern induced by Fe-stress are well documented (Van Egmond and Aktas, 1977). Sunflower is one of the species known to enhance proton extrusion at Fe-stress, induced by preferential cation uptake (Römheld *et al.*, 1984). This root-induced acidification of the rhizosphere is assumed to be of considerable ecological importance for mobilization of sparingly soluble Fe sources.

Changes in uptake pattern of rape, induced by P-starvation, have been recorded by Grinsted *et al.* (1982). When rape plants were grown at high root densities in the absence of P, soil pH declined from 6.5 to 4.1 within 2 weeks. Hedley *et al.* (1982) concluded that this pH decline was the result of a change in the cation-anion balance: P-starvation should induce a steady decline in NO_3 uptake and a slight increase in Ca uptake. Moorby *et al.* (1985) confirmed that reduced NO_3 uptake resulted in rhizosphere acidification. However, later experiments of Hedley *et al.* (1983) revealed that total anion uptake was similar in P-starved and P-supplied rape plants; the net H-ion extrusion was thought to have been caused by increased cation uptake (especially Ca) as a reaction to P-starvation. Bekele *et al.* (1983), Schjørring (1986) and Moorby *et al.* (1988) were unable to reproduce the alkaline uptake pattern of rape during P-deficiency with NO_3 as N-source. Moorby *et al.* (1988) suggested that rhizosphere acidification was due to a reduced NO_3 uptake, together with a local high Ca and/or Mg uptake. This should result in a local rhizosphere acidification, whereas the over-all uptake pattern was still causing a pH increase.

High Ca uptake by plants can also stimulate solubilization of rock phosphate by removing Ca from the solubilization equilibrium (Johnston and Olsen, 1972). Bekele *et al.* (1983) observed that of all species investigated, rape showed the highest Ca uptake. They suggested that effective use of rock phosphate by rape might be the result of high Ca uptake.

This uncertainty about the mechanism of rock phosphate mobilization by rape was the reason to investigate whether the capacity of rape to mobilize rock phosphate can indeed be explained in terms of cation-anion uptake or by high Ca uptake. For this purpose, sunflower was used as a reference crop. In addition, the effect of spatially separated supply of rock phosphate and other nutrients and of high Ca concentrations in the root medium on the rock phosphate mobilization by rape was investigated by means of a horizontally split root system.

Materials and Methods

Comparison of rock phosphate mobilization

Eight plants of rape (*Brassica napus* L. cv. Jetneuf) or sunflower (*Helianthus annuus* L. cv. Relax) were grown in 3-l pots on a quartz sand/perlite mixture (4/1, v/v). To each pot, 630 ml of −P nutrient solution (−P treatment), +P nutrient solution (+P treatment), or −P nutrient solution plus 0.57 g × pot^{-1} Mali rock phosphate (apatite, 13.6% P ; RP treatment) was added and thoroughly mixed. The

−P nutrient solution consisted of: 5.0 mM $Ca(NO_3)_2$, 5.0 mM KNO_3, 2.0 mM $MgSO_4$ and trace elements, in mg × l^{-1}: Fe (as FeEDTA) 4.6 ; B 0.5 ; Mn 0.5 ; Zn 0.05 ; Cu 0.02 ; Mo 0.01. In the +P nutrient solution, KH_2PO_4 (4.0 mM) was added. The plants were grown in a growth chamber at 20°C, a 16 h light (70 W × m^{-2})/8 h dark cycle and a relative humidity of ± 80%. After harvesting, plants were dried and subsequently analyzed for P.

Cation-anion balance

Seeds of rape and sunflower were germinated in quartz sand and after 6 days the seedlings (250 rape and 110 sunflower) were placed on 50-l volumes of a −P or +P nutrient solution (−P: 1.25 mM $Ca(NO_3)_2$, 1.25 mM KNO_3, 0.5 mM $MgSO_4$, trace elements as described above; +P: 0.25 mM KH_2PO_4 was added). The plants were grown in a growth chamber (conditions: see above). After 4, 7, 9 and 11 days of growth, about 60 plants of rape and 25 plants of sunflower were harvested and analyzed for Ca, K, Mg, N, P, S and Cl.

Split pot experiments

Rape and sunflower plants were grown on split pots (Janssen, 1974; 3 plants × pot^{-1}; Fig. 1) in a glasshouse at 20°C. The upper half of the root system grew in quartz sand, the lower half in nutrient solution. In the −P treatment, the upper half of the pot (ϕ 7 cm, height 7 cm) contained 295 g quartz sand, mixed with 45 ml demineralized water. The lower half contained 3 l −P nutrient solution (see "*cation-anion balance*"). In the RP treatment, 0.32 g Mali rock phosphate was mixed with the quartz sand, while the lower half contained −P nutrient solution. In the +P treatment, no Mali rock phosphate was added, but the lowest compartment contained +P nutrient solution (see "*cation-anion balance*"). In the experiments with Ca addition, 2.5 g $CaSO_4$ was added to the quartz sand mixture.

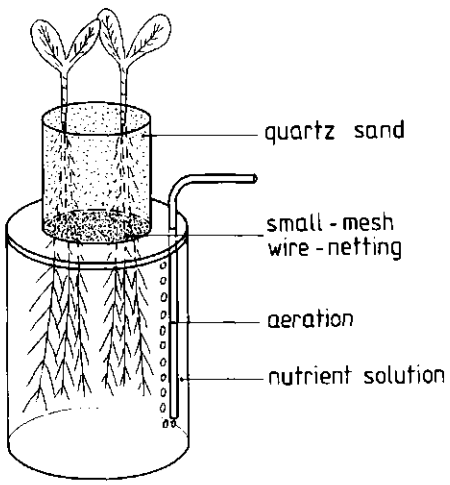

Fig. 1. The split pot setup.

The plants were grown for 31 days. The nutrient solutions were regularly replenished during the experiment. After harvesting, dry weight and P concentration in the plants were determined.

Analytical methods

Total N, P, K, Ca and Mg were determined after wet digestion of dried

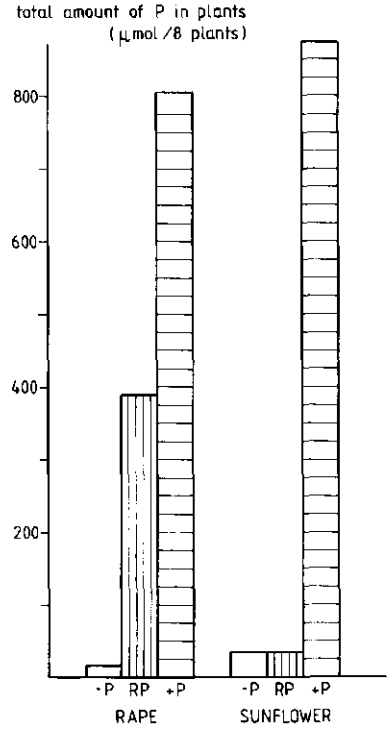

Fig. 2. Rock phosphate mobilization by rape: total amount of P in plants grown on quartz sand without P (−P), with Mali rock phosphate (RP) or with KH_2PO_4 (+P). Rape was grown for 22 days, sunflower for 27 days.

subsamples in a H_2SO_4-Se-salicylic acid mixture with addition of H_2O_2. Total N was determined by the indophenol blue method, total P by the molybdenum-blue method, K and Ca by flame photometry and Mg by atomic absorption spectrometry. Total S was determined by inductively coupled plasma atomic emission after wet digestion of dried subsamples in concentrated HNO_3. For the determination of Cl, dried subsamples were extracted with demineralized water. Cl was determined coulometrically with an Ag anode at constant current.

Results and Discussion

Rock phosphate mobilization by rape and sunflower

The comparison of rock phosphate mobilization by rape and sunflower grown on quartz sand revealed that rape was able to mobilize rock phosphate, while sunflower was not (Fig. 2). The total amount of P in the sunflower plants grown with rock phosphate was not higher than that in the −P plants, and

Table 1. Dry matter yields and ion concentrations in dry matter of rape and sunflower plants, grown for 9 days on nutrient solution with or without added P.

P-status	Dry matter (mg × plant^{-1})	Concentrations in dry matter (mmol × kg^{-1} dm)					
		Ca	K	Mg	N	P	S
Rape							
+ P	40	560	1601	204	3731	236	299
− P	29	484	1075	192	3352	56	260
Sunflower							
+ P	195	426	1928	184	3641	293	135
− P	150	315	1682	160	2177	56	152

Table 2. Uptake of cations (ΣC, sum of Ca, Mg, and K) and anions (ΣA, sum of N, P and S; no Cl was taken up) during successive periods of growth on nutrient solution with or without added P.

P-status	Period (days)	Dry weight increase (mg × plant^{-1})	Uptake of ions (μeq × plant^{-1})		
			ΣC	ΣA	ΣC minus ΣA
Rape					
+ P	0-4	4	22	28	-6
+ P	4-7	12	34	54	-20
+ P	7-9	19	64	86	-22
+ P	9-11	54	142	239	-97
Rape					
− P	0-4	4	22	28	-6
− P	4-7	8	22	36	-14
− P	7-9	11	22	35	-13
− P	9-11	8	18	40	-22
Sunflower					
+ P	0-4	10	152	144	+8
+ P	4-7	91	321	411	-90
+ P	7-9	47	117	151	-39
+ P	9-11	163	407	500	-93
Sunflower					
− P	0-4	21	158	160	-2
− P	4-7	28	82	42	+40
− P	7-9	54	130	133	-3
− P	9-11	52	54	43	+11

about the same as already present in the seeds (9 μmol P × seed^{-1}).

Role of cation-anion balance

After 9 days of growth on nutrient solution with or without P, the dry weight of −P plants of both rape and sunflower was about 75 % of the dry weight of +P plants (Table 1). Both P-supplied rape and sunflower plants had taken up more anion than cation equivalents (Table 2). Therefore, alkalinization of the rooting medium has to be expected.

At P-starvation, the uptake pattern of rape did not change greatly: anion uptake still exceeded cation uptake (Table 2). The decline in H_2PO_4 and NO_3 uptake coincided with a decline in Ca and K uptake. Therefore, no remarkable changes of concentrations in dry matter appeared at P-starvation (Table 1) and still an increase in nutrient solution pH was to be expected. This is consistent with the results of Bekele *et al.* (1983), Schjørring (1986) and Moorby *et al.* (1988).

With sunflower, P-starvation led to a change in nutrient uptake balance (Table

2): the acidic uptake pattern changed into an alkaline one, and solution acidification could be expected. This change was mainly due to a strong decrease in NO_3 uptake by P-starved plants. The N concentration in dry matter declined drastically (Table 1).

Therefore, if a decrease in pH of the rhizosphere caused by imbalanced nutrient uptake would be the mechanism of solubilizing rock phosphate (Hedley *et al.*, 1982; 1983), then sunflower should be more effective than rape in mobilization of rock phosphate. Yet, this was not the case (Fig. 2).

In the split pot experiments, the difference in rock phosphate mobilization by rape and sunflower was maintained even though effects of variations in rhizosphere pH due to cation-anion uptake on rock phosphate solubilization were excluded by separating the supplies of rock phosphate and of other nutrients (Fig. 3). Under such conditions, rape was still able to use rock phosphate as a P-source. This is another indication that rock phosphate mobilization by rape grown with NO_3 as N source is not due to (a shift in) its nutrient uptake pattern.

Role of Ca uptake

The role of Ca uptake in rock phosphate mobilization by rape was evaluated in a split pot experiment. In this experiment, the Ca concentration in the upper half of the pot was artificially kept high, by adding an excess of the sparsely soluble $CaSO_4$. If the maintenance of a low Ca concentration in the rhizosphere would be the mechanism for the solubilization of rock phosphate, this treatment should decrease the ability of rape to grow on rock phosphate. However, rock phosphate mobilization by rape did not differ significantly with and without $CaSO_4$.

In contrast to the results of Hedley *et al.* (1983), P-deficiency induced a decrease in internal Ca concentration and uptake in our experiment with nutrient solution (Table 1). This neither supports the idea of a role of an enhanced Ca uptake induced by P-stress in rock phosphate mobilization by rape.

In summary, neither the ion uptake

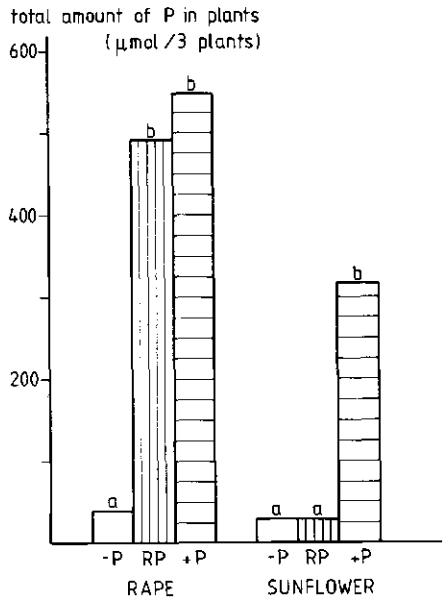

Fig. 3. P uptake by plants grown on split pots without P (−P), with Mali rock phosphate in the upper half of the pot (RP) or with KH_2PO_4 in the nutrient solution (+P). Rape and sunflower were grown for 55 and 34 days, respectively. Different letters indicate significant differences within one species (Student's T-test, P=0.05).

pattern nor the extent of Ca uptake can fully explain the capacity of rape to mobilize rock phosphate in our experiments. However, rhizosphere acidification in the absence of P, independent of the uptake of nutrient cations and anions cannot be excluded. In this respect, exudation of organic acids has to be considered. Exudation of citrate and/or malate has been demonstrated as a response of lupin (Gardner *et al.*, 1983) and alfalfa (Lipton *et al.*, 1987) to P-starvation. Therefore, further research should concentrate on this subject.

Acknowledgements

The authors would like to thank Mr Karel van Gaalen for technical support during plant cultivation and Mr Erik Heij for analytical assistance.

References

Bekele T, Cino B J, Ehlert P A J, Van der Maas A and Van Diest A 1983 An evaluation of plant-borne factors promoting the solubilization of alkaline rock phosphates. Plant and Soil 75, 361-378.

Brewster J L, Bhat K S and Nye P H 1976a The possibility of predicting solute uptake and plant growth response from independently measured soil and plant characteristics. IV. The growth and uptake of rape in solutions of different phosphorus concentration. Plant and Soil 44, 279-293.

Brewster J L, Bhat K S and Nye P H 1976b The possibility of predicting solute uptake and plant growth response from independently measured soil and plant characteristics. V. The growth and phosphorus uptake by rape in soil at a range of phosphate concentrations and a comparison of results with the predictions of a simulation model. Plant and Soil 44, 295-328.

Foehse D and Jungk A 1983 Influence of phosphate and nitrate supply on root hair formation of rape, spinach and tomato plants. Plant and Soil 74, 359-368.

Gardner W K, Barber D A and Parbery D G 1983 The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. Plant and Soil 70, 107-124.

Gerdemann J W 1968 Vesicular-arbuscular mycorrhizae and plant growth. Ann. Rev. Phytopathol. 6, 394-418.

Grinsted M J, Hedley M J, White R E and Nye P H 1982 Plant-induced changes in the rhizosphere of rape (*Brassica napus* cv. Emerald) seedlings. I. pH change and the increase in P concentration in the soil solution. New Phytol. 91, 19-29.

Hedley M J, Nye P H and White R E 1982 Plant-induced changes in the rhizosphere of rape (*Brassica napus* cv. Emerald) seedlings. II. Origin of the pH change. New Phytol. 91, 31-44.

Hedley M J, Nye P H and White R E 1983 Plant-induced changes in the rhizosphere of rape (*Brassica napus* cv. Emerald) seedlings. IV. The effect of rhizosphere phosphorus status on the pH, phosphatase activity and depletion of soil phosphorus fractions in the rhizosphere and on the cation-anion balance in plants. New Phytol. 95, 69-82.

Janssen B H 1974 A double pot technique for rapid soil testing. Trop. Agric. (Trinidad) 51, 161-166.

Johnston W B and Olsen R A 1972 Dissolution of fluorapatite by plant roots. Soil Sci. 114, 29-36.

Lipton G S, Blanchar R W and Blevins D G 1987 Citrate, malate and succinate concentration in exudates from P-sufficient and P-stressed *Medicago sativa* L. seedlings. Plant Physiol. 85, 315-317.

Moorby H, Nye P H and White R E 1985 The influence of nitrate nutrition on H^+ efflux by young rape plants (*Brassica napus* cv. Emerald). Plant and Soil 84, 403-415.

Moorby H, White R E and Nye P H 1988 The influence of phosphate nutrition on H ion efflux from the roots of young rape plants. Plant and Soil 105, 247-256.
Römheld V, Müller Ch and Marschner H 1984 Localization and capacity of proton pumps in roots of intact sunflower plants. Plant Physiol. 76, 603-606.
Schjørring J K 1986 Nitrate and ammonium absorption by plants growing at sufficient or insufficient level of phosphorus in nutrient solution. Plant and Soil 91, 313-318.
Van Egmond F and Aktas M 1977 Iron-nutritional aspects of the ionic balance of plants. Plant and Soil 48, 685-703.

Chapter 3

Solubilization of Rock Phosphate by Rape

II. Local root exudation of organic acids as a response to P-starvation

Plant and Soil 113, 161-165 (1989)

with: Günter R. Findenegg and
Jacob A. Nelemans

Solubilization of rock phosphate by rape
II. Local root exudation of organic acids as a response to P-starvation

Key words: agar plate technique, *Brassica napus*, citric acid, malic acid, phosphate nutrition, rhizosphere acidification

Abstract

Local rhizosphere acidification by rape as a reaction to P-starvation was visualized by means of an agar plate technique. By means of a modification of this technique local differences in cation-anion uptake and organic acid exudation along intact roots of rape were observed for plants grown on nutrient solution with or without added P. No differences in uptake rates of K-, NO_3- and Ca-ions could be detected between P-starved and P-supplied plants. However, exudation of malic and citric acid was distinctly higher in acidified root zones of P-starved plants, coinciding with higher levels of malate in the corresponding root tissue. Organic acid exudation is indicated as the cause of local rhizosphere acidification by rape as a reaction to P-starvation and as a possible mechanism of its phosphate-solubilizing capacity.

Introduction

Unlike most other nonmycorrhizal species, rape (*Brassica napus*) is able to grow with rock phosphate as P-source, even when NO_3 is the N-source. In a previous paper (Hoffland *et al.*, 1989) we provided evidence that this property cannot be explained by rhizosphere acidification due to an over-all imbalanced cation-anion uptake (Hedley *et al.*, 1982; 1983), nor by high Ca uptake (Bekele *et al.*, 1983). Therefore, other mechanisms, which are independent of nutrient uptake, have to be regarded.

Rhizosphere acidification in relation to mineral nutrition has been reviewed by Marschner *et al.* (1986). Local rhizosphere acidification has been shown for a number of dicotyledonous species as a reaction to Fe-deficiency and for lupin as a response to P-deficiency. Gardner *et al.* (1983) demonstrated that the proteoid roots of P-deficient lupin plants secreted large quantities of citric acid. P-starved alfalfa seedlings also exuded organic acids (Lipton *et al.*, 1987) but in the latter case it is unknown whether this is restricted to certain root zones. Moorby *et al.* (1988) demonstrated that P-starved rape acidifies its rhizosphere only just behind the root tip, but suggested that this was due to a localized shift in ion uptake. In earlier experiments, no significant quantities of organic acids have been detected in the rhizosphere of rape (Hedley *et al.*, 1982).

In this paper, we present the results of experiments dealing with the occurrence of local exudation of organic acids from P-starved rape plants, being a potential mechanism underlying their capacity to mobilize rock phosphate.

Materials and Methods

Growth of plants

Seeds of rape (*Brassica napus* cv. Jetneuf) germinated in moist quartz sand were after 6 days transferred to a nutrient solution with or without 0.25 mM KH_2PO_4. The nutrient solution consisted of: 1.25 mM $Ca(NO_3)_2$, 1.25 mM KNO_3, 0.50 mM $MgSO_4$ and trace elements (in mg $\times$ l^{-1}): Fe (as FeEDTA) 4.6; B 0.5; Mn 0.5; Zn 0.05; Cu 0.02; Mo 0.01. After 7 days of growth in a growth chamber on nutrient solution, the plants were used for experiments. At that moment, clear symptoms of P-deficiency were visible. Growth conditions: day/night regime 16/8 h; light intensity 70 W $\times$ m^{-2}; temperature 20°C; relative humidity $\pm$ 80%.

Visualization of rhizosphere acidification

To visualize acidification and/or alkalinization along single roots of intact +P and −P rape plants *in situ*, an agar technique, similar to that described by Weisenseel *et al.* (1979), was applied. After having been laid out on a glass plate, the roots were covered with a 2-3 mm thick agar layer. Bromocresol purple (0.015%) was used as pH indicator, dissolved in an agar medium (1.0% agar) containing the normal nutrient solution without phosphate. The solution was adjusted to pH 5.8 with NaOH and kept liquid at 45°C before being poured over the roots. Acidification and/or alkalinization became visible within 1 h.

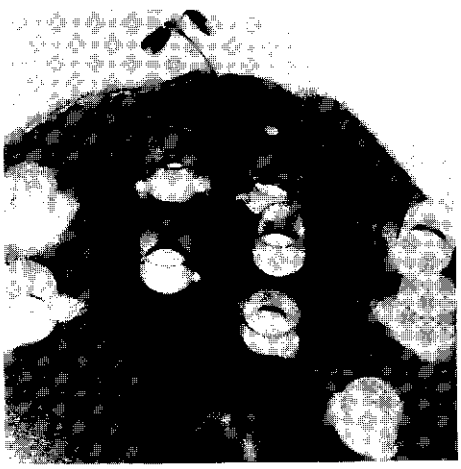

Fig. 1. Method used to determine exudation of organic acids and ion uptake at different distances from root tips of intact plants. The plants were partly covered with an agar solution.

Collection and analyses of root exudates

Root exudates were collected by means of an adapted agar plate technique. While spreading the roots on a glass plate, three lateral roots were placed next to each other, with the root tips in adjacent positions (Fig. 1). Before covering the roots with agar solution, small plastic rings (ϕ 1.2 cm) were placed over the root zone just behind the root tips, and another one over the same three roots, as closely as possible to the root base. After covering the roots outside the rings with agar solution, 0.25 ml −P nutrient solution was pipetted into the rings. After 2 h incubation at room temperature and high humidity, the contents of the rings were collected ("root tip" and "root base"

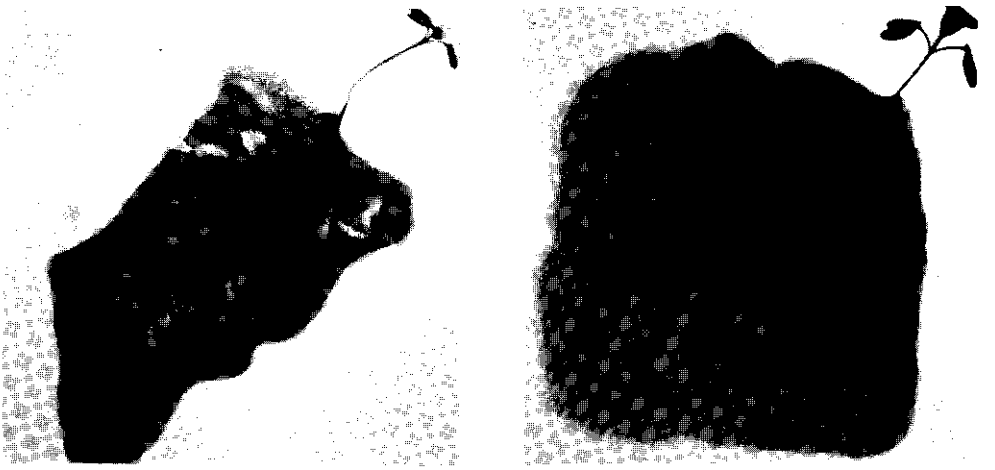

Fig. 2. Alkalinization (dark) and/or acidification (light) of a thin agar layer covering roots of rape plants grown for 7 days on nutrient solution without P (*left*) or with P (*right*). The agar (pH 5.8) contained bromocresol purple and a −P nutrient solution.

separated) and immediately analyzed for citrate, malate and fumarate by enzymatic procedures (Anonymous, 1984).

Uptake of K-, Ca- and NO_3-ions by root tips in situ

About the same procedure as described for root exudates was followed. Larger rings (ϕ 1.8 cm) were used, containing 0.5 ml −P nutrient solution (see above). After 24 h incubation, the contents of the rings were analyzed. Quantities absorbed by the zone just behind the root tip of −P and +P plants were compared. The uptake rate was assumed to be constant in time. K and Ca were determined by flame photometry and NO_3 by automatic spectrophotometry after reduction to NO_2.

Internal malate and citrate concentrations in root sections

From plants grown on −P and +P nutrient solution, two root sections were collected: one 0.0-1.5 cm and another 1.5-3.0 cm behind the root tip. After collection of about 25 mg dry weight, the samples were ground, extracted in 15 ml demineralized water and these extracts were analyzed for malate and citrate.

Results

Changes in pH on agar plates depending on the plant P-status

Within a few hours, clear yellow zones could be detected in the agar plates with

Table 1. Amounts of organic acids detected after 2 h in exudates from different root zones of rape plants, grown for 7 days on nutrient solution with or without P. Values are means ± sd (n=9).

P-status	Root zone	Amounts of organic acids in exudates after 2 h (nmol × cm^{-1} root)	
		Malate	Citrate
− P	Behind root tip	0.87 ± 0.11	0.27 ± 0.15
	Near root base	0.20 ± 0.10	0.13 ± 0.10
+ P	Behind root tip	0.15 ± 0.09	0.06 ± 0.03
	Near root base	0.03 ± 0.02	0.01 ± 0.02

plants grown without P (Fig. 2). The acidification was limited to a root zone of about 1.5 cm length, just behind the root tip. After 22 h incubation the yellow spots could have increased to spots of about 2 cm diameter. Along the remainder of the −P root system, only alkalinization could be detected. No acidification occurred along the +P roots (Fig. 2).

Exudation of organic acids

Malate and citrate were detected in the exudates of −P and +P plants (Table 1). The amounts in exudates of −P roots just behind the root tips were significantly higher than those of the other zones. No fumarate could be detected.

Uptake of K-, Ca- and NO_3-ions by root tips in situ

For K, Ca and NO_3, uptake rates of +P plants were about twice as high as those for −P plants (Table 2). However, for the electrical charge balance these differences did not have any consequence (Table 2).

Internal organic acid concentrations of two root zones

The malate and citrate tissue concentrations of root zones 0.0-1.5 cm behind the root tip of −P plants were clearly higher than those of the zone further from the tip (Table 3). Generally, the malate and citrate concentrations of +P plants were lower than those of −P plants.

Table 2. Amounts of nutrients taken up during 2 h by a 1.8 cm root zone behind the root tip of rape plants, grown for 7 days on nutrient solution with or without P. Values are means ± sd (n=14).

P-status	Uptake of ions (nmol × cm^{-1} root)			(neq × cm^{-1} root)
	K	Ca	NO3	Σ(K + Ca - NO_3)
− P	1.1 ± 0.3	1.9 ± 0.3	5.6 ± 0.7	-0.8 ± 0.4
+ P	2.5 ± 0.6	3.4 ± 0.3	10.1 ± 1.4	-0.8 ± 0.6

Discussion

In case of NO_3 nutrition, the uptake pattern of rape grown with P is acidic, *i.e.* more anions than cations are taken up (Hoffland *et al.*, 1989). Alkalinization of the agar along the roots (Fig. 2) is consistent with this phenomenon. The uptake is not affected by P-starvation. Nevertheless, it has become clear that the rhizosphere of −P plants undergoes acidification, but only in a restricted root zone (Fig. 2; Moorby *et al.*, 1988).

Table 3. Malate and citrate concentrations in different root sections of rape plants, grown for 7 days on nutrient solution with or without P. Values of 2 replicates (A and B) are given.

P-status	Distance from root tip (cm)	Concentrations in roots (μmol × g^{-1} dry matter) Malate A	Malate B	Citrate A	Citrate B
− P	0.0 - 1.5	256	178	84	78
	1.5 - 3.0	92	116	54	55
+ P	0.0 - 1.5	35	32	9	13
	1.5 - 3.0	42	21	44	10

On the basis of the ionic composition of the plants (Hoffland *et al.*, 1989), NO_3, K and Ca were regarded as the relevant nutrients with respect to rhizosphere acidification. Even in the acidified root zones (Fig. 2), no H-ion extrusion, caused by an excess of cation over anion uptake can be expected (Table 2). This is in contrast to the suggestion of Moorby *et al.* (1988) that local acidification might be due to a locally high Ca uptake. We observed that acidification also occurred when no nutrients were added to the agar solution. Therefore, local acidification caused by P-stress cannot be explained in terms of changes in nutrient uptake pattern along the root surface, which is consistent with earlier results (Hoffland *et al.*, 1989).

Organic acid exudation (Table 1), acidification of the rhizosphere (Fig. 2) and organic acid concentrations in the relevant root sections (Table 3), are all highest for the root tips of P-starved plants. When the effect of NO_3, K and Ca uptake (Table 2) is superimposed to the effect of organic acid exudation (Table 1), only in the root tips of P-starved plants a net acidification can be expected. This is in line with the results of the agar plate technique (Fig. 2). Thus, the major part of the local rhizosphere acidification by P-stressed rape plants has to be attributed to exudation of malate and, to a lower extent, citrate.

Exudation of soluble amino acids and reducing sugars induced by P-stress was demonstrated by Ratnayake *et al.* (1978) for sudangrass. They postulated that permeability of root membranes is increased during P-deficiency, due to decreased phospholipid levels. If this would be the explanation for the increased excretion of organic acids by P-stressed rape plants, then lower rather than higher root tissue concentrations would be expected. Our results (Table 3) suggest an increased rate of organic acid synthesis in P-stressed rape plants.

An increased root tissue organic acid concentration has been demonstrated for Fe-stressed bean plants (Landsberg, 1984). The local rhizosphere acidification in this case was caused by an extrusion of protons, in exchange for cations (Van Egmond and Aktas, 1977). The protons originated from organic acids (De Vos *et al.*, 1986). In contrast, in our P-stressed rape plants no increased cation uptake

could be detected in the acidifying root zones (Table 2). Instead, organic acids were detected in the root environment (Table 1).

A high local exudation rate of organic acids may be of ecological benefit, compared with lower exudation rates along the complete root system. In well-buffered soils, a pH decline can only be achieved by high flux densities of organic acids. Further, a strong decrease of soil pH may inhibit growth of microorganisms, and consequently prevent microbial degradation of the exuded substances. This view, which leaves no space for a possible favorable role of microorganisms in rock phosphate mobilization, is in agreement with the results of Laheurte and Berthelin (1988) and Hedley *et al.* (1982). The latter did not find any relation between the number of hydroxyapatite-solubilizing bacterial colonies and P-mobilization by rape. We have indications that microbial degradation of exudates has to be taken into account: in our samples malate and citrate were degraded within a few hours with concomitant CO_2 production. Microbial degradation may also be the reason why Hedley *et al.* (1982) did not find significant amounts of organic acids. Further research is necessary to establish the role of microorganisms in rock phosphate mobilization by rape.

References

Anonymous 1984 Methods of Enzymatic Food Analysis Using Single Reagents. Boehringer Mannheim GmbH, Mannheim, Germany.

Bekele T, Cino B J, Ehlert P A J, Van der Maas A A and Van Diest A 1983 An evaluation of plant-borne factors promoting the solubilization of alkaline rock phosphates. Plant and Soil 75, 361-378.

De Vos C R, Lubberding H J and Bienfait H F 1986 Rhizosphere acidification as a response to iron deficiency in bean plants. Plant Physiol. 81, 842-846.

Gardner W K, Barber D A and Parbery D G 1983 The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. Plant and Soil 70, 107-124.

Hedley M J, Nye P H and White R E 1982 Plant-induced changes in the rhizosphere of rape (*Brassica napus* cv. Emerald) seedlings. II. Origin of the pH change. New Phytol. 91, 31-44.

Hedley M J, Nye P H and White R E 1983 Plant-induced changes in the rhizosphere of rape (*Brassica napus* cv. Emerald) seedlings. IV. The effect of rhizosphere phosphorus status on the pH, phosphatase activity and depletion of soil phosphorus fractions in the rhizosphere and on the cation-anion balance in plants. New Phytol. 95, 69-82.

Hoffland E, Findenegg G R and Nelemans J A 1989 Solubilization of rock phosphate by rape. I. Evaluation of the role of the nutrient uptake pattern. Plant and Soil 113, 155-160.

Laheurte F and Berthelin J 1988 Effect of a phosphate solubilizing bacteria on maize growth and root exudation over four levels of labile phosphorus. Plant and Soil 105, 11-17.

Landsberg E-Ch 1981 Organic acid synthesis and release of hydrogen ions in response to Fe deficiency stress of mono- and dicotyledonous plant species. J. Plant Nutr. 3, 579-591.

Lipton G S, Blanchar R W and Blevins D G 1987 Citrate, malate and succinate concentration in exudates from P-sufficient and P-stressed *Medicago sativa* L. seedlings. Plant Physiol. 85, 315-317.

Marschner H, Römheld V, Horst W J and Martin P 1986 Root-induced changes in the rhizosphere: Importance for the mineral nutrition of plants. Z. Pflanzenernähr. Bodenk. 149, 441-456.

Moorby H, White R E and Nye P H 1988 The influence of phosphate nutrition on H ion efflux from the roots of young rape plants. Plant and Soil 105, 247-256.

Ratnayake M, Leonard R T and Menge J A 1978 Root exudation in relation to supply of phosphorus and its possible relevance to mycorrhizal formation. New Phytol. 81, 543-552.

Van Egmond F and Aktas M 1977 Iron-nutritional aspects of the ionic balance of plants. Plant and Soil 48, 685-703.

Weisenseel M H, Dorn A and Jaffe L F 1979 Natural H^+ currents traverse growing roots and root hairs of barley (*Hordeum vulgare* L.). Plant Physiol. 64, 512-518.

Chapter 4

Origin of Organic Acids Exuded by Roots of Phosphorus-Stressed Rape (*Brassica napus*) Plants

In *Plant Nutrition - Physiology and Applications. Ed. M. L. van Beusichem (1990). pp 179-183. Kluwer Academic Publishers, Dordrecht, The Netherlands.*

with: Jacob A. Nelemans and
Günter R. Findenegg

Origin of organic acids exuded by roots of phosphorus-stressed rape (*Brassica napus*) plants

Key words: *Brassica napus* L., citrate, $^{14}CO_2$, malate, organic acids, P deficiency, phloem transport, phosphoenolpyruvate carboxylase, root exudation

Abstract

To determine the origin of organic acids exuded by the roots of P deficient rape plants, phosphoenolpyruvate carboxylase (PEPC) activity was measured in plants after deprivation of P in the nutrient solution. PEPC activity in the shoot increased as a reaction to P stress. This increase coincided with accumulation of citrate in the shoot and with a higher citrate/sugar ratio in the phloem. Application of $^{14}CO_2$ to the shoots resulted in a ninefold increase in specific activity of organic acids exuded by −P roots as compared with +P roots. These results indicate that exuded organic acids resulted from increased PEPC activity in the shoot of P-stressed rape plants.

Introduction

Roots of phosphorus-deficient rape plants acidify their rhizosphere by exudation of malic and citric acid. The efficient use of rock phosphates by rape has been attributed to this phenomenon. Exudation is restricted to a root segment of 1.5 cm behind the root tip and coincides with higher tissue concentrations of malic and citric acid in this segment (Hoffland *et al.*, 1989).

The purpose of this study was to investigate whether root exudation originates from an increased rate of organic acid synthesis induced by P deficiency. Phosphoenolpyruvate (PEP) carboxylation and subsequent reduction of oxaloacetate to malate by malate dehydrogenase was regarded as the most obvious anaplerotic pathway for organic acid synthesis in plant tissue (Latzko and Kelly, 1983).

We determined the origin of organic acids exuded by P-deficient rape plants by measuring PEP carboxylase (PEPC) activity in root tissue. PEPC activity in shoot tissue and phloem levels of organic acids were measured to establish the role of shoot-borne organic acids in root exudation.

Materials and Methods

Plant cultivation

Seeds of rape (*Brassica napus* L. var. Jetneuf) were germinated in quartz sand. After 6 days the seedlings were placed on 50-l containers filled with nutrient solution. The plants were grown in a growth chamber at 20°C, a light intensity of

70 W × m^{-2}, a photoperiod of 16 h and a relative humidity of ± 80%.

Nutrient media

The −P nutrient solution consisted of 1.25 mM $Ca(NO_3)_2$, 1.25 mM KNO_3, 0.5 mM $MgSO_4$, and trace elements, in mg × l^{-1}: Fe (as FeEDTA) 4.6 ; B 0.5 ; Mn 0.5 ; Zn 0.05 ; Cu 0.02 ; Mo 0.01. In the +P nutrient solution 0.25 mM KH_2PO_4 was added.

P concentration

Plant material was analyzed for P after wet digestion in a H_2SO_4-Se-salicylic acid mixture with addition of H_2O_2 (Novozamsky *et al.*, 1983). P was determined by the molybdenum-blue method (Murphy and Riley, 1962).

PEPC activity

The determination of PEPC activity by coupling the carboxylation reaction to NADH oxidation was described elsewhere (Arnozis *et al.*, 1988). MDH and LDH were added to the standard essay medium according to Meyer *et al.* (1988).

Collection of phloem sap

Phloem sap was collected by the method of King and Zeevaart (1974). The stems of three rape plants were cut just above the roots while submerged under 20 mM K-EDTA, pH 7.5. These shoots were attached in an Eppendorf reaction vessel filled with 500 µl of the K-EDTA solution. After 1 h incubation (24°C, 100% humidity, and light intensity 65 W × m^{-2}) the solution was immediately analyzed for malate, citrate and sugar concentrations.

Malate, citrate and sugar concentration

Sample preparation To determine malate and citrate in plant material, the extracts for the determination of PEPC activity were used (see above).

Nutrient solutions of the labeling experiment were freeze dried, resolved in 0.5 ml 80% methanol and centrifuged. From the supernatants 25 µl was used for chromatography (see below). The other part was dried under N_2, resolved in 500 µl water and analyzed for malate and citrate.

Determinations Enzymatic procedures provided by Boehringer Mannheim GmbH were used for the determination of malate, citrate (Anonymous, 1989) and glucose+sucrose (Anonymous, 1986) concentrations. The glucose+sucrose concentration will further be referred to as sugar concentration.

Labeling of the shoots

Two shoots of intact rape plants grown for 7 days on nutrient solution were enclosed with gum in a 100-ml glass tube. From three or two sets of two plants the roots were put in 15 ml nutrient solution. $^{14}CO_2$ was liberated inside the tubes by adding HCl to $Na_2{}^{14}CO_3$ (specific activity 49.3 MBq × mmol^{-1}). To two shoots 100 kBq was added.

The shoots were exposed to $^{14}CO_2$ for 6 h during the light period. All analyses were carried out with material collected 27 h after the start of the labeling.

Radioactivity

To count radioactivity in plant material, the samples were ground in a mortar in 96% ethanol. To part of the samples of nutrient solutions 0.1 ml 1 M HCl was added per ml to remove CO_2.

Radioactivity of plant extracts and nutrient solutions was counted in a Packard Liquid Scintillation Counter.

Chromatography

From the above mentioned supernatants of nutrient solutions (see '*Malate, citrate and sugar concentration*') 25 µl was chromatographed on a cellulose TLC plate developed with 2-pentanol/formic acid/water (48.8/48.8/2.4). Formic acid was removed by heating the plate at 120°C for 1 h. The plate was stained with bromocresolegreen (0.04% (w/v) in 96% ethanol, pH 13). The acid and radioactive spots with r_f values equal to those of standards of radioactive malic acid and citric acid were collected and radioanalyzed.

Results

PEPC activity and organic acids in tissue and phloem

Rape plants were precultured for 7 days on +P nutrient solution. Then, at the moment defined as t=0, the roots were washed and the plants were transferred to a −P nutrient solution. Analyses in shoots and roots were done 0, 6, 11 and 14 days after transfer.

Tissue P concentration declined steadily after t=0 in both shoots and roots (Fig. 1), but more rapidly in the shoots. In the shoots this decline coincided with a substantial increase in PEPC activity and an accumulation of citrate while the malate concentration declined slightly. No such effects could be detected in the roots.

Citrate accumulation in the shoot might cause, with an overflow mechanism, increased transport of organic acids towards the roots via the phloem. Therefore

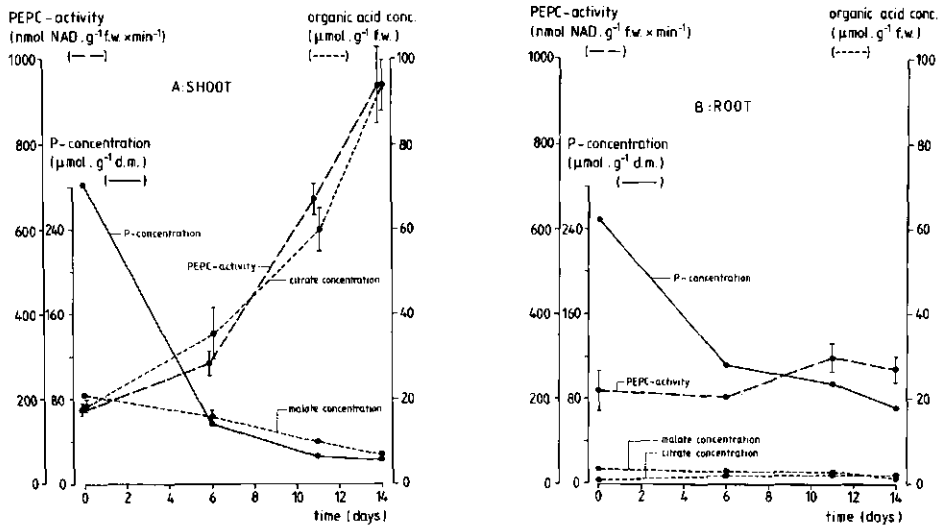

Fig. 1. P concentration, PEPC activity and organic acid concentrations in shoots (A) and roots (B) of rape plants as a function of time. The plants were precultured on +P nutrient solution and at t=0 transferred to −P nutrient solution. Values of PEPC activity and organic acids are means ± s.d. (n=3).

phloem sap was analyzed for malate, citrate and sugars 0, 4, 6 and 8 days after the transfer from +P to −P nutrient solution.

After t=0 an increase in the citrate/sugar ratio was determined (Table 1). This was caused by both a decrease in the amount of sugar and an increase in the amount of citrate detected. No significant change in the malate/sugar ratio was found.

In other experiments plants were precultured for 7 days on −P nutrient solution and then transferred to +P nutrient solution. In such plants PEPC activity and citrate concentration in the shoot and phloem transport of citrate decreased after transfer (results not shown). The above described phenomena can therefore be considered as reactions to P stress, and not as being a consequence of aging.

Table 1. Citrate, malate and sugar in phloem exudates of three rape plants, collected in 500 µl 20 mM K-EDTA during 1 h. At t=0 rape plants were transferred from +P to −P nutrient solution. Values are given ± s.d. (n=6).

Time (days)	Sugar (nmol)	Citrate (nmol)	Citrate / Sugar	Malate (nmol)	Malate / Sugar
0	732 ± 78	32 ± 4	0.04 ± 0.00	104 ± 29	0.14 ± 0.04
4	601 ± 254	42 ± 14	0.07 ± 0.03	106 ± 37	0.18 ± 0.07
6	468 ± 107	72 ± 21	0.16 ± 0.06	97 ± 26	0.21 ± 0.11
8	296 ± 126	101 ± 7	0.34 ± 0.13	53 ± 12	0.18 ± 0.05

Table 2. Results of the labeling experiment. Shoots of rape plants grown for 7 days on nutrient solution were exposed to $^{14}CO_2$. If two values are given, the first one results from an experiment with 6 plants, the second one from 4 plants per 15 ml nutrient solution. If one value is given, it results from the first experiment.

Material	Quantity	Unit	Sample	Treatment			
				+P		−P	
Plant material	Fresh weight	mg plant^{-1}	shoot	525	486	237	213
			root	75	76	109	76
	Radioactivity	kBq g^{-1}	shoot	47	35	103	119
			root	14	12	40	41
		kBq plant^{-1}	shoot	24.7	17.0	24.4	25.3
			root	1.0	0.9	2.9	3.1
Nutrient solution	Radioactivity	kBq plant^{-1}		0.6	0.7	1.9	2.1
		Bq plant^{-1}	malate + citrate	5.9	4.4	90.8	72.5
	Amounts exuded	nmol plant^{-1}	malate	4.7		7.5	
			citrate	2.7		5.0	
	Specific activity	kBq mmol^{-1}	malate + citrate	0.80		7.27	

Labeling experiment

Rape plants were grown for 7 days with or without P. It has been shown that PEPC activity in −P shoots was 214% of that in +P shoots. Shoots of those plants were exposed to $^{14}CO_2$. Results of this experiment are given in Table 2.

The radioactivity fixed per gram fresh weight was about 2.5 times higher in −P shoots than in +P shoots. This proportion was even higher in the roots. This indicates an enhanced transport of labeled compounds from the shoot towards the root in −P plants. Because no significant difference in radioactivity of samples of the nutrient solution with and without addition of HCl was found, no $^{14}CO_2$ had been available to the roots. The radioactivity in the roots therefore had to originate from the shoots.

More organic acids, especially malate, were exuded by −P roots than by +P roots, which is in accordance with earlier results (Hoffland *et al.*, 1989).

Part of the nutrient solutions was chromatographed on a TLC plate. No discrimination between the spots of malate and citrate (r_f values 0.47 and 0.37, respectively) was possible on the chromatogram. Therefore, one large radioactive spot with a r_f value of about 0.42 was radioanalyzed. In combination with the results of the concentration determinations the specific activities of malate+citrate could be calculated. The −P plants exuded malate and citrate with a much higher specific activity than the +P plants (Table 2).

Discussion

The results presented indicate that organic acids exuded by roots of P-stressed rape plants originate from enhanced PEPC activity in the shoot. Increase of P stress coincided with increase of PEPC activity and accumulation of citrate in the shoot and subsequent increased phloem levels of citrate. In addition, $^{14}CO_2$ application to shoots resulted in higher specific activities of organic acids exuded by −P roots than by +P roots.

We showed that P stress induces enhanced PEPC activity in the shoot (Fig. 1). The causal relation between P stress and PEPC activity is unknown. When nitrate reductase activity (NRA) increases, PEP carboxylation can be increased, resulting in the production of malate, which plays a key-role in the intracellular pH-stat (Smith and Raven, 1979) or in the supply of reductants for NRA (Naik and Nicholas, 1986). However, Moorby *et al.* (1988) demonstrated that the P concentrations found in our P-stressed rape plants cause a reduction in NRA. A direct influence of P_i on PEPC is speculative, but cannot be excluded. Data on the effect of P_i on PEPC from C_4 plants *in vitro* are conflicting (Walker *et al.*, 1988). So far, effects of P deficiency on PEPC activity in C_3 plants have not been reported.

Malate must be regarded as the major product of PEP carboxylation (Lance and Rustin, 1984). Therefore, citrate accumulation in P-stressed rape shoots and subsequent root exudation of malate seems remarkable. However, a change from malate to citrate as the predominant organic acid in combination with higher tissue concentrations was demonstrated before by Landsberg (1981) in roots of several C_3 plants as a response to Fe deficiency. The same appeared to occur in P-stressed rape shoots. In the exuding root zones, however, where increased malate concentrations were determined (Hoffland *et al.*, 1989), the opposite should occur.

Accumulation of organic acids in P-stressed rape shoots could be caused by decreased oxidation rates due to decreased transport into the mitochondria. Wiskich (1975) reported that influx of malate and especially citrate into isolated mitochondria of *Brassica oleracea* L. is stimulated by P_i, and that the rate of entry of these acids can limit the rate of mitochondrial oxidation. It remains to be investigated whether this phenomenon plays a role in accumulation of organic acids in P-stressed rape plants.

The suggestion that exudation is not caused by leakage but by increased synthesis of organic acids is confirmed by the fact that the ratio of labeled compounds in the root to that in the nutrient solution did not differ between −P and +P plants. The increased transport of labeled compounds from shoot to root in −P plants and the increased specific activity of organic acids exuded by −P roots are strong indications that these acids originate from increased PEP carboxylation in the shoot.

Further research should establish which of the above reactions to P stress are specific for rock phosphate mobilizing species like rape in order to understand fully the exudation of organic acids.

Acknowledgements

The authors are very greatful to Mr Hans Overbeek for his hospitality and his essential suggestions during the performance of the labeling experiment. This was carried out entirely at his labortory at the Centre for Agrobiological Research, Wageningen, which is thanked for the supply of chemicals.

Mr Erik Heij and Mr Karel van Gaalen are thanked for analytical and technical assistance, and Dr Rien van Beusichem for his comments on the manuscript.

References

Anonymous 1986 Methods of biochemical Analysis and Food Analysis using Test-combinations. Boehringer Mannheim GmbH, Mannheim, Germany.

Anonymous 1989 Methods of biochemical Analysis and Food Analysis using single Reagents. Boehringer Mannheim GmbH, Mannheim, Germany.

Arnozis P A, Nelemans J A and Findenegg G R 1988 Phosphoenolpyruvate carboxylase activity in plants grown with either NO_3^- or NH_4^+ as inorganic nitrogen source. J. Plant Physiol. 132, 23-27.

Hoffland E, Findenegg G R and Nelemans J A 1989 Solubilization of rock phosphate by rape. II. Local root exudation of organic acids as a response to P-starvation. Plant and Soil 113, 161-165.

King R W and Zeevaart J A D 1974 Enhancement of phloem exudation from cut petioles by chelating agents. Plant Physiol. 53, 96-103.

Lance C and Rustin P 1984 The central role of malate in plant metabolism. Physiol. Vég. 22, 625-641.

Landsberg E-Ch 1981 Organic acid synthesis and release of hydrogen ions in response to Fe deficiency stress of mono- and dicotyledonous plant species. J. Plant Nutr. 3, 579-591.

Latzko E and Kelly G J 1979 The many-faceted function of phosphoenolpyruvate carboxylase in C_3 plants. Physiol. Vég. 21, 805-815.

Meyer C R, Rustin P and Wedding R T 1988 A simple and accurate spectrophotometric assay for phosphoenolpyruvate carboxylase activity. Plant Physiol. 86, 325-328.

Moorby H, Nye P H and White R E 1988 The effect of phosphate nutrition of young rape plants on nitrate reductase activity and xylem exudation, and their relation to H ion efflux from the roots. Plant and Soil 105, 257-263.

Murphy J and Riley J P 1962 A modified single solution method for the determination of phosphate in natural waters. Anal. Chim. Acta 27, 31-36.

Naik M S and Nicholas D J D 1986 Malate metabolism and its relation to nitrate assimilation in plants. Phytochem. 25, 571-576.

Novozamsky I, Houba V J G, Van Eck R and Van Vark W 1983 A novel digestion technique for multi-element plant analysis. Commun. Soil Sci. Plant Anal. 14, 239-248.

Smith F A and Raven J A 1979 Intracellular pH and its regulation. Ann. Rev. Plant Physiol. 30, 289-311.

Walker G H, Ku M S B and Edwards G E 1988 The effect of phosphorylated metabolites and divalent cations on the phosphatase and carboxylase activity of maize leaf phosphoenolpyruvate carboxylase. J. Plant Physiol. 133, 144-151.

Wiskich J T 1975 Phosphate-dependent substrate transport into mitochondria. Plant Physiol. 56, 121-125.

Chapter 5

Simulation of Nutrient Uptake by a Growing Root System Considering Increasing Root Density and Inter-Root Competition

Plant and Soil 124, 149-155 (1990)

with: Henny S. Bloemhof
Peter A. Leffelaar
Günter R. Findenegg and
Jacob A. Nelemans

Simulation of nutrient uptake by a growing root system considering increasing root density and inter-root competition

Key words: *Brassica napus* L., CSMP, diffusion, inter-root competition, nitrate, mass flow, nutrient uptake, quartz sand, simulation model, zero-sink

Abstract

A simulation model is presented which describes uptake of a growth limiting nutrient from soil by a growing root system. The root surface is supposed to behave like a zero-sink. Uptake of the nutrient is therefore determined by the rate of nutrient supply to the root surface by mass flow and diffusion. Inter-root competition and time dependent root density are accounted for by assigning to each root a finite cylindrical soil volume that delivers nutrients. The radius of these cylinders declines with increasing root density. Experiments with rape plants grown on quartz sand were used to evaluate the model. Simulated nitrogen uptake agreed well with observed uptake under nitrogen limiting conditions. In case no nitrogen limitation occurred nitrogen uptake was overestimated by the model, probably because the roots did not behave like a zero-sink any more.

Introduction

Simulation models for nutrient uptake have frequently been used in the evaluation of the effect of soil and root characteristics on nutrient uptake. Uptake models allowing for growing roots were developed by Nye *et al.* (1975), Claassen and Barber (1976) and Cushman (1979). No effects of time dependent root density on inter-root competition are included in these models. This hampers their use in the simulation of experiments with rapidly increasing root densities, as usually found in pot experiments.

Claassen and Barber did not include inter-root competition because they assumed that roots exploit a cylindrical soil volume with a constant solute concentration at the outer boundary. Their model overestimates nutrient uptake if nutrients are mobile (Silberbush and Barber, 1983). In Cushman's model the cylinder wall is impermeable to nutrients and the nutrient concentration at the cylinder wall declines in consequence of uptake. Though root growth is considered in this model, root density is kept constant in time *i.e.* the soil volume available per unit root length does not change with increasing root length. Therefore, in a situation with high root growth rates, this model also overestimates nutrient uptake (Silberbush and Barber, 1983). Baldwin *et al.* (1973) proposed an equation to extend Nye's model in which the effect of increasing root density on inter-root competition is described: the radius of each finite soil volume surrounding a root is a function of root density, assuming that each newly formed root samples the mean nutrient concentration. The validity of this assumption is questionable, because it is well established that roots branch

mostly in zones of highest nutrient concentration (Russell, 1977). Yet, Nye's model was only tested under conditions where inter-root competition was not expected so that the equation of Baldwin *et al.* was not included in the model. Overestimation of nutrient uptake by Nye's model was again attributed to inter-root competition (Brewster *et al.*, 1975).

The objective of this paper is to present a new simulation methodology that considers inter-root competition in a soil with increasing root density. Simulation results and experimental data will be compared.

Methods

Theoretical

The simulation model developed describes nutrient uptake by a root system that grows in a restricted soil volume. Each root is assigned a finite cylindrical soil volume delivering nutrients, and the soil volume per unit root length declines with increasing root density. Uptake of nutrients by the root, transport of nutrients to the root surface and the effect of increasing root density are considered as the three main components of this model and will be described subsequently.

Each timestep, the equation of continuity for cylindrical co-ordinates is solved:

$$\frac{\delta C}{\delta t} = -\frac{1}{r} \times \frac{\delta}{\delta r} (r \times F) + S \quad \text{(Eq. 1.9 in Nye and Tinker, 1977)}$$

The initial boundary condition is described by:

$$t = 0 \quad r > r_0 \quad C = C_i$$

The sink term S represents nutrient uptake by the root that is situated in the centre of a soil cylinder. All nutrients arriving at the root surface are supposed to be absorbed, *i.e.* the root surface is supposed to behave like a zero-sink. Root hairs are assumed to be so abundantly present that they are regarded to enlarge the root surfacial area to one located near their tips (Nye, 1966). The boundary condition at the tips of the root hairs is therefore:

$$t > 0 \quad r = r_0 \quad C_0 = 0$$

The rate of nutrient supply to the root surface by mass flow and diffusion is described according to

$$F = -D_e \times \frac{dC}{dr} + v \times C \quad \text{(Eq. 1.5 in Nye and Tinker, 1977)}$$

with

$$D_e = \Theta \times f \times D_0 \quad \text{(Nye, 1968)}$$

List of symbols

symbol	definition	units
A	total surface area of the pot	cm^2
C	ion concentration in soil solution	$\mu mol \times cm^{-3}$
C_0	ion concentration in the soil solution at the root surface	$\mu mol \times cm^{-3}$
C_i	initial ion concentration in the soil solution	$\mu mol \times cm^{-3}$
D_0	diffusion coefficient of ion in free solution	$cm^2 \times day^{-1}$
D_e	effective diffusion coefficient	$cm^2 \times day^{-1}$
F	flux of nutrients to root surface	$\mu mol \times cm^{-2} \times day^{-1}$
F_1	total flux of the nutrient across the outer boundary of the soil cylinder	$\mu mol \times cm^{-2} \times day^{-1}$
f	tortuosity factor	-
L_c	critical root length	cm
M_t	amount of P on infinite sink	$\mu mol \times cm^{-2}$
n	number of plants per pot	-
r	radial distance from root axis	cm
r_0	root radius + root hair length	cm
r_1	radius of the soil cylinder surrounding each root	cm
S	sink term	$\mu mol \times cm^{-3} \times day^{-1}$
t	time	day
V_1	total volume of the pot or soil layer considered	cm^3
v	inward water flux	$cm^3 \times cm^{-2} \times day^{-1}$
v_0	water flux across the root surface	$cm^3 \times cm^{-2} \times day^{-1}$
v_1	water flux across the outer boundary of the soil cylinder	$cm^3 \times cm^{-2} \times day^{-1}$
Θ	volumetric moisture content	$cm^3 \times cm^{-3}$

Buffering of the solute by the soil is not included.

Inter-root competition for nutrients is accounted for by assigning finite cylindrical volumes with radius r_1 to each root. Water but no nutrients can pass the cylinder wall, similarly to Cushman's model. The boundary condition at r_1 is therefore:

$$t > 0 \quad r = r_1 \quad F_1 = 0 \quad v_1 = r_0 \times v_0 / r_1$$

The initial radius of each soil cylinder is calculated by

$$t = 0 \quad r_1 = \sqrt{(A / (\pi \times n))}$$

assuming that each plant starts with one root growing in vertical direction. Each of these parallel soil cylinders is divided into a number of concentric compartments (shells). The time course of the concentration of nutrients in the soil solution in each of these shells is described according to the above mentioned equations.

Each time the actual root length exceeds a certain critical root length L_c, the outer shell of each soil cylinder is stripped off and their material is used to form new soil cylinders that are assigned to the newly developing roots. These new cylinders have the same radius as the older ones after stripping and they are divided into the same number of shells as left over in the stripped cylinders.

The solute concentration in the newly formed soil cylinders is initially the same as that in the outer stripped shells, where the nutrient concentrations are highest.

This can be interpreted as new roots penetrating between older ones, in zones of highest nutrient concentration. After formation, a new time dependent nutrient concentration gradient is calculated in each soil cylinder.

The critical root length L_c is a function of the current radius of the soil cylinder surrounding each root, according to the following equation:

$$L_c = V_1 / (\pi \times r_1^2)$$

Up to the first moment that a critical root length is reached and the stripping procedure is executed, the uptake calculation takes place with a root length that is half of the first critical root length. After reaching the first critical root length, the calculations are performed with the arithmetic average of the previous and newly calculated critical root length. Therefore, during the first half of each period nutrient uptake is overestimated, whereas during the last one it is underestimated. On the average this should yield reasonable results. This method spares CPU-time in comparison with a method by which uptake is calculated with the actual root length.

Additional conditions for which the model was developed are: neither temporal nor spatial gradients in volumetric moisture content occur, there is no nutrient production, no spatial gradient in root density and nutrient uptake is homogeneous along the root.

Plant parameters needed to run the model are: root length as a function of time, radius of the root, root hair length and the water uptake per unit root length as a function of time. Soil parameters needed are: the volumetric water content Θ, the tortuosity factor f, D_0 and the initial concentration C_i of the nutrient.

The CSMP-III simulation model was executed on a VAX computer using the variable time step integration method of Runge-Kutta Simpson. A copy of the model is available at request from the first authoress (E.H.).

Experimental

Experiments were done to provide the above mentioned soil and plant parameters for the model and to evaluate the model by comparing predicted nitrate uptake with observed nitrate uptake by rape plants growing in cylindrical pots on quartz sand.

Measurement of f as function of Θ The relation between the tortuosity factor f and Θ was determined by a method similar to that described by Vaidyanathan and Nye (1966). Iron oxide paper (2 × 4 cm; see Van der Zee *et al.*, 1987) was used as an "infinite sink" for phosphate ions. Quartz sand was washed with 1.5 M HCl and demineralized water respectively and subsequently heated at 900°C to make it inert with respect to phosphate so that the behaviour of phosphate did not differ essentially from that of nitrate. The pretreated sand was mixed with a nutrient solution containing 5.0 mM KH_2PO_4 and packed into Petri dishes (dry bulk density 1.28 g × cm^{-3}) with a piece of iron oxide paper on the bottom. The moisture content of the iron oxide paper had previously been equilibrated with quartz sand that was

moistened with demineralized water up to the desired Θ. To prevent sagging of the nutrient solution, the dishes were placed in an end-over-end shaker. After about 3 hours contact time the iron oxide paper was removed, washed in demineralized water, air dried, and extracted in 5 ml 0.2 M H_2SO_4. The extract was analyzed for phosphate by the molybdenum-blue method. D_e was then calculated from the amount of phosphate on the paper by

$$D_e = \frac{\pi \times M_t^2}{4 \times C^2 \times t}$$ (derived from Eq. 3.15 in Crank, 1975)

and f by

$$f = \frac{D_e}{\Theta \times D_0}$$ (derived from Nye, 1968).

Plant growth Rape plants (*Brassica napus* L. cv. Jetneuf) were grown for 16 days on quartz sand in cylindrical pots at two nitrate levels. Ten plants of rape were grown in 3-l pots (ϕ 12 cm, height 27 cm). Each pot contained a mixture of 3.2 kg quartz sand (dry bulk density 1.28 g $\times$ cm^{-3}) and 575 ml (high moisture level) or 385 ml (low moisture level) nutrient solution. In the 1.5 mM-treatment 1.5 mM KNO_3 and 3.5 mM KCl were added to the nutrient solution which consisted of 5.0 mM $CaCl_2$, 2.0 mM $MgSO_4$, 2.0 mM KH_2PO_4 and trace elements (in mg $\times$ l^{-1}): Fe (as FeEDTA) 4.6; B 0.5; Mn 0.5; Zn 0.05; Cu 0.02; Mo 0.01. In the 5.0 mM-treatment 5.0 mM KNO_3 was added. The plants were grown in a growth chamber at 20°C, a 16 h light (70 W $\times$ m^{-2}) - 8 h dark cycle and a relative humidity of about 80%. Evaporation (from blanc pots) and evapotranspiration were measured daily and the moisture level was readjusted daily. The beginning of the experiment, t=0 was defined as the moment at which half of the plants was germinated. At each harvest, a number of pots was deep frozen and divided into five layers of 4.5 cm height by sawing. The layers are referred to as layer I through V, from top to bottom. Root length, Θ and nitrate concentration in the soil solution were measured in each layer. Nitrate was measured after extraction of dried sand by automatic spectrophotometry after reduction to nitrite. From other pots, plant material was dried and analyzed for N after wet digestion in a H_2SO_4-Se-salicylic acid mixture with addition of H_2O_2. Total N was determined by the indophenol blue method.

Results

Experimental

Measurement of f as function of Θ The value of f for different volumetric moisture contents is shown in Figure 1.

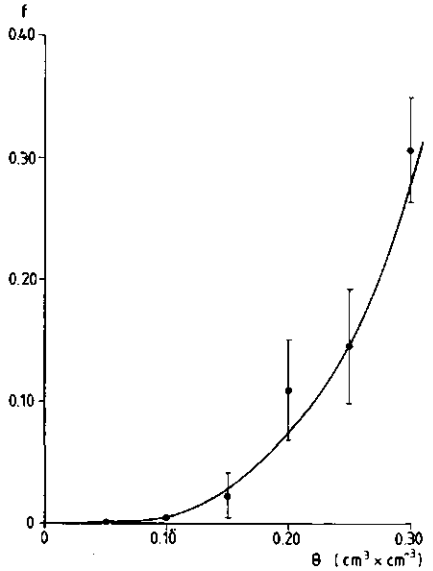

Fig. 1. Experimentally determined relation between f and Θ (means ± s.d., $n=5$) and relation used in the simulation model (——).

Plant growth Substantial differences in volumetric moisture content Θ (Table 1) and in the nitrate concentration in the soil solution (Table 2) were found among the five layers of one pot. In pots without plants neither of these two gradients changed significantly during the experiment. Differences of Θ with depth resulted from the poor water holding capacity of the quartz sand used. The difference in N concentration among layers is probably caused by the fact that evaporation from layer I was compensated by supplying demineralized water via a tube positioned with its bottom in layer II.

Also considerable difference in root length among the layers (Table 3) was found within one treatment. There was no difference among the five layers with respect to root radius (0.02 cm) and root hair length (± 0.05 cm).

Water uptake per cm root declined from 0.028 $cm^3 \times day^{-1}$ at the beginning of the experiment to 0.002 $cm^3 \times day^{-1}$ at the end. No significant differences were found among the three treatments.

Total N uptake per pot and tissue N concentration as a function of time for each treatment are depicted in Figures 2 and 3, respectively. In the high moisture level treatments no nitrate was left in the soil solution at the end of the experiment, while in the low moisture level treatment about 30 % of the added nitrogen was left in

Table 1. Volumetric water content per layer for each treatment. Values are means of two replicates.

N level (mM):	1.5	5.0	5.0
Moisture level:	high	high	low
Layer	Volumetric water content ($cm^3 \times cm^{-3}$)		
I	0.13	0.13	0.10
II	0.19	0.19	0.08
III	0.23	0.23	0.15
IV	0.28	0.28	0.17
V	0.30	0.30	0.21

Table 2. Concentration N in the soil solution per layer at t=0 for each treatment. Values are means of two replicates.

N level (mM):	1.5	5.0	5.0
Moisture level:	high	high	low
Layer	N concentration in soil solution (mM)		
I	2.6	9.6	7.7
II	0.5	2.7	1.9
III	1.2	3.9	4.9
IV	1.3	5.6	6.4
V	1.6	5.1	4.8

Table 3. Root length per layer as a function of time for each treatment. Values are means of two replicates.

Layer	1.5 mM N high moisture level					5.0 mM N high moisture level					5.0 mM N low moisture level				
	Root length (m) after 0, 3, 7, 10 or 16 days														
	0	3	7	10	16	0	3	7	10	16	0	3	7	10	16
I	0.3	1.6	5.0	6.4	12.5	0.3	1.2	6.4	8.4	24.8	0.3	0.3	4.3	7.2	10.0
II	0.1	0.5	3.7	4.1	6.6	0.1	0.3	3.5	7.2	10.9	0.1	0.1	2.5	7.1	5.5
III	0.0	0.2	2.8	4.5	8.3	0.0	0.2	3.3	7.2	17.0	0.0	0.0	1.9	9.3	11.8
IV	0.0	0.1	1.4	4.2	11.7	0.0	0.1	2.3	5.7	19.1	0.0	0.0	1.4	8.0	17.7
V	0.0	0.0	0.8	2.0	19.7	0.0	0.1	0.8	4.0	41.7	0.0	0.0	0.6	3.9	25.1

the nutrient solution (mainly in layers IV and V).

Simulation

To approximate the model conditions of absence of spatial gradients in moisture level and root density, the simulation model was run for each of the five soil layers considered in the experiment. Total N uptake per pot was calculated by summation.

Soil parameters presented in Table 1 and Figure 1 were used to run the model. The simulation was initialized with respect to the N concentration in the soil solution with data given in Table 2. Root length (Table 3) and water uptake per cm root as a function of time were used as forcing functions. The initial number of shells surrounding a root was set to 27. During the simulation period this number declined to 1 at t=16 days in consequence of root growth.

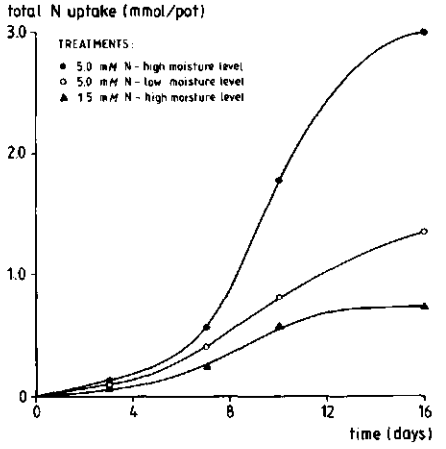

Fig. 2. N uptake by rape plants grown on quartz sand as a function of time. Values are means of three replicates.

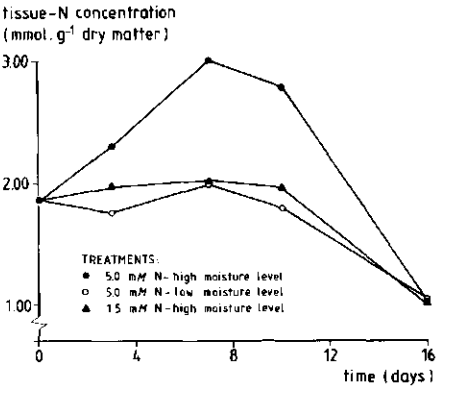

Fig. 3. N concentration in dry matter of rape plants grown on quartz sand as a function of time. Values are means of three replicates.

Predicted and observed N uptake are shown in Figure 4.

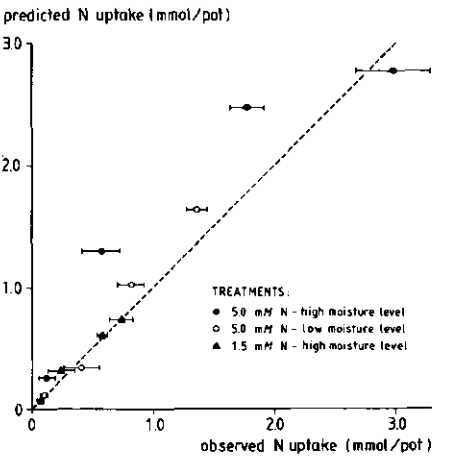

Fig. 4. **Comparison of observed N uptake (means ± s.d., $n=3$) by rape with predicted N uptake for three treatments. The dashed line is where predicted uptake equals observed uptake.**

Discussion

Comparison of predicted N uptake with observed uptake (Fig. 4) shows good agreement for the 1.5 mM-high moisture level and 5.0 mM-low moisture level treatment. When predicted and observed N uptake are compared per layer (data not shown) for the 1.5 mM-high moisture level treatment, there is a close agreement in each layer. This means that both nutrient transport to the root surface and the effect of increasing root density on nutrient uptake are simulated well. In the 5.0 mM-low moisture level treatment a slight overestimation of N uptake occurs after 10 days of growth. This overestimation originates from layer IV and V, with relatively high amounts of N available in the soil solution.

The model overestimates N uptake for the 5.0 mM-high moisture level treatment (Fig. 4). Figure 3 shows that tissue N concentration in this treatment at $t=7$ and $t=10$ days is about 3 mmol $\times$ g^{-1} dry matter, which is the concentration of N sufficient rape plants (Hoffland *et al.*, 1989). Nitrate was apparently not growth limiting and therefore, it is very likely that the model assumption that roots act like a zero-sink is not met under these conditions. This will cause an overestimation of N uptake. The model should be extended with a description of biologically controlled nutrient uptake to simulate uptake under these conditions. No effort has been made to describe nutrient uptake by first order or Michaelis-Menten kinetics, because too little is known about the required parameters and their dependence on root age, state of development of the plant and nutrient status of the plant.

The satisfactory prediction of N uptake in cases where N is growth limiting throughout the experiment indicates that the presented equations that describe the effects of inter-root competition and increasing root density on nutrient uptake are powerful.

Acknowledgements

The authors are very grateful to Dr ir J. Goudriaan for his valuable suggestions in the development of the simulation model. Thanks are also due to Mr E. Heij for

analytical assistance and to Dr ir B. H. Janssen for his useful comments on the manuscript.

References

Baldwin J P, Nye P H and Tinker P B 1973 Uptake of solutes by multiple root systems from soil. III. A model for calculating the solute uptake by a randomly dispersed root system developing in a finite volume of soil. Plant and Soil 38, 621-635.

Brewster J L, Bhat K K S and Nye P H 1975 The possibility of predicting solute uptake and plant growth response from independently measured soil and plant characteristics. III. The growth and uptake of onions in a soil fertilized to different initial levels of phosphate and a comparison of the results with model predictions. Plant and Soil 42, 197-226.

Claassen N and Barber S A 1976 Simulation model for nutrient uptake from soil by a growing plant root system. Agron. J. 68, 961-964.

Crank J 1975 The Mathematics of Diffusion, 2nd edition. Clarendon Press, Oxford, 414 p.

Cushman J H 1979 An analytical solution to solute transport near root surfaces for low initial concentration: I. Equations development. Soil Sci. Soc. Am. J. 43, 1087-1090.

Hoffland E, Findenegg G R and Nelemans J A 1989 Solubilization of rock phosphate by rape. I. Evaluation of the role of the nutrient uptake pattern. Plant and Soil 113, 155-160.

Nye P H 1966 The effect of the nutrient intensity and buffering power of a soil, and the absorbing power, size and root hairs of a root, on nutrient absorption by diffusion. Plant and Soil 25, 81-105.

Nye P H 1968 The use of exchange isotherms to determine diffusion coefficients in soil. 9th Int. Cong. Soil Sci. Trans. (Adelaide) 1, 117-126.

Nye P H and Tinker P B 1977 Solute Movement in the Soil-Root System. Blackwell Scientific Publications, Oxford, 342 p.

Nye P H, Brewster J L and Bhat K K S 1975 The possibility of predicting solute uptake and plant growth response from independently measured soil and plant characteristics. I. The theoretical basis of the experiments. Plant and Soil 42, 161-170.

Russell R S 1977 Plant Root Systems: Their Function and Interaction with the Soil. McGraw-Hill Book Company, London, 298 p.

Silberbush M and Barber S A 1983 Prediction of potassium uptake by soybeans with a mechanistic mathematical model. Soil Sci. Soc. Am. J. 47, 262-265.

Vaidyanathan L V and Nye P H 1966 The measurement and mechanism of ion diffusion in soils. II. An exchange resin paper method for measurement of the diffusive flux and diffusion coefficient of nutrient ions in soils. J. Soil Sci. 17, 175-183.

Van der Zee S E A T M, Fokkink L G J and Van Riemsdijk W H 1987 A new technique for assessment of reversibly adsorbed phosphate. Soil Sci. Soc. Am. J. 51, 599-604.

Chapter 6

Use of a Simulation Model to Quantify the Amount of Phosphate Released from Rock Phosphate by Rape

Transactions of the 14th International Congress of Soil Science (Kyoto) II, 170-175 (1990)

with: Günter R. Findenegg
Peter A. Leffelaar and
Jacob A. Nelemans

Use of a simulation model to quantify the amount of phosphate released from rock phosphate by rape

Abstract

The simulation model described by Hoffland *et al.* (1990) was used to predict P uptake by rape from inert quartz sand mixed with nutrient solution. The model was evaluated by comparing observed and predicted uptake of dissolved P, which agreed well. In case P uptake from rock phosphate was simulated without considering the effect of rhizosphere acidification on rock phosphate solubility, predicted P uptake was only about 6% of the observed P uptake after 16 days of growth. It was concluded that about 94% should originate from the rock phosphate that dissolves as a result of exudation of organic acids by roots of rape.

Introduction

In earlier papers it was established that rape (*Brassica napus* L.) is, in contrast to other species, an effective user of sparsely soluble rock phosphates (Hoffland *et al.*, 1989a). It was demonstrated that rape acidifies its rhizosphere along a root zone of about 1.5 cm behind the root tip by exudation of organic acids as a reaction to P deficiency (Hoffland *et al.*, 1989b). This rhizosphere acidification was assumed to be the cause of increased solubilization of rock phosphate.

In this paper we estimate the amount of P released from rock phosphate by rape as the difference between results of model simulations of P uptake without considering solubilization of rock phosphate by root exudation with experimentally determined P uptake by rape grown on rock phosphate.

Therefore, first the validity of the model for the simulation of nutrient uptake described by Hoffland *et al.* (1990) is checked with respect to its ability to predict uptake of dissolved P by a growing root system of rape at low P levels. Most other simulation models (Nye *et al.*, 1975; Claassen and Barber, 1976; Cushman, 1979) underestimate the uptake of P from low P soils, even in case of non-rock phosphate-mobilizing plant species. This underestimation is attributed to lack of inclusion of the effect on P uptake of root hairs (Brewster *et al.*, 1976; Schenk and Barber, 1979; Fontes *et al.*, 1986) or of the effects of inter-root competition in the model (Brewster *et al.*, 1975). In the model of Hoffland *et al.* both phenomena are accounted for.

In a pot experiment the amount of P absorbed by rape grown on quartz sand mixed with a nutrient solution containing soluble P (KH_2PO_4) has been measured. The experiment provided plant and soil parameters for the simulation model. The P uptake measured in the pot experiment and the P uptake calculated by the model are compared and the differences are discussed in this paper.

Methods

Experiment

Rape plants (*Brassica napus* L. cv. Jetneuf) were grown for 16 days after germination on cylindrical pots (10 plants per pot; ϕ 12 cm) filled to a height of 20 cm with 3.1 kg quartz sand mixed with 560 ml nutrient solution. To make the quartz sand as inert as possible it was previously washed in 1.5 M HCl, rinsed with water and subsequently heated at 900°C for a few hours. The nutrient solution consisted of 5.0 mM $Ca(NO_3)_2$; 5.0 mM KNO_3; 2.0 mM $MgSO_4$ and trace elements (Hoffland *et al.*, 1989a). In the 0.01 mM P and the 0.05 mM P treatment 0.01 mM and 0.05 mM KH_2PO_4 was added, respectively. In the rock phosphate (rock P) treatment 560 mg Mali rock phosphate (13.4 % P) was mixed with the quartz sand. The moisture content was adjusted daily for each treatment. Growth conditions were: temperature: 20°C; light period: 16 h (70 W $\times$ m^{-2}); relative humidity: $\pm$ 80%.

Plant material from part of the pots was dried and analyzed for P at each harvest. Other pots were deep frozen and divided into five layers of 4 cm height, referred to as layer I to V, from top to bottom. Root length and volumetric moisture content were determined in each layer.

The initial P concentrations in the soil solution of each layer were determined at t=0 (moment of germination) and t=16 after collecting the soil solution by extraction under vacuum from pots without plants. Evapotranspiration was measured daily. The tortuosity factor as dependent on the volumetric moisture content was determined as described by Hoffland *et al.* (1990).

Simulation

The main part of the simulation model used was described earlier (Hoffland *et al.*, 1990). Only essential characteristics are given here. The root is located in the centre of a soil cylinder from which a limited amount of nutrients can be withdrawn. The root surface behaves like a zero-sink, *i.e.* each phosphate ion arriving at the root surface is absorbed, resulting in a zero P concentration at the root surface. Therefore, uptake of phosphate is the resultant of phosphate supply to the root surface by mass flow and diffusion, described according to Nye and Tinker (1977; eqn 1.5). Interaction of phosphate with the soil is not described in the model.

Inter-root competition is accounted for in the model similarly as in Cushman (1979): the wall of the considered soil cylinder surrounding a root is impermeable to nutrients. This results in a decreasing phosphate concentration at the cylinder wall in consequence of uptake.

To describe the effect of increasing root density on phosphate uptake the soil cylinders are divided into a number of shells. Each time the root length exceeds the length of the current soil cylinder, the outer shell of the cylinder is stripped off and

its material is used to form a new soil cylinder, assigned to the newly grown roots. The new soil cylinder is divided into the same number of shells as left over in the stripped cylinders. Initially the phosphate concentration in the newly formed soil cylinders equals the weighted mean concentration of the shells from which they originate.

Root hairs are considered as an extension of the root radius.

To predict P uptake from rock phosphate without considering rhizosphere acidification, the model was extended. It is assumed that the P concentration in the soil solution of each shell remains constant as long as the amount of rock phosphate in that shell is not depleted. The amount of phosphate transported from a shell towards the root is replenished from the rock phosphate immediately, *i.e.* the chemical equilibrium which keeps the phosphate concentration at a certain constant level is adjusted without delay. The zero-sink concept is maintained, resulting in a concentration gradient of zero at the root surface to the equilibrium concentration within the innermost shell.

The soil parameters necessary to run the simulation model are: the volumetric moisture content, the diffusion coefficient of phosphate in water, the tortuosity factor, and the initial concentration of phosphate in the soil solution and in rock phosphate. Required plant parameters are: root length and water uptake per unit root length as a function of time, root radius and root hair length. No spatial gradients in volumetric moisture content and root density are accounted for in the model.

The CSMP-III model was run on a VAX computer using the variable time step integration method of Runge-Kutta Simpson. The initial number of shells was set to 20.

Results

Experiment

The initial P concentrations in the soil solution and the root lengths determined in the experiment are given in Table 1 and 2, respectively. The volumetric moisture contents were 0.15, 0.21, 0.25, 0.28 and 0.31 in layer I to V in all treatments due to redistribution of water by gravity. The initial P concentration differed considerably from one layer to another. Part of the phosphate added with the nutrient solution is apparently adsorbed on the quartz sand. If the amount adsorbed per gram sand would be the

Table 1. Initial phosphate concentration (mM) in the soil solution of each layer for all treatments in pots without plants. Results are means of two.

	Treatment		
Layer	0.01 mM P	0.05 mM P	rock P
I	0.003	0.009	0.004
II	0.007	0.029	0.001
III	0.010	0.041	0.001
IV	0.011	0.042	0.001
V	0.012	0.044	0.001

Table 2. Root length (m) per layer as a function of time for each treatment. Values are means of two.

	0.01 mM P					0.05 mM P					rock P				
Time (days)	0	4	7	11	16	0	4	7	11	16	0	4	7	11	16
Layer															
I	0.3	2.7	5.2	8.6	11.9	0.3	2.7	5.8	7.7	9.5	0.3	2.6	7.4	12.5	25.2
II	0.1	1.2	5.3	7.3	11.8	0.1	1.9	4.0	9.0	11.6	0.1	0.6	3.6	11.7	24.5
III	0.0	0.6	2.0	4.8	9.7	0.0	0.2	1.2	7.4	12.9	0.0	0.4	1.3	8.8	17.3
IV	0.0	0.0	0.4	1.7	4.0	0.0	0.0	0.1	2.4	11.3	0.0	0.0	0.1	3.3	13.7
V	0.0	0.0	0.0	0.2	0.7	0.0	0.0	0.0	0.1	3.3	0.0	0.0	0.0	0.7	3.9

same in each layer, the different initial concentrations could partly be caused by the different volumetric moisture contents.

The amounts of P absorbed by the plant as a function of time are shown in Figure 1. In the 0.01 mM P and 0.05 mM P treatment growth was strongly limited by P: the tissue concentration of P declined in both treatments from about 90 mmol × kg^{-1} dm after 4 days of growth to about 25 mmol × kg^{-1} dm after 16 days. In case of the rock P treatment the tissue concentration of P remained constant at about 85 mmol × kg^{-1} dm throughout the experiment.

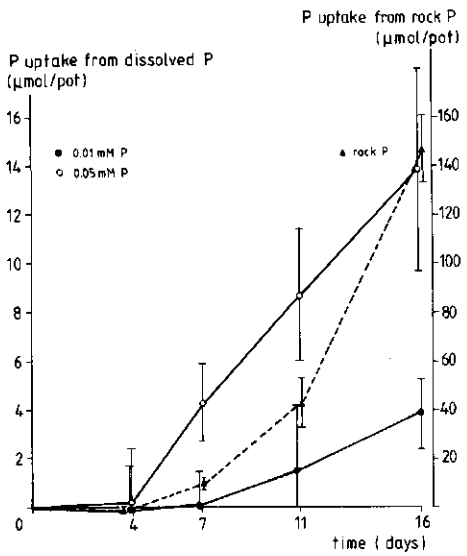

Figure 1. P uptake (means ± s.d.; n=3) by rape plants grown on quartz sand as a function of time. Note the different scales for the different treatments.

Simulation

Soil and plant parameters derived from the pot experiments were used to run the model. In case of the rock P treatment the concentrations of dissolved P as given in Table 1 were taken as the equilibrium concentrations.

The simulation model was run for each of the considered soil layers. Total P uptake was calculated by summation.

Predicted and observed P uptake are shown in Figure 2.

Discussion and Conclusions

The results of the experiments show that there are considerable differences in initial P concentration (Table 1), root length (Table 2) and volumetric moisture content between the five layers of one pot. Since the model does not account

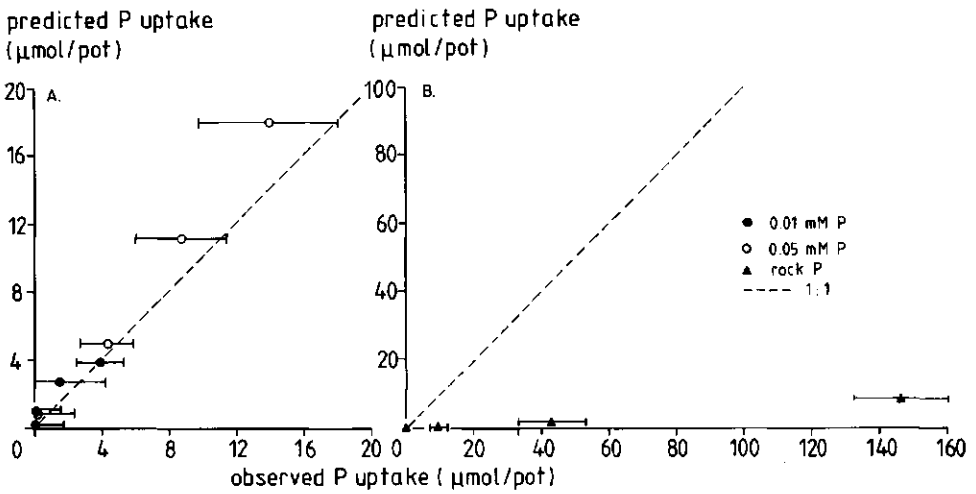

Figure 2. Comparison of observed P uptake (means ± s.d.; n=3) by rape with predicted uptake for the 0.01 and 0.05 mM P (A) and the rock P treatment (B).

for spatial gradients in these parameters, the necessity to distinguish five layers instead of one for simulations is evident.

There is a relatively large scatter in the observed P uptake (Fig. 1). This is partly due to the fact that from the small amounts of P in the plants the amounts already available in the seeds (11.41 ± 1.14 µmol) had to be subtracted to calculate P uptake.

Figure 2A shows good agreement between predicted and observed P uptake in case of the 0.01 mM P and the 0.05 mM P treatment although the model tends to overestimate P uptake slightly. This may be caused by the model assumption that at any time the roots are regularly distributed within one layer. In the experiment the roots will exploit the upper part of a layer first before penetrating into the lower part. Distinguishing more layers would be an improvement in this respect. In case the roots would be distributed random in the experiment instead of regular as supposed in the model, this could also cause an overestimation by the model (De Willigen and Van Noordwijk, 1987). The model descriptions of the effect of root hairs, inter-root competition and increasing root density seem powerful.

Figure 2B shows that the model underestimates P uptake from rock phosphate by rape considerably. This must be due to the fact that the effect of organic acid exudation on rock phosphate solubility was not included into the model. From Fig. 2A it can be concluded that the model would predict P uptake well in case no interaction with rock phosphate would occur. Therefore, the amount of P absorbed in the rock phosphate experiment diminished by the predicted amount absorbed must be the amount of P dissolved as a result of exudation of organic acids. Sixteen days after germination this is about 140 µmol P per pot, equivalent to 94% of the total

amount absorbed.

References

Brewster J L, Bhat K K S and Nye P H 1975 The possibility of predicting solute uptake and plant growth response from independently measured soil and plant characteristics. III. The growth and uptake of onions in a soil fertilized to different initial levels of phosphate and a comparison of the results with model predictions. Plant Soil 42, 197-226.

Brewster J L, Bhat K K S and Nye P H 1976 The possibility of predicting solute uptake and plant growth response from independently measured soil and plant characteristics. V. The growth and phosphorus uptake of rape at a range of phosphorus concentrations and a comparison of results with the predictions of a simulation model. Plant Soil 44, 295-328.

Claassen N and Barber S A 1976 Simulation model for nutrient uptake from soil by a growing root system. Agron.J. 68, 961-964.

Cushman J H 1979 An analytical solution to solute transport near root surfaces for low initial concentration: I. Equations development. Soil Sci. Soc. Am. J. 43, 1087-1090.

De Willigen P and Van Noordwijk M 1987 Uptake potential of non-regularly distributed roots. J. Plant Nutr. 10, 1273-1280.

Fontes P C R, Barber S A and Wilcox G E 1986 Prediction of phosphorus uptake by two tomato cultivars growing under insufficient and sufficient phosphorus soil conditions using a mechanistic mathematical model. Plant Soil 94, 87-97.

Hoffland E, Findenegg G R and Nelemans J A 1989a Solubilization of rock phosphate by rape. I. Evaluation of the role of the nutrient uptake pattern. Plant Soil 113, 155-160.

Hoffland E, Findenegg and J.A. Nelemans 1989b Solubilization of rock phosphate by rape. II. Local root exudation of organic acids as a reaction to P stress. Plant Soil 113, 161-165.

Hoffland E, Bloemhof H S, Leffelaar P A, Findenegg G R and Nelemans J A 1990 Simulation of nutrient uptake by a growing root system considering increasing root density and inter-root competition. Plant Soil 124, 149-155.

Nye P H, Brewster J L and Bhat K K S 1975 The possibility of predicting solute uptake and plant growth response from independently measured soil and plant characteristics. I. The theoretical basis of the experiments. Plant Soil 42, 161-170.

Nye P H and Tinker P B 1977 Solute Movement in the Soil-Root System. Blackwell Scientific Publications, Oxford, United Kingdom. 342 pp.

Schenk M K and Barber S A 1979 Root characteristics of corn genotypes as related to P uptake. Agron.J. 71, 921-924.

Chapter 7

Quantitative Evaluation of the Role of Organic Acid Exudation in the Mobilization of Rock Phosphate by Rape

Submitted for publication in Plant and Soil

Quantitative evaluation of the role of organic acid exudation in the mobilization of rock phosphate by rape

Key words: *Brassica napus* L., citric acid, malic acid, pH gradient, phosphate uptake, rhizosphere, rock phosphate, root exudation, root hairs, simulation

Abstract

Phosphorus-deficient rape plants appear to acidify part of their rhizosphere by exuding malic and citric acid. A simulation model was used to evaluate the effect of measured exudation rates on phosphate uptake from Mali rock phosphate. The model used was one on nutrient uptake, extended to include both the effect of ion uptake and exudation on rhizosphere pH and the effect of rhizosphere pH on the solubilization of rock phosphate. Only the youngest segments of the root system were assumed to exude organic acids. The transport of protons released by organic acids was described by mass flow and diffusion. An experimentally determined relation describing pH and phosphate concentration in the soil solution as a function of total soil acid concentration was used. Model parameters were determined in experiments on organic acid exudation and on the uptake of P by rape from a mixture of quartz sand and rock phosphate.

Simulation results indicated that the exudation rates measured in rape plants deficient in P can provide the roots with more phosphate than is actually taken up. Presence of root hairs enhanced the effect of organic acid exudation on calculated phosphate uptake. However, increase of root hair length without exudation as an alternative strategy to increase phosphate uptake from rock phosphate appeared to be less effective than exudation of organic acids.

It was concluded that organic acid exudation is a highly effective strategy to increase phosphate uptake from rock phosphate, and that it is unlikely that other rhizosphere processes play an important role in rock phosphate mobilization by rape.

Introduction

In earlier papers it was shown that roots of P-deficient rape plants decrease the pH of their rhizosphere by exuding malic and citric acid (Hoffland *et al.*, 1989b). The relatively high phosphate uptake from poorly soluble rock phosphates by rape was attributed to this rhizosphere acidification. The work reported here aimed to quantify the possible effect of organic acid exudation on phosphate uptake from rock phosphate (rock P) in order to evaluate the effectiveness of this reaction of rape to P deficiency. The exudation rates of plants grown without phosphate were assumed to represent the maximum phosphate mobilizing potential of rape plants grown with nitrate as N source.

A simulation model was used to calculate phosphate uptake from rock P when organic acids are exuded, using independently measured plant, soil and rock P parameters. This paper describes how organic acid exudation and its effect on the solubilization of rock P and phosphate uptake were incorporated into a previously

described model (Hoffland *et al.*, 1990a) and reports the results of experiments to measure the required parameters. The validity of the model and its sensitivity to parameters on organic acid exudation and root hair length will be discussed.

Methods

Simulation

The model developed by Hoffland *et al.* (1990a) describes nutrient uptake by a growing root system on the basis of nutrient transport towards a root by mass flow and diffusion. Nutrient uptake by root hairs, inter-root competition and the effect of increasing root density are considered. It has been demonstrated that this model predicts the uptake of dissolved phosphate well under phosphate-limiting conditions (Hoffland *et al.*, 1990b). Therefore, it was used as the basis for an extended model that describes how pH gradients develop around roots as a result of organic acid exudation and ion uptake, and what effect these gradients have on the solubility of rock P and on phosphate uptake by rape.

When rock P dissolves as a consequence of acid exudation by roots, two interacting ions are transported in opposite directions: protons are transported from the root surface towards the bulk solution whereas phosphate ions move from the bulk solution towards the root surface. Meanwhile, protons solubilize rock P. Nye (1983) developed equations to describe this situation in the case that two solutes with known or measurable interaction coefficients diffuse. He applied his theory to pH changes and phosphate solubilization near roots (Nye, 1984).

There are two limitations to applying Nye's equations. Firstly, not only diffusive transport but also mass flow occurs. Secondly, the precise composition of rock phosphates is often unknown: they consist of microcrystalline carbonate fluorapatite, all the ionic components (Ca^{2+}, PO_4^{3-}, CO_3^{2-} and F^-) of which may be substituted for other ions. Furthermore, the apatite is associated with a variety of impurities, accounting for 5 to 20% of the rock P and these can have a profound influence on its solubility (Khasawneh and Doll, 1978). Therefore, often no solubility product or interaction coefficient with protons can be given. In the model developed, an experimentally determined relationship between the concentration of total soil acid and the phosphate concentration in the soil solution is used to overcome this problem. "Total soil acid" is taken to mean the total amount of H_3O^+, *i.e.* the amount buffered by the solid phase plus the amount dissolved.

The model considers two ways of changing the rhizosphere pH. On the one hand roots excrete OH^- (or HCO_3^-) to maintain electrical neutrality across the soil-root interface when nitrate is the N source. This results in alkalinization of the rhizosphere. The magnitude of this OH^- flux was determined experimentally (see *Growth experiment*). It is assumed that this excretion is homogeneous along the root. To simplify model calculations, the model simulates this flux as H_3O^+ uptake instead

of OH^- excretion. On the other hand, the youngest segments of roots of P-deficient rape plants exude malic and citric acid, resulting in acidification of their rhizosphere (Hoffland *et al.*, 1989b). Both the exudation rates of malic and citric acid and the proportion of the root system that is exuding were determined experimentally (see *Experiments*). To simplify model calculations the organic acid flux was transformed into a proton flux. The pH of the inner shell surrounding the exuding root segment is assumed to determine the number of protons released per mole of malic and citric acid exuded. Transport of malate and citrate is not described in the model.

The geometry of the soil-root system described in the existing model (Hoffland *et al.*, 1990a) is adapted to account for the differentiation of organic acid exudation along the root. In the model the soil cylinder surrounding a root is divided into shells. Each time the actual root length exceeds the current length of the soil cylinder, the outer shell is stripped off and its material is used to form a new cylinder section which is assigned to the youngest part of the root system. The length of this new cylinder section is determined by the volume of the outer shell from which it originated and by the radius of the stripped cylinder. By altering the thickness of the shells, it can be achieved that the length of the youngest cylinder section always accounts for a constant proportion of the total cylinder length, corresponding with the length of the exuding root segment.

H_3O^+ transport, like phosphate transport, is described by mass flow and diffusion. Each time step, phosphate and H_3O^+ fluxes are calculated and integrated, resulting in new amounts of phosphate and total soil acid in each shell surrounding the root. Then, the new equilibrium concentrations of phosphate and H_3O^+ in the soil solution of each shell are read from the previously mentioned empirical relationship between the total soil acid concentration on the one hand and the concentration of H_3O^+ and of phosphate in the soil solution on the other hand. It is assumed that the equilibrium between soil solution and surface of rock P is established instantaneously and that no delay is caused by the diffusion of phosphate ions from the dissolving surface towards the bulk solution. The new equilibrium concentration of phosphate in the soil solution determines how much rock P dissolves or how much phosphate precipitates. New fluxes are calculated on the basis of these new concentrations of H_3O^+ and phosphate in the soil solution of the shells. Maintenance of electroneutrality during ion transport is ignored.

Experiments

Phosphate and H_3O^+ concentration in the soil solution as a function of the total soil acid concentration Experiments were done to find the effect of the total soil acid concentration on the phosphate concentration and pH in the soil solution in a mixture of rock phosphate and quartz sand. Various conditions were used to examine the solubility of Mali rock phosphate; they corresponded with those expected in the growth experiment (see below).

Mali rock phosphate (13.4% P) was mixed with quartz sand (180 mg $\times$ kg^{-1}) and

nutrient solution containing no phosphate but with varying H_3O^+ concentrations, obtained by the addition of HCl. This H_3O^+ concentration before mixing is referred to as total soil acid concentration. The soil solution was extracted under vacuum after incubation at 20°C for one week. In this soil solution the phosphate concentration was determined (molybdenum-blue method) and the pH was measured.

Table 1. Pretreatment of Mali rock phosphate for the determination of the relation between total soil acid concentration and H_3O^+ and phosphate concentration in the soil solution.

Number of extractions	Extractant	Proportion of P removed from rock P (%)
1	demin. water	0
1	HCl pH 2.5	13
1	HCl pH 2.0	30
2	HCl pH 2.0	50

This experiment was done at different moisture contents (Θ 0.15, 0.20 and 0.30) and different pretreatments of rock P to investigate the solubility of Mali rock P under varying conditions. To mimic aging of rock P it was pretreated in order to obtain types of rock P from which different proportions of P had been dissolved. The original rock P was extracted in different concentrations of HCl (18 cm^3 HCl in different molarities × 18 mg^{-1} rock P), centrifuged (20 min. at 48,000 × *g*), eventually extracted and centrifuged again, and air dried. The phosphate concentration in the extractant was measured (molybdenum-blue method) to find the proportion of P dissolved. Table 1 shows the different pretreatments and the resulting types of rock P used in the experiment described above.

Growth experiment Rape plants were grown in cylindrical pots filled with a mixture of quartz sand, Mali rock P and a nutrient solution containing nitrate as N source but no phosphate. The pH of the nutrient solution before mixing was 5.2. For details see Hoffland *et al.* (1990b).

Five pots were harvested 0, 4, 7, 11 and 16 days after germination. Dry weight and total N, P, K, Ca and Mg content of the plants from three pots were determined after wet digestion of dried subsamples in a mixture of H_2SO_4, Se and salicylic acid with added H_2O_2. Total N was determined by the indophenol blue method, total P by the molybdenum-blue method, K and Ca by flame photometry and Mg by atomic absorption spectrometry. The S concentration in plant tissue was estimated using a previously observed relation between S and N concentration (S concentration = 0.08 × N concentration; Hoffland *et al.*, 1989a).

Two pots were divided into five equally thick horizontal layers, in which root length and volumetric moisture content (Θ) were determined.

Data on Θ, root length and transpiration were used as model parameters. To calculate OH^- excretion rates per cm root the difference between the sum of cations and the sum of anions taken up during a time interval was calculated and divided by the average root length during that interval and by the length of the time interval. Data on phosphate uptake were used as a reference in the evaluation of model calculations.

Rates of organic acid exudation Plants were grown on nutrient solution without phosphate (1.25 mM $Ca(NO_3)_2$, 1.25 mM KNO_3, 0.50 mM $MgSO_4$ and trace elements). After 8 days of growth, sets of 6 plants were transferred to 5 cm^3 fresh nutrient solution. Beforehand, their roots were washed with demineralized water. After 30 min. this nutrient solution was analyzed for malic and citric acid by enzymatic procedures (Anonymous, 1989). The results were used to calculate the exudation rates per plant. To calculate exudation rates per cm root, the length of the acidifying root segment of 8-day-old plants, grown under the same conditions, was measured on an agar plate. For a description of this agar plate technique, see Hoffland *et al.* (1989b).

In situ *determination of the exuding proportion of the root system and of the pH of the rhizosphere* To find the exuding proportion of a root system growing in quartz sand plants were grown between two parallel glass plates (Fig. 1). The volume between the plates was filled with quartz sand (dry bulk density 1.28 g × cm^{-3}) mixed with nutrient solution containing no phosphate and with rock P in the same proportion as in the growth experiment. Growth conditions were the same as in the growth experiment. Each day between 3 and 17 days after germination the lower plate was removed from 3 containers. The exposed sand and roots were sprayed with a bromocresole purple solution (1 mg × cm^{-3}; pH 5.8; yellow below pH 4). The proportion of the acidifying roots was determined by estimating the total length of visible roots and the total length of the root segments that showed a yellow rhizosphere.

The pH of the rhizosphere was estimated in a similar way. After 8 days of growth the roots of similarly grown plants and the surrounding quartz sand were sprayed with either bromocresole purple, bromocresole green or phenol red (1 mg × cm^{-3}; pH 5.5). A mixture of quartz sand and nutrient solution with a known soil solution-pH was sprayed with the same indicators and used as a reference to interpret the colour changes of the rhizosphere.

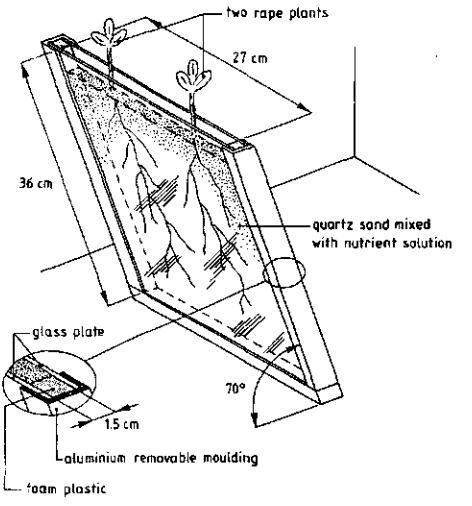

Figure 1. Containers used to examine the proportion of the root system that exudes organic acids and the pH of the rhizosphere of rape plants.

Results

Experiments

Phosphate and H_3O^+ concentration in the soil solution as a function of the total soil acid concentration Results given in Figure 2. The proportion of Mali rock P dissolved in the pretreatment affected neither the phosphate concentration nor the pH in the soil solution. Therefore, the results of differently pretreated rock phosphates were averaged, so that data in Figure 2 are means of four. This seems to contradict the results of the pretreatment, shown in Table 1. However, the amounts dissolved in this experiment were much smaller than those dissolved in the pretreatment.

The volumetric moisture content (Θ) affected both the phosphate concentration and the pH in the soil solution. At small Θ the pH was larger and the phosphate concentration less. The effect of Θ on pH at small total soil acid concentrations was considerable.

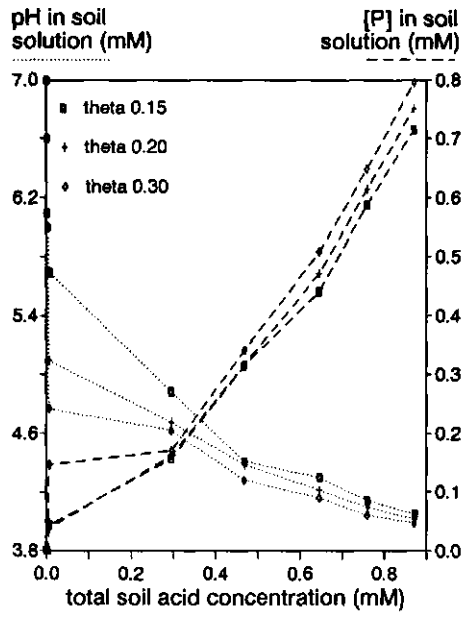

Figure 2. Relation between total soil acid concentration and pH in the soil solution (dotted line; left Y axis) and phosphate concentration in the soil solution (dashed line; right Y axis) at different values of Θ when Mali rock phosphate is mixed with quartz sand; the symbols represent experimental data (means of 4), the lines represent the relations used in model calculations.

At total soil acid concentrations below 0.003 mM the pH of the soil solution was buffered at 7.0 at all values of Θ. At a total soil acid concentration below 0.001 mM the phosphate concentration was buffered at 0.001 mM.

Growth experiment The concentrations of N, P, K, Ca and Mg in plant tissue did not change during the growth period and were (in mmol × kg^{-1} dm): N 2500 ± 300; P 84 ± 14; K 880 ± 200; Ca 590 ± 140 and Mg 190 ± 20. S was assumed to be 200 mmol × kg^{-1} dm. The mean difference between cations and anions absorbed was -550 mmol × kg^{-1} dm on equivalent basis. These data and data on total root length (Hoffland *et al.*, 1990b) and dry matter increase were used to calculate the OH^- flux resulting from ion uptake. The values are given in Table 2.

Hoffland *et al.* (1990b) presented the results of determinations of Θ and root length per layer and transpiration rate and phosphate uptake. Phosphate uptake is also given in Figure 3.

Rates of organic acid exudation The exudation rate measured in P-deficient rape

plants grown on nutrient solution was 29 ± 7 nmol × h^{-1} × $plant^{-1}$ for malic acid and 10 ± 4 nmol × h^{-1} × $plant^{-1}$ for citric acid (n=9). The sum of the length of the acidifying root segments of a plant grown for 8 days on nutrient solution without phosphate measured on an agar plate was 23 ± 6 cm per plant (n=10). Consequently, the mean exudation rates calculated per cm root were 30.2 nmol × cm^{-1} × day^{-1} (malic acid) and 10.4 nmol × cm^{-1} × day^{-1} (citric acid).

The concentration of P in the tissue of the plants sampled was 60 mmol × kg^{-1} dm.

Table 2. **Fluxes of OH^- from the root surface towards the bulk solution, calculated on basis of experimental data, resulting from the growth experiment. Means of 3 pots.**

Time interval (days)	Dry matter increase (g pot^{-1})	Mean total root length (cm pot^{-1})	Calculated OH^- flux (μmol cm^{-1} day^{-1})
0 - 4	0.078	202	0.068
4 - 7	0.155	806	0.045
7 -11	0.368	2475	0.026
11 -16	1.177	6089	0.027

In situ *determination of the exuding proportion of the root system and of the pH of rhizosphere* The proportion of the root system of plants grown with Mali rock P that had acidified the rhizosphere did not change significantly during the growth period considered; it was 25 ± 10% of the total visible root length. A comparison of the results for consecutive days from the same plants revealed that half of this 25% was about one day old. Probably the other half was no longer exuding organic acids, but still had an acidified rhizosphere caused by exudation on the previous day, and therefore the proportion of the root system that was actively exuding was assumed to be 12.5%.

When the root systems of plants grown on rock P were sprayed with bromocresole purple they turned yellow for a distance of about 0.5 cm from the root surface of the youngest root parts, indicating that the pH had fallen below 4. There were no orange zones when bromocresole green was used, indicating that the pH was always more than 2.5.

The rhizosphere of the older part of the root system was stained blue with bromocresole green and red with phenol red, indicating that the pH was about 7.

Simulation

To calculate the possible effect of organic acid exudation on phosphate uptake, the simulation model was firstly run with measured parameter values (*Standard run*). Then, its sensitivity was tested by changing parameter values on exudation rates of organic acids (*Exudation rates*), on the proportion of the root system that exudes organic acids (*Distribution of exudation...*) and on root hairs (*Root hairs*).

The parameter values used and the results of the simulation are summarized in Table 3, but will be detailed below.

Table 3. Simulated phosphate uptake after 16 days of growth after germination on a quartz sand/rock P mixture, using varying parameter values. The first row reflects the standard run. Observed phosphate uptake was 147 ± 17 μmol per pot.

Model parameters			Calulated phosphate uptake
Root hair length (cm)	Exudation rate per plant related to measured rates (%)	Proportion of root system that exudes organic acids (%)	(μmol per pot)
0.040	100	12.5	568
0.040	50	12.5	202
,,	25	,,	27
,,	0	,,	22
0.040	100	7.5	9*
,,	,,	10	332
,,	,,	30	46
0.000	0	12.5	16
,,	100	,,	210
0.065	0	,,	39

*after 9 days of growth

Standard run The simulation model was run with parameters on root length, root hair length, transpiration and volumetric moisture content given by Hoffland *et al.* (1990b). The exudation rates used were those measured in P-deficient plants grown on nutrient solution (see *Organic acid exudation rates*), *i.e.* 30.2 nmol × cm^{-1} × day^{-1} for malic acid and 10.4 nmol × cm^{-1} × day^{-1} for citric acid. It was assumed that 12.5% of the root system actively exudes organic acids (see In situ *determination...*). The number of shells initially surrounding a root was set at 20. Further increase of this number did not affect model output. The results are given in Figure 3 ("standard run") and Table 3.

The simulated pH-gradient in the rhizosphere around the youngest segments of the root system is given in Figure 4.

Exudation rates The model was run with lower exudation rates (0.5 and 0.25 × measured rates and no exudation) to establish the effect on calculated phosphate uptake. The results are given in Figure 3 and in Table 3. The calculated phosphate uptake was strongly dependent on the exudation rates. When the rates were halved (50% of measured rates) it did not deviate greatly from observed phosphate uptake. When the rates were halved again (25% of measured rates) it was only slightly more than when exudation was set at zero. When exudation rates were set at zero, the calculated phosphate uptake fell to only 4% of that calculated in the standard run.

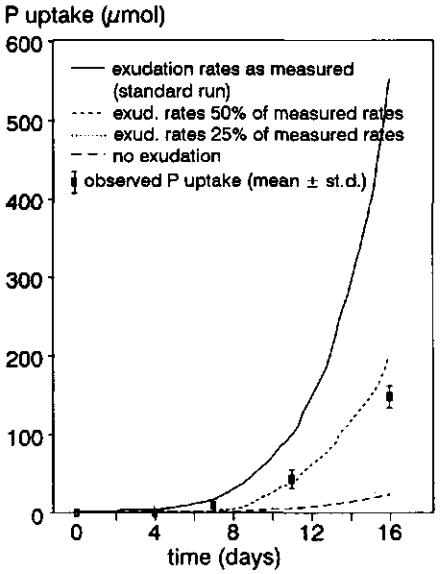

Figure 3. Simulated (lines) and observed (symbols) phosphate uptake by rape grown on quartz sand and Mali rock phosphate. The exudation rates of malic and citric acid were varied.

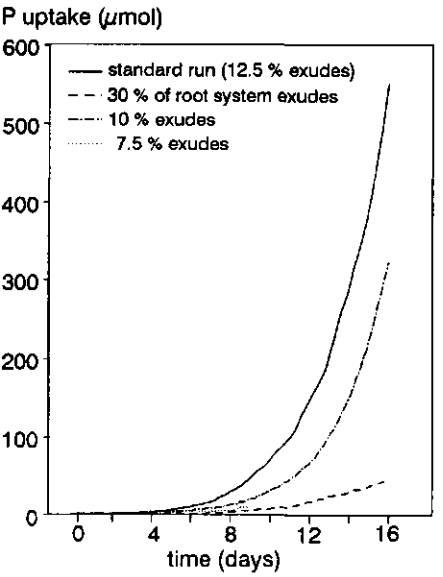

Figure 5. Simulated phosphate uptake from Mali rock phosphate. The proportion of the root system that exudes organic acids was varied, but the exudation rate per plant was not.

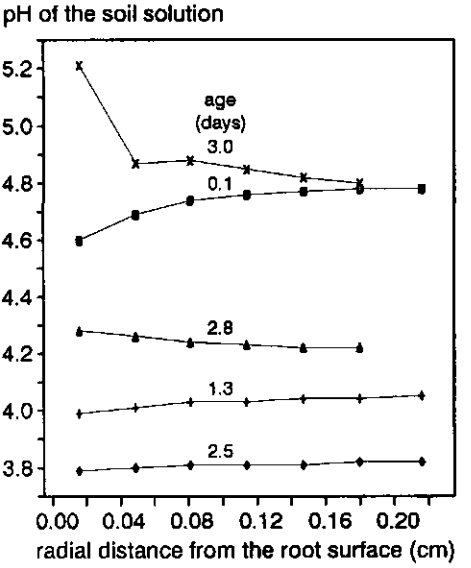

Figure 4. Simulated pH gradient around a segment of a root at different ages. When 0 to 2.6 days old the root segment exudes organic acids. When 2.6 days old the outer shell is stripped off and the root segment is no longer the youngest. Before formation of the root segment and when older than 3.5 days, the pH is 7.0 at any distance within the soil cylinder surrounding the root. The pH observed around the exuding root segments with the aid of several pH indicators was more than 2.5 but less than 4.0 and around the root segments that did not exude it was 7.

Distribution of exudation over the root system The extent of the exuding proportion of the root system was varied to check the effect of distributing the exudation over a smaller or larger part of the root system. Total exudation per plant was not changed, *i.e.* if the proportion of the root system that was exuding was increased from 12.5% to 30% (× 2.4) the exudation rates were reduced by a factor of $^{1}/_{2.4}$. The results of changing the proportion of the root system that was exuding to 30%, 10% and 7.5%, respectively, are given in Figure 5 and

Table 3. To realize the latter situation (7.5%) 44 shells would be required to account for the maximum root length measured in the growth experiment. To avoid having to change the geometry the simulation was halted after 9 days, so that 20 shells were sufficient.

The calculated phosphate uptake fell when the exudation was spread over a larger proportion of the root system. A more concentrated exudation also appeared to reduce calculated phosphate uptake.

Root hairs The root hair length measured in the pot experiment was 0.040 cm (Hoffland *et al.*, 1990b), which was used in the standard run. To establish the role of root hairs in phosphate uptake, the model was run under the assumption that there were no root hairs. The resulting phosphate uptake was 37% of that in the standard run (Table 3), which means that in the standard run root hairs accounted for 63% of total phosphate uptake. Comparison of the effect of organic acid exudation on simulated phosphate uptake at presence and absence of root hairs, revealed that root hairs increase the effect of organic acid exudation.

To determine the effectiveness of increasing root hair length as an alternative strategy to enhance phosphate uptake from rock P the phosphate uptake was calculated with exudation rates set at zero and with longer root hairs. The maximum root hair length reported for rape plants grown in soil was used (0.065 cm; Brewster *et al.*, 1976). The calculated phosphate uptake was 39 μmol, which is less than the observed uptake.

Discussion

From Figure 2 it can be concluded that Mali rock phosphate does not dissolve like an ideal solid. As expected, no constant relationship could be found between pH_2PO_4 and pH and pCa. In addition, the phosphate concentration and the pH in the soil solution are dependent on θ, which indicates that the surface area of rock P per unit volume of soil solution is an important factor. Because the values presented in Figure 2 were measured after the equilibrium value had been established, the different concentrations at different values of θ cannot be caused by differing diffusion rates of the phosphate ions, moving from the dissolving particle into the bulk solution. This effect of θ can be attributed to re-adsorption of dissolved phosphate ions on the dissolving surface, resulting in a lower phosphate concentration at a lower ratio of soil solution to adsorbing surface *i.e.* at smaller θ. Precipitation of dissolved phosphate with metal cations on the mineral surface might also occur.

The use of the empirical relations as given in Figure 2 limits the use of the model to one type of rock P. If another type of rock P is used, the relations must be re-established. However, much research would be needed to describe all the processes involved in the dissolution of rock P. Precise composition, solubility product and

sorption characteristics would have to be determined for each type of rock P. If these parameters are known, it might be worth incorporating the model presented by Kirk and Nye (1986) into our model. An empirical relation is sufficient for the research described here.

It has been established that the model satisfactorily describes uptake of dissolved P at low levels of P (Hoffland *et al.*, 1990b). Therefore, here I will focus on an evaluation of the model's description of factors affecting the dissolution of rock P. The most important factor of these is the pH gradient surrounding the root.

Only one acid-base pair (H_2O-H_3O^+) is considered in the model; the H_2CO_3-HCO_3^- pair is not accounted for. Nye (1972) demonstrated that below pH 5.5 H_2O-H_3O^+ is the most important pair. The range above pH 5.5 is less important in this model, because the phosphate concentration is very low (Fig. 2). Errors caused by this simplification will therefore quantitatively be of no importance with respect to phosphate uptake.

Parameters derived from experimental data were used to describe the exudation of organic acids. The assumption that the pH of the innermost shell determines the amount of protons released per mol of organic acid exuded will cause the effect of exudation on phosphate uptake to be underestimated: in reality, organic acids and their anions will diffuse away from the root and protons will be released further away too, because of the higher pH; as a result the pH will be lower and the phosphate concentration higher there. This would result in a greater uptake of phosphate if the rock P in the innermost shell is depleted, which occurs in a considerable part of the root system.

Besides pH, the calcium concentration is an important factor determining the solubility of rock P (Johnston and Olsen, 1972). This factor was not taken into account in the model. Two processes affect the Ca concentration in the rhizosphere. The first is that the Ca in the rhizosphere might be depleted because of uptake. If, in the growth experiment, only the Ca added with the soil solution is taken into consideration, then about 20% more Ca was absorbed than could be supplied to the roots by mass flow. However, the dissolution of rock P will have enhanced the Ca concentration (Ca/P $\approx$ 2 in Mali rock P), which makes it questionable whether depletion did indeed occur. The second process, complexing of Ca by citrate, which reduces the concentration of free Ca in the soil solution, might be more important. The impact of these two processes depends on the composition of the rock P (Khasawneh and Doll, 1978). Ignoring the effect of Ca complexation might cause phosphate uptake to be underestimated. However, it has been shown (Hoffland *et al.*, 1989a) that this process is quantitatively unimportant in rape.

Given the abovementioned limitations to the model, it is concluded that exudation of organic acids can be a very effective strategy for plant roots to enhance phosphate uptake from rock P. The exudation rates measured are far more than adequate to explain the relatively large phosphate uptake from rock P by rape (Fig. 3). Conse-

quently, it is unlikely that other rhizosphere processes than the ones described in the model are involved in rock phosphate mobilization by rape.

It is reasonable to assume that exudation rates are related to concentrations of P in the root or plant tissue. The rates used in the standard run were measured in plants with a lower tissue P concentration than that of the plants in the growth experiment and therefore the model probably overestimated phosphate uptake. But it is important to realize that enough phosphate could be absorbed even if the rates were halved (Fig. 3; Table 3). Reducing the exudation rates to 25% of those used in the standard run should approximate the rates of phosphate-sufficient rape plants (Hoffland *et al.*, 1989b).

The model might also have overestimated phosphate uptake because the roots may no longer act like a zero-sink due to enhanced phosphate availability. Furthermore, the model ignores microbial degradation of exuded organic acids. However, it can be concluded that the potential of rape plants to acidify the rhizosphere by exuding organic acids is sufficient to release the amounts of phosphate taken up by plants grown on rock P.

Although the phosphate uptake calculated in the standard run is nearly four times higher than the observed phosphate uptake, the simulated pH gradients (Fig. 4) do not contradict those observed with the aid of several pH indicators. This might be caused by converting the organic acid exudation into a proton flux on the basis of the pH in the innermost shell with the result that the extent of the acidification is underestimated.

Calculated phosphate uptake declines dramatically if the proportion of the root system that exudes organic acids is changed, while the exudation per plant is kept constant (Fig. 5; Table 3). If this proportion is increased to *e.g.* 30%, the pH effect of exudation is neutralized to a large extent by alkalinization caused by the uptake of excess anions. Apparently a more concentrated exudation (*e.g.* 10 or 7.5%) is less effective too, probably because the larger decline in pH is overcompensated by the fact that the immediate vicinity of the exuding root part becomes depleted of rock P more quickly. The observed proportion of exuding root (12.5%) seems to be very effective.

The presence of root hairs affected calculated phosphate uptake greatly, mainly by enhancing the effect of organic acid exudation (Table 3). In the model the root hairs are considered to be an extension of the root radius. Although this is a rather simple approach, it has proved useful when describing the uptake of dissolved phosphate (Bhat and Nye, 1973; Hoffland *et al.*, 1990b). Nevertheless, in the case of rock P this approach might underestimate the effect of root hairs on phosphate uptake because in reality rock P present within the root hair cylinder might easily be dissolved and absorbed due to high root hair density and uptake activity. It can be calculated that in the growth experiment the amount of P in rock P within the root hair cylinder is 75 μmol after 16 days. If this were absorbed completely in addition to the calculated phosphate uptake, the phosphate uptake would be 97 μmol

if organic acid exudation would be set at zero; this is less than the observed uptake (147 $\pm$ 17 μmol). If the radius of the root hair cylinder would be increased to 0.065 cm, the maximum amount of P present within the root hair cylinder would be 171 μmol. So, only if the root hair cylinder is depleted of rock P instantaneously after a root samples a new soil volume, extending the root hair length would be sufficiently effective to explain the observed phosphate uptake, but far less effective than the observed rates of exudation of organic acids.

Acknowledgements

The authoress would like to acknowledge Mr Jaap Nelemans, who did most of the experiments presented in this paper. Dr Peter A. Leffelaar and Prof. dr Günter R. Findenegg are thanked for constructively criticizing the manuscript.

References

Anonymous 1989 Methods of biochemical Analysis and Food Analysis using single Reagents. Boehringer Mannheim GmbH. Mannheim. Germany.

Bhat K K S and Nye P H 1973 Diffusion of phosphate to plant roots in soil. I. Quantitative autoradiography of the depletion zone. Plant and Soil 38, 161-175.

Brewster J L, Bhat K K S and Nye P H 1976 The possibility of predicting solute uptake and plant growth response from independently measured soil and plant characteristics. V. The growth and phosphorus uptake of rape at a range of phosphorus concentrations and a comparison of results with the predictions of a simulation model. Plant and Soil 44, 295-328.

Hoffland E, Findenegg G R and Nelemans J A 1989a Solubilization of rock phosphate by rape. I. Evaluation of the role of the nutrient uptake pattern. Plant and Soil 113, 155-160.

Hoffland E, Findenegg G R and Nelemans J A 1989b Solubilization of rock phosphate by rape. II. Local root exudation of organic acids as a response to P-starvation. Plant and Soil 113, 161-165.

Hoffland E, Bloemhof H S, Leffelaar P A, Findenegg G R and Nelemans J A 1990a Simulation of nutrient uptake by a growing root system considering increasing root density and inter-root competition. Plant and Soil 124, 149-155.

Hoffland E, Findenegg G R, Leffelaar P A and Nelemans J A 1990b Use of a simulation model to quantify the amount of phosphate released from rock phosphate by rape. Trans. 14th Int. Congr. Soil Sci. (Kyoto) II, 170-175.

Johnston W B and Olsen R A 1972 Dissolution of fluorapatite by plant roots. Soil Sci. 114, 29-36.

Khasawneh F E and Doll E C 1978 The use of phosphate rock for direct application to soils. Adv. Agron. 30, 159-206.

Kirk G J D and Nye P H 1986 A simple model for predicting the rates of dissolution of sparingly soluble calcium phosphates in soil. I. The basic model. J. Soil Sci. 37, 529-540.

Nye P H 1972 The measurement and mechanism of ion diffusion in soils. VIII- A theory for the propagation of changes of pH in soils. J. Soil Sci. 23, 82-92.

Nye P H 1983 The diffusion of two interacting solutes in soil. J. Soil Sci. 34, 677-691.

Nye P H 1984 pH changes and phosphate solubilization near roots - an example of coupled diffusion processes. *In* Roots, Nutrient and Water Influx, and Plant Growth. Eds. S A Barber, D R Bouldin and D M Kral. pp 89-100. ASA Spec. Publ. 49, Soil Sci. Soc. Am., Madison, Wisconsin.

Chapter 8
Epilogue

Epilogue

This chapter assesses the agronomic relevance of the conclusions of the research reported in this thesis, evaluates the use of modelling in this type of research and concludes with a discussion on the prospects for future research.

Agronomic relevance

Serious shortages of phosphate fertilizers are not expected to occur in the near future, but high grade rock phosphates are present only in limited amounts and are becoming more expensive. This means they must be managed with great care. Moreover, excessive fertilization with phosphates is causing serious environmental problems in some developed countries. Underlying this situation is the fact that only a small proportion of the phosphorus applied in fertilizers is recovered by crops; the remainder accumulates in soil. Therefore, any means of increasing phosphate availability can be both economically and environmentally beneficial and this justifies investigating all the possibilities of making poorly soluble phosphates more available for plant uptake. The work reported in this thesis must be regarded as a contribution to this.

The confirmation that rape plants exude organic acids to increase phosphate uptake from rock phosphate can have implications for agronomy. This property could be exploited in crop rotation, mixed cropping systems and, if it can be genetically transferred, in plant-breeding.

It might be possible to use rape to mobilize phosphate for the next crop in the rotation, *i.e.* as a green manure which converts poorly soluble rock phosphates into more readily available organic phosphates. Using rape in this way might raise the yields of the following crop by increasing the availability of phosphate, especially in temperate countries where superphosphate is not readily available but rock phosphate is (*e.g.* the USSR). However, a leguminous plant that not only mobilizes rock phosphate but also enhances nitrogen availability, *e.g.* lupin, might be more suitable than rape.

Using rape as a phosphate mobilizer in a mixed cropping system is more doubtful. It has been demonstrated in pot experiments that white lupin is able to mobilize more phosphate from rock phosphate than it needs, so that phosphate uptake by oats (Prjanischnikow, 1934) and wheat (Horst and Waschkies, 1987) is enhanced when grown in combination with lupin. It is doubtful whether such results can be obtained with rape in the field. Firstly, it is questionable whether the roots of two species will intertwine to the same extent in the field as was found in the pot experiments.

If not, it can be expected that phosphate, released by the mobilizing species will be re-adsorbed on the solid phase before being taken up by the other species. Secondly, our results (Chapter 3) reveal that the rate at which rape exudes organic acids is a function of the phosphorus concentration in root or plant tissue. It is therefore unlikely that rape will release more phosphate from rock phosphate than it needs. To do so would be ecologically unsound, because it must be assumed that a considerable proportion of photosynthates would be needed to achieve the exudation rates measured. From their experiment on white lupin Dinkelaker *et al.* (1989) calculated that the amount of citrate released as a reaction to phosphorus deficiency represented about 23 % of the plant dry matter production. There are no data on the size of this fraction in the case of rape.

To be able to assess the agronomic relevance of the exudation phenomenon more thoroughly, it would be necessary to establish its occurrence among other species. The results presented in Chapter 1 (Fig. 2) indicate that rape is not the only crucifer that uses rock phosphate efficiently. In our laboratory we demonstrated that the capacity of *Brassica nigra*, *Brassica rapa*, *Raphanus sativus* and *Sinapis arvensis* to mobilize rock phosphate is also related to the exudation of malic and citric acid induced by phosphorus deficiency (Van den Boogaard, unpublished results). This suggests that the phenomenon is more widespread, at least among crucifers. Moreover, organic acid exudation induced by phosphorus deficiency has been shown to occur in white lupin (Gardner *et al.*, 1983) and *Medicago sativa* (Lipton et al., 1987).

Evaluation of the use of a simulation model

This study was inspired by the combination of experimental and simulation work, as are some of our conclusions. Brewster *et al.* (1976) found that simulated phosphate uptake was considerably less than the observed uptake of phosphate by rape grown at low phosphorus supply. Their suggestion that this discrepancy could be caused by root exudates increasing the phosphate concentration in the soil solution has been confirmed by this research.

A simulation model was used to check our understanding of the processes involved in nutrient uptake by a growing root system, and to evaluate the effectiveness of rape's reactions to phosphate deficiency. The fact that the model satisfactorily describes the uptake of dissolved nutrients under varying but growth-limiting nutrient supply suggests that the most relevant processes involved are described and understood and that the model can be used to evaluate the effect of changing soil and root parameters on nutrient uptake, observing restrictions given in Chapter 5. Without model calculations on phosphate uptake from rock phosphate it would have been impossible to quantify the effect of the exudation of organic acids on phosphate

uptake or to conclude that rhizosphere acidification caused by organic acid exudation is probably the only process of note affecting the mobilization of rock phosphate by rape (Chapter 7). Thus, modelling has been very helpful in planning experimental work, which no longer needs to concentrate on other reactions of rape to phosphate deficiency.

Prospects for further research

Although many questions on the mobilization of rock phosphate by rape have been addressed in this thesis, a number of questions remain to be answered, especially about the role of bacteria and the physiological basis of the process. These two aspects will be elaborated below.

Role of bacteria

A factor that might play a role in the mobilization of rock phosphate by rape and has been ignored in this thesis is rhizosphere bacteria. The interaction between roots of phosphate-deficient rape plants and bacteria is especially interesting because bacteria tend to use organic acids as a substrate. It would be worthwhile investigating whether this does occur. It is conceivable that the growth of some bacterial species is inhibited when the pH falls below 4 in the rhizosphere of the exuding root segments.

The presence of bacteria could have both an inhibiting and a stimulating effect on phosphate uptake from rock phosphate. If bacteria consume organic acids or their anions, it can be assumed that this mitigates the pH decrease of the rhizosphere, and hence the dissolution of rock phosphate. Such a reducing effect of microorganisms on the activity of secretions from white lupin was demonstrated by Gardner *et al.* (1982). If the increased availability of substrate stimulates bacterial growth, then bacteria and plant roots might compete for phosphate, hence reducing the phosphate available for plant uptake. On the other hand, the increased availability of substrate could enhance the growth of the number of phosphate-dissolving bacteria, although, as stated in Chapter 1, it is doubtful whether they can beneficially affect plant uptake of phosphate. Bacteria could produce organic acids, thus enhancing the solubilization of rock phosphates (Moghimi *et al.*, 1978). Our results (Chapter 3 and 4) exclude the possibility that the organic acids detected in the rhizosphere of rape plants are produced by bacteria.

As well as consuming the organic acids exuded, bacteria can stimulate root exudation of organic substances. This has often been reported (Barber and Martin, 1976; Barber and Lynch, 1977; Přikryl and Venčura, 1980; Heulin *et al.*, 1987). It can be assumed that if bacteria increase exudation of organic acids by rape, they would

enhance phosphate solubilization.

Enhancing the solubilization of rock phosphate by bacterial activity could be an extra way of increasing the efficiency of fertilizer use. Therefore, interactions by plant roots and rhizosphere bacteria are worth investigating. A first step could be to establish whether the presence of bacteria stimulates or inhibits rape to absorb phosphate from rock phosphate; this could be done by comparing phosphate uptake under sterile and non-sterile conditions.

Physiological reactions to phosphate deficiency

It is still not clear why phosphate-deficient rape plants exude organic acids. The results reported in Chapter 4 only lift the tip of the veil. Although there are indications that the acids exuded are produced in the shoot and subsequently transferred to the root, it should be investigated whether increased phosphoenolpyruvate carboxylase and citrate accumulation in the shoot are prerequisites for organic acid exudation by the roots of phosphate-deficient rape plants. For further understanding, it is necessary to know the sequence of reactions involved and to know why malate and citrate accumulate in the root tip. With respect to the exudation process itself, it should be established whether proton pumps driven by plasma membrane ATPases are involved in rhizosphere acidification induced by phosphate stress, as has been shown to be the case in rhizosphere acidification induced by iron stress (Römheld *et al.*, 1984).

Intriguing and confusing is the observation (not published) that in the case rock phosphate is applied only to a small part of the root system of rape, the roots acidify only that part of the rhizosphere, irrespective of the distance from the root tip. Exudation behind the root tips of the rest of the root system stops. This phenomenon should be taken into account in unravelling the physiological characteristics responsible for the exudation of organic acids.

The striking similarity between the reaction of rape to phosphate deficiency and the reaction of dicotyledons and non-grass monocotyledons to iron deficiency (*see* Chapter 3 and Marschner *et al.* (1982), respectively) should be paid attention to. In both cases, acidification of the rhizosphere by the youngest root segments occurs, and has the same ecological significance, *i.e.* mobilization of a mineral nutrient from sparingly soluble sources. In the case of iron deficiency it is generally assumed that the acidification is caused by proton efflux from rhizodermal transfer cells (Landsberg, 1986). To date there have been no reports of the occurrence of transfer cells induced by phosphate deficiency. Neither has the release of organic acids by roots of iron-deficient plants been detected (Venkat Raju *et al.*, 1972). However, the agar plate technique described in Chapter 3 might give more reliable information on this. Yet, proton extrusion induced by iron deficiency has been found to be coupled to

the production of malic and citric acid (Landsberg, 1981; De Vos *et al.*, 1986; Landsberg, 1986) and thus, like rhizosphere acidification induced by phosphate deficiency, to be related to organic acid metabolism. Knowledge of the role of organic acid metabolism in rhizosphere acidification induced by iron deficiency might be of value in elucidating the physiological processes involved in rhizosphere acidification by phosphorus-deficient rape plants.

References

Barber D A and Martin J K 1976 The release of organic substances by cereal roots into soil. New Phytol. 76, 69-80.

Barber D A and Lynch J M 1977 Microbial growth in the rhizosphere. Soil Biol. Biochem. 9, 305-308.

Brewster J L, Bhat K K S and Nye P H 1976 The possibility of predicting solute uptake and plant growth response from independently measured soil and plant characteristics. V. The growth and phosphorus uptake of rape in soil at a range of phosphorus concentrations and a comparison of results with the predictions of a simulation model. Plant Soil 44, 295-328.

De Vos R C, Lubberding H J and Bienfait H F 1986 Rhizosphere acidification as a response to iron deficiency in bean plants. Plant Physiol. 81, 842-846.

Dinkelaker B, Römheld V and Marschner H 1989 Citric acid excretion and precipitation of calcium in the rhizosphere of white lupin (*Lupinus albus* L.). Plant Cell Environ. 12, 285-292.

Gardner W K, Barber D A and Parberry D G 1982 Effect of microorganisms on the formation and activity of proteoid roots of *Lupinus albus* L.. Aust. J. Bot. 30, 303-309.

Gardner W K, Barber D A and Parberry D G 1983 The acquisition of phosphorus by *Lupinus albus* L. III. The probable mechanism by which phosphorus movement in the soil/root interface is enhanced. Plant Soil 70, 107-124.

Heulin T, Guckert A and Balandreau J 1987 Stimulation of root exudation of rice seedlings by *Azospirillum* strains: carbon budget under gnotobiotic conditions. Biol. Fert. Soils 4, 9-14.

Horst W J and Waschkies C 1987 Phosphatversorgung von Sommerweizen (*Triticum aestivum* L.) in Mischkultur mit Weißer Lupine (*Lupinus albus* L.). Z. Pflanzenernaehr. Bodenkd. 150, 1-8.

Landsberg E-C 1981 Organic acid synthesis and release of hydrogen ions in response to Fe deficiency stress of mono- and dicotyledonous plant species. J. Plant Nutr. 3, 579-591.

Landsberg E-C 1986 Function of rhizodermal transfer cells in the Fe stress response mechanism of *Capsicum annuum* L. Plant Physiol. 82, 511-517.

Lipton D S, Blanchar R W and Blevins D G 1987 Citrate, malate and succinate concentration in exudates from P-sufficient and P-stressed *Medicago sativa* L. seedlings. Plant Physiol. 85, 315-317.

Marschner H, Römheld V and Ossenberg-Neuhaus H 1982 Rapid method for measuring changes in pH and reducing processes along roots of intact plants. Z. Pflanzenernaehr. Bodenkd. 105, 407-416.

Moghimi A, Tate M E and Oades J M 1978 Characterization of rhizosphere products especially 2-ketogluconic acid. Soil Biol. Biochem. 10, 283-287.

Přikryl Z and Venčura V 1980 Root exudates of plants. VI. Wheat root exudation as dependent on growth, concentration gradient of exudates and the presence of bacteria. Plant Soil 57, 69-83.

Prjanischnikow D 1934 Über das Aufschließen der Rohphosphate durch die Wurzelausscheidungen von Lupinen. Phosphorsäure 4, 1-23.

Römheld V, Müller C and Marschner H 1984 Localization and capacity of proton pumps in roots of intact sunflower plants. Plant Physiol. 76, 603-606.

Venkat Raju K, Marschner H and Römheld V 1972 Effect of nutritional status on ion uptake, substrate pH and production and release of organic acids and riboflavin by sunflower plants. Z. Pflanzenernaehr. Bodenkd. 132, 178-191.

Samenvatting

Mobilisatie van ruwfosfaat door koolzaad (*Brassica napus* L.)

Het doel van het werk dat beschreven is in dit proefschrift was om vast te stellen welke eigenschap koolzaad in staat stelt om ruwfosfaten in vergelijking met andere gewassen efficiënt te benutten als fosfaatbron. Na een algemene inleiding (Hoofdstuk 1) is daartoe vastgesteld welke planteigenschappen een rol spelen (Hoofdstuk 2 tot en met 4). Vervolgens is een simulatiemodel ontwikkeld waarmee kon worden berekend hoe groot het effekt van deze eigenschappen op de fosfaatopname uit ruwfosfaat is (Hoofdstuk 5 tot en met 7). Het proefschrift wordt beloten met een evaluatie van de relevantie van de konklusies voor de praktijk en met suggesties voor nader onderzoek (Hoofdstuk 8).

Ruwfosfaat is een gemalen sediment, dat veelal wordt gebruikt als grondstof voor kunstmestfosfaten. Het wordt echter ook als zodanig als fosfaatmeststof toegepast, in het bijzonder in ontwikkelingslanden en in de Sovjetunie. Ruwfosfaat bestaat voornamelijk uit apatiet. Dit kan variëren van samenstelling, maar bevat in ieder geval calcium, fosfaat en fluoride. De meeste ruwfosfaten zijn onder niet-zure omstandigheden slecht oplosbaar, waardoor hun waarde als fosfaatmeststof beperkt is.

Planten verschillen sterk in het vermogen ruwfosfaat te benutten als fosfaatbron. In Hoofdstuk 1 wordt uiteengezet welke eigenschappen en omstandigheden hierbij een rol spelen. Een belangrijke faktor is de mate waarin planten in staat zijn om de rhizosfeer te verzuren. Dit hangt onder meer af van de vorm waarin stikstof wordt opgenomen.

Koolzaad (*Brassica napus* L.) stond al in de vorige eeuw bekend als een efficiënte benutter van ruwfosfaat. Uit recente experimenten bleek dat de fosfaatopname uit Mali ruwfosfaat ongeveer 55% bedraagt van de fosfaatopname uit eenzelfde hoeveelheid opgelost kaliumfosfaat, terwijl dit percentage voor veel andere plantesoorten nihil is wanneer stikstof wordt opgenomen in de vorm van nitraat.

Ook was reeds bekend dat de vergroting van het wortelоppervlak, die wordt gerealiseerd door toename van de lengte en dichtheid van wortelharen, onvoldoende is om het ruwfosfaatmobiliserend vermogen van koolzaad te verklaren. Uit de literatuur waren twee additionele verklaringen bekend: Engelse onderzoekers konkludeerden dat koolzaad bij fosfaatgebrek meer kationen dan anionen opneemt, ook wanneer nitraat de stikstofbron is. Hierdoor zou de rhizosfeer-pH dalen en het ruwfosfaat oplossen. Anderen gaven relatief hoge calciumopname aan als verklaring. Onttrekking van calcium aan de bodemoplossing leidt eveneens tot verhoogde oplosbaarheid van ruwfosfaat.

Deze beide theorieën werden in Hoofdstuk 2 geëvalueerd. De opname van een overmaat kationen bij fosfaatgebrek en nitraatvoeding kon niet worden gereproduceerd. De tweede theorie werd getest door de calciumkoncentratie in de rhizosfeer

kunstmatig hoog te houden door toevoeging van calciumsulfaat. Koolzaad kon ook onder deze omstandigheden fosfaat opnemen uit ruwfosfaat, zodat het ruwfosfaatmobiliserend vermogen niet verklaard kan worden met een hoge calciumopname.

Met behulp van een dubbele-pot-techniek werd aangetoond dat ruwfosfaatmobilisatie ook plaatsvindt wanneer ruwfosfaat ruimtelijk gescheiden van andere nutriënten wordt aangeboden. Daarom werd gekonkludeerd dat het ruwfosfaatmobiliserend vermogen niet is gerelateerd aan nutriëntenopname.

In Hoofdstuk 3 werd met behulp van een agartechniek vastgesteld dat de uiteinden van wortels van fosfaatgebrekkige koolzaadplanten de rhizosfeer verzuren. Deze verzuring treedt alleen op in een zone van ongeveer 1,5 cm lengte, direkt achter de wortelpunt. Het overige deel van het wortelstelsel, evenals het totale wortelstelsel van fosfaatverzadigde planten, verhoogt de rhizosfeer-pH. Deze alkalisering was verwacht omdat stikstof werd aangeboden in de vorm van nitraat.

Na vergelijking van de kationen- en anionenopname door verzurende en alkaliserende wortelzones werd opnieuw vastgesteld dat de verzuring niet veroorzaakt wordt door een lokale verandering in het nutriëntenopnamepatroon. Wel is de hoeveelheid appel- en citroenzuur in de rhizosfeer van de verzurende wortelzones van fosfaatgebrekkige planten een faktor drie à vier hoger dan die in de rhizosfeer van vergelijkbare wortelzones van fosfaatverzadigde planten. Bovendien is de koncentratie appel- en citroenzuur in het weefsel van de verzurende wortelzones verhoogd. Zowel in het weefsel van deze wortelzones als in de rhizosfeer is de appelzuurkoncentratie een faktor twee à drie hoger dan de citroenzuurkoncentratie.

Er werd gekonkludeerd dat fosfaatgebrek bij koolzaad leidt tot verhoogde synthese en exudatie van appel- en citroenzuur, hetgeen een lokale verzuring van de rhizosfeer veroorzaakt. Deze exudatie werd aangemerkt als mogelijke verklaring voor het fosfaatmobiliserend vermogen van koolzaad.

De herkomst van de geëxudeerde organische zuren werd nader onderzocht in Hoofdstuk 4. Omdat reeds was vastgesteld dat de exudatie niet (alleen) het gevolg is van membraanlek, maar (ook) van verhoogde synthese-aktiviteit, werd de aktiviteit van fosfoenolpyruvaat-carboxylase (PEPC) onderzocht. Dit enzym katalyseert de carboxylatie van fosfoenolpyruvaat, welke leidt tot de vorming van oxaalacetaat. Oxaalacetaat is een *precursor* van appel- en citroenzuur in de citroenzuurcyclus.

Fosfaatgebrek bleek een verhoogde aktiviteit van PEPC in de spruit te induceren. Bovendien treedt ophoping van citraat in de spruit op en wordt de verhouding citraat/suikers in het floeem hoger. In de wortel verandert de PEPC aktiviteit niet. Dit leidde tot de hypothese dat het appel- en citroenzuur dat wordt uitgescheiden door de wortelpunten van fosfaatgebrekkige koolzaadplanten afkomstig is uit de spruit.

Deze hypothese werd getoetst door de spruit radioaktief kooldioxide aan te bieden. De specifieke aktiviteit van het geëxudeerde appel- en citroenzuur bij fosfaatgebrekkige planten werd vergeleken met die van fosfaatverzadigde planten.

Deze is bij fosfaatgebrekkige planten negen maal hoger. Dit werd beschouwd als een aanwijzing dat de extra hoeveelheid appel- en citroenzuur die als reaktie op fosfaatgebrek wordt uitgescheiden inderdaad afkomstig is uit de spruit.

Om vast te stellen of de waargenomen uitscheiding van appel- en citroenzuur voldoende effektief is om de hoge fosfaatopname uit ruwfosfaat te verklaren, werd een simulatiemodel ontwikkeld. In de eerste versie van dit model (Hoofdstuk 5) werd de opname van een opgelost, groeibeperkend nutriënt uit een bodemoplossing beschreven. Vervolgens werd met behulp van dit model berekend hoeveel fosfaat koolzaad uit ruwfosfaat zou opnemen als er geen rhizosfeerverzuring zou plaatsvinden (Hoofdstuk 6). Tenslotte werd het effekt van uitscheiding van organische zuren berekend (Hoofdstuk 7).

In het model wordt verondersteld dat ieder nutriënt dat het worteloppervlak bereikt, onmiddellijk wordt opgenomen. Hierdoor wordt de koncentratie aan het worteloppervlak nul. Het transport van nutriënten naar het worteloppervlak toe wordt beschreven met behulp van de vergelijking voor massastroming en diffusie.
Het model beschrijft nutriëntenopname door een wortelstelsel dat in een beperkt volume groeit. Daarom wordt rekening gehouden met het effekt van toenemende worteldichtheid. Hiertoe wordt verondersteld dat iedere wortel is omgeven door een bodemcylinder, waaruit nutriënten kunnen worden opgenomen. Deze cylinder is onderverdeeld in een aantal koncentrische schillen. Bij toenemende worteldichtheid neemt de straal van de bodemcylinder af doordat de buitenste schil verwijderd wordt. Het materiaal van deze buitenste schil vormt een nieuw cylinderdeel waarin de jongste wortels gaan groeien.
De geldigheid van het model werd getest door de resultaten van een potproef te vergelijken met modelberekeningen. In de potproef werd de nitraatopname uit een mengsel van kwartszand en voedingsoplossing gemeten. De nitraatkoncentratie in de voedingsoplossing werd gevarieerd. Het model bleek de nitraatopname goed te beschrijven bij een laag, groeibeperkend aanbod. Bij een hoger nitraataanbod overschat het model de werkelijke opname, waarschijnlijk omdat in dat geval in werkelijkheid niet alle nitraat dat het worteloppervlak bereikt, wordt opgenomen.

In Hoofdstuk 6 werd het model gebruikt om te schatten hoeveel fosfaat koolzaad uit Mali ruwfosfaat zou opnemen, indien er geen organische zuren zouden worden uitgescheiden. Daarbij wordt verondersteld dat de fosfaatkoncentratie in de bodemoplossing konstant, op evenwichtsnivo, blijft zolang er ruwfosfaat aanwezig is. De op deze wijze berekende fosfaatopname bedraagt slechts 6% van de werkelijke opname, terwijl de opname van fosfaat afkomstig van opgelost kaliumfosfaat bij een groeibeperkend aanbod goed wordt gesimuleerd. Hieruit werd gekonkludeerd dat rhizosfeerverzuring leidt tot een bijna vervijftienvoudiging van de fosfaatopname uit ruwfosfaat indien koolzaad wordt gekweekt op een mengsel van kwartszand en Mali ruwfosfaat.

In Hoofdstuk 7 werd beschreven hoe het bestaande model is uitgebreid om het effekt van uitscheiding van organische zuren te berekenen. In het model wordt een empirisch vastgestelde relatie gebruikt om het verband te beschrijven tussen de koncentratie van toegevoegd zuur en de fosfaatkoncentratie en pH in de bodemoplossing. De bij de simulatie gebruikte uitscheidingssnelheden werden experimenteel vastgesteld aan koolzaadplantjes die zonder fosfaat op voedingsoplossing waren opgekweekt.

Het bleek dat de aldus gesimuleerde fosfaatopname ongeveer vier maal hoger is dan de experimenteel vastgestelde opname uit ruwfosfaat. Daarom werd gekonkludeerd dat de zuuruitscheiding zoals die bij fosfaatgebrekkige planten is gemeten, voldoende effektief is om de hoge fosfaatopname door koolzaad uit ruwfosfaat te verklaren. Bovendien bleek dat het lokaliseren van de uitscheiding tot een klein deel van het wortelstelsel bijzonder effektief is.

In de epiloog (Hoofdstuk 8) werd vastgesteld dat voor een goed begrip nader onderzoek noodzakelijk is naar de fysiologische reakties op fosfaatgebrek die leiden tot de exudatie van organische zuren, naar de rol van rhizosfeer-bakteriën in ruwfosfaat mobilisatie door koolzaad en naar de verspreiding van het verschijnsel binnen het plantenrijk.

Levensloop

Ellis (Elizabeth) Hoffland werd geboren op 18 mei 1962 in Haastrecht. Zij doorliep het Atheneum aan de Rijksscholengemeenschap in Gouda en startte in 1980 met de studie Biologie aan de Rijksuniversiteit te Utrecht. Als doctoraalvakken werden gedaan Oecofysiologie (hoofdvak), Fytopathologie en Microbiologie (bijvakken) en Theoretische Teeltkunde (nevenrichting). Het doctoraalexamen werd afgelegd in januari 1987.

In de periode van januari 1987 tot maart 1991 werd het in dit boekje beschreven promotie onderzoek uitgevoerd bij de vakgroep Bodemkunde & Plantevoeding van de Landbouwuniversiteit te Wageningen.

Sinds 1 februari 1991 is zij aangesteld als universitair docent bij de vakgroep Botanische Oecologie en Evolutiebiologie (projektgroep Fytopathologie) van de Rijksuniversiteit te Utrecht.