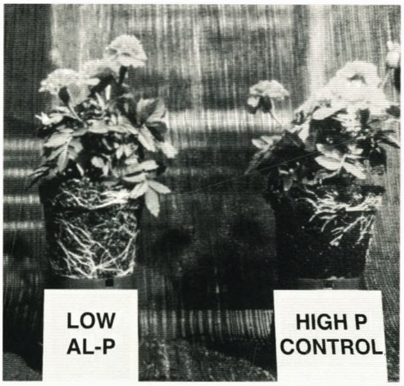
DIAS
report

December 1999

No. 10 • Horticulture



Kristian Borch

Ph.D. Dissertation, 1998
Odense University

Plant Stress and Root Growth Response to Low Phosphorus Nutrition and the Implication of Ethylene

Ministry of Food, Agriculture and Fisheries
Danish Institute of Agricultural Sciences

Ph.D. Dissertation:
Plant Stress and Root Growth Response to Low Phosphorus Nutrition and the Implication of Ethylene

Kristian Borch
Department of Ornamentals
Kirstinebjergvej 10
DK-5792 Årslev

DIAS report Horticulture no. 10 • December 1999

Publisher: Danish Institute of Agricultural Sciences Tel. +45 89 99 19 00
Research Centre Foulum Fax +45 89 99 19 19
P.O. Box 50
DK-8830 Tjele

Sale by copies: (incl. VAT)	up to 50 pages	50,- DKK
	up to 100 pages	75,- DKK
	more than100 pages	100,- DKK

Subscription: Depending on the number of reports sent but equivalent to 75% of the price of sale by copies.

Preface

The work leading to this dissertation was partly carried out at The Danish Institute of Agricultural Sciences, the Department of Ornamentals with Drs. Carl-Otto Ottosen and Mette Miller as supervisors.

At Penn State I would like to thank Drs. Kathleen Brown, Jonathan Lynch, and David Beattie for guiding me scientifically and for the good friendship during my long stay at the Department of Horticulture. I am also thankful for the good treatment and warm smiles I received from the faculty and staff at the Department of Horticulture. Especially thanks to those of you who understood my strange humor and let me become your friend. You know who you are - just remember the bonfires, lumbering, ice making, good food, wine and beer and whatever fun we had.

Also thanks to Carter Miller and Tjeerd Bouma whom I had the pleasure to work and laugh with at Penn State. Thanks to Kai Nielsen for giving me good advice at Penn State and for staying a good friend. Thanks to Solveig for having such a wonderful laugh.

A lot of thanks to Carl-Otto Ottosen and Mette Miller for taking care of the administrative complications during the past three years, and for keeping me straight when things got out of hand.

My very good colleagues at Aarslev deserve all the best wishes because they are great to work with - and often know a lot that I don't.

Finally I'm most grateful to my family and close friends who, despite they never really understood what I was wasting my time with, supported me with their love - especially my beautiful wife who has all the wonderful talents that I am lacking.

This Ph.D. study has been financially supported by the Danish Institute of Agricultural Sciences, and by a grant from The Danish Academy of Science and from the Department of Horticulture, Pennsylvania State University.

Aarslev, January 1998 Kristian Borch

Contents

I. Plant stress and root growth response to low phosphorus nutrition and the implication of ethylene

II. Appendix

A. **Borch, K.** and Beattie, D.J. (199x) Drought Avoidance in Potted Miniature Roses and Significance of Root Structure. HortScience (submitted).

B. **Borch, K.**, Brown, K.M. and Lynch, J. (199x) Improvement of bedding plant quality and stress resistance with low phosphorus HortTec (accepted).

C. **Borch, K.**, Brown, K.M., Lynch, J. and Miller, C. (199x) Improved drought avoidance by manipulation of root growth with low P nutrition (in preparation).

D. **Borch, K.**, Bouma, T., Brown K.M. and Lynch, J. (199x) Ethylene: a regulator of root architectural responses to soil phosphorus availability (submitted).

E. **Borch, K.** (1998) Growing pot plants with reduced phosphorus can improve root structure and avoid drought stress. Short communication. IPPS comb. proc. (in press).

English summary

The focus of this work has been on the growth response of roots exposed to low phosphorus availability in the soil and on the interaction of ethylene in this response. Moreover, the importance of root structure for drought tolerance or avoidance in container grown plants was studied. In this context it was investigated if the improved root growth by low but adequate phosphorus availability could enhance plant stress tolerance and especially drought tolerance.

The hidden nature of roots have made studies of root structure and specially root architecture very challenging. Environmental factors such as water and nutrient availability and composition of the soil (or the soilless growth medium) will influence the growth and shape of the root system. Root structure is important for efficient acquisition of nutrients and water. However, depending on the mobility of the resources in question, different types of root structures can be predicted for optimal uptake.

Phosphorus is one of the essential mineral nutrients involved directly in plant metabolism and cannot be replaced by other elements. Normally phosphorus is present only in very low concentrations in the soil solution. This means that phosphorus is transported to the root surface by diffusion and is thus very immobile and, hence, root structure is very important for the exploration and exploitation of this element. A common response to low phosphorus fertilization is an arrested shoot growth while root growth is maintained. Due to this it can be reasoned that low phosphorus grown plants might be more tolerant to drought stress and therefore have a better postharvest quality. Results have been presented that when the growth medium is amended with a solid phase phosphorus buffer, creating a low but constant phosphorus concentration in the root zone (as it is the case in natural soils), a better root distribution can be achieved. This led to an improved water acquisition and thus enabled the plant to avoid drought stress for an extended period.

Ethylene is a multifunctional plant hormone and has been shown to be implicated in several aspects of root development. However, the role of ethylene in root development is not well understood. It is possible that changes in root architecture in response to low levels of phosphorus are mediated by ethylene. Using different ethylene inhibitors several attempts have been made to investigate if the endogenously produced ethylene in roots has any physiological effect on root growth. Thus, evidence has been found that e.g. decreased lateral root density in low phosphorus grown common bean (*Phaseolus vulgaris* L.) is mediated by ethylene.

The results presented in this work suggest that we need to reconsider the traditional strategies of P fertilization that have been used in horticulture as well as in agriculture so far. It has here been demonstrated how reduced P fertilization

can have positive effects on root growth, transpiration and plant quality in container grown plants. However, natural colloid rich soils have their own natural P buffer. Therefore, major improvements probably can be achieved by reducing P fertilization in field crops with respect to plants resistance towards environmental stress, as well as a reduction in eutrophication of streams and lakes due to a reduced P loss from fields.

The experimental work that has been conducted in connection with my Ph.D. study is presented in five papers and attached as appendix to this dissertation.

Appendix A

Two comparable varieties of miniature pot roses (*Rosa x hybrida*), one of them using excessive amounts of irrigation water, were used to investigate the importance of root structure in water acquisition. The increased demand for irrigation could be explained by differences in the root topology.

Appendix B

By growing *Tagetes* and *Impatiens* in low phosphorus buffered medium it was shown that these plants can grow well with more than 50 times reduced phosphorus, compared to what is traditionally used in commercial horticulture. It was observed that roots were confined to a small volume beneath the drip tube in high phosphorus control plants, in contrast to low buffered phosphorus plants where they were well distributed throughout the medium. Also the amount of flowers which were initiated and maintained during low light conditions was increased in low but adequate phosphorus plants. This was possibly due to increased photosynthesis per unit leaf area because of reduced leaf area in low buffered phosphorus plants and reduced internal canopy shading.

Appendix C

Tagetes grown with low phosphorus had a reduced whole plant transpiration that could be explained by a reduced leaf area. When irrigation was withheld, the stomata closed at a higher relative water content in the pots of plants grown with high phosphorus compared to plants grown with low but adequate phosphorus. The improved drought avoidance could probably be explained by improved root growth and distribution. *Tagetes* grown with low, but not deficient phosphorus was less drought stressed, when irrigation was reduced to 50% of water use prior to applied drought stress compared to control plants grown with traditional levels of phosphorus.

Appendix D
To investigate if ethylene is involved in the root growth response to low phosphorus, common beans (*Phaseolus vulgaris* L.) were grown with adequate or deficient phosphorus. Simultaneously, the roots were subjected to an inhibitor of ethylene production and a range of ethylene concentrations. It could be shown that ethylene is involved in lateral root extension and that ethylene is involved in the reduced lateral root density response in roots grown with deficient phosphorus concentrations.

Appendix E
A summary of the results from growing bedding plants with reduced P using the alumina-P buffer method. It is discussed how reduced P will improve root structure and the space filling capacity enhancing water utilisation. Moreover, reduced P decreased leaf area which will reduce momentary water uptake and thereby save water resources. Both these features can improve water stress avoidance by the plant.

Danish Summary - Dansk Resumé

Dette arbejde har koncentreret sig omkring rødders vækstrespons på lave fosfat koncentrationer i voksemediet, og ætylens rolle i dette respons. Endvidere blev vigtigheden af rodstruktur for tørketolerance, eller mere korrekt tørkeundvigelse, i potteplanter studeret. I denne forbindelse blev det undersøgt, om den forbedrede rodvækst ved lav men tilstrækkelig fosfortilgængelighed kunne øge planters stresstolerance og specielt tørketolerance.

Det faktum, at rødder er skjult i jorden, har gjort studier af rodstruktur udfordrende. Miljøfaktorer, såsom tilgængeligheden af vand og næringsstof samt sammensætningen af jorden eller vækstmediet, vil påvirke væksten og formen af rodsystemet. Rodstrukturen er vigtig for en effektiv optagelse af næringsstoffer og vand, men afhængig af mobiliteten af de aktuelle ressourcer kan forskellige typer rodstrukturer forventes at have den mest optimale udnyttelses grad.

Fosfor er en af de essentielle mineraler, som er involveret direkte i plantens metabolisme, og kan ikke erstattes af andre elementer. Normalt er fosfor til stede i meget lave koncentrationer i den frie jordvæske. Dette betyder, at fosfor transporteres til røddernes overflade ved hjælp af diffusion, og er således meget lidt mobilt. Derfor er rodstrukturen meget vigtig for plantens afsøgning og udnyttelse (optagelsen) af dette element. En velkendt respons på lav fosfatgødning er retarderet skudvækst, mens rodvæksten opretholdes. På grundlag af dette kan man forestille sig, at planter, dyrket med reduceret men tilstrækkelig fosfat, kunne være mere tolerante over for tørkestress, og således have en forbedret holdbarhed. Resultater viser, at når voksemediet iblandes en fosfatbuffer, der bevirker en lav men konstant fosfat tilgængelighed for rødderne (hvilket ofte er tilfældet i naturlig jord), kan en forbedret rodfordeling opnås. Dette vil medføre en forbedret vand udnyttelse, og dermed tillade planten at undgå tørkestress i længere tid.

Ætylen er et multifunktionelt plantehormon, og det er vist, at det er involveret i adskillige aspekter i rodudviklingen. På trods af dette er forståelsen af ætylens rolle i rodudviklingen ikke særlig god. Det er muligt, at forandringer i rodstrukturen, som følge af lav fosfattilgængelighed, er medieret af ætylen. Ved at bruge forskellige ætyleninhibitorer er der gjort adskillige forsøg på at undersøge, om den endogent producerede ætylen i rødder har en fysiologisk effekt på rodvækst. Således er der fundet tegn på, at bl.a. nedsat lateral-roddensitet er i rødder fra almindelig bønne (*Phaseolus vulgaris* L.) dyrket ved lav fosfat er medieret af ætylen.

De præsenterede resultater betyder, at vi må overveje den traditionelle opfattelse af optimal P gødskning i havebrug såvel som i agerbrug. Det er her blevet demonstreret, hvordan reduceret P gødskning kan have en positiv effekt på rodvækst, transpiration og kvalitet i potteplanter. Imidlertid har naturlige kolloid rige jorde deres egen P buffer. Derfor kan man forvente betydelige fordele ved at reducere P gødskning i markafgrøder m.h.t. planters modstandskraft over for naturlige stress faktorer. Ligeledes kan en forventet reduktion i P tab fra landbrugsjorde afhjælpe de omfattende problemer omkring eutrofiering af vandløb og søer.

Det eksperimentelle arbejde, som er udført i forbindelse med mit Ph.D.-studium, er præsenteret i fem artikler, der er vedlagt som appendiks til denne afhandling.

Appendiks A

To sammenlignelige sorter af pottede miniatureroser (*Rosa x hybrida*), hvoraf den ene bruger ekstra mængder vandingsvand, blev brugt til at studere rodens betydning for vandudnyttelsen. Det forøgede vandforbrug kunne forklares med forskelle i rodens topologi.

Appendiks B

Ved at dyrke *Tagetes hybrid* og *Impatiens x hybrid* i lav fosfat buffered vækstmedie kunne det vises, at disse planter kan gro udmærket med mere end 50 gange reduceret fosfatgødning, sammenlignet med niveauer, der traditionelt anvendes i gartnerier. Det blev observeret, at rødderne var begrænset til et lille område under drypslangen i planter dyrket, med høj fosfat, mens rødderne var jævnt fordelt i planter, dyrket med lav fosfat-buffer. Endvidere havde planter dyrket, med lav men tilstrækkelig fosfat, flere blomster når, de blev stresset med lavt lys, sammenlignet med høj fosfat dyrkede planter. Dette kunne sandsynligvis forklares med mere effektiv fotosyntese, på grund af det reducerede bladareal, og dermed mindsket intern skygning.

Appendiks C

Tagetes hybrid, dyrket med lav fosfat, havde en reduceret transpiration, som kunne forklares med et reduceret bladareal. Når vandingen blev afbrudt, lukkede stomata ved et højere vandindhold i potten i planter, dyrket med høj fosfat, sammenlignet med planter, dyrket med lav men tilstrækkelig fosfat. Den forbedrede evne til at undgå tørkestress kunne sandsynligvis forklares med øget rodvækst og en forbedret rodfordeling, og dermed bedre udnyttelse af tilgængeligt vand. Endvidere var *Tagetes hybrid,* dyrket med lav men ikke mangelfuld fosfat, ikke så tørkestressede, når vandingen var reduceret til 50%, sammenlignet med planter, dyrket med høj fosfat.

Appendiks D

For at undersøge, om ethylene er involveret i rødders vækstrespons på lav fosfat, blev almindelig bønne (*Phaseolus vulgaris*) dyrket med tilstrækkelig og mangelfuld fosfat. Samtidig blev rødderne eksponeret til en inhibitor af ætylenproduktion og en række ætylenkoncentrationer. Det kunne herved vises, at ætylen var involveret i den reducerede lateral-roddensitet respons i rødder dyrket, med mangelfuld fosfatkoncentration.

Appendiks E

En oversigt over resultater fra forsøg med dyrkning af planter med reduceret P ved hjælp af alumina-P-buffermetoden. Det diskuteres, hvordan reduceret P forbedrer rodstrukturen og rodens evne til at fordele sig jævnt i et volumen hvilket vil forbedre udnyttelsen af vandressourcerne. Yderligere medfører reduceret P, at bladarealet formindskes, hvilket igen vil reducere vandforbruget og således spare vandressourcer. Begge disse karakteristika kan hjælpe planten med undgå at vandstress.

I. Plant stress and root growth response to low phosphorus nutrition and the implication of ethylene

1. Introduction

How roots respond to shortcomings in the soil is of obvious botanical interest and important in the context of agricultural crop performance. Understanding the changes in root architecture and morphology during low P conditions, can enable us to prepare an ideal root zone which will allow maximum growth and development of the root system and thereby improve plant tolerance to stress factors. This is important in our efforts to improve crop yield in soils poor in available P which is the case in many third world countries.

One of the prime functions of roots is acquisition of soil based resources, namely water and dissolved ions. Other functions are anchorage, storage, synthesis of growth regulators, propagation and dispersal.

Root systems and their structure vary widely even within species as the root growth of the individual plant will respond specifically to numerous environmental challenges. Besides the genetic regulation, the structure of a given root system will be a question of structural changes and biomass allocation, depending on the mobility and location of the most abundant resource(s) in the heterogeneous soil (e.g. Fitter, 1994; 1996; Drew, 1990).

Phosphate is a poorly mobile resource that is present only in micromolar concentrations in the soil (Rending and Tylor, 1989). Add to this that the P cycle is open ended in most terrestrial systems and will ultimately be exhausted. Today's high-value crops have traditionally been fertilized heavily and agricultural P loss to surface waters has become a major pollutant concern in large parts of the industrialized world. However, research has shown that plants can grow well at P levels as low as 10 µM P (Lynch et al., 1991; Hansen and Lynch, 1998; **appendix b**) when a P buffered medium or solution is used. In addition it may be possible to improve plant quality and stress tolerance by encouraging strong root growth during production by reducing the phosphorus (P) levels in the root zone.

The development of root systems is a highly regulated process responsive to environment and a key factor to the well being of plants (Jackson, 1991). There is now bulk evidence indicating that hormones produced in the roots play an important role in the root development and overall hormonal physiology of the plant (e.g. Torrey, 1976; Marschner, 1995; Itai and Birnbaum, 1996). Roots contain and synthesize the major known plant hormones and many aspects of shoot behavior, such as stomatal closure and leaf senescence, are influenced by the status of the roots in ways that cannot be explained simply by alterations in water and mineral nutrient supply.

Ethylene is a potent plant hormone and has demonstrated numerous effects on the growth and development of roots (Jackson, 1991), and many of these responses are mirrored in the behavior of roots exposed to different kinds of environmental stress. Despite the apparently numerous effects on structure, morphology and anatomy of roots (e.g. Jackson, 1991), evidence of the role of ethylene in root growth remains rudimentary.

In the following I will discuss the importance of root structure for water and nutrient acquisition and environmental factors influencing root growth. Moreover, the role of low P in root growth and the possibility of improving plant stress tolerance (especially drought stress) in container grown plants will be dealt with. Finally the role of ethylene in root development and especially its implication in the low P growth response of roots will be discussed. The P acquisition topics: mycorrhizal infection of roots and root exudes are extremely important for plants grown in natural soils. However, the research that I have conducted here is done on container grown plants where mycorrhizal infection of roots and root exudes are of less importance because the plants grow in a soilless medium which is the reason why I have not included these subjects in this overview. Besides the scope of this dissertation would have been too wide if these otherwise interesting topics would have been included.

2. Root structure

The importance of root structure for acquisition of nutrients and water is intuitively obvious. Roots inhabit a sphere which is highly heterogeneous where the ability to explore and exploit nutrients and water is a key factor of a successful existence for most plants. There is a great variety in the form of root systems explained by a complex interplay between genotype and the roots adaptive plastic response to environmental factors. There is a great variation in the spatial and temporal distribution of resources and their mobility. Therefore the question; which branching and growth pattern will result in the best root distribution for optimal return of resources with the lowest construction cost for the plant is quite complex.

2.1. Methods of root studies

One of the biggest challenges in the study of roots is their out of sight nature and problems of retrieving an intact root system from the soil. Therefore, many systems have been designed to circumvent this problem:

- Rhizotrones allow direct visual study of the roots by letting the roots grow against a glass wall or tube with visual access for the observer or a camera.

- Hydroponics (roots grown in a nutrient solution) and aeroponics (moist air where dissolved nutrients are sprayed on the roots) allow a direct study of the morphology and topology, but does not allow for architectural studies. Moreover, these systems do not offer any impedance to the roots which have a profound influence on the root structure.
- Sand has fair water holding capacity and most plants grow well in this medium and the roots are easy to retrieve. However, the impedance fluctuates with the wetness of the sand, which influences the growth pattern of the roots.
- Porous materials as calcined clay offer fair water holding capacities, the impedance is very constant and it is very easy to separate from the roots.
- Trench excavations, core samples etc. can give some information about root distribution e.g. by fractal analysis (Nielsen et al., 1997). The advantage of this technique is that the root can grow in a natural soil.

Except for trench excavations all the soilless media described above are usually not heterogeneous in contrast to most natural soils, a fact which have to be considered when conclusions are drawn (Snapp et al,. 1995; **appendix D**).

Lately powerful computers have allowed for researchers to construct computer models to simulate root growth (e.g. Berntson, 1994; Clausnitzer and Hopmans 1994; Nielsen et al., 1997). Unfortunately only few of these researchers have engaged simulating models and empirical experimentation although they are highly complementary (Lynch and Nielsen, 1996).

2.2 Root structure terminology

As the term root structure involves distinct aspects of the form or shape of root systems, and as it is of importance in the following I shall elaborate the term here (condensed from Lynch and Nielsen 1996):

- Morphology: refers to the surface features of a single root axis, including characteristics of the epidermis such as root hairs, root diameter, root cap etc.
- Topology: describes branching of root axes and how they are connected to each other through branching.
- Architecture: is the spatial configuration of the root system.

Distribution: describes the space distribution of a root system in a grid for example roots per cm^2.

The different types of root making up the root structure are classified as:

- Tap root - the extended radicle or its replacement (in some species, e.g. maize (*Zea mays* L.), this is a seminal root which replaces the radicle)
- Basal root - a root originating from the hypocotyl or mesocotyl i.e. seminal roots other than the tap root.

- Adventitious root (shoot borne root) - a root originating from shoot tissues (stem, petiole, leaf, etc.)
- Lateral root - a root originating from another root.

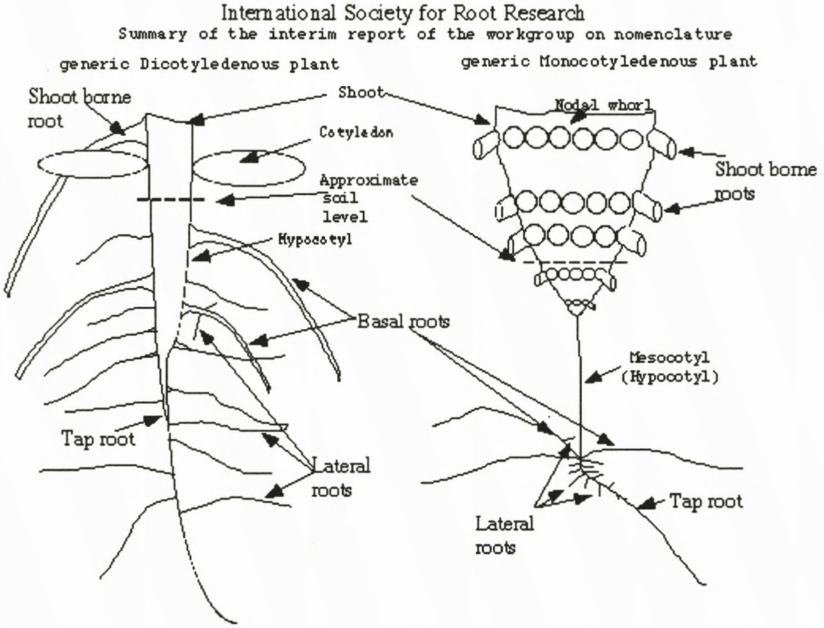


Fig 2.1. Root nomenclature as suggested at ISRR Symposium, Clemson University, July, 1996.

2.3 Environmental factors influencing the root structure

Environmental factors will influence the root structure, either because of restriction or because of adaptive response of the root. The influence of P nutrition will be dealt with in section 3.

2.3.1. Water

Water will never be distributed uniformly in a soil profile and while surface desiccation enhances rooting depth, frequent irrigation encourages proliferation of roots at the surface. Moreover, soils, especially sand, generally develop a greater resistance to deformation at lower water potentials so that root extension is inhibited by mechanical resistance (e.g. Hamblin, 1985; Almaras et al., 1988).

2.3.2. Flooding and waterlogging

When the gas filled porous system in the soil becomes blocked by water the roots can become hypoxic causing ethylene to develop and in extreme cases cause injury to roots (Russell, 1977) and reduce longevity in container grown plants

(Borch, 1994). Some plants e.g. corn (*Zea mays* L.) develop cortical air spaces (Aerenchyma) in the roots and stems in response to O_2 deficiency during waterlogging (Drew et al., 1979; Justin and Armstrong, 1991). This accelerates the transfer of O_2 from the aerial tissues to the O_2-deficient tissues in the roots (Armstrong, 1979). The cell lysis that leads to aerenchyma formation (Campbell and Drew, 1983) can be promoted by exogenous ethylene under fully aerobic conditions and blocked by inhibitors of ethylene action (e.g. AVG) or ethylene action (e.g. Ag^+). Therefore it is believed that aerenchyma formation is caused by an enhanced rate of ethylene production stimulated by hypoxia (He et al., 1996).

2.3.3. *Impedance*

The root tip is forced through the growth media which consist of more or less incompressible particles by expansion of cells in the elongation zone. However, the root cap is compressible and is not forced to take a preset direction through the soil, but follows the path of least resistance, e.g. through channels left by worms or decaying roots (Hamblin, 1985). Kays et al. (1974) reported that when impedance become high or the root tip of *Vicia faba* encounter an obstacle the ethylene production increased within one hour and was maintained for 20 - 30 hours. As ethylene induce thickening of roots (Sarquis et al., 1992; **appendix D**) it is possible that ethylene help penetration of the substrate by the root tip (Zacarias and Reid, 1992; Barley and Graecen, 1967).

2.3.4. *Temperature*

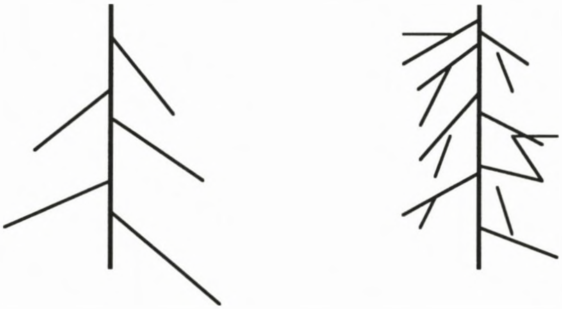
Cell division and expansion are temperature dependent which will influence root growth (Barlow, 1987). High temperatures in the surface layers of soil will slow root growth and cell division ceases at 35 - 40 °C in barley and onion (Barlow, 1987). Low temperatures also reduce root growth and frozen layers in the soil will be a barrier for root penetration.

2.3.5. *Nutrients and pH*

Nutrients either have to be transported to the root surface or the root will have to intercept the nutrients (Barber, 1995). In natural heterogeneous soil P will often be found in patches. The roots will explore for these P rich patches, and when encountered they will proliferate vigorously to exploit the available P (Snapp et al., 1995). However, in containerized plants, that grow in a homogenous growth media, is it generally more important to have an uniform distributed root system, which will give a better water acquisition capacity (**appendix B, C**). It is often very difficult to describe the soil-root interface conditions with a mechanistic model sufficiently, since they vary considerably with the distance from the roots (Marschner, 1995).

Nutrients can be transported to the root surface in two ways: mass flow and diffusion (diffusion coefficient less than 10^{-8} cm^2 s^{-1}). Typically mass flow supplies water, Ca^+, Mg^{++}, Cl^-, SO_4^{--} and NO_3^- while K^+ and $H_2PO_4^-$ are supplied by diffusion because of the very low concentration in the free soil solution (Barber, 1995).

For acquisition of mobile resources a herringbone like topology (Fig. 2.2) with a low root density (longer links i.e. long main roots with reduced lateral root density) may be beneficial (Fitter, 1996; **appendix A**). Optimum root structure with regard to acquisition of less mobile resources is dependent on abundance and distribution of these resources (Snapp et al., 1995; **appendix D**). Thus, if K^+ and $H_2PO_4^-$ concentrations are low in the immediate vicinity of the root it could be beneficial to develop a root with a herringbone like structure with long links increasing exploration capacity. In a patch with high concentrations of the immobile resource a more branched roots structure (e.g. dichotomous (Fig. 2.2)) where root density is highly reducing the distance of diffusion, is more beneficial.



Herringbone

Dichotomous

Fig. 2.2. Illustration of a root structure with a low root density (herringbone) and a root with high root density (dichotomous).

2.4 Root structure and water acquisition

As transpiration is an inevitable consequence of photosynthesis, water acquisition is an essential part of the function of roots. Plants can avoid water deficits by obtaining access to water deep in the soil or more efficiently extract water from the occupied part of the profile (Ludlow, 1989), only the later being a possibility for container grown plants. Improved water acquisition will allow the shoot to postpone the short-term and long-term cost of elastic and plastic drought responses (Ludlow, 1989). Elastic drought response, being paraheliotropic leaf movement and stomatal closure, will reduce light interception and carbon assimilation and in turn reduce vegetative growth. Whereas the plastic drought response, the shedding of older leaves, requires long time to recover as new leaves have to be formed. Moreover, the visual impression of the plants may be reduced, which is important to avoid for ornamental growers.

Root architecture and distribution, influence water uptake (Subbarao et al., 1995; **appendix A**). Hence, growers report that the pot rose 'Bianca Parade' (*Rosa x hybrida*), when grown under the same conditions as numerous other cultivars like 'Charming Parade' frequently shows drought stress symptoms which may reduce plant quality. It was shown that the relative water content in the growth medium of container grown 'Bianca Parade' was higher compared to 'Charming Parade' when wilting point was reached (**appendix A**). The analysis of the root structure of the two cultivars revealed that the two root systems were very different. The individual branches and links were longest in 'Charming Parade'. This may result in a better capacity of the roots to explore the medium for water, with a lower probability of overlap between the depletion zones of adjacent roots (Fitter et al., 1991). Moreover, the soil water flow is often the largest resistance for the soil-plant-atmosphere continuum (Nobel, 1991), thus, a short diffusion distance will reduce the resistance. As 'Bianca Parade' roots have a high lateral root density compared to 'Charming Parade' the water diffusion distance will be lower allowing a higher transpiration rate during well watered conditions with high soil water content. Of course increased root surface area will also reduce the resistance to water flow across the root surface, thus, roots of 'Bianca Parade' had numerous root hairs, while 'Charming Parade' only had very few (**appendix A**). As desiccation progresses the diffusion distance become larger and increased lateral root density is expected to become less relevant for the water uptake resistance because the depletion zones overlap. Then the length of the individual root segments become more important as it can explore a bigger volume (**appendix B**).

3. Low phosphorus nutrition and root structure

Phosphorus is one of the essential mineral nutrients, which are directly involved in plant metabolism. Phosphorus has a strong influence on the development of plant growth and plants that are deficient in P will be stunted and low yielding (Marschner, 1995). Phosphate is tightly bound in the soil, especially at either high or low pH, where the solubility of P is reduced, and most available in neutral to slightly acidic soils. Once P is taken up into the plant, it becomes quite mobile. It will be allocated to the youngest parts of the plant. If there is a shortage of P, it will be reallocated from older leaves to tissue where it is needed.

When discussing plant nutrition it is important to distinguish between P concentrations in excess, low but *adequate* P concentration and low but *deficient* P. Ornamental plants are typically grown with phosphorus levels in excess, which may have detrimental effects (Hansen and Lynch, 1998). For example can high P concentration in the soil induce zink deficiency and probably also iron deficiency (Marschner, 1995). It has been demonstrated that plants can grow well with more than 50 times reduced P (adequate P) compared to levels traditionally used in horticulture (Hansen and Lynch, 1998; **appendix B,C**) as long as it is applied in such a way, that internal P levels do not become so low that metabolic functions are disturbed (deficient P).

3.1 Phosphorus

Phosphorus is one of the essential mineral nutrients involved directly in plant metabolism and cannot be replaced by other elements (Marschner, 1995).

Phosphorus is involved in numerous functions:

- structural element (sugar phosphate backbone) in nucleic acids,
- construction of the phospholipids for maintaining the integrity of the cell membrane;
- formation of high-energy phosphate compounds (ATP, ADP, and NADPH);
- substrate, end product, or regulator in some enzyme reactions necessary for the regulation of metabolic pathways in the cytoplasm and chloroplast (Marschner, 1995).
- Phosphorylated metabolits (Stryer, 1988).

The general requirement of P for optimal plant growth is in the range of 0.3 to 0.5% of the total plant dry weight during the vegetative growth (Marschner, 1995). Plants suffering from P deficiency exhibit retarded and erect growth (dwarf and stiffness), dark green foliage, and often reddish to purplish coloration on stems and veins (Bergmann, 1992). Since P is involved in major metabolic processes, P deficiency effects photosynthesis, respiration, and cell division and expansion. The dark green coloration is due to the cell and leaf expansion being more retarded than chlorophyll formation, and the reddish color is due to enhanced anthocyanin formation (Marschner, 1995). Phosphorus deficient plants can have an enhanced root growth (Marschner, 1995) and root to shoot biomass ratio increases as relatively more dry mater is allocated to the roots to optimize P uptake (Aung, 1971; Glass, 1989).

3.2 Phosphorus acquisition

The ability of the root system to explore the growth media efficiently in time and space is very important for P acquisition because of the very low concentration in the free soil water solution (Barber, 1995). In the acquisition of mobile resources it is not essential to have a high root density because they more easily can be transported over relatively larger distances. Rather it is important to have an extended root system which can reach deep layers e.g. for water. Acquisition of less mobile resources is a more open question. High root density (root length per soil volume), as is the case with a more branched root structure could be predicted to optimize P acquisition because the phosphate ion has to diffuse a shorter distance than for a lower root density (e.g. herringbone root structure) (Baldwin, 1975). Fitter (1996) on the other hand argued that topology is not important for less mobile resources (diffusion coefficient less than 10^{-8} $cm^2 s^{-1}$) because the depletion zone is so narrow. Therefore, rate of root extension into as yet unexplored volumes of soil may predict the rate of phosphorus absorption better (Koide, 1990; Newman and Andrews 1973). In *Tagetes x hybrida* (marigold) grown with low but adequate P nutrition we found that the roots had almost twice the length of control plants grown with excess P levels. When grown with deficient P root length was not increased compared to control plants (**appendix C**). However, natural soils are highly heterogeneous and in particular the low mobile resources will be found in patches (Snapp et al., 1995). If the immediate vicinity of the root is low in P, it is not beneficial for the root to develop a high root density. Rather the root need to invest in elongation and this way increase the possibility of finding a P rich patch (**appendix D**) in which it will branch vigorously to exploit the P (Fig. 3.1). It has been observed that P-deficient common bean (*Phaseolus vulgaris*) had inhibited xylem development, secondary wall thickening and reduced root diameter. This may reflect a strategy to reduce structural cost during P deficiency (Eshel et al., 1995). Moreover, either hypoxia, which stimulated ethylene biosynthesis, or temporary N or P starvation enhanced lysigenic aerenchyma formation in adventitious roots (Drew et al., 1989; He et al., 1992; 1994). Moreover, aerenchyma formation in the cortex has been observed in P starved roots (Borch, unpublished; Eshel, personal communication). Formation of aerenchyma during flooding is believed to be an adaptation strategy to hypoxia (Jackson, 1991). It is less clear whether aerenchyma formation in nutrient stressed plants has a similar adaptation, or if aerenchyma formation during low P is just a byproduct of increased ethylene sensitivity (Lynch and Brown, in press).

Root hairs are generally believed to assist in phosphorus acquisition because they can substantially increase root-soil contact (Bhat and Nye, 1974; Foehse and Jungk, 1983; Anghinoni and Barber, 1980; Gahoonia et al., 1997). In *Arabidopsis thaliana*, it was observed that root hair length was regulated by phosphorus availability (Bates and Lynch, 1996).

Thus, root diameter (Eissenstat, 1992), root structure, architecture (Fitter, 1991; Lynch and Beebe, 1995), root extension (Barber, 1995) and elongation rate (Koide 1990), structural changes in the root tissue (Eshel et al., 1995) and root surface area (Bates and Lynch, 1996) can influence the uptake of P (Lewis and Quirk, 1967a,b; Marschner, 1995). These factors are all part of the roots adaptive plastic response during low P conditions in the root zone.

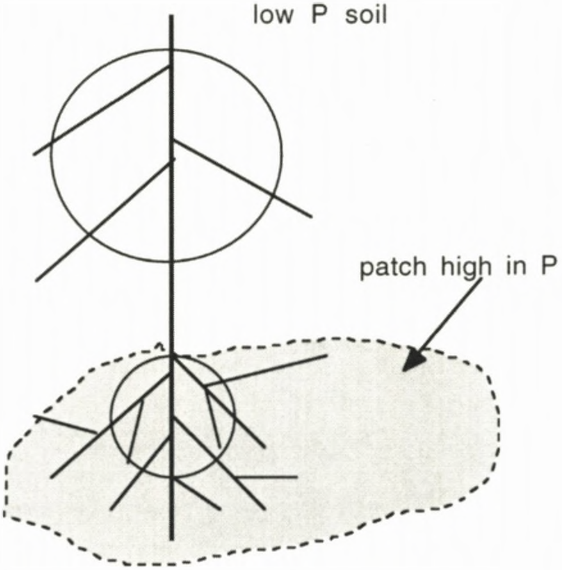


Fig. 3.1. Illustration of hypothesized root branching in a high P patch surrounded by low P soil. The circles indicate the volume soil including the same root length.

3.3 Low P nutrition

To increase consumer satisfaction, growers have to produce high-quality plants which are compact, stress tolerant, and free from diseases. This often does not harmonise with growers attempts to keep the production period as short as possible by growing bedding plants with optimum light, temperature and a surplus of fertiliser to maximise growth rate. Such conditions often produce plants with elongated, lush shoots. However, this is on the expense of root development, and in turn poor stress tolerance. Therefore, it is recommended that before shipping growers harden their plants with a short period of lower temperature and reduced fertiliser and water at the end of the production cycle (Serek, 1990). This practice encourages root growth at the expense of shoot growth and is advantageous because plants with well developed root systems which exploit the medium uniformly and with room for further growth are best at withstanding the fluctuations in soil moisture which occur during shipping, handling, and in the hands of the consumers.

Even though this is helpful, it might be possible to improve plant quality and stress tolerance even more by encouraging strong root growth during production by reducing the P levels in the root zone. Research has shown that the high P levels (1-2 mM P) commonly used in commercial floriculture inhibit root elongation (Hansen and Lynch, 1998) and studies using P-buffered medium or solutions show that plants can grow well at P levels as low as 10 µM P (Lynch et al., 1991; Hansen and Lynch, 1998; **appendix B**).

We have observed that roots of plants grown in media amended with a solid-phase P buffer providing 5-35 µM P explored the media more uniformly than plants grown under a conventional 1 mM P fertilization regime (**appendix B**). In addition, the plants grown under reduced P were more successful in avoiding drought stress (**appendix B**). This suggests that root growth can be manipulated to obtain optimal media exploration thereby allowing a better water utilization. In an experiment with *Tagetes x hybrida* grown with excess and adequate P concentrations it could be shown that at the wilting point the relative water content in the pot was lower in plants grown with adequate P concentration compared to excess P. Simultaneously, root elongation increased and lateral root density was reduced in adequate P plants indicating that an improved spatial distribution of the roots tend to improve water acquisition (**Appendix B**).

Since plants grown under low but adequate P have a higher root to shoot ratio, it could be reasoned that they might be more tolerant to drought stress due to reduced transpiration and improved root growth and thereby obtaining more robust plants. This hypothesis was supported by longevity experiments were *Tagetes x hybrida* grown with low but adequate P had more flowers when growing in reduced light (**appendix B,C**). Even when the P concentration in the growth media was more than 100-fold lower than in the high-P plants no detrimental effects on either *Tagetes x hybrida* nor *Impatient x hybrida* was found. The reduced leaf area in plants grown with reduced P nutrition may be a factor which contribute to an increased postharvest stress (e.g. low light) tolerance due to reduced canopy self shading an thus a higher photosynthesis per leaf area (**appendix C**).

4. Ethylene and root growth

The development of root systems is a highly regulated process responsive to the environment and a key factor to the well being of plants (Jackson, 1991). Simultaneously, roots contain and synthesize the major known plant hormones (e.g. Torrey, 1976), which have been shown to play a major role in the regulation and control of whole plant development. It is therefore a reasonable assumption that hormones produced in the roots play an important role in the root development and overall hormonal physiology of the plant.

One of the hormones produced by roots is gaseous ethylene, and already in 1933, Zimmerman and Hitchcock demonstrated that adventitious root formation was promoted by ethylene. Until recently it was thought that ethylene was only involved in stress related processes in the shoot as well as in the root. However, ethylene is also involved in normal plant development e.g. diverse array of cellular, developmental and adaptive related processes. Hence, ethylene has been shown to be involved in adaptations to chemical toxicity, water stress, and interactions with symbionts and nutrient deficiency (reviewed by Lynch and Brown, in press), and as a positive regulator of root hair development (Tanimoto et al., 1995).

Still, the role of ethylene in roots is not well understood despite the apparently various effects on structure, morphology and anatomy of root tissue.

4.1 The plant hormone ethylene

Considering it is the most multifunctional of plant hormones ethylene is a remarkably simple molecule (Fig. 4.1). It was identified as a gaseous growth factor by Neljubov in 1901 who observed that ethylene in the air of the laboratory was causing horizontal growth of pea seedlings (Neljubov, 1901). Ethylene is a two-carbon compound with one double binding and a molecular weight of 28.05. Solubility of ethylene in water at room temperature is 131 10^{-6} g per g of water (McAuliffe, 1966), and is considered very low.

Although ethylene acts in the liquid phase of the cell it is normally measured as a component of the gas phase and the most common way to measure the physiological low concentrations of ethylene is by gas chromatography (GC). A flame ionization detector (FID) has a lower detection limit of 15 ppb if settings are optimal. With a photoionization detector (PID) a slightly lower detection level can be achieved. A laser-based photoacoustic method for ethylene analysis has been developed with detection down to 0.03 ppb (Woltering et al., 1988), but besides being expensive the laser-based photoacoustic apparatus is very tedious to operate (Woltering, personal communication).

4.2 Ethylene biochemistry and binding in higher plants

The existence of the MSAE pathway (l-methionine, S-adenosylmethionine, 1-aminocyclopropane-1-carboxylic acid, ethylene pathway) (Fig. 4.1) has been demonstrated by the use of inhibitors, precursors, and labeled compounds (Yang and Hoffman, 1984).

The two carbons of ethylene are derived from number 3 and 4 carbons of the amino-acid L-methionine, which is first converted to S-adenosyl methionine (AdoMet or SAM), then to 1-aminocyclo-propane-1-carboxylic acid (ACC) by ACC-synthase before it is oxidized to ethylene by ACC-oxidase (Fig. 4.1) (see also Yang and Hoffman 1984). The rate of ethylene production is controlled by the formation of ACC by ACC-synthase and by its conversion to ethylene by the ethylene-forming enzyme (EFE) also called ACC-oxidase. Normally ACC-oxidase is present to a sufficient level in plant tissue making the conversion of AdoMet to ACC the rate limiting reaction.

ACC is water soluble and moves over great distances in the apoplast from root to shoot (Bradford and Yang, 1980). Therefore, a role for ACC exported from anoxic roots as a positive message that influences shoot development and morphology has been supported (reviewed by Jackson, 1993).

Evans et al. (1982) found that the ethylene binding site was located in endoplasmatic reticulum and Sisler (1982) estimated that leaf cells of tobacco have 4000 binding sites per cell. Similar numbers were found for bean, citrus and *Ligustrum* leaves (Goren and Sisler, 1986).

Ethylene production is feedback regulated and both an autoinhibitory (e.g. Sawamura and Miyazaki, 1989), and autocatalytic (e.g. Schierle et al., 1989) action of ethylene has been found.

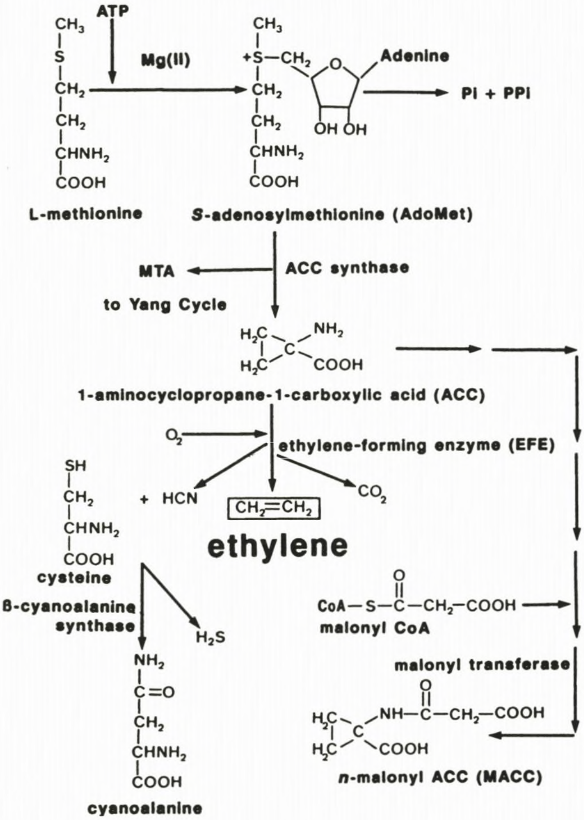


Fig. 4.1. The ethylene (MSAE) pathway showing conversion of L-Methionine into ethylene. The ACC conjugate Malonyl ACC (MACC) which is an inactive end product of ACC (Botella et al., 1995) is shown to the lower right. Also the detoxification of HCN by cysteine is shown to the lower left. The continued regeneration of methionine by the Yang cycle (Yang and Hoffman, 1984) is indicated in the middle of the figure. After Abeles (1992).

The genetic dissection of the ethylene signal transduction pathway has given powerful insights into the molecular mechanism of how plants perceive and respond to ethylene. This has been possible mainly from studies using mutants of *Arabidopsis thaliana* that either are ethylene insensitive in shoot and/or roots or show a constitutive ethylene response (Ecker, 1995). A series of genes has been identified which products control ethylene responses (Chang and Meyerowitz, 1995; Ecker, 1995). This is an important point since any change in their expression or activity might produce an ethylene-like response without changing the ethylene biosynthesis rate.

Ethylene production is promoted by several physical, chemical and environmental factors in whole plants (Fig 4.2). Several recent studies of the genes encoding ACC synthase and ACC oxidase, which are rapidly induced by these factors, have been identified and characterized (reviewed by Morgan and Drew, 1997). For example did repeated bending of leaves of mung bean induce ACC synthase within 10 min. (Botella et al. 1995). Thus, as most experimental work with plants imply handling, this has to be considered when results are interpreted as even gentle touch can promote ethylene production (touch stress) (Finlayson et al., 1996).

4.3 Evidence of ethylene implication in root growth

Ethylene is a volatile plant hormone, which has received considerable attention as a growth regulator in above ground parts of plants. However, the development of roots is as least as sensitive to ethylene as shoots, and they grow in a medium with the capacity of trapping ethylene, producing it, or oxidizing it depending on the circumstances (Jackson, 1991).

Ethylene has been shown to be involved in several aspects of root development. During waterlogging and flooding or mechanical compression of the soil lysogeneous aerenchyma formation (continuous gas-filled channels) in the roots and stems of many dicots and monocots takes place in response to O_2 deficiency (Drew et al. 1979; Kawase, 1981a, 1981b; Justin and Armstrong 1991; Huang et al., 1997), accelerating O_2 transfer to the depleted tissues in the roots (Armstrong, 1979). In nitrogen- or phosphate-starved roots, enhanced sensitivity of ethylene-responsive cells lead to cell lysis and aerenchyma (He et al. 1992). Ethylene may also be involved in gravitropic response of roots (Lee et al., 1979), and penetration of the substrate by root tips (Zacarias and Reid, 1992).

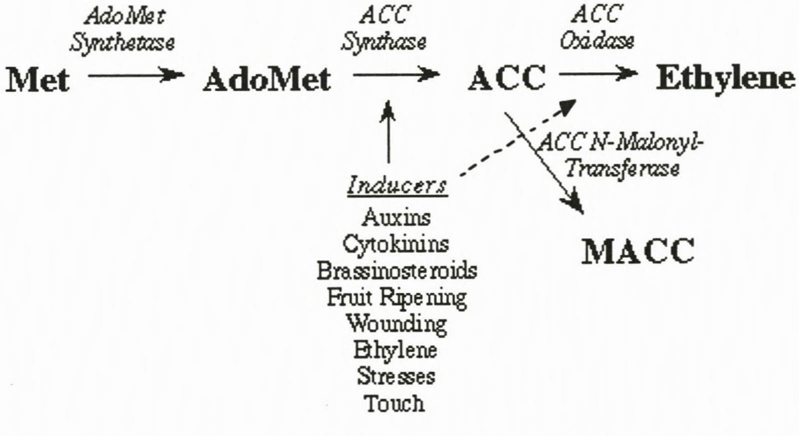


Fig. 4.2. Chemical and environmental inducers of ethylene production in whole plants and the possible steps being regulated.

4.4 Ethylene regulation of root growth under deficient P

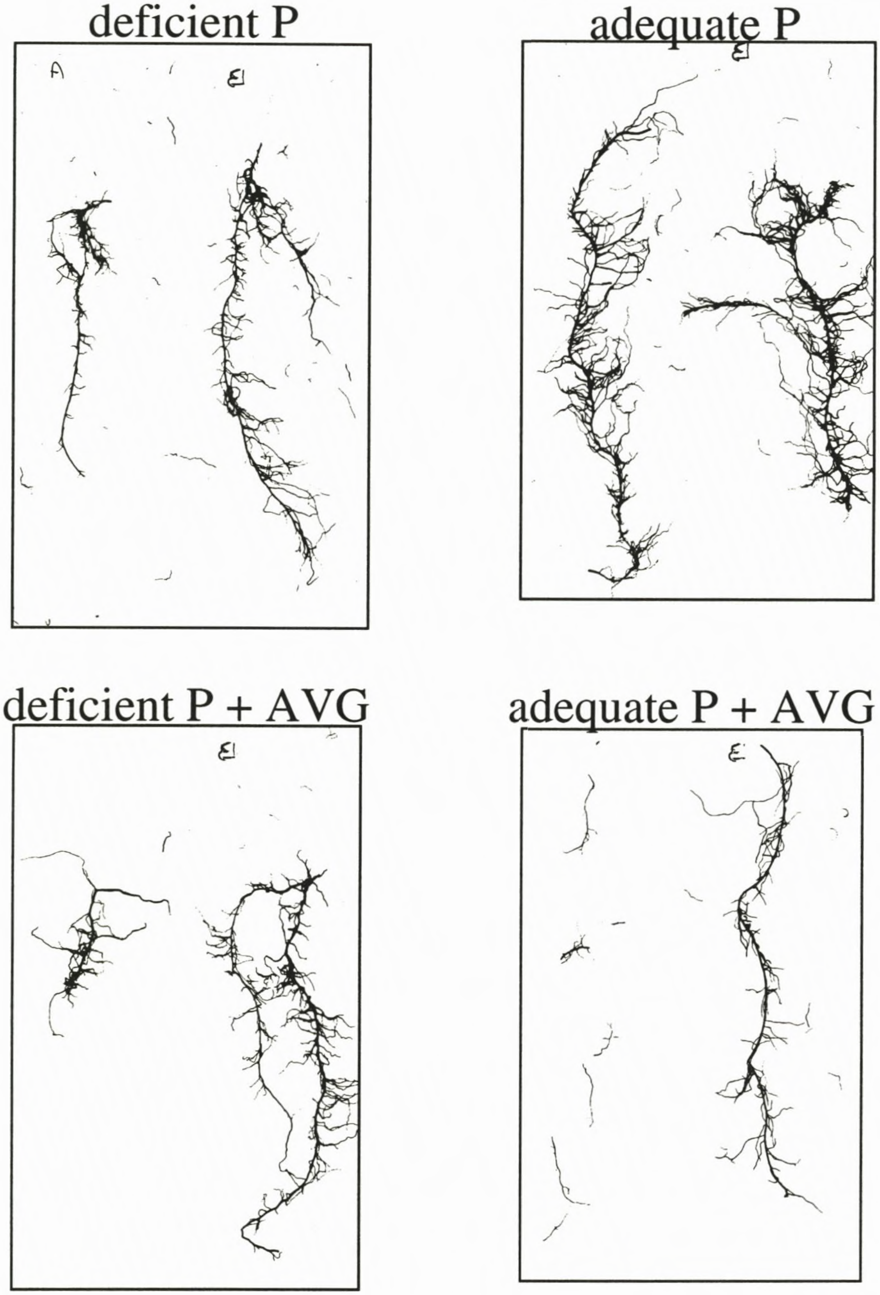
The low concentrations normally present in the root zone have made studies of the implication of ethylene in root growth troublesome. This is mainly due to problems in measuring the very low concentrations in the physiologically interesting range and, thus, controlling application of exogenous ethylene.

Attempts to find out whether the production endogenous ethylene is sufficient to have any physiological effect on root growth has been carried out using inhibitors of ethylene biosynthesis (e.g. amino-ethoxyvinylglycine (AVG)) or blocking of ethylene receptors (e.g. 1-methylcyclopropene (1-MCP), 2.5-norbornadiene (NBD) and silver thiosulfate (STS)). However, only few experiments have involved dose-response curves (**appendix D**) to determine concentrations small enough to avoid toxic effects (Abeles and Wydoski, 1987; Jackson 1991). Thus, the role of endogenous production of ethylene by roots is still ambiguous.

The production of ethylene vary within different portions of the root and the highest production on a fresh weight basis is found in the tip (Hew et al., 1995; Finlayson et al., 1996). However, ethylene produced by the root tip is small compared to the other root parts, when total ethylene evolution from the whole root was considered (Finlayson et al., 1996).

Also ethylene production and sensitivity may vary with different environmental stimuli. For example, in excised maize roots starved of phosphorus or nitrogen, ethylene production decreased but the root tissue became more sensitive to aerenchyma formation promoted by ethylene (Drew et al., 1989; He et al., 1992). On the other hand, we found that intact undisturbed roots of common beans (*Phaseolus vulgaris* L.), that where deficient in P, produced more ethylene per g dry matter compared to adequate P roots (**appendix D**). It is problematic, however, to compare ethylene production of excised roots with the equilibrated concentration of ethylene in the root zone. Internal ethylene concentrations would most likely be higher in deficient P roots than in adequate-P roots, since the production rate is higher (**appendix D**). This ethylene could mediate some of the observed growth and development responses to P deficiency. Even when endogenous ethylene production was greatly reduced by AVG, there was a significant interaction between P concentration and ethylene treatment, indicating differences in ethylene responsiveness under deficient P (**appendix D**). Therefore we can not rule out that ethylene could mediate changes in root morphology both by changes in synthesis and by changes in tissue responsiveness.

In several species it was found that ethylene concentrations well below 1 ppm promoted root elongation while higher concentrations inhibited elongation (Kays at al., 1974; Smith and Robertson, 1971; Bucher and Pilet, 1982; Goeschl and Kays, 1975). Konings and Jackson (1979) found a relationship between the rate of elongation and ethylene production. Simultaneously, plants with the highest ethylene production were the least sensitive to the promotive effect of low concentrations of exogenous ethylene. So far studies of ethylene in whole root systems have involved high concentrations (Crosset and Campbell, 1975; Jackson and Campbell, 1979; Jackson et al., 1981; Huang et al., 1997) and only few studies of the effect of low ethylene have been performed (**appendix D**). We did not find increased root length of the whole root system of common bean in an experiment where a range of very low concentrations of ethylene was applied to the root zone for several weeks while non toxic concentrations of AVG were used to block endogenous ethylene production (**appendix D**). However, in the same experiment an inhibitory AVG effect on lateral root growth could be restored by addition of ethylene indicating that ethylene is somehow implicated in lateral root growth. Promoting effects of low ethylene application on isolated root segments has previously been demonstrated (Smith and Robertson, 1971; Kays at al., 1974; Goeschl and Kays, 1975; Bucher and Pilet, 1982). Our experiment also suggested that ethylene sensitivity change with P availability since only deficient P plants were sensitive to AVG. Thus, in P deficient but not in adequate P common bean plants treated with AVG exhibited reduced main root length and increased lateral root density. The effect of inhibited internal ethylene production could almost be restored by exogenous applied ethylene indicating that ethylene reduces lateral root density during low P conditions by maintaining main root elongation with a reduced number of lateral roots (Fig. 4.3)(**appendix D**). The observed increase in lateral root spacing in a P deficient media will decrease the possibility of the roots overlapping their depletion zones. This will again increase the exploration of available P increasing the probability of finding a P rich patch, with the limited resources for growth during P deficiency. When a P rich patch is encountered the growth pattern will change and root density will increase (Drew and Saker, 1978; Snapp et al., 1995).



deficient P

adequate P

deficient P + AVG

adequate P + AVG

Fig. 4.3. Samples of five week old common bean roots (*Phaseolus vulgaris* L.) grown with adequate and deficient P nutrition and with and without AVG. Note the increased lateral root density in the deficient P root treated with AVG.

5. Application of low P fertilization and future perspectives

Increased environmental problems with eutrophication of the water resources has forced greenhouse growers to reduce the nutritional waste, e.g. by recirculation of the irrigation water. This may cause problems with the control of the nutrient content in the recirculated water, and especially with nutrients that are only required in low concentrations as e.g. P. However, our and others (Hansen and Lynch, 1998) research has shown that it is possible to grow plants with considerable reduced P concentrations using a technique with Al_2O_3 charged P amended in the soilless medium.

5.1 Root structure in soilless media amended with P-Al_2O_3

In field situations the depth of rooting and the ability to sustain an uninterrupted supply of water are important factors for drought avoidance for plants (Gregory, 1988). When the plants only have access to a limited soil volume, as it is the case in container grown crops, the space filling properties of the roots become a comparatively more important factor for drought avoidance (Fitter, 1986). We therefore compared the root systems of a drought tolerant and a drought intolerant miniature potted rose (**appendix A**). The results showed that lateral roots of the drought tolerant variety were longer. This will enable the plants to explore the medium for water more efficiently (Fitter, 1986), with a lower probability of overlap between the depletion zones of adjacent roots (Fitter et al., 1991).

When plants are grown with low but adequate concentrations of P, they will have an increased root elongation, thinner roots, and reduced lateral root density (**appendix C**). This allow exploration for available P with as little dry matter invested as possible. In turn this may increase drought avoidance capacity of the plant. Thus, in *Tagetes x hybrid* grown with low but adequate P it was possible to postpone wilting when irrigation was withheld. This phenomena could partly be explained by an increased water acquisition due to increased root length (**appendix D**) and improved root distribution in the growth media that allowed larger volumes of the container to be explored. This is often not achieved in traditional P fertilized plants (**appendix B**) and besides reduced drought avoidance it also can cause problems, when plants are transplanted e.g. into the garden.

By amending the growth media with P-charged Al_2O_3 it is possible to imitate the P characteristics of natural soils and obtain a low but constant supply of P to the roots, promoting the roots natural response such as increased elongation in search of patches high in P (Snapp et al., 1995), without the plant becoming P deficient. Simultaneously, as P is the limiting resource which is distributed uniformly in the growth media, there is no P gradient to guide the root. This will in turn create a more uniform root distribution allowing the root to more optimal utilize water resources (Furtura et al., 1977; Van de Werken 1989) as well as nutrients.

With the knowledge that containerized plants can grow well with considerable reduced P levels, we have a good starting position to reduce the increasing problem with eutrophication of streams and lakes.

5.2 The role of ethylene in the low P root growth response

We have shown that ethylene play a role in controlling lateral root density in deficient P plants (**appendix D**). Because of ethylene's profound influence on post harvest quality and longevity different chemical treatments has been used to control ethylene production (e.g. Serek, 1993) and microbial control of ethylene has been suggested (Elsgaard, 1996). By understanding the role of ethylene in the root zone we may be able to predict complications and advantages in future strategies of controlling this important hormone. Moreover, as ethylene clearly is involved in several root growth responses to nutrient stress, knowledge of this hormone and its interaction with other plant hormones will help us understanding root growth responses to environmental stimuli.

With the development of gene transfer systems for plants molecular biologist can genetically engineer plants with desirable traits. For example has transformation to delay fruit ripening using antisense ACC synthase and ACC oxidase been successful. A genetic approach with *Arabidopsis thaliana* has considerably increased our understanding of ethylene signal transduction (reviewed by Ecker, 1995). Using these mutants an ETR1 gene has been identified (Chang and Meyerowitz, 1995) and evidence that it encodes for an ethylene receptor has been presented (Schaller and Bleecker, 1995). Using the recent breakthroughs with ethylene receptor it is now possible to use this technology to create transgenic plants which are insensitive to ethylene. The *Arabidopsis thaliana* mutant (eir1-1) which is insensitive to ethylene in the root (Roman et al., 1995), has provided us with a very strong tool to study the role of ethylene in root development under different nutritional regimes in the future. Accordingly, I already have conducted preliminary experiments using these ethylene mutants in combination with AVG and different P levels in vitro.

Many plants secrete acid phosphatase in response to phosphate starvation. Characterization of *Arabidopsis thaliana* mutants that produce reduced amounts of acid phosphatase in response to phosphate starvation and related mutants should provide information about the phosphate concentration sensing mechanism (Trull et al., 1996), as well as lead to cloning the acid phosphatase gene. Both of these elements of the plant response to phosphate starvation will be useful tools for development of plants tolerant to phosphate-deficient soils.

5.3 Postharvest quality in plants grown with low but adequate P

Low P fertilization has often been suggested to control shoot compactness, however, with traditional P fertilization by irrigation this is problematic (Grantzau, 1996). By amending soilless growth media with P-charged Al_2O_3, a low but constant supply of P to the shoot can be achieved. Depending on the nature of the soilless media, we have shown that by using P-Al_2O_3 plants can grow well with more than 50 times reduced P compared to traditional P fertilization (Lynch et al., 1991, **appendix B,C**), without extending the production period in *Tagetes x hybrid* and *Impatient x hybrid.* However preliminary results with potted roses have shown that the technique needs to be fine tuned depending on the cultivar and growth media (Borch, unpublished results). The low but adequate P fertilization reduced the leaf area, but in *Tagetes x hybrid* this did not reduce the overall appearance, and in *Impatiens x hybrid* the internodial distance was reduced giving the plants a more compact appearance (**appendix B**). The reduced leaf area lowered transpiration losses considerably allowing the plant to avoid drought stress for extended time periods (**appendix C**). This also have the advantage that irrigation frequency can be reduced and thereby saving resources. Moreover, it will facilitate planning of irrigation since the time window where the plants need to be watered will be enlarged.

Besides improved drought avoidance *Tagetes x hybrid* that were grown with reduced P fertilization had more flowers during postharvest. This may be explained by improved photosynthesis per leaf area because of reduced canopy self shading (**appendix C**) and increased chlorophyll content per unit leaf (Hecht-Buchholts, 1967), however, this was not investigated in this work. The possibility of improving postharvest quality and longevity by improved photosynthesis and reduced transpiration need further attention. Moreover, the increased compactness of low P grown plants have promising perspectives in the effort to reduce use of growth retarding chemicals, which are of environmental concern.

References

Abeles, F.B. and Wydosky, S.G. (1987) Inhibition of ethylene synthesis and action: a comparison of their activities in a lettuce root growth model system. J. Am. Soc. Hortic. Sci. 112:133.

Abeles, F.B, Morgan, P.W and Saltveit, M.E. (1992) Ethylene in plant biology. 2nd ed., Academic Press, ISBN 0-12-041451-1.

Almaras, R.R., Kraft, J.M. and Miller, P.E. (1988) Effects of soil compaction and incorporated crop residues on root health. Ann. Rev. Phytopathol. 26:219-243.

Armstrong, W. (1979) Aeration in higher plants. Adv. Bot. Res. 7:225-331.

Anghinoni, I. and Barber, S.A. (1980) Phosphorus application rate and distribution in the soil and phosphorus uptake by corn. Soil Sci. Soc. Am. J. 44:1041-1044.

Aung, L.H. (1971) Root-shoot relationships. In: The Plant Root and Its Environment, Ed: E.W. Carson. University Press of Virginia, Charlottesville, VA.

Barber, S.A. (1995) Soil nutrient bioavailability: a mechanistic approach. Wiley, NY. ISBN: 0471587478 p 202-230.

Barley, K.P. and Graecen, E.L. (1967) Mechanical resistance as a soil factor influencing the growth of roots and underground shoots. Adv. Agron. 19:1-43

Baldwin, J.P. (1975) A quantitative analysis of the factors affecting plant nutrition uptake from some soils. J. Soil Sci. 26:195-206.

Barlow, P.W. (1987) The cellular organization of roots and its response to the physical environment. In: Root development and function, Ed: Gregory, P.J., Lake, J.V. and Rose, D.A. Cambridge University Press, Cambridge, pp. 1.

Bates, T.R., and Lynch, J. (1996) Stimulation of root hair elongation in *Arabidopsis thaliana* by low phosphorus availability. Plant Cell, and Environment 19:529-538.

Bergmann, W. (ed.). (1992) Nutritional Disorders of Plants: visual and analytical diagnosis. Gustav Fisher Verlag, New York.

Berntson, G.M. (1994) Modeling root architecture: are there tradeoffs between efficiency and potential of resource acquisition? New Phytol. 127:483-494.

Bhat, K.K.S. and Nye P.H. (1974) Diffusion of Phosphate to Plant Roots in Soil III. Depletion around onion roots without root hairs. Plant Soil 41:383-394.

Borch, K. (1994) Potteroser som Mærkevare. Gartnertidende 109:376-377.

Botella, J.R., Arteca, R.N. and Frangos, J.A. (1995) A mechanical strain-induced ACC synthase gene. Proc. Natl. Acad. Sci. USA, 92:1595-1598.

Bradford, K.J. and Yang, S.F. (1980) Xylem transport of 1-aminocyclopropane-1-carboxylic acid, an ethylene precursor, in waterlogged plants. Plant Physiol. 65:322-326.

Bucher, D. and Pilet, P.-E. (1982) Ethylene effects on growing and gravireacting maize root segments. Physiol. Plant. 55:1.

Campbell, R. and Drew, M.C. (1983) Electronic microscopy of gas spaces (aerenchyma) formation in adventitious roots of *Zea mays* L. subjected to oxygen shortage. Planta 157:350-357.

Chang, C. and Meyerowitz, E.M. (1995) The ethylene hormone response in *Arabidopsis*: A eukaryotic two-component signaling system. Proc. Natl. Acad. Sci. USA 92: 4129-4133.

Clausnitzer, V and Hopmans, J.W. (1994) Simultaneously modeling of transient three-dimensional root growth and soil water flow. Plant Soil 164:299-314.

Crosset, R.N. and Campbell, D.J. (1975) The effects of ethylene in the root environment upon the development of barley. Plant Soil 42:453.

Drew, M.C. (1990) Root function development, growth and mineral nutrition. In: The Rhizosphere, Eds.: Lynch, J.M., John Wiley & sons Ltd. W. Sussex, England. pp. 35.

Drew, M.C., Jackson, M.B. and Giffard, S. (1979) Ethylene-promoted adventitious rooting and development of cortical air spaces (Aerenchyma) in roots may be adaptive responses to flooding in *Zea mays* L. Planta 147:83-88.

Drew, M.C., He, C.J. and Morgan, P.W. (1989). Decreased ethylene biosynthesis, and induction of aerenchyma, by nitrogen- or phosphate-starvation in adventitious roots of Zea mays L. Plant Physiol. 91:266-271.

Drew, M.C. and Saker, L.R. (1978) Nutrient supply and the growth of the seminal root system in barley. III. Compensatory increase in growth of lateral toots, and in rates of phosphate uptake, in response to a localized supply of phosphate. Journal of Experimental Botany. 29:435-451.

Ecker, J.R. (1995) The ethylene signal transduction pathway in plants. Science 268: 667-675.

Eissenstat, D.M. (1992) Costs and benefits of constructing roots of small diameter. Journal of Plant Nutrition. 15:763-782.

Elsgaard, L (1996) Ethylene degradation in peat-soil for horticultural practice. Abstract of the NATO Advanced Research Workshop on Biology and Biotechnology of the Plant Hormone Ethylene. Chania, Greece p.97.

Eshel, A. Nielsen, K. and Lynch, J. (1995) Response of bean root systems to low level of P. In: 14th Long Ashton International Symposium, Bristol.

Evans, D.E., Dodds, J.H., Lloyd, P.C., ap Gwyn, I. and Hall, M.A. (1982) A study of subcellularlocalization of an ethylene binding site in developing cotylidons of *Phaseolus vulgaris* L. by high resolution autoradiographi. Planta. 154:48-52.

Finlayson, S.A., Liu, J. and Ried, D.M. (1996) Localization of ethylene biosynthesis in roots of sunflower (*Helianthus annuus*) seedlings. Physiol. plant. 96:36-42.

Fitter, A.H. (1986) The topology and geometry of plant root systems: Influence of watering rate on root system topology in *Trifolium pratense.* Ann. Bot. 58:91-101.

Fitter, A.H. (1994) Architecture and biomass allocation as components of the plastic response of root systems to soil heterogeneity. In: Exploitation of environmental heterogeneity, Academic Press. pp. 305.

Fitter, A. (1996) Characteristics and functions of root systems. In: Plant roots - The hidden half, Eds.: Waisel, Y., Eshel, A., and Kafkafi, U. Marcel Dekker, Inc., NY, USA. ISBN: 0824796853. pp. 1.

Fitter, A.H., Stickland, T.R., Harvey, M.L. and Wilson, G.W. (1991) Architectural analysis of plant root systems. I. Architectural correlates of exploitation efficiency. New Phytol. 118:375-382.

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.

Furuta, T., T. Mock, and R. Coleman (1977) Estimating the water need for container-grown nursery stock. Amer. Nurseryman 145:68.

Gahoonia, S.T., Care, D. Nielsen, N.E. (1997) Root hairs and phosphorus acquisition of wheat and barley cultivars. Plant and Soil. 191:181-188.

Glass, A.D.M. (1989) Plant Nutrition: an introduction to current concepts. Jones and Bartlett publishers, Boston, MA.

Goeschl, J.D. and Kays, S.J. (1975) Concentration dependencies of some effect of ethylene on etiolated pea, peanut, bean and cotton seedlings. Plant. Physiol. 55:670.

Goren, R. and Sisler, E. (1986) Ethylene-binding characteristics in *Phaseolus*, citrus and *Ligustrum* plants. Plant Growth Reg. 4:43-54.

Grantzau, E. (1996) Poinsetten mögen phosphat. De. Ga. 32:1752-1754.

Gregory, P.J. (1988) Root growth of chickpea, fababean, lentil, and pea and effects of water and salt stress. In: World Crops: Cool season Food Legumes., Ed: Summerfield, R.J., Kluwer Academic Publishers, London, UK. pp. 857.

Hamblin, A.P. (1985) The influence of soil structure on water movement, crop root growth, and water uptake. Adv. Agron. 38:95-158.

Hansen, C.W. and Lynch, J. (1998) Response to phosphorus availability during vegetative and reproductive growth of *Chrysanthemum*. J Amer. Soc. Sci. (in press).

He, C-J., Morgan, P.W. and Drew, M.C. (1992). Enhanced sensitivity to ethylene in nitrogen- or phosphate-starved roots of *Zea mays* L. during aerenchyma formation. Plant Physiol. 98:137-142.

He, C-J., Drew, M.C. and Morgan, P.W. (1994) Induction of enzymes associated with lysigenous aerenchyma formation in roots of *Zea mays* during hypoxia or nitrogen starvation. Plant Physiol. 105:861-865.

He, C.-J., Morgan, P.W. and Drew, M.C. (1996) Transduction of an ethylene signal is required for cell death and lysis in the root cortex of maize during aerenchyma formation induced by hypoxia. Plant Physiol. 112:463-472.

Hecht-Buchholtz, C. (1967) Über die Dunkelfärbung des Blattgrüns bei Phosphormangel. Z. Pflantzenernaehr. Bodenkd. 118:12-22.

Hew, C.S., Gouk, S.S., Lin, W.S. and Yong, J.W.H. (1995) Ethylene production by orchid roots. Lindeyana 10:43-48.

Huang, B. Johnson, J.W., Box, J.E., and NeSmith, D.S. (1997) Root characteristics and hormone activity of wheat in response to hypoxia and ethylene. Crop Sci. 37:812-818.

Itai, C and Birnbaum, H. (1996) Synthesis of plant growth regulators by roots. In: The hidden half, Eds: Waisel, Y., Eshel, A. and Kafkafi U. Marcel Dekker, New York. ISBN 0824796853 pp. 273.

Jackson, M.B. (1991) Ethylene in root growth and development. In: The Plant Hormone Ethylene, Eds.: Matto and Suttle. CRC Press. ISBN 0-8493-4566-9.

Jackson, M.B (1993) Are plant Hormones involved in root to shoot communication?: Advances in Botanical Research, Ed: J.A. Callow. Academic Press, London. 19:103-187.

Jackson, M.B. and Campbell, D.J. (1979) Effect of benzyadenine and giberellic acid on the responses of tomato plants to anaerobic root environments and to ethylene. New Phytol. 83:331.

Jackson, M.B., Drew, M.C. and Giffard, S.C. (1981) Effects of applying ethylene to the root system of *Zea mays* on growth and nutrient concentration in relation to flooding tolerance. Physiol. Plant. 52:23.

Justin, S.H.F. and Armstrong, W. (1991) The anatomical characteristics of roots and plant responses to soil flooding. New Phytol. 106:465-495.

Kawase, M. (1981a) Anatomical and morphological adaptations of plants to waterlogging, HortScience 16:30-34.

Kawase, M. (1981b) Effect of ethylene on aerenchyma development. Am. J. Bot. 68:651-658.

Kays, S.J., Nicklow, C.W. and Simsons, D.H. (1974) Ethylene in relation to the response of roots to physical impedance. Plant and Soil 40:565.

Koide, R.T. (1990) Nutrient supply, nutrient demand and plant response to mycorrhizal infection. New Phytol. 117:365-386.

Konings, H. and Jackson, M.B. (1979) A relationship between rates of ethylene production by roots and the promoting and inhibiting effects of exogenous ethylene and water on root elongation. Z. Planzenphysiol. 92:385.

Lee, J.S. Chang, W-K. and Evans, M.L. (1979) Effects of ethylene on the kinetics of curvature and auxin redistribution in gravistimulated roots of *Zea mays*. Plant Physiol. 94:1770-1775.

Lewis, D.G. and Quirk J.P. (1967a). Phosphate diffusion in soil and uptake by plants. I. Self-diffusion. Plant and soil 26:99-118.

Lewis, D.G. and Quirk J.P. (1967b) Phosphate diffusion in soil and uptake by plants. III. P^{31}-movement and uptake by plants as indicated by P^{32}-autoradiography. Plant and Soil 27:445-453.

Ludlow, M.M. (1989) Strategies of response to water stress. In: Structural and functional responses to environmental stresses, Eds.: Kreeb, K.H., Richter, H., and Hinckley, T.M. SPB Academic Publishing, The Hague, NL. pp. 269.

Lynch, J., A. Läuchli, and E. Epstein. (1991) Vegetative growth of the common bean in response to P-nutrition. Crop Science 31:380-387.

Lynch, J.P. and Beebe, S.E. (1995) Adaptation of beans (*Phaseolus vulgaris* L.) to low phosphorus availability. HortScience. 23:1165-1171.

Lynch, J. and Nielsen, K.L. (1996) Simulation of root system architecture. In: Plant roots: The hidden half, Eds.: Waisel, Y., Eshel, A. and Kafkafi U. Marcel Dekker, New York. 0824796853 2nd edition, pp. 247.

Lynch, J. and Brown, K. (199X) Ethylene and nutritional stress. Physiol. Plant (in press).

Marschner, H. (1995) Mineral Nutrition of Higher Plants, second ed. Academic Press, London. ISBN 0124735436.

McAuliffe, C. (1966) Solubility in water of paraffin, cycloparaffin, olifin, acetylene cycloolefin, and aromatic hydrocarbons. J Physic. Chem. 70:1267-1275.

Morgan, P.W. and Drew, M.C (1997) Ethylene and plant responses to stress. Physiol. Plant 100:620-630.

Neljubov, D.N. (1901) Uber die Horizontale Nutatiuon der Stengel von *Pisum sativum* und einiger anderen Pflanzen. Beihefte zum Botanischen Zentralblatt 10:128-139.

Newman, E.I. and Andrews, R.E. (1973) Uptake of phosphorus and potassium in relation to root growth and root density. Plant and Soil 38:49-69.

Nielsen, K.L., Lynch, J. and Weiss, H.N. (1997) Fractal geometry of bean root systems: correlation's between spatial and fractal dimension. Am. J. Bot. 84:26-33.

Nobel, P.S. (1991) Physicochemical and environmental plant physiology. Academic Press, San Diego, CA, pp. 257.

Rending, V.V. and Tylor, M.M. (1989) Principles of soil-plant relationships. McGraw-Hill, New York.

Russell, R.W. (1977) Plant root systems. Their function and interaction with the soil. 193-218. McGrawe-Hill, London.

Roman, G., Lubarsky, B., Kieber, J.J., Rothenberg, M. and Ecker, J.R. (1995) Genetic analysis of ethylene signal transduction in *Arabidopsis thaliana*: five novel mutant loci integrated into a stress response pathway. Genetics 139:1393-1409.

Sarquis, J.I., Morgan, P.W. and Jordan, W.R. (1992) Metabolism of 1-aminocyclopropane-1-carboxylic acid in etiolated maize seedlings grown under mechanical impedance. Plant Physiol. 98:1342-1348.

Sawamura, M and Miyazaki, T. (1989) Effects of exogenous ethylene on ethylene production in discs of mum fruits. J. Hort. Sci. 64:633-638.

Schaller, G.E. and Bleecker, A.B. (1995) Ethylene-binding sites generated in yeast expressing the *Arabidopsis* ETR1 gene. Science 270: 1809-1811.

Schierle, J., Rohwer, F. and Bopp, M. (1989) Distribution of ethylene synthesis along the etiolated pea shoot and its regulation by ethylene. J. Plant Physiol. 134:331-337.

Serek, M. (1990) Effects of pre-harvest fertilization on the flower longevity of potted *Campanula carpatica* 'Karl Foerster'. Scientia Hortic. 44:119-126.

Serek, M. (1993) Ethephon and silver thiosulfate affect postharvest characteristics of *Rosa hybrida* 'Victory Parade'. HortScience 28(3):199-200.

Sisler, E.C. (1982) Ethylene-binding properties of a Triton X-100 extract of mung bean sprouts. J. Plant Growth Regul. 1:211-218.

Smith, K.A. and Robertson, P.D. (1971) Effect of ethylene on root extension of cereals. Nature 243:148.

Snapp, S. Koide, R. and Lynch, J. (1995) Exploitation of localized phosphorus patches by common bean roots. Plant and Soil. 177:211-218.

Stryer, L. Biochemistry (Third Edition), W.H. Freeman and Co., New York, 1988.

Subbarao, G.V., Johansen, C., Slinkard, A.E., Nageswara Rao, Saxena, N.P. and Chauhan, Y.S. (1995) Strategies for improving drought resistance in grain legumes. Crit. Rev. Plant Sci. 14:469-523.

Tanimoto, M., Roberts, K. and Doland, L. (1995) Ethylene as a positive regulator of root hair development in *Arabidopsis thaliana.* The Plant Journal 8:943-948.

Torrey, J.G. (1976) Root hormones and plant growth. Ann. Rev. Plant Physiol. 27:435-459.

Trull, M.C., Guiltinan, M.J. Lynch, J. and Deikman, J. (1996) The response of wild type and ABA mutant *Arabidopsis thaliana* plants to phosphorus starvation. Plant Cell and Environ. in press.

Van de Werken, H. (1989) A new way with containers. Amer. Nurseryman 170:43-51.

Woltering, E.J. Haren, F. and Boerrichter, H.A.M. (1988) Use of a laser driven photo acoustic detection system for measurements of ethylene production in *Cymbidium* flowers. Plant Physiol. 88:506.

Yang, S.F and Hoffman, N.E. (1984) Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Physiol. 35:155-189.

Zacarias, L. and Reid, M.S. (1992) Inhibition of ethylene action prevents root penetration through compressed media in tomato (*Lycopersicon esculentum*) seedlings. Physiol. Plant 86:301-307.

Zimmerman, P.W. and Hitchcock, A.E. (1933) Initiation and stimulation of adventitious roots caused by unsaturated gases. Contri. Boyce Thompson Inst. Plant Res. 5:351.

Drought Avoidance in Potted Miniature Roses and Significance of Root Structure

Kristian Borch
Department of Ornamentals, Danish Institute of Agricultural Sciences,
Kirstinebjergvej 10, Aarslev DK-5792.

David J. Beattie
Department of Horticulture, The Pennsylvania State University, University Park,
PA -16802.

Abbreviations

LWP: leaf water potential; RWC_{leaf}: Relative water content in the leaf; RWC_{pot}: relative water content in the pot; IE: instant transpiration.

Acknowledgments

We would like to thank Poulsen Roses Aps, Ove Nielsen A/S, Denmark, for providing us with rose plants and cuttings . This work was partly financed by The Danish Academy of Science.

Drought Avoidance in Potted Miniature Roses and Significance of Root Structure

Additional index words. root structure, *Rosa hybrida*, drought avoidance.

Abstract Postharvest water relations of two commercially important potted miniature rose plants, Charming Parade® (Charming), and Bianca Parade® (Bianca) were examined during drought stress. Plants were propagated from cuttings and grown in either sphagnum moss peat or in the clay granular 'SERAMIS'. At anthesis, transpiration, root:shoot ratio, leaf area, stomate number, and root length and structure were determinate. Drought tolerance was determined gravimetrically by slowly drying the growth medium. 'Bianca' transpired more water than 'Charming' on a whole plant basis although their leaf areas were not significantly different. However, the structure and morphology of 'Bianca' roots probably offer less resistance to water uptake compared to 'Charming' roots, due to increased lateral root density and the presence of root hairs. 'Charming' continued to transpire and maintain leaf water potential at a lower soil water content than 'Bianca'. Because the individual root segments are longer in 'Charming' they probably explore the growing medium more throroghly than do 'Bianca' roots. The volume of medium explored may have been a factor contributing to avoidance of drought stress in 'Charming' for extended periods.

Miniature roses are an important pot crop in Denmark. Each year, more than 50 million plants are produced, primarily in 10 cm pots, and sold throughout Europe. In addition to selecting attractive post harvest characteristics, it is important that cultivars are able to withstand adverse growing conditions. For instance, the cultivars 'Charming Parade®' (Charming) and 'Bianca Parade®' (Bianca) have excellent keeping qualities (Andersen et al., 1994). However, growers report that 'Bianca', when grown under the same conditions as numerous other cultivars like 'Charming', frequently shows drought stress symptoms which may reduce plant quality.

Many factors influence how plants take up, translocate, and transpire water. Root architecture and distribution, especially in container-grown plants with restricted water reserves, will influence water uptake (Borch et al., in preparation). Water movement within the vascular system may be limited by differences in xylem flow or root uptake. Transpirational losses may be due to anatomical or physiological causes. Leaf morphology characteristics such as pubescence, cuticle thickness, stomate number, the plant's inability to regulate stomatal opening or closing may also influence water loss. For instance, some standard roses do not completely close their stomates during the dark period (Assaf and Zieslin, 1996).

In this research we present evidence that structural differences in the roots may explain why 'Charming' appears to use irrigation water more efficiently than 'Bianca'.

Materials and methods

Plants of *Rosa hybrida* L. Charming Parade® Poulhappy^{N} and Bianca Parade®, Paulbian^{N} were obtained from a commercial rose grower (Ove Nielsen A/S, Denmark).

Gravimetrical recordings Plants at anthesis (2-5 fully open flowers) in 10 cm pots with four cuttings in each pot were delivered from the grower and placed randomly in a growth chamber. The temperature was maintained at 20±2 °C, with a photosynthetic photon flux density (PPFD) of 8 μmol m^{-2} s^{-1}. The photoperiod was 12h (06:00 to 18:00) and the relative humidity (RH) was 55-65%. Instantaneous transpiration (IE) was determined in a climate chamber were the PPFD was 500 μmol m^{-2} s^{-1}, 25 °C and 40 % relative humidity. IE was measured using an infra-red gas analyzer (IRGA, ADC-LCA-3, Analytical Development Co. Ltd., UK) connected to a leaf cuvette (PLC-B, Analytical Development Co. Ltd., UK) in an open system set up. Number of stomates was determined by applying nail polish (Hsiao and Fischer, 1975; Wang and Clark, 1993), to the surface of the terminal leaflet of the uppermost fully expanded 5-leaflet leaf. After 30 min. the hardened nail polish was removed and transferred to a microscope where stomatal number was estimated by counting number of stomates inside five 0.2 by 0.2 mm squares per leaf.

The surface of the growing medium of six plants of each cultivar were covered with plastic foil to minimize evaporation. After the plants were thoroughly watered they were then allowed to desiccate and the water loss for each plant was recorded by daily weighing at 08:00 and again at 16:00 hours until the plants noticably wilted. After eight days the plants were harvested to determine the leaf area. This procedure was repeated after 10 days with six new plants of each cultivar.

Drought stress and root recordings Cuttings of 'Charming' and 'Bianca' obtained from the grower were rooted in sphagnum moss-peat (Pindstrup 3, Pindstrup Mosebrug a/s, Ryomgaard, Denmark). After ca. two weeks when the roots were 1 cm long the cuttings were transplanted into calcined clay Seramis (Effem, Mogendorf, Germany) in 9 cm pots.

Plants were subirrigated with a half-strength Epstein nutrient solution (Epstein, 1972) in a greenhouse environment with supplemented light (70 W/m^{2}). Minimum greenhouse temperature was 21 °C and vented at 24 °C. Relative humidity (RH) was maintained between 30 and 40%. The plants were pruned twice at 5 cm above the medium surface. At anthesis all plants were moved to a growth chamber and placed randomly on two sub irrigation tables. IE was measured using an infra-red gas analyzer (IRGA, ADC-LCA-3, Analytical Development Co. Ltd., UK) connected to an glass chamber (5800 ml) containing only the sealed shoot portion of the plant in a open system set up. Flow through the chamber was 0.37 L min.^{-1}. Climatic parameters were the same as described earlier.

Ten plants of each cultivar were then water stressed by withholding irrigation until the mid-day stomatal conductance dropped below 0.1 cm/s (from a normal 0.67 ±0.02 as determined by a Delta-T MK3 porometer, Cambridge, England) and visible wilting had occurred. At this time leaf water potential (LWP) was also recorded with a pressure bomb (SKMP 1400, Skye instruments, UK). At wilting the youngest fully matured 5-leaflet leaf was used to determine RWC_{leaf}. Using, $RWC_{leaf} = [(w_w-w_d)/(w_t-w_d)]$ x100 (Hsiao, 1990), where w_t is the turgid leaf weight, w_w the leaf weight at the wilting point, and w_d is leaf dry weight. The excised leaf was weighed, then submerged in distilled water at 22°C and placed under a light source. After 24 hours the leaf was blotted dry, and reweighed to determine turgid weight, w_t. Finally, the leaves were oven dried for 48 hours at 60 °C to determine dry weight, w_d. The remainder of the leaves were excised and leaf area determined. At the wilting point, the growing medium was weighed, and then dried to calculate RWC_{pot} ($RWC_{pot} = [(w_w-w_d)/(w_t-w_d)]$ x100, where w_t is the pot weight at field capacity, w_w is pot weight at wilting and w_d is weight of the pot media and pot after 48 hours of oven drying at 70 °C). Root topology was evaluated by removing the plant from the pot, lightly shaking the clay from the roots, then washing the roots in tap water (Starkey and Pedersen, 1997). This procedure resulted in the recovery of the entire root system with minimal root damage. After cleaning, the roots were stained in a 0.2% neutral red dye, then placed in tap water in a shallow water bath on top of a flat bed scanner. Digital image analyis if root length recordings where performed with a Delta-T SCAN package, Delta Devices LTD (Cambridge, England). Lateral root density was estimated from the scanned image of the root.

Data were analyzed using the PC-statistical package JMP® (SAS Institute 1989-91) evaluating the significance between treatment means with ANOVA (randomized block design). Values presented are means of ten plants ± standard error of mean (SE), except the gravimetric transpiration recordings, where values are mean of twelve plants and two replicates.

Results

Transpiration

Leaf area did not differ significantly between 'Charming' and 'Bianca' in either experiment (Table 1, 2). When pot weight was recorded twice a day until noticable wilting over several days, 'Bianca' initially lost more water to transpiration compared to 'Charming' (Fig. 1). However, the difference in IE rate could not be detected between cultivars (Table 1, 2). As expected, stomates where found only on the lower leaf in both cultivars (e.g. Rajapakse et al., 1988) but no difference in stomate number was found between 'Charming' and 'Bianca' (Table 1).

At pot capacity, LWP and stomatal conductance of 'Bianca' and 'Charming' plants were identical (data not shown). However, as the growth medium desiccated, stomatal conductance dropped below 0.1 cm/s in 'Bianca' at a higher RWC_{pot} compared to 'Charming' when LWP was approximately equal (Fig. 2).

Root measurements

'Charming' roots were significantly longer compared to 'Bianca', but lateral root density was significantly higher in 'Bianca' (Table 2, Fig. 3). 'Bianca' roots had numerous root hairs while 'Charming' only had very few (not shown). The root:shoot ratio was greater in 'Charming' compared to 'Bianca' (Table 1).

Discussion

Over time 'Bianca' transpired more water compared to 'Charming' when recorded gravimetrically over several hours (Fig. 1) as also reported by growers. The increased transpiration could not be explained by differences in leaf area between the two cultivars (Table 1) However, no difference was found when IE was recorded either on a whole plant basis or on a single leaf. An explanation for this may be that the short duration (15 minutes) for the IE recordings is insufficient to detect any difference. Another reason could be that stoma fail to close fully durring darknes as reported by Assaf and Zieslin (1996), Which inded seem to be the case (Beattie and Borch, 1998). It was expected that increased stomatal number could explain the increased transpiration as suggested by Tan and Buttery (1995), but no difference in number of stomates per unit leaf area where found between the two cultivars.

Turning to the roots zone, root architecture, proliferation, and mass affect the rate at which root systems remove moisture from soils (Faber et al., 1991). The water flow in soil is often the largest resistance for the soil-plant- atmosphere continuum (Nobel, 1991), thus, a short diffusion distance will reduce the resistance. As 'Bianca' roots have a high lateral root density compared to 'Charming' the water diffusion distance will be lower allowing a grater water uptake rate and thus a higher transpiration rate during well watered conditions with high soil water content (fist 12 hours in Fig. 1). The tendency of the water transpiration rate in 'Bianca' to approach that of 'Charming' after approximately 30 hours represented by the slope of the trend lines in figure 1 may be explained by reduction in water content in the pot due to transpiratory depletion. The diffusion distance then become larger and increased lateral root density is expected to become irrelevant for the water uptake resistance because the depletion zones overlap. In addition, roots of 'Bianca' had numerous root hairs, while 'Charming' only had very few. This will increase the root surface area in 'Bianca' roots in turn reducing the resistance to water flow across the root surface.

The decreased water diffusion distance in the soil, together with the increased root surface in 'Bianca' can to some extend explain the increased transpiration compared to 'Charming'.

'Bianca' wilted at the same LWP as 'Charming', but at a higher RWC_{pot} (Fig. 2) indicating that 'Bianca' root system was more dehydrated than 'Charming' root system at the same soil water potential. We did not find indications that 'Charming' was more drought resistant than 'Bianca' due to osmotic adjustment (Turner et al., 1987) since they have equal tissue water content at the same water potentials (Sinclair and Ludlow, 1986). The root system of 'Charming' is longer than the root system of 'Bianca' but 'Bianca' has a higher lateral root density (Table 1, Fig. 3). This will give 'Charming' roots a better 'space filling' capacity and better be able to explore the medium for e.g. water (Fitter, 1986), with a lower probability of overlap between the depletion zones of adjacent roots (Fitter et al., 1991). With the root structure dominant in 'Bianca' the possibility of leaving areas of the pot unexplored is increased, which would allow proportionately more water to be lost by evaporation and reduce efficiency of water use (Furuta et al. 1977; Van de Werken 1989). The presence of root hairs on 'Bianca' did not seam to be of importance for water acquisition, although root hairs have been suggested to compensate for loss of soil contact when the soils dry out and soil matrix shrink (Mackay and Barber, 1987).

In summary, there are at least two major root structural reasons why the drought problems of 'Bianca' reported by growers are greater than those of 'Charming'. Here we suggest that 'Bianca' transpires more water over time than 'Charming' partly because the water diffusion distance in the root zone is reduced due to increased lateral root density, and partly because root surface area is increased due to root hairs. Moreover, 'Bianca' wilts at a higher water content in the growing medium probably because root structure of 'Bianca' gives it a less efficient distribution in the growing medium and consequently a lower water acquisition ability when soil water is limited.

These result show that when selecting for water use efficient cultivars it is important to consider root structure. However, effects from the shoot on the transpiration can not be ignored, and we are now analyzing results indicating differences in night water consumptionthe aperture durring darknes between the two cultivars.

Literature Cited

Andersen, L., M. Williams and L. Høyer. 1994. Charming Parade® a new miniature potted rose with a very good keeping quality. Danish institute of Plant and Soil Science, Dept. of Horticulture, 2 pp.

Assaf, G and N. Zieslin. 1996. Night water consumption by rose plants. J. Hort. Sci. 71:673-678.

Beattie, D.J. and Borch, K. (1998) Failure to close stomata durring darkness can explain excess use of irrigation water in *Rosa hybrida* 'Bianca Parade'. (in preparation)

Faber, B.A., D.N. Muuns, K. Schackel, and R.J. Zasoski. 1991. A method of measuring hyphal nutrient and water uptake in mycorrhizal plants. Can. J. Bot. 69:87-94.

Fitter, A.H. 1986. The topology and geometry of plant root systems: Influence of watering rate on root system topology in *Trifolium pratense* Ann. Bot. 58:91-101.

Fitter, A.H., T.R. Stickland, M.L. Harvey, and G.W. Wilson. 1991. Architectural analysis of plant root systems 1. Architectural correlates of exploitation efficiency. New Phytol. 118:383-389.

Furuta, T., T. Mock, and R. Coleman. 1977. Estimating the water need for container-grown nursery stock. Amer. Nurseryman 145:68.

Hsiao T.C. and R.A. Fischer. 1976. 2. Microscopic Measurements. Bulletin of the Washington Agricultural Experimental Station pp. 807-838.

Hsiao T.C. 1990. Measurements of plant water status. In: Irrigation of Agricultural Crops. Eds: B.A. Steward, D.R. Nielsen. Agronomy No. 30, Amer. Soc. Agron., Madison, pp 243-279.

Mackay, A.D. and S.A. Barber. 1987. Effect of cyclic wetting and drying of a soil on root hair growth of maize roots. Plant Soil 104:291-293.

Nobel, P.S. 1991. Physicochemical and environmental plant physiology. Academic Press, San Diego, CA, pp.257.

Rajapakse, N.C., J.W. Kelly and D.Wm. Reed. 1988. Transpiration and Water Use of Potted Floricultural Plants Under Low-light Conditions. J. Amer. Soc. Hort. Sci. 113:910-914.

Sinclair, T.R and M.M. Ludlow. 1986. Influence of soil water supply on the plant water balance of four tropical grain legumes. Austral. J. Plant Physiol. 13:329-341.

Starkey, K.R. and A.R. Pedersen 1997. Increased levels of calcium in the nutrient solution improves the postharvest life of pot roses. J. Amer. Soc. Hort. Sci. (in press).

Tan, C.S. and B.R. Buttery. 1995. Determination of the Water Use of two Pairs of Soybean Isolines Differing in Stomatal Frequency using a Heat Balance Stem Flow Gauge. Can. J. Plant Sci. 75:99-103.

Turner, N.C., W.R. Stern and P. Evans. 1987. Water relations and osmotic adjustment of leaves and roots of lupins in response to water deficits. Crop Sci. 27:977-983.

van de Werken, H. 1989. A new way with containers. Amer. Nurseryman 170:43-51.

Wang, H. and J.M. Clarke. 1993. Relationship of Excised-leaf Water Loss and Stomatal Frequency in Wheat. Can. J. Plant Sci. 73:93-99

Table 1. Morphological and physiological parameters in 'Charming' and 'Bianca' potted miniature roses used for gravimetrical recordings. Values are mean of twelve plants.

		Cultivars		
Parameter	units	'Charming'	'Bianca'	p^z
Transpiration	$\mu mol\ m^{-2}s^{-1}$	2.1 $\pm0.07^y$	2.1 ±0.07	ns
Leaf area	mm^2	964 ±28	951 ±28	ns
Stomate number	mm^{-2}	54.9 ±2.7	59.7 ±2.7	ns

[z] Student paired t-test

[y] Standard error of mean.

Table 2. Morphological and physiological parameters in 'Charming' and 'Bianca' potted miniature roses used for root structure recordings. Values are mean of eight plants.

		Cultivars		
Parameter	units	'Charming'	'Bianca'	p^z
Transpiration	$\mu mol\ m^{-2}s^{-1}$	1.9 $\pm0.2^y$	2.1 ±0.2	ns
RWC_{leaf}:	%	75.3 ±2.4	71.2 ±2.3	ns
Leaf DW	g	0.74 ±0.08	0.90 ±0.08	ns
Root DW	g	0.236 ±0.02	0.171 ±0.02	ns
Root:shoot ratio		0.33 ±0.02	0.19 ±0.01	.05
Total root length	mm	3825 ±284	2835 ±284	.05
lateral root density	cm^{-1}	1.33 0.08	1.89 0.08	0.01

[z] Student paired t-test

[y] Standard error of mean.

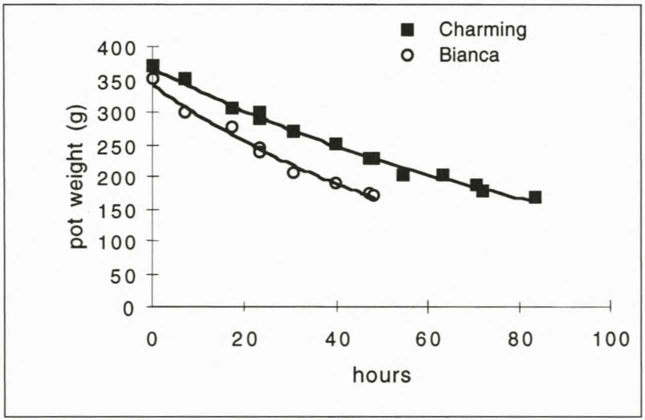


Fig. 1 . Difference in reduction in pot weight over time due to transpiration at anthesis in 'Bianca' and 'Charming' until wilting occurred ($p>0.01$, F test for repeated measures, a=.5, N=12, standard error of mean = 4.5).

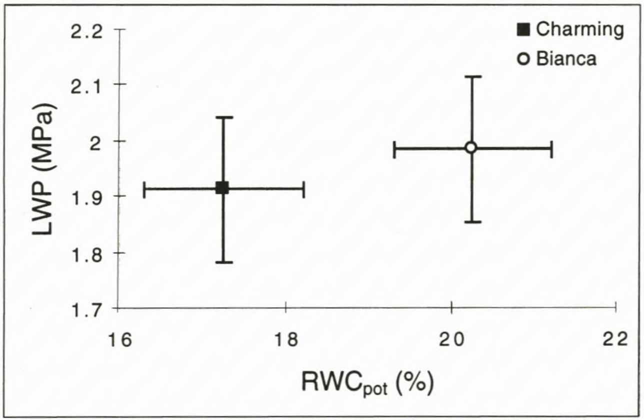


Fig. 2 . Relative water content in the pot (RWC_{pot}) as a function of leaf water potential (LWP) in the miniature potted rose varieties 'Bianca' and 'Charming' recorded at wilting. There is a significant difference in RWC_{pot} between cultivars ($p<0.01$, student t-test, a=0.5, N=8.). Bars indicate standard error of mean.

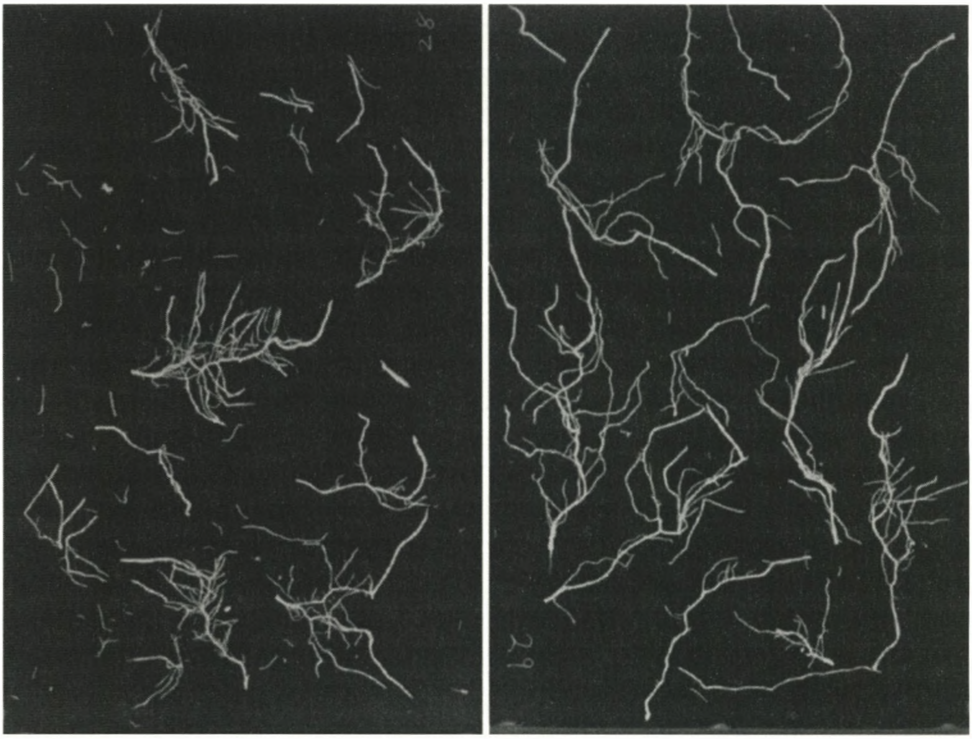


Fig. 3. 'Bianca' roots (left) compared to 'Charming' roots (right). Note how 'Bianca' roots have a high lateral root density while 'Charming' roots have the longest lateral roots.

Improvement of Bedding Plant Quality and Stress Resistance with Low Phosphorus

Kristian Borch[1], Kathleen M. Brown[2], and Jonathan P. Lynch[2]

[1]Department of Ornamentals, Danish Institute of Agricultural Sciences, Årslev, 5792, Denmark

[2]Department of Horticulture, The Pennsylvania State University, University Park, PA -16802, USA

This work was funded by Bedding Plants Foundation, Inc. and the Danish Academy of Science. We thank Kai L. Nielsen, Dr. Carl-Otto Ottosen and Dr. David Beattie for valuable comments.

Additional index words. drought, *Impatiens x hybrida* , plant nutrition, postproduction quality, *Tagetes x hybrida*, phosphorus

Summary. Bedding plants are frequently exposed to water stress during the postproduction period, resulting in reduced quality. We have demonstrated that alumina-buffered phosphorus fertilizer (Al-P) provides adequate but much lower phosphorus concentrations than conventionally used in soilless mixes. When impatiens (*Impatiens x hybrida* 'Impulse Orange') and marigold (*Tagetes x hybrida* 'Janie Tangerine') plants were grown under reduced phosphorus using the buffered fertilizer, P leaching was greatly reduced and plant quality was improved. Impatiens plant diameter was significantly reduced by Al-P, and leaf area was reduced by Al-P in both species. Marigold plants grown under Al-P had more flowers and fewer wilted flowers. Flower wilting was also reduced in impatiens plants grown under Al-P. In marigold plants, roots were confined to a small volume beneath the drip tube in control plants, while in Al-P plants they were well distributed through the medium. Impatiens roots showed no obvious differences in root distribution. When plants at the marketing stage were exposed to drought, the Al-P plants of both species wilted more slowly than the high-P controls. The reduced leaf area in both species and the improved root distribution in marigold may account for the improvement in drought tolerance in the Al-P plants.

To increase consumer satisfaction, growers must produce high-quality plants which are compact, stress tolerant, and free from diseases. Plants with well-developed root systems which exploit the medium uniformly and with room for further growth are best at withstanding the fluctuations in soil moisture which occur during shipping and handling and in the hands of the consumers.

To keep the production period as short as possible, bedding plants are grown with optimum light, temperature and a surplus of fertiliser to maximise growth rate. However, these conditions often produce plants with elongated, lush shoots, poorly developed roots, and poor stress tolerance. Therefore, it is recommended that before shipping growers harden their plants with a short period of lower temperature and reduced fertiliser and water at the end of the production cycle (eg. Serek, 1990). This practice is believed to increase stress resistance by encouraging root growth at the expense of shoot growth. It might be possible to improve plant quality and stress tolerance even more by encouraging strong root growth during production by reducing the phosphorus (P) levels in the root zone. Our previous research has shown that the high P levels (30-60 $mg{\cdot}L^{-1}$ P) commonly used in commercial floriculture inhibit root growth (Lynch, unpublished), and studies using P-buffered solutions show that plants can grow well at P levels as low as 0.09 $mg{\cdot}L^{-1}$ P (Lynch et al., 1991). When various concentrations of solid phase alumina-buffered P were tested on marigold plants, there was no evidence of aluminum toxicity unless 12% or more P-Al_2O_3 was added to the media (Lin et al., 1996). There was also some evidence for increased branching, indicating that lower P may have positive effects on plant quality. In this project, we have investigated the effects of low P levels on development and quality of impatiens and marigold plants. We specifically tested the hypothesis that low P would improve resistance of these bedding plants to postproduction drought stress.

Materials and methods

Two species, impatiens (*Impatiens x hybrida* 'Impulse Orange') and marigold (*Tagetes x hybrida* 'Janie Tangerine') were germinated in low-P-peat media, and grown in a greenhouse environment without supplemental light. Day and night temperature were 24.4 ±2.4 °C and 20.9 ±0.5 °C respectively. Seeds were germinated in December and transplanted to 12 cm containers after 2 weeks.

Control plants (high P) were grown in peat and fertilised with a nutrient solution containing a P concentration of 46.5 $mg{\cdot}L^{-1}$ (Johnson et al., 1957; Epstein, 1972). The experimental plants were grown in peat medium amended with P-charged alumina (P-Al_2O_3) (Lynch et al., 1990) and fertilised with the same nutrient solution as supplied to the control plants, but using $(NH_4)_2SO_4$ instead of $NH_4H_2PO_4$. Thus, the only P source for the experimental treatments was from the P-Al_2O_3. Alumina was charged with two levels of P giving two levels of P-desorption (low-Al and high-Al give the actual concentrations) (Lynch et al., 1990). The P-Al_2O_3 amendment was added at a rate of 2% by volume of the medium. No additional nutrients were supplied after anthesis (day 0).

Leachate was sampled once a week during each of the 3 weeks preceding flowering and at flowering (combined to give "day 0" data) and 3 weeks later and analysed using Murphy-Riley's phosphomolybdenum blue method (Murphy and Riley, 1962). There were 4 samples collected on each preflowering sampling date and 12 samples collected 3 weeks after flowering.

Quality and postharvest longevity. When the second flower opened (day 0, representing the onset of the marketing period) height and diameter were recorded. Six plants per treatment were placed in darkness at 16-17 °C for 4 d to simulate a transportation environment and then placed in a postproduction environment consisting of low light (8 μmol·m^{-2}·s^{-1}) for 12 h per d and constant 21-23 °C. An additional six plants were placed directly in the postproduction environment. Since the simulated transportation treatment had no significant effect, the two groups were pooled in the statistical analyses.

Once a week the height, width, and numbers of open flowers and wilted flowers were recorded for each plant. The experiment terminated after 6 weeks for marigolds and after 7 weeks for impatiens.

Drought treatment . To investigate whether the low-P treatments would increase drought tolerance, irrigation was withheld from plants in the greenhouse until wilting of foliage was observed (2-4 d). The medium was then rehydrated and the plants were allowed to recover completely (at least 2 d), as evidenced by full leaf turgor, before a second drought cycle was started. After two cycles of drought treatment, data on plant quality were collected as described above. In addition, leaf area was recorded for control plants. Plants were subjected to these treatments 4 weeks after anthesis. Six plants per treatment were exposed to drought, and five were irrigated regularly as controls.

Severity of drought stress was assessed by measuring the average midday stomatal conductance from the two youngest fully expanded leaves with a Delta-T Mk3 porometer (Cambridge, England). Stomatal conductance of irrigated plants was recorded at the same time. After drought treatments the experiment was terminated and leaf area was recorded.

Statistical treatment: Data were analysed using the PC-statistical package JMP® (SAS Institute 1989-91). The data collected weekly in the postproduction environment (number of flowers and % wilted flowers) were analysed by repeated measures analysis. Multiple comparisons were performed using ANOVA and mean separations with a paired student t-test.

Results

The P levels in leachate from control plants decreased 7-fold during the three weeks after initial flowering (day 0), when the plants were irrigated only with distilled water (Table 1). Phosphorus in the leachate from media amended with solid phase-

P buffer decreased about 2-fold in the high Al-P pots and was constant in the low Al-P pots. The pH of the media increased over the experimental period in all treatments.

High levels of Al in the growth media can be toxic to roots, especially at low pH (de Lima & Copeland, 1994). However, there were no visible signs of damage due to the solid-phase phosphorus-charged alumina (P-Al_2O_3) amendment in the growth media (in agreement with Lin et al., 1996). The pH of the leachate ranged from 4.5-5 in all P treatments.

Quality and longevity. Impatiens plants fertilised with low and high Al-P were significantly more compact than control plants, evidenced by their smaller diameter compared with control plants at flowering (day 0) (Table 2). Neither height nor diameter was affected by P treatment in marigold (Table 2). There was no difference among the treatments in time to bud break (Table 2). The number of flowers developing in the postproduction environment was significantly increased by low-P treatments in marigold but not in impatiens (Fig. 2). The percentage of wilted flowers was reduced by the low-P treatment in both marigold and impatiens (Fig. 3). There was no significant effect of simulated transportation (dark treatment) on postharvest quality (data not shown).

Drought tolerance. When irrigation was withheld, plants grown with Al-P wilted more slowly than the control plants during each of two consecutive drought cycles. Stomatal conductance was used as an indicator of water status. Fig. 4 shows the difference among treatments in stomatal conductance two days after the start of the second drought cycle. Plants grown with Al-P had higher stomatal conductance and had not yet wilted, while stomatal conductance of conventionally fertilized plants was only 10-20% of irrigated controls and plants were wilted.

In irrigated marigold plants, stomatal conductance was inversely correlated with P level (Fig. 4). Stomatal conductance of irrigated impatiens plants was lower than for marigold, but P treatment had no significant effect. Leaf area was reduced by low P treatments for both species (Table 2).

Roots were distributed uniformly in the pots amended with P-charged alumina. The medium held together in a very compact root ball which could be removed from the pot without falling apart. On the other hand, the root ball of control plants was small and insufficient to hold the medium together, and large areas of the pot were not even explored by the roots (Fig. 1).

Discussion

By using a solid-phase buffer technique it has been shown that plants grow well at P-concentrations 100-fold lower than traditionally used in horticulture (Lynch et al., 1991; Lin et al., 1996). The solid-phase aluminum buffers phosphorus at a low but constant level, unlike fertigation with low P, which causes the P-concentrations to fluctuate with the irrigation cycles and with P depletion through root uptake.

In this experiment, impatiens plants grown with low-P fertilization were more compact than plants grown at conventional, high-P concentrations. Marigolds grown with low P had more flowers which wilted more slowly in the postproduction environment, but compactness was not affected. There were no detrimental effects on plant quality from low-P treatments for either species, even when the P concentration in the growth media was more than 100-fold lower than the control plants.

Impatiens and marigold plants grown with low P were more drought tolerant than plants grown with high P. Low-P plants had 25-50% less leaf area than control plants, which would be expected to reduce transpiration and water use considerably. The low-P plants closed stomates more slowly, coincident with slower desiccation of the growth medium. The stomatal conductance of low-P marigold plants was higher than that of control plants before the plants were subjected to drought (Fig. 4). This may have been a physiological or anatomical compensation for the reduced leaf area of low-P plants. The leaf area of low-P plants was about half that of control plants (Table 2), while the stomatal conductance was about double (in well-irrigated plants), giving appproximately equal gas exchange capacity. The stomatal conductance of low-P marigolds may also have been a manifestation of the improved water status of these plants even under irrigation. In impatiens plants, leaf area was lower in plants grown under high-Al or low-Al (Table 2), but there were no differences in stomatal conductance among P treatments in well-irrigated plants (Fig. 4).

Root architecture, proliferation and mass affect the rate at which root systems remove moisture from soils (Faber et al., 1991). When P was supplied by amending the peat medium with P-Al_2O_3, the roots explored the medium uniformly. Marigold roots in pots fertilized with high-P solution (controls) tended to remain in a constricted area leaving large areas of the pot unexplored, which would allow proportionately more water to be lost by evaporation and reduce efficiency of water use (Furtura et al., 1977; Van de Werken, 1989). Although there were no obvious differences in root distribution in the media of high and low-P impatiens plants, there could have been subtle differences in, for example, the distribution of fine roots. The volume of media explored could have been a factor contributing to water use efficiency in low-P plants.

Use of buffered phosphorus fertilizer may provide bedding plant growers with a means to improve plant quality and stress tolerance while reducing fertilizer leaching and attendant waste-management problems. This research clearly shows that marigold and impateins plants grown with lower concentrations of P provided by the buffered-P fertilizer have equal or better quality and improved tolerance to drought.

References

Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. John Wiley and Sons, Inc. New York.

Faber, B.A., D.N. Muuns, K. Schackel, and R.J. Zasoski. 1991. A method of measuring hyphal nutrient and water uptake in mycorrhizal plants. Can. J. Bot. 69:87-94.

Furuta, T., T. Mock, and R. Coleman. 1977. Estimating the water need for container-grown nursery stock. Amer. Nurseryman 145:68.

Johnson, C.M., P.R. Stout , T.C. Broyer, and A.B. Carlton. 1957. Comparative chlorine requirements of different plant species. Plant Soil 8:337-353.

Lima, de M.L. and L. Copeland. 1994. Changes in the ultrastructure of the root tip of weat following exposure to aluminium. Aust. J. Plant Physiol. 21:85-94.

Lin, Y-l. P., E.J. Holcomb, and J. Lynch. 1996. Marigold growth and phosphorus leaching in a soilless medium amended with phosphorus-charged alumina. HortScience 31:94-98.

Lynch, J., E. Epstein, A. Läuchli, and G. Weigt. 1990. An automated greenhouse sand culture system suitable for studies of P nutrition. Plant Cell Environ. 13:547-554.

Lynch, J., A. Läuchli, and E. Epstein. 1991. Vegetative growth of the common bean in response to P-nutrition. Crop Science 31:380-387.

Murphy, J. and J.P. Riley. 1962. A modified single solution reagent for the determination of phosphate in natural waters. Anal. Chim. Acta 27:3136.

Serek, M. 1990. Effects of pre-harvest fertilization on the flower longevity of potted *Campanula carpatica* 'Karl Foerster'. Scientia Hortic. 44:119-126.

Van de Werken, H. 1989. A new way with containers. Amer. Nurseryman 170:43-51.

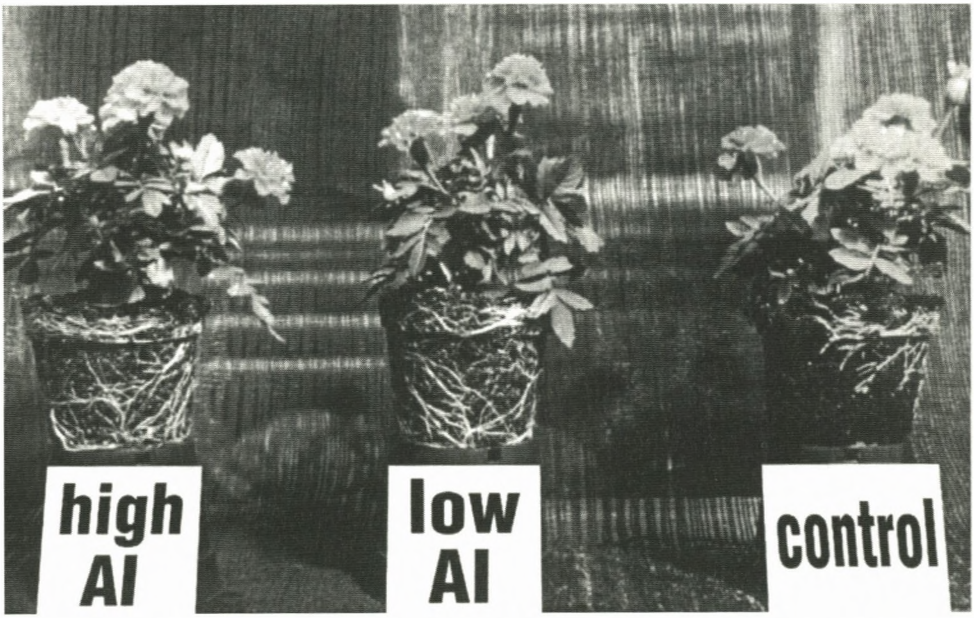


Fig 1. Mature marigold plants grown with conventional P fertilizer (control) or two concentrations of Al-P. Note the uniform distribution of the roots in alumina-buffered P (high-Al and low-Al) plants.

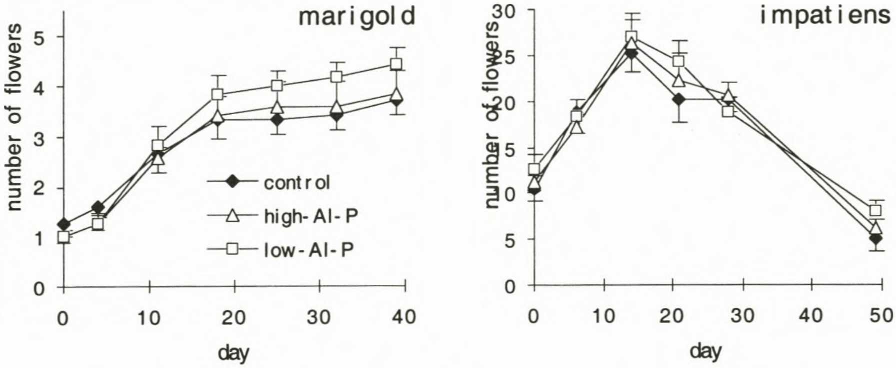


Fig. 2. Flower number on marigold and impatiens plants held in a postproduction environment. Plants were grown with conventional P fertilizer (control) or two concentrations of Al-buffered P. Phosphorus treatment significantly ($P<0.01$) affected flower number for marigold, but had no significant effect on impatiens. Bars indicate SE.

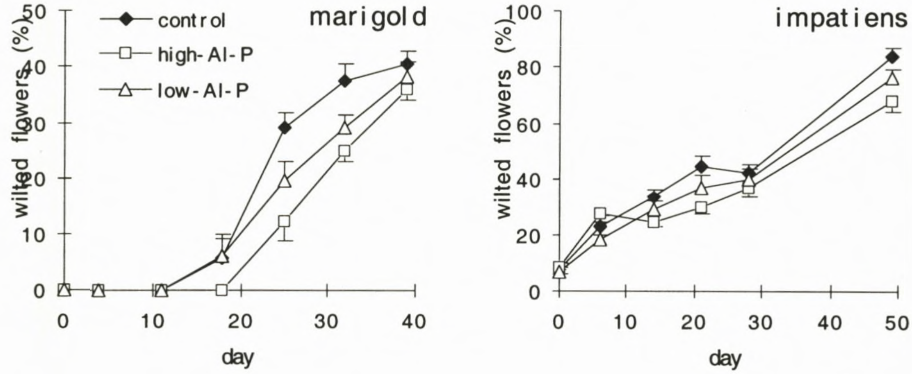


Fig. 3. Wilting of marigold and impatiens flowers in the postproduction environment. Plants were grown with conventional P fertilizer (control) or two concentrations of Al-buffered P. Phosphorus treatment significantly (P<0.01) reduced flower wilting for both species. Bars indicate SE.

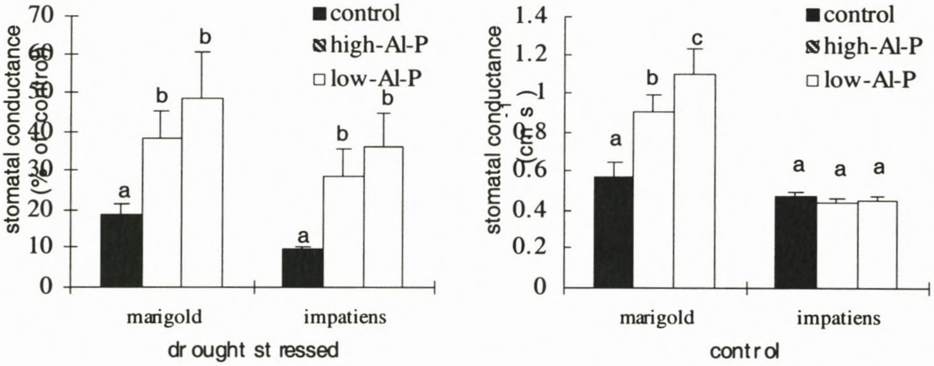


Fig. 4. Mid-day stomatal conductance in droughted and irrigated (control) marigold and impatiens plants grown under different P regimes. Drought was imposed by withholding irrigation for 2 d. Stomatal conductance is shown for irrigated plants on the same day. Bars indicate SE and pairs labeled with the same letter are not significantly different (P<0.05).

Table 1. Phosphorus and pH in leach water from conventionally fertilized (control) and alumina-buffered P-treated (high Al-P and low Al-P) plants before flowering (day 0) and after 3 weeks in the postproduction environment. During the postproduction period the plants were irrigated with distilled water only. Values shown are means of 4 samples collected during each of the 4 weeks preceding flowering (day 0) or 12 samples collected on one date (day 21) ± SE.

	Day 0			**Day 21**		
	[P] mg·L^{-1}		pH	[P] mg·L^{-1}		pH
Control	28.3	± 5.4	4.5	4.12	± 0.46	6.8
High Al-P	1.07	± 0.15	4.5-5.0	0.50	± 0.16	6.4
Low Al-P	0.19	± 0.09	4.5-5.0	0.17	± 0.01	6.5

Table 2. Time to bud break, size, and leaf area of impatiens and marigold plants grown at 3 P levels. Height and diameter were recorded when the second flower opened (day 0), which was the beginning of the postproduction period. Leaf area was recorded 4 weeks after anthesis. Numbers within columns and species followed by the same letter are not significantly different according to the Student t-test (P< 0.05).

Species	**P level**	**Bud break** days	**Height** (cm)	**Diameter** (cm)	**Leaf area** (cm^2)
Impatiens	Control	50.7	11.5	33.8 a	1698 a
	High Al-P	50.8	10.5	28.9 b	1094 ab
	Low Al-P	50.5	11.0	28.8 b	1285 b
LSD		0.3	3.1	3.1	413
Marigold	Control	45.4	11.7	14.1 a	573 a
	High Al-P	46.0	11.5	15.1 a	274 b
	Low Al-P	46.2	11.7	14.0 a	290 b
LSD		1.0	1.1	2.0	228

Improved Drought Avoidance by Manipulation of Root Growth with Low-Phosphorus Nutrition

Kristian Borch[1], Carter Miller[2] Kathleen M. Brown[2] and Jonathan Lynch[2]
[1]Department of Ornamentals, Danish Institute of Agricultural Sciences, 5792 Aarslev, Denmark, k.borch@aarslev.ou.dk. [2]Department of Horticulture, Pennsylvania State University, University Park 16802, USA

Prepared for JASHS

Abbreviations: P: phosphorus; RWC_{pot}: relative water content in the pot media; RWC_{leaf}: relative water content in the leaf; LOP: leaf osmotic potential; P_n: net photosynthesis; g_s: stomatal conductance

Abstract

A series of experiments was conducted to investigate the response to drought stress of marigold (*Tagetes patula L.* 'Janie Tangerine') plants grown with reduced phosphorus. Plants were grown with conventional phosphorus fertilization (1 mM, control) or one of two levels of reduced, alumina-buffered phosphorus, sufficient (20 μM) or deficient (5 μM). Sufficient phosphorus fertilization produced plants with more flowers and a reduced leaf area compared to control plants. Photosynthetic CO_2 assimiation was x% higher in phosphorus-sufficient plants than in controls, possibly due to decreased internal canopy shading. In phosphorus-sufficient plants, smaller leaf area resulted in reduced transpiration. Moreover, the relative water content of the pot (RWC_{pot}) was significantly lower at wilting. The improved water acquisition could be explained by increased root proliferation via longer main roots and less densely distributed laterals under sufficient phosphorus availability, whereas phosphorus-deficient plants have a reduced root proliferation and thus high RWC_{pot} when wilting. The results indicate that optimizing phosphorus nutrition with solid-phase buffered-phosphorus fertilizer improves drought tolerance by reducing transpiration and increasing water use efficiency.

Introduction

Plants grown in soilless media are typically grown with excessive phosphorus levels, which may have detrimental effects on plant growth and performance (Hansen and Lynch, 1998). Studies using buffered-phosphorus solutions show that plants can grow well at phosphorus levels as low as 10 μM (Borch et al., 1998a; Lin et al., 1996; Lynch et al., 1991). We have shown that impatiens and marigold plants grown under reduced, alumina-buffered phosphorus (Al-P) were more resistant to drought stress (Borch et al., 1998a). Marigold plants showed better distribution of roots throughout the medium, which may have partially accounted for slower wilting, but impatiens plants showed no obvious changes in root distribution and also showed slower wilting (Borch et al., 1998a). Both species had smaller leaf area, which could reduce transpiration, slowing the rate of water loss and delaying wilting.

There are similarities in plant adaptations to low phosphorus availability and to water stress. Plants grown with low phosphorus usually have a higher root-to-shoot ratio (Anuradha and Narayanan, 1991; Lynch and Beebe, 1995; Hansen et al., 1997). Biomass allocation to roots may improve phosphorus acquisition in natural soils when phosphorus mobility is strongly limited. Likewise, under conditions of low soil water potential, abscisic acid (ABA) accumulation induces the plant to both maintain primary root growth and inhibit shoot growth (Saab et al., 1990). These changes in root to shoot ratio are important in acclimation of the plant to water deficiency. since the shoot is the water consuming organ and the roots must elongate to reach soil layers with water. Thus, the changes in growth induced by reduced phosphorus availability might improve resistance to drought, since the mechanisms of adaptation are similar. This hypothesis was supported by our preliminary work showing slower wilting during drought stress of marigold and impatiens (Borch et al., 1998a).

Phosphorus has previously shown to impact stomatal behavior which is believed to be a short term response in plants subjected to drought. This response is mediated by physiologically active messages from the roots to minimize damage to shoot tissues by reducing transpiration (e.g. Davies and Zhang, 1991). Radin (1984) reported that when water was withheld from the pots, stomates closed at a leaf water potential (LWP) of approximately -1.6 and -1.2 MPa in the normal and phosphorus -deficient plants, respectively. The mechanism behind this is not clear, however, it has been considered that plant nutrition in general, might affect root-to-shoot signaling of soil drying particularly because it influences xylem sap constituents (Saab and Sharp, 1989; Jones, 1990). Moreover, Augé et al. (1995) found that leaf phosphorus concentration appeared to affect this signaling.

This suggests that by manipulating nutrient availability it is possible to encourage root growth for optimal media exploration thereby achieving improved water utilization in container grown plants. The objective of this experiment was to investigate increased root proliferation and inhibition of shoot growth in response to low- phosphorus stress and to investigate a possible improvement of drought avoidance in plants grown under different phosphorus regimes, due to improved root growth.

Methods

Marigolds ('Janie Tangerine') were germinated in low-phosphorus peat media and grown in a greenhouse environment without supplemental light. Day and night temperatures were 24.4 ±2.4 °C and 20.9 ±0.5 °C, respectively. Seeds were germinated in December and transplanted to 12 cm containers after 2 weeks. Except for plants used in 'experiment 3,' plants were grown in sphagnum-moss peat (Premier Horticulture, 326 Main St., Red Hill, PA 18076) and fertilized by irrigation with Peters Professional Dark Weather Feed 15-0-15 (Scotts-Sierra Horticultural Products Co. Marysville, Ohio) containing (in mM) 1.40 NO_3, 2.12 K, 1.83 Ca, 1.4 SO_4, 0.006 Fe, 0.004 B, 0.007 Mn, 0.0003 Zn, 0.00006 Mo, and 0.0004 Cu. Control plants was additianal fertilized with 1.5 mM K_2PO_4. The reduced phosphorus plants were grown in the same peat medium amended with phosphorus -charged alumina (P-Al_2O_3) (Elliott et al., 1983) and subirrigated with the same nutrient solution as supplied to the control. Alumina was charged with two levels of phosphorus giving two levels of phosphorus desorption (se below). The amounts of P-Al_2O_3 amendment for the treatments were 1% by volume of the medium. Thus, the only phosphorus source for the experimental treatments was from the P-Al_2O_3. Plants analyzed for root structure in experiment 2 were grown a calcined clay 'SERAMIS' (Effem, Mogendorf, Germany) (Starkey and Pedersen, 1997). Other aspects of cultivation were identical to the peat experiments.

Phosphorus levels: Leachate was sampled weekly until anthesis and analyzed using a phospho-molybdenum blue method (Murphy and Riley, 1962). Phosphorus level for control plants was 1.07 (±0.162) mM; for sufficient phosphorus 21 (±5.6) µM and for deficient- phosphorus plants 5 (±2.0) µM.

Experimental treatment

1) Transpiration experiment

The aim of this experiment was to describe transpiration and evaporation from plants grown with three different phosphorus treatments. At anthesis the plants were moved from the greenhouse to a growth chamber with 8 µmol $m^{-2} s^{-1}$ irradiance for 12 hours per day and a constant temperature of 25 ±2.1°C and humidity 30 ±3.4% RH. Plants were acclimatized to the growth chamber environment for 24 hours and irrigated to field capacity. Water loss was continuously monitored gravimetrically for 72 hours using twelve thin beam load cells (LCD-816G, Omega Technology Co., Stamford, CT) mounted on an aluminum beam. From each load cell a light-weight platform was suspended on which the potted plants were placed. The load cells were excited with 6 volt DC low noise electrical supply and connected via a general purpose terminal panel

(T71-GP, Strawberry Tree Inc., Sunnyvale, CA). The plants were weighed continuously and the data was logged to a DynaRes-16 data acquisition board (DR-16, Strawberry Tree Inc., Sunnyvale, CA). The software package QuickLog PC ver. 2.4 was used to monitor data acquisition and store data. A standard curve was obtained for individual load cells using mass standards yielding a 0.2 g precision. To distinguish between transpiration and evapotranspiration, recordings were repeated with the growth surface covered to eliminate evaporation. The difference between these measurements was assumed to be evaporation, and transpiration was calculated by subtracting evaporation from water loss measurements in uncovered pots. After the load cell recordings the plants where harvested to measure leaf area.

2) Root structure and water utilization

The aim of this experiment was to identify the relationship between root structure, phosphorus concentration and water utilization. To make this possible, plants were grown in SERAMIS which is easy to separated from the roots. These plants were produced as described earlier. At anthesis they were transferred to a growth chamber with low light (45 µmol m^{-2}), constant temperature of 21°C and 35% RH and acclimatized for two days before drought treatment was started. The plants were then irrigated to field capacity, whole plants were weighed, and stomatal conductance (g_s) of two fully expanded leaves in the upper half of the canopy was recorded with a Delta-T Mk3 porometer (Cambridge, England). The plants were not irrigated while they were in the chamber. The g_s and weight of the plants were recorded once every day until wilting. Photosynthesis of whole shoots was recorded before and two days after the initiation of drought stress with a LI-COR 6200 portable photosynthesis system (LI-COR Inc., Lincoln, USA) operated as a closed system. Photosynthesis was measured in a 36 L cuvette for 60 sec. When mid-day g_s dropped below 0.05 cm s^{-1} (as compared to controls at 0.20-0.15 cm s^{-1}) and visible wilting occurred, final measurements were taken and the experiment was terminated. Plants were harvested to record leaf area and the roots were separated from the growth media. A subsample of the roots was stained in a 2% neutral red solution for at least two hours and digitally analyzed for root length and diameter using DT-Scan (Cambridge, England). The subsample and the rest of the root system were oven dried separately at 70 °C for 48 hours to determine dry weight. The growth media was dried to calculate RWC_{pot} ($RWC_{pot} = [(w_w-w_d)/(w_t-w_d)]$ x100 (Hsiao, 1990), where w_t is the pot weight at field capacity, w_w is pot weight at wilting and w_d is weight of the pot media and pot after 48 hours of oven drying).

3) Long term drought and longevity

Peat grown plants were placed in a growth chamber as described in experiment 1. Daily water use was determined gravimetrically for each plant over a 24 hour period. This was repeated after rewatereing. Drought stress was applied to ten plants by daily irrigation with half the volume of water used prior to the drought treatment. Ten plants of each phosphorus treatment were irrigated as needed and refered to as unstressed controls. After three weeks drought stress was assessed by recording g_s . The two youngest fully matured leaves were used to record RWC_{leaf} using $RWC_{leaf} = [(w_w-w_d)/(w_t-w_d)]x100$, where w_t is leaf turgid weight, w_w refers to leaf weight at the wilting point and w_d is leaf dry weight. The leaf was then submerged in distilled water at 22°C , placed under a light source for 24 hours, blotted dry and weighed to determine turgid weight, w_t. After drying for 48 hours at 60 °C it was weighed again to determine dry weight w_d. Leaf osmotic potential (LOP) was recorded with a vapor pressure osmometer (Wiscor, 5200). Flowers, wilted flowers and buds were counted and the shoots were oven dried for 48 hours to determine dry weight.

Statistical analysis: Data were analyzed using the PC-statistical package JMP® (SAS Institute 1989-91). Data was analyzed with ANOVA (split plot design). Contrasts were used to test for significant differences among means with a t-test. n.s. denotes no significant difference at a 5% level. Values are presented as mean ± standard error of mean.

Results

1) Transpiration

Transpiration over a 72 hour period was decreased significantly with reduced phosphorus concentrations, but the fraction of water lost to evaporation was highest in phosphorus-deficient plants (Fig. 1). Leaf area and total shoot dry weight decreased with decreasing phosphorus (Table 1). When mid-day transpiration was expressed on a leaf area basis, there was no difference among the phosphorus treatments (control phosphorus, 36.9 ± 9.0; sufficient phosphorus, 38.4 ± 8.4 ; deficient phosphorus, 34.9 ± 8.4 g m^{-2} h^{-1}).

2) Root structure and water utilization experiment

Leaf area was reduced with decreasing phosphorus availability to a similar degree as in experiment 1 (Table 2). Root diameter decreased with decreasing phosphorus and total root length was highest in phosphorus-sufficient roots (Table 2). When irrigation was withheld, all control plants wilted (mid-day g_s below 0.05 cm s^{-1}) after 4 days, while phosphorus-sufficient plants wilted after roughly 4.5 days and phosphorus-deficient plants wilted after 5 days (data not shown). RWC_{pot} was significantly lower in phosphorus-sufficient plants compared to control and phosphorus-deficient plants, although stomatal conductance was the same (Fig. 2). Density (cm^{-1}) of 1st and 2nd order lateral roots was about one third less in Al-P fertilized roots (Fig. 3). A correlation (R^2=0.40, $p<0.05$) was found between total root length and RWC_{pot}.

Net photosynthesis (P_n) was highest per leaf area in phosphorus-sufficient plants and lowest in control plants. After drought treatment followed by irrigation and recovery of turgor, $P_n$was increased in adequate and deficient-phosphorus plants, but not in control plants (Fig. 5).

3) Longevity and long term drought

At the end of three weeks of reduced irrigation, leaves were not visibly wilted but the lower leaves were yellowing or had abscised, indicating severe drought stress. In well irrigated plants, LOP increased as phosphorus decreased (Fig. 6). After three weeks of drought, control plants had accumulated more solutes (increased LOP) whereas no changes were found in phosphorus-sufficient plants and phosphorus-deficient plants (Fig. 6). RWC_{pot} was significantly higher in phosphorus-deficient plants than the two other phosphorus treatments, while RWC_{leaf} was not significantly affected by phosphorus treatments (Fig. 7).

The number of flowers was significantly higher in phosphorus-sufficient plants after three weeks in low light conditions (Table 3). Long-term drought had no effect on flower number in control plants, but decreased it in phosphorus-sufficient plants and even more in phosphorus-deficient plants (Table 3). Wilting of flowers was highest in phosphorus-deficient plants and lowest in control plants, and drought treatment increased flower wilting in all treatments (Table 3). Dry weight of the shoot was much lower in phosphorus-deficient plants than in plants from other phosphorus treatments (Table 3). Drought decreased shoot DW by nearly 50% in phosphorus-deficient plants but did not significantly affect shoot DW of control and phosphorus-sufficient plants (Table 3). In addition, the number of buds was the same in control-phosphorus and phosphorus-sufficient plants but lower in phosphorus-deficient plants (data not shown).

Discussion

There is accumulating evidence that phosphorus nutrition is implicated in water acquisition and drougt tolerance in plants (Borch et al., 1998; Radin, 1984; Nelsen and Safir, 1982; Rodriguez et al. 1996). However, the mechanism behind this is unclear and together with dificulties in applying low phosphorus to the growth media has conclusions inconsistent. We have previously found that plants grow in a low phosphorus buffered media could avoid drought stress for extended periods (Borch et al., 1998). However, the effects of low phosphorus nutrirtion can be split in to root related effects and shoot related effects.

The plants transpired the same amount of water per leaf area regardless of phosphorus treatment, but due to the reduced leaf area in the low-phosphorus treatments these plants transpired less thus desiccated more slowly. Phosphorus-deficient plants lost more water to evaporation, probably because the soil surface was more exposed by the reduced leaf area of these plants (Fig. 1).

Plants can avoid water deficits by accessing water deep in the soil or by more efficiently extracting water from the occupied part of the profile (Ludlow, 1989). Thus, greater root elongation and wider distribution of lateral roots of phosphorus-sufficient plants (Fig. 3, Table 2) may have resulted in more thorough use of the available water in the pot. Plants grown with sufficient phosphorus had a lower RWC_{pot} at the wilting point than control plants (Fig. 2), indicating their superior ability to make use of available water. The phosphorus-deficient and control plants had less root length and a relatively high RWC_{pot} at wilting (Fig. 2). The significant correlation between root length and RWC_{pot} lends further support to the view that increased root length is associated with improved drought stress avoidance in phosphorus-sufficient plants (Fig. 4). However, the relationship can probably be improved by considering other aspects of root structure e.g root topology and architecture

Phosphorus-deficient roots had lower lateral root density than controls and maintained root elongation equal to that of control plants. The reduction in leaf area and shoot dry weight (Tables 1 & 2), as well as the lack of enhancement of root elongation that was observed in phosphorus-sufficient plants (Fig. 3), suggests that phosphorus-deficiency severely limited the capability of the plant to undertake adaptive responses. Previous work with sorghum and bean also showed that phosphorus-deficient plants had shorter roots and poor drought resistance than control plants (Al-Karaki et al. 1996).

Phosphorus deficiency is known to reduce net photosynthesis (P_n), (Terry and Rao, 1991), possibly by disturbing thylakoid function (Abadia et al., 1987), the regulation of carbon fluxes in the mesophyll cells (Dietz and Harris, 1997), and RuBP activity limitation. (Dietz and Harris, 1997) In experiment 2, phosphorus-deficient plants had lower P_n than phosphorus-sufficient and control plants (Fig. 5). Surprisingly, phosphorus-sufficient plants had increased net photosynthesis, despite the fact they were grown with much lower phosphorus levels compared to control (Fig. 5). The key explanation to this is prbably the reduction of internal canopy shading resulting from the smaller leaf area of the phosphorus-sufficient plants. This could explain our findings that P_n was highest in sufficient phosphorus plants despite the reduced leaf area (Fig. 5). Moreover, Hecht-Buchholts (1967) found that low-phosphorus plants had a higher chlorophyll content per unit leaf area since cell and leaf expansion are retarded more than chlorophyll formation during low-phosphorus nutrition.

During drought stress, control plants increased the LOP by approximately 10% whereas phosphorus-sufficient and phosphorus-deficient plants did not adjust. The reason for lack of osmotic adjustment could simply be that the concentration of solutes in the leaf tissue is already high as a consequence of lower phosphorus nutrition. Therefore, we may conclude that the internal drought stress was less severe in phosphorus- sufficient plants compared to control plants because they could maintain RWC_{leaf} at a relative high level (Fig. 7) without osmoregulation. The reason for this may be an improved water acquisition ability of the larger root system in sufficient phosphorus plants. The reason why LOP was increased in deficient P plants at all times as also reported by Schröppel-Meier and Kaiser (1988), is unclear and need more research to be explained.

Phosphorus-sufficient plants were able to maintain more flowers with a reduced leaf area compared to control plants also during drought stress (Table 3). On the other hand, was wilting of flowers increased in adequate phosphorus plants compared to control phosphorus plants but increased wilting due to drought stress was less significant in adequate phosphorus plants (Table 3). We have earlier observed that decreased phosphorus nutrition increase number of flowers (Borch et al., 1997a). The increased photosynthesis on whole plant basis in sufficient phosphorus plants may give them an advantage during low light conditions allowing them to support more flowers.

In summary, decreased phosphorus availability achieved by amending soilless media with a solid-phase phosphorus-buffer improved drought avoidance. Several mechanisms could have contributed to this, including smaller leaf area. Low, but sufficient, phosphorus availability also resulted in longer roots with lower lateral root density, which increased root distribution within the container and improved water acquisition and drought avoidance. Phosphorus-deficient plants do not have an increased root elongation compared to control plants, which explains why they wilt at higher relative water content in the pot compared to adequate phosphorus plants. Moreover, sufficient phosphorus plants initiated more flowers in a shorter time period compared to control phosphorus plants, which may be attributable to the increased P_n in these plants. More research is needed to investigate the role of the observed increase in leaf water osmotic potential for drought avoidance in deficient phosphorus plants.

Acknowledgments

We would like to thank Drs. Kai Nielsen, Michelle Williams, Conny Hansen and Bob Ebel for valuable comments and suggestions. This work was supported by The Danish Academy of Science, the Pennsylvania Department of Agriculture, The American Floral Endowment, The Fred C. Gloeckner Foundation, and Bedding Plants Incorporated.

Literature

Abadia, J., Rao, I.M. and Terry, N. (1987) Changes in leaf phosphate status have only small effects on the photochemical apparatus of sugar beet leaves. Plant Science 50:49-55.

Al-Karaki, G.N., Clark, R.B. and Sullivan, C.Y. (1996) Phosphorus nutrition and water stress effects on proline accumulation in sorghum and bean. J. Plant Physiol. 148:745-751.

Anuradha, M. and Narayanan, A. (1991) Promotion of root elongation by phosphorus deficiency. Plant and Soil 136:273-275.

Augé, R.M., Stodola, A.J.W., Ebel, R.C. and Duan, X.R. (1995) Leaf elongation and water relations of mycorrhizal sorghum in response to partial soil drying: Two Glomus species at varying phosphorus fertilization. J. Exp. Bot. 46: 297-307.

Baldwin, J.P. (1975) A quantitative analysis of the factors affecting plant nutrient uptake from some soils. J. Soil Sci. 26:195-206.

Borch, K., Brown, K. and Lynch, J. (1998a) Improvement of plant stress resistance with low phosphorus. HortTech (accepted).

Borch, K., Bouma, T. Brown, K. and Lynch, J. (1998b) Interactions of ethylene and phosphorus nutrition on root growth. Plant, Cell, Environ. (submitted Feb. 1998).

Crafts-Brandner, S.J. (1992) Phosphorus nutrition influences on starch and sucrose accumulation, and activities of ADP-clucose pyrophosphorylase and sucrose-phosphate synthase during the grain filling period of in soybean. Plant Physiol. 98: 1128-1132.

Davies, W.J. and Zhang J. (1991). Root signals in the regulation of growth and development of plants in drying soil. Annual Review of Plant Physiology and Plant Molecular Biology 42:55-76.

Dietz, K-J. and Harris, G.C. (1997) Photosynthesis under nutrition deficiency. In: Handbook of Photosynthesis. Ed: M. Pessarakli, Marcel Dekker, N.Y., pp. 951

Elliott, G.C., Lynch, J., Läuchli, A., and Anderson, E.L. (1983) Solid-phase buffering of phosphate in sand cultures. Journal of Plant Nutrition 10:1499.

Fredeen, A., Rao, I.M. and Terry, N. (1989) Influence of phosphorus nutrition on growth and carbon partitioning in *Glycine max*. Plant Physiol. 89:225-230.

Fitter, A.H., Stickland, T.R., Harvey, M.L. and Wilson, G.W. (1991) Architectural analysis of plant root systems. I. Architectural correlates of exploitation efficiency. New Phytol. 118:375-382.

Hansen, C.W. (1997) Response to phosphorus availability during vegetative and reproductive growth of *Dendranthema grandiflorum- hybris* 'Coral Charm'. SP report no.13, Danish Institute of Agricultural Sciences.

Blackwell. ISBN 0632027576. pp. 229-243.

Hansen, C.W. and Lynch, J. (1998) Response to phosphorus availability during vegetative and reproductive growth of chrysanthemum: II. Biomass and phosphorus dynamics. *Journal of the American Society for Horticultural Science* 123:223-229.

Hecht-Buchholtz, C. (1967) Über die Dunkelfärbung des Blattgrüns bei Phosphormangel. Z. Pflantzenernaehr. Bodenkd. 118:12-22.

Hsiao, T.C. (1990) Measurements of plant water status, In : Irrigation of Agricultural Crops. Eds: Steward, D.R. Nielsen, Agronomy No. 30, Amer. Soc. Agron., Madison, pp. 243-279.

Jones, H.G. (1990) Control of growth and stomatal behavior at the whole plant level: effects of soil drying. In: Davis, W.J., Jeffcoat, B., Eds: Importance of root to shoot communication in the responses to environmental stress. Procedings of the British Society for Plant Growth Regulation, Monograph 21. Oxford: Parchments Ltd., 81-93.

Kondracka, A. and Rychter, A.M. (1997) The role of P_i recycling processes during photosynthesis in phosphate-deficient bean plants. J. Exp. Bot. 48:1461-1468.

Lin, Y-l. P., E.J. Holcomb, and J. Lynch. (1996) Marigold growth and phosphorus leaching in a soilless medium amended with phosphorus-charged alumina. HortScience 31:94-98.

Ludlow, M.M. (1989) Strategies of response to water stress. In: Structural and functional responses to environmental stresses, Eds: Kreeb, K.H., Richter, H., and Hinckley, T.M. SPB Academic Publishing, The Hague, NL. pp. 269.

Lynch, J., Lauchli, A. and Epstein, E. (1991). Vegetative growth of the common bean in response to phosphorus nutrition. Crop Science 31:380-387.

Lynch, J. and Beebe, S.E. (1995) Adaptations of beans (*Phaseolus vulgaris* L.) to low phosphorus availability. HortScience, 30:1165-1171.

Murphy, J. and Riley, J.P. (1962) A modified single solution reagent for the determination of phosphate in natural waters. Analytica Chemica Acta 27:3136.

Qui, J. and Israel, D.W. (1992) Diurnal starch accumulation and utilization in phosphorus-deficient soybean plants. Plant Physiol. 98:316-323.

Radin, J.W. (1984). Stomatal responses to water stress and to abscisic acid in phosphorus-deficient cotton plants. Plant Physiology 76:392-394.

Rao, I.M., Fredeen, A.L. and Terry, N. (1993) Influence of phosphorus limitation on photosynthesis, carbon al ocation and partitioning in sugar beat and soybean grown with a short photoperiod. Plant Physiol. Biochem. 31:223-231.

Saab, I.N., Pritchard, J. and Voetburg, G.S. (1990). Increased endogenous abscisic acid maintains primary root growth and inhibits shoot growth at low water potentials. Plant Physiology 93:1329-1336.

Saab, I.N. and Sharp, P. (1989) Non-hydraulic signals from maize roots in drying soil: inhibition of leaf elongation but not stomatal conductance. Planta 179:466-476.

Schröppel-Meier, G. and Kaiser, W.M. (1988). Ion homeostasis in chloroplasts under salinity and mineral deficiency. II. Solute distribution between chloroplasts and extrachloplastic space under excess or deficiency of sulfate, phosphate, or magnesium. Plant Physiol. 87:828-832.

Snapp, S. Koide, R. and Lynch, J. (1995) Exploitation of localized phosphorus patches by common bean roots. Plant and Soil. 177:211-218.

Starkey, K.R. and Pedersen, A.R. (1997) Increased levels of calcium in the nutrient solution improves the postharvest life of pot roses. J. Amer. Soc. Hort. Sci. 122:863-868.

Stitt, M (1990) Fructose-2,6-bisphosphate as a regulatory molecule in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. 41:153-185.

Terry , N and Rao, I.M. (1991) Nutrients and photosynthesis: iron and phodphorus as case studies. In: Plant Growth: interactions with nutrition and environment. Ed. Porter, J.R and Lawlor, D.W. pp. 54-79.

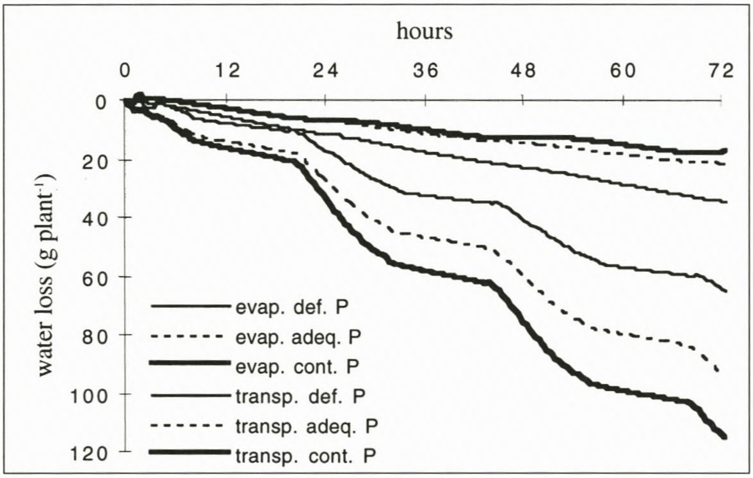


Fig. 1. Transpiration monitored as gravimetric water loss (transpiration and evaporation) from the soilless media of marigold at anthesis grown with three levels of P (control-P, adequate-P and deficient-P) for 72 hours. Values are means of 8 plants.

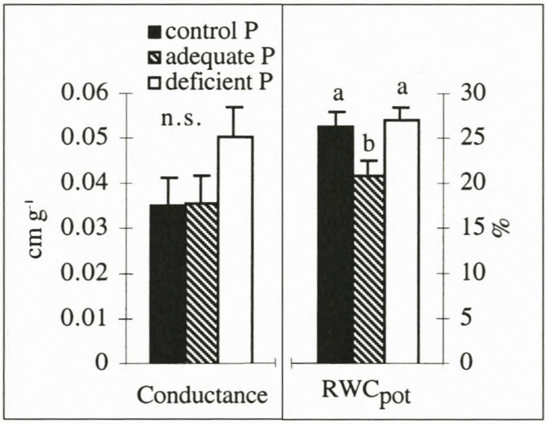


Fig. 2. Stomatal conductance and relative water content in the pot (RWC_{pot}) at the wilting point. Stomatal conductance was not significant different between P treatments (F=1.78) indicating equal water stress. There was a significant effect on RWC_{pot} from the P treatments (F=4.65, $p<0.05$) where RWC_{pot} was significantly lower in plants grown with adequate P compared to control and deficient P plants. Different letters indicate significanr difference between P treatments on a 5% level. Each point represents 10 plants and bars indicate standard error of mean.

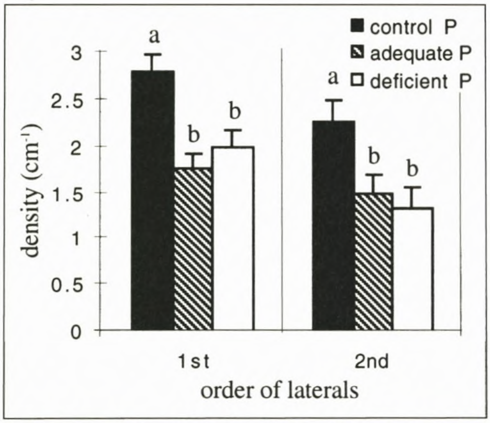


Fig 3. Density of 1st and 2nd order laterals on marigold roots grown with 3 different P levels. ANOVA: 1st order latterals, F=9.99, $p<0.01$; 2nd order latterals, F=5.37, $p<0.01$. Values are mean of 10 plants and different letters indicate significant difference on a 5% level (t-test). Bars represent standard error of mean.

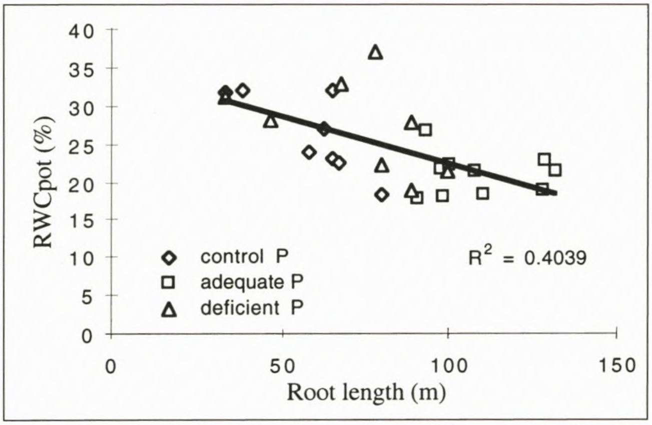


Fig 4. Relationship between root length and RWC_{pot} of control-P, adequate-P and deficient P plants at wilting. Each point represents one of 30 plants, 10 plants per P treatment. Line is fitted by linear regression.

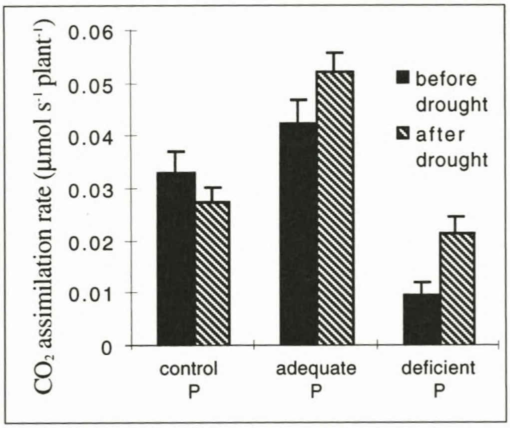


Fig 5. Photosynthesis rate before and after drought in whole marigold shoots grown with three different P levels. Photosynthesisis expressed on a whole plant basis. ANOVA: P-treatment , F=12.1, p<0.01; drought treatment, F=3.3, n.s. and P x drought, F=1.2, n.s. N=10. Bars indicate standard error of mean.

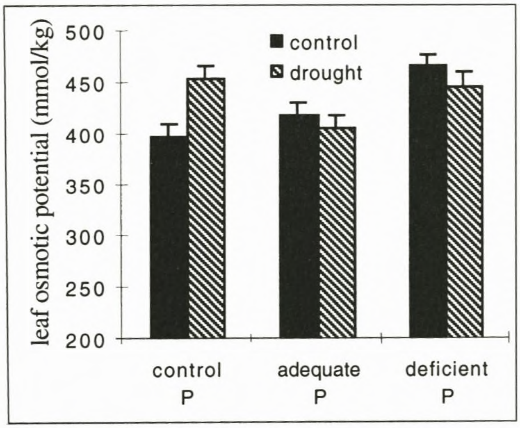


Fig 6. Leaf osmotic potential in (LOP) from drought stressed plants for three weeks and control plants grown with three different P levels. F-test: P-treatment, F=8.4, p<0.01; drought-treatment, F=5.4, p<0.01 and P x drought, F=4.6, p<0.05. N=10. Bars indicate standard error of mean.

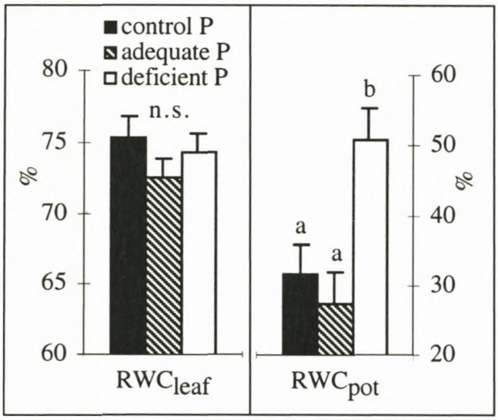


Fig 7. RWC$_{pot}$ and RWC$_{leaf}$ for plants grown with control-P, adequate-P and deficient P-nutrition after three weeks of drought stress. RWC$_{leaf}$ in well irrigated plants was: control 77.5 ±0.9; adequate P 81.5 ±1.5 and deficient P 80.1 ±1.8. Values are mean of 10 plants and different letters indicate significant difference on a 5% level (t-test). Bars indicate standard error of mean.

Table 1. Shoot dry weight and leaf area from the transpiration experiment on plants grown with different P levels. Values are mean of 10 plants, followed by ± standard error of mean. Different letters significant difference on a 5% level (t-test).

P	Shoot DW g	leaf area cm^2
control	1.07 (±0.08) a	45.2 (±0.4) a
sufficient	0.74 (±0.03) b	31.3 (±0.2) b
deficient	0.63 (±0.03) c	27.4 (±0.3) b

Table 2. Leaf area, mean root diameter and estimated root length from experiment with root structure and water utilization on plants grown with different P levels. Values are mean of 10 plants, followed by ± standard error of mean. Different letters significant difference on a 5% level (t-test).

P	leaf area (cm^2)	root diam. (mm)	root length (m)
control	59.9 (±4.7) a	0.39 (±.01) a	59.1 (±6.4) a
sufficient	47.3 (±3.2) b	0.39 (±.01) a	108.3 (±4.9) b
deficient	26.9 (±3.0) c	0.36 (±.01) b	65.1 (±8.0) a

Table 3. Number of flowers, % of wilted flowers and shoot dry weight determined on well watered plants following a 3 week period of drought stress on plants grown with different P availability. Values are mean of 10 plants, followed by standard error of mean, were different letters indicate significant difference (t-test, a=5%).

P	water stress	number of flowers		wilted flowers (%)		dry weight (g)	
control	irrigated	9.67	(±0.76)	15.12	±(2.01)	7.77	(+1.44-1.42)*
	water stress	9.50	(±0.72)	45.13	±(1.99)	6.07	(+1.27-1.26)
sufficient	irrigated	13.57	(±1.11)	25.15	±(2.34)	7.91	(+1.50-1.47)
	water stress	11.43	(±0.75)	41.52	±(3.14)	6.27	(+1.34-1.32)
deficient	irrigated	8.88	(±0.81)	36.11	±(2.02)	2.08	(+1.10-1.09)
	water stress	5.90	(±0.38)	73.65	±(4.82)	1.16	(+1.05-1.05)
	N	p	F	p	F	p	F*
irrigation	10	0.01	10.98	0.01	129	0.01	98.2
P	10	0.01	12.18	0.01	41.9	0.01	811
P x irrig.	10	n.s.	2.51	0.01	5.97	0.01	10.8

*data ln-transformed for statistical analysis.

Ethylene: a regulator of root architectural responses to soil phosphorus availability

KRISTIAN BORCH[1], TJEERD J. BOUMA[2,3], JONATHAN P. LYNCH[2], & KATHLEEN M. BROWN[2]

[1]Danish Institute of Agricultural Sciences, 5792 Aarslev, Denmark [2]Department of Horticulture, Penn State University, University Park, PA 16802, USA [3]Present address: Netherlands Institute of Ecology, Center for Estuarine and Coastal Ecology, 4400 AC Yerseke, The Netherlands.

Published 1999 in Plant Cell and Environment, 22: 425.

ABSTRACT

The involvement of ethylene in root architectural responses to phosphorus availability was investigated in common bean (*Phaseolus vulgaris* L.) plants grown with sufficient and deficient phosphorus. Although phosphorus deficiency reduced root mass and lateral root number, main root length was unchanged by phosphorus treatment. This resulted in decreased lateral root density in phosphorus-deficient plants.

The possible involvement of ethylene in growth responses to phosphorus deficiency was investigated by inhibiting endogenous ethylene production with amino-ethoxyvinylglycine (AVG) and aerating the root system with various concentrations of ethylene. Phosphorus deficiency doubled the root-to-shoot ratio, an effect which was suppressed by AVG and partially restored by exogenous ethylene. AVG increased lateral root density in phosphorus-deficient plants but reduced it in phosphorus-sufficient plants. These responses could be reversed by exogenous ethylene, suggesting ethylene involvement in regulation of main root extension and lateral root spacing. Phosphorus-deficient roots produced twice as much ethylene per gram dry matter as phosphorus-sufficient roots. Enhanced ethylene production and altered ethylene sensitivity in phosphorus-deficient plants may be responsible for root responses to phosphorus deficiency.

Key-words: *Phaseolus vulgaris* L., Leguminosae, common bean, ethylene, phosphorus nutrition, root architecture

INTRODUCTION

The rate of phosphorus diffusion in soil solutions is generally slower than the absorption rate needed to sustain maximum growth rate (Nye and Tinker, 1977). Hence, the ability of the roots to explore the growth media efficiently in time and space is very important for phosphorus uptake in low phosphorus soils. Factors believed to directly influence phosphorus uptake by roots are root diameter (Eissenstat, 1992), root topology (Fitter, 1991), root extension rate (Barber, 1995), root length and density (Baldwin, 1975), and root hairs (Jungk, 1987).

Little is known about how root growth responses to phosphorus deficiency are mediated. However, the plant hormone ethylene is known to be involved in elongation of roots, lateral and adventitious rooting, root extension, radial expansion and aerenchyma formation (reviewed by Jackson, 1991; Doland, 1997). There is evidence for ethylene involvement in stress-related and adaptive root responses to chemical toxicity, water stress, interactions with symbionts, and nutrient deficiency (Lynch and Brown, 1997). Such responses may involve changes in ethylene synthesis and/or responsiveness. For example, although phosphorus or nitrogen starvation reduced ethylene production in maize roots, ethylene responsiveness, measured as aerenchyma formation, increased (Drew et al., 1989; He et al., 1992). On the other hand, wheat seedling roots grown under nitrogen deficiency and low pH displayed increased ethylene production and reduced root elongation (Tari and Szén, 1995). Ethylene at high concentrations has been reported to inhibit root growth and root gravitropic responses (Ycas and Zobel, 1983).

The objective of our study was to test the hypothesis that ethylene is involved in the growth responses of intact bean root systems to phosphorus deficiency. Our approach was to measure ethylene production and responsiveness in bean plants grown in sand amended with a solid-phase-phosphorus buffer providing sufficient or deficient phosphorus. A range of ethylene concentrations was achieved by aereating the root zone for 4 weeks with various amounts of ethylene while inhibiting endogenous ethylene production with amino-ethoxyvinylglycine (AVG).

MATERIALS AND METHODS

Plant material

Seeds of common bean (*Phaseolus vulgaris* L. CIAT breeding line DOR364) were obtained from CIAT in Cali, Colombia. Seeds were surface sterilized in 7 mM NaOCl and 0.1% Triton X-100 (Sigma Chemical Co., St. Louis, MO) for 10 min., and germinated in 0.5 mM $CaSO_4$ for 36 hours at 25°C. Seedlings were then planted at a depth of 3 cm into 1400-ml containers. All plants were grown in a greenhouse in University Park, Pennsylvania, USA (40° 85′ N, 77° 83′ W). Temperature ranged from a maximum of 26 °C (day) to a minimum of 19 °C (night). Natural light was supplemented from 900-1700 h with 110 ± 10 μmol m^{-2} s^{-1} from 400 W metal-halide bulbs (Energy Technics, York, Pennsylvania, USA). Maximum midday photosynthetically active photon flux densities reached 1400 μmol m^{-2} s^{-1} on clear days and 500 μmol m^{-2} s^{-1} on days with heavy cloud cover.

Phosphorus treatments

Plants were grown in silica sand containing solid-phase-phosphorus buffer (alumina) as described by Lynch *et al.* (1990) with two different desorption concentrations allowing precise management of phosphorus concentrations. Once a day, pots were irrigated with nutrient solution containing (in mM) 3.1 NO_3, 1.8 K, 1.2 Ca, 1.4 SO_4, 1.0 NH_4, 0.825 Mg, 0.05 Cl, 0.005 Fe-EDTA, 0.002 B, 0.0015 Mn, 0.0015 Zn, 0.000143 Mo, and 0.0005 Cu. Sufficient and deficient phosphorus treatments contained 50 μM and 1 μM KH_2PO_4, respectively, in the nutrient solution, in addition to the phosphorus desorbed from the alumina-phosphorus buffer. Leachate sampled from the pots was analysed using the phosphomolybdenum blue method (Murphy and Riley, 1962) giving phosphorus levels in the soil solution of 8.6 (±0.9) μM for sufficient and 1.1 (±0.03) μM for deficient treatments.

Ethylene and AVG treatments

In a preliminary experiment, common bean plants were irrigated with a phosphorus-sufficient nutrient solution mixed with a range of AVG concentrations (Fig. 1) to determine the lowest AVG concentration which would inhibit ethylene production from the roots without affecting growth. Roots were excised from 3 week old plants, cut into 1 cm pieces and placed in a 150 ml flask containing the same AVG concentration as used for irrigation during growth. The flask was sealed with a rubber stopper and held for 5 hours in darkness at 20 °C. A 1 ml sample was then collected from the head space for ethylene analysis by gas chromatography (GC). The GC (Hewlett-Packard 6890, Palo Alto, California) was fitted with a flame ionization detector and a column of activated alumina. The detection limit was 0.013 μL L^{-1}. To examine the effect of ethylene on root development, bean plants were grown under sufficient (50 μM) and deficient (1 μM) phosphorus in the presence of AVG plus one of 4 ethylene concentrations (0, 0.02, 0.1, 1 μL L^{-1}). Control plants were grown without AVG or ethylene. Roots were exposed to different ethylene concentrations without affecting the shoot atmosphere by growing each bean plant in the apparatus shown in Fig. 2 (Bouma et al., 1997). Different ethylene concentrations were obtained by mixing ethylene (from 0.1 % or 0.004% gas bottle) into a stream of ambient air, using a mass flow controller (Brooks 5800 series, Brooks Instrument B.V., Veenendaal, Holland) for the two low concentrations and rotameters for the high concentration. Each container was provided with a flow of 500 ml min^{-1} except for the control (no AVG) and AVG + 0 [C_2H_4] treatment.

Measuring ethylene concentrations in the root zone

Ethylene concentrations in the root zone were measured to determine the effectiveness of our ethylene perfusion treatments, and to determine ethylene production rates by intact root systems in the absence of AVG. To allow gas sampling, small tubes were inserted through the sides of the containers at 14 cm depth before planting (Fig. 2). These were open to the root zone on the inside and sealed with a serum stopper on the outside so that the gas space inside would be in equilibrium with the gas space in the root zone. Ethylene samples (1 ml) were withdrawn from this space and analyzed by GC. There was no observable diurnal fluctuation in ethylene production, based on sampling every 4-6 hours.

Root growth and development

Plants grown with the two different phosphorus availabilities were harvested after 4 weeks of ethylene exposure (just before anthesis). Leaf area, root length and biomass of leaves, stem and roots were determined. Roots were excavated by rinsing the sand with deionized water. A subsample of the root (including 2 whole basal roots) was collected randomly from each plant and stained for 1 h prior to scanning to create optimal contrast (0.16 g L^{-1} neutral red dye, Sigma Chemical Co., St. Louis, MO). Leaves and the root subsamples were scanned using a flat bed scanner (HP ScanJet II, resolution = 140 dots per mm^2, Hewlett Packard, USA). Leaf area, root length, and root diameter distribution were estimated using image analysis software (Delta-T SCAN, Delta-T Devices Ltd., Cambridge, England). Total root length was estimated by multiplying the subsample length by the dry weight (DW) ratio of the scanned subsample and the total dry weight of the root. Average lateral root length and density were determined from the scanned subsample. Main root length was estimated from the ratio between lateral root length and basal root length in the scanned subsample. Specific root length was calculated by dividing total root length by root dry weight. Plant material was dried at 70 °C for 48 h prior to dry matter determinations.

Statistical analysis

Data were analyzed using the PC-statistical package JMP® (SAS Institute 1989-91). Data with one variable were analyzed using student t-test for each pair at $P<0.05$. Data with more variables were analyzed with ANOVA (randomized block design) and mean separations were performed by contrast for each parameter by t-test and F-test for all parameters tested jointly. When data were not normally distributed, they were log transformed before conducting the ANOVA.

RESULTS

Effect of phosphorus deficiency on root and shoot growth

Phosphorus-deficiency reduced total root length (main plus lateral roots) by more than a third (Fig. 3). Total lateral root length (product of mean lateral root length (LRL) and number of lateral roots) was significantly reduced by phosphorus deficiency (Fig. 3). This was a result of the reduction in lateral root number (Fig. 3), since mean LRL was unaffected by phosphorus treatment (Table 1). Lateral root density (number of laterals per cm of main root) was significantly reduced in phosphorus-deficient roots compared to phosphorus-sufficient roots (Fig. 4). Phosphorus deficiency reduced dry weight (DW) of roots by about one-third (Table 2), but there was a proportionately greater reduction in shoot DW, nearly doubling the root-to-shoot ratio (Fig. 4).

There was no difference between the sufficient and deficient phosphorus treatments in ethylene concentrations in the root zones. However, ethylene production per g DW was significantly higher in phosphorus-deficient roots compared to phosphorus-sufficient roots (Table 2).

Manipulation of ethylene with AVG and ethylene perfusion

A preliminary dose-response experiment was used to determine the effect of AVG on growth and ethylene production by roots of bean plants grown with sufficient phosphorus. Ethylene production by excised roots was reduced to 10% of that of control roots when plants were irrigated with at least 1.28 μM AVG (Fig. 1). Consequently 1.3 μM AVG was used in our experiments, resulting in ethylene concentrations below detection level (<0.013 $\mu L\ L^{-1}$ ethylene) in pots irrigated with AVG and without ethylene perfusion (Table 3).

Ethylene treatments to the root zones of AVG-treated plants resulted in stable ethylene concentrations bracketing the physiological range (Table 3). There were no significant differences between phosphorus-sufficient and deficient plants in ethylene concentrations in the root zones.

Interactions between phosphorus and ethylene

AVG and AVG+ethylene treatments resulted in a small increase in shoot DW in both phosphorus treatments (Fig. 4). The small increase in shoot DW (from 0.49 to 0.54 g, Fig. 4) combined with a small decrease in root DW (0.23 to 0.19 g) resulted in a significant reduction in the root-to-shoot ratio when phosphorus- deficient plants were treated with AVG (Fig. 4). There was a significant phosphorus x ethylene interaction for root to shoot ratio (Table 1).

Total root length (all main and lateral roots) was reduced by phosphorus deficiency and by ≥ 0.1 μL L^{-1} ethylene (Fig. 3, 5), but there was no significant interaction between phosphorus and ethylene for this variable (Table 1).

AVG treatment increased main root length of phosphorus-sufficient plants, an effect which was reversed by added ethylene (Fig. 4). The ethylene-modifying treatments had opposite, though smaller, effects on phosphorus-deficient plants (Fig. 4). Unlike the effects on total root length, high ethylene concentrations did not significantly reduce length of main roots compared with controls (no AVG, no ethylene) (Fig. 4). The effects of AVG and ethylene on total lateral root length resembled their effect on total root length, with no significant interaction between phosphorus and ethylene (Fig. 5, Table 1).

The reduction in main root length caused by AVG in phosphorus-deficient plants (Fig. 4) was not accompanied by a significant change in lateral root number (Table 1), resulting in an increase in lateral root density (Fig. 4). The AVG effect was reversed by low ethylene concentrations. AVG had the opposite effect on phosphorus-sufficient roots: it reduced lateral root density, probably by increasing main root elongation (Fig. 4).

AVG and ethylene reduced mean LRL regardless of phosphorus treatment (Fig. 5, Table 1). Although root diameter was unaffected by phosphorus treatment, the high ethylene treatment increased root diameter and reduced specific root length (cm mgDW^{-1}) (data not shown).

DISCUSSION

Effect of phosphorus deficiency on root architecture

The changes in root architecture observed under phosphorus deficiency suggest that resources are redistributed to the roots to allow continued exploration for phosphorus. Phosphorus deficiency resulted in inhibition of both root and shoot growth, but the disproportionate reduction in shoot growth relative to root growth led to a higher root-to-shoot ratio (Fig. 3). Main root length (Fig. 3, 4), and average lateral root length were not significantly affected by phosphorus deficiency (Table 1). Maintenance of growth of these roots would be expected to maximize the soil volume colonized by roots, and therefore access to phosphorus resources.

In real soils, phosphorus availability is non-uniform. From an economic perspective (Fitter, 1991; Bloom, 1985), an efficient root system would explore the soil extensively in order to locate phosphorus-rich patches or microsites, but once such a patch is found, should intensively exploit that patch. This strategy could be implemented if roots responded differently to the phosphorus availability in their immediate soil environment, such that branching was more intense in high phosphorus soil environment, whereas extension of main root axes was accentuated in low phosphorus soil environment. In previous research on plants grown with heterogeneous phosphorus distribution, roots growing in a low phosphorus patch elongate but do not proliferate (develop quantities of fine roots), while in a high-phosphorus patch, the reverse occurs: main root elongation is reduced, but proliferation is enhanced (Drew and Saker, 1978; Snapp et al., 1995). In this study, we find a similar response to phosphorus availability in homogeneous substrate, as has also been reported for *Chrysanthemum* (Hansen, 1997) and some families of *Pinus radiata* (Theodorou and Bowen, 1993). The ability of plants to sense and respond to local phosphorus availability suggests the presence of a signaling mechanism to regulate changes in plant growth.

Does ethylene mediate root architectural responses to nutrient stress?

In non-AVG treated plants, phosphorus-deficient roots produced twice as much ethylene per gram as phosphorus-sufficient roots, resulting in similar root zone ethylene concentrations despite reduced root biomass in phosphorus-deficient plants (Table 2). Ethylene concentrations in the root tissues would be higher in phosphorus-deficient roots than in phosphorus-sufficient roots, since the production rate is higher. Increased internal ethylene could mediate some of the observed growth and development responses to phosphorus deficiency.

When endogenous ethylene production was greatly reduced by AVG, there was a significant interaction between phosphorus availability and ethylene treatment, indicating that phosphorus deficiency changes tissue responsiveness to ethylene. Ethylene could therefore mediate changes in root morphology via both changes in synthesis and changes in tissue responsiveness. Low endogenous ethylene has been shown to promote root extension in seedling roots for some species, although higher concentrations generally inhibit root growth (Abeles, 1992; Konings, 1979; Jackson, 1985; Jackson 1991; Smith and Robertson, 1971). We show here that the effect of ethylene on root length depends on the root type (main or lateral root) and on phosphorus nutrition. In phosphorus-sufficient plants, main root length was increased by AVG treatment (Fig. 4), indicating that endogenous ethylene production limits main root growth. In phosphorus-deficient plants, AVG inhibited main root growth and exogenous ethylene reversed this effect (Fig. 4), indicating that ethylene promotes root extension under phosphorus deficiency. This is consistent with the hypothesis that ethylene action makes possible the maintenance of root extension under phosphorus-deficient conditions, despite an overall reduction in root growth.

Ethylene appears to be important for the reduction in lateral root density observed in phosphorus-deficient plants. AVG reduced lateral root density in phosphorus-sufficient plants but increased it in phosphorus- deficient plants; both effects were reversible with exogenous ethylene. Because lateral root number was unaffected in both phosphorus treatments (Fig. 3), the observed AVG effect on lateral root density (Fig. 5) is probably a result of the opposite effects of AVG on main root length (Fig. 4). Previous research on the effect of ethylene on root growth utilized plants supplied with sufficient or surplus phosphorus. The present study shows that response of roots to ethylene depends on phosphorus availability. By irrigating with low, non-toxic AVG concentrations and flushing the root zone with ethylene, we succeeded in exposing roots to ethylene concentrations in the physiologically relevant range. This method allowed us to show that ethylene permits continued root extension, even under phosphorus deficiency. Moreover, ethylene seems to be involved in the reduction of lateral root density that occurs in low phosphorus soil environments, which may be advantageous for efficient soil exploration. These findings support the hypothesis that ethylene may be a global regulator of root responses to soil nutrient availability (Lynch and Brown, 1997).

REFERENCES

Abeles F.B., Morgan P.W. & Saltveit M.E. (1992) *Ethylene in Plant Biology*. San Diego, Academic Press, Inc. p. 113-114.

Baldwin J.P. (1975) A quantitative analysis of the factors affecting plant nutrition uptake from some soils. *Journal of Soil Science* **26**:195-206.

Barber S.A. (1995) *Soil nutrient bioavailability: a mechanistic approach*. 2nd ed. Wiley, NY.

Bloom A., Chapin F.S. III & Mooney H. (1985) Resource limitation in plants - an economic analogy. *Annual Review of Ecology and Systematics* 16:363-392.

Bouma T.J., Neilsen K.L., Eissenstat D.M. & Lynch J.P. (1997) Soil CO_2 concentration does not affect growth or root respiration in bean or citrus. *Plant, Cell and Environment* 20: 1495-1505.

Doland L. (1997) The role of ethylene in the development of plant form. *Journal of Experimental Botany* **48**:201-210.

Drew M.C. & Saker L.R. (1978) Nutrient supply and the growth of the seminal root system in barley. III. Compensatory increase in growth of lateral toots, and in rates of phosphate uptake, in response to a localized supply of phosphate. *Journal of Experimental Botany* **29**: 435-451.

Drew M.C., He C.J. & Morgan P.W. (1989) Decreased ethylene biosynthesis, and induction of aerenchyma, by nitrogen- or phosphate-starvation in adventitious roots of *Zea mays* L. *Plant Physiology* **91**: 266-271.

Eissenstat D.M. (1992) Costs and benefits of constructing roots of small diameter. *Journal of Plant Nutrition* **15**:763-782.

Fitter A.H. (1991) The ecological significance of root system architecture: an economic approach. In: *Plant Root Growth: An Ecological Perspective*. (ed: D. Atkinson). pp. 229-243. Blackwell Scientific Publications, Oxford.

Hansen C.W. & Lynch J. (1998) Response to phosphorus availability during vegetative and reproductive growth of chrysanthemum: II. Biomass and phosphorus dynamics. *Journal of the American Society for Horticultural Science* 123:223-229.

He C.-J., Morgan P.W. & Drew M.C. (1992). Enhanced sensitivity to ethylene in nitrogen- or phosphate-starved roots of *Zea mays* L. during aerenchyma formation. *Plant Physiology* **98**:137-142.

Jackson M. (1985) Ethylene and the responses of plants to excess water in their environment - a review. In *Ethylene and Plant Development* (eds. J. Roberts & G. Tucker), pp. 241-265. Butterworths, London.

Jackson M.B. (1991) Ethylene in root growth and development. In: *The Plant Hormone Ethylene.* (eds. A. Mattoo & J. Suttle), pp. 159-181. CRC Press, Boca Raton, FL.

Jungk A. (1987) Soil-root interactions in the rhizosphere affecting plant availability of phosphorus. *Journal of Plant Nutrition* **10**:1197-1204.

Konings H. & Jackson M.B. (1979) A relationship between rates of ethylene production by roots and the promoting and inhibiting effects of exogenous ethylene and water on root elongation. *Zeitschrift für Pflanzenphysiology* **92**:385-397.

Lynch J. & Brown K.M. (1997) Ethylene and plant responses to nutritional stress. *Physiologia Plantarum* **100,** 613-619.

Lynch J., Epstein E., Läuchli A. & Weigt G. I. (1990) An automated greenhouse sand culture system suitable for studies of phosphorus nutrition. *Plant, Cell and Environment* **13**: 547-554.

Murphy J. & Riley J.P. (1962) A modified single solution reagent for the determination of phosphate in natural waters. *Analytica Chemica Acta* **27**:3136.

Nye P.H. & Tinker P.B. (1977) *Solute movement in the soil-root system.* Studies in Ecology, Vol 4. Blackwell Scientific Publications, Oxford.

Smith K.A. & Robertson P.D. (1971) Effect of ethylene on root extension of cereals. *Nature* **243**:148.

Snapp S., Koide R. & Lynch J. (1995) Exploitation of localized phosphorus patches by common bean roots. *Plant and Soil* **177**:211-218.

Tari I. & Szén L. (1995) Effect of nitrite and nitrate nutrition on ethylene production by wheat seedlings. *Acta Phytopathologica et Entomologica Hungarica* **30**:99-104

Theodorou C. & Bowen G.D. (1993) Root morphology, growth and uptake of phosphorus and nitrogen of *Pinus radiata* families in different soils. *Forest Ecology and Management* **56**: 43-56.

Ycas J.W. & Zobel R.W. (1983) The response of maize radicle orientation to soil solution and soil atmosphere. *Plant and Soil* **70**:27-35.

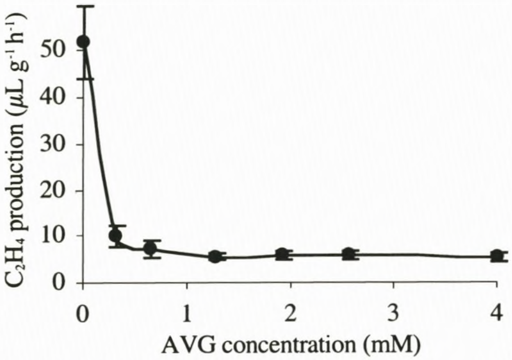


Fig. 1. Ethylene production rate by excised roots irrigated with different concentrations of AVG (aminoethoxyvinylglycine), an ethylene biosynthesis inhibitor. Bars indicate standard error of mean.

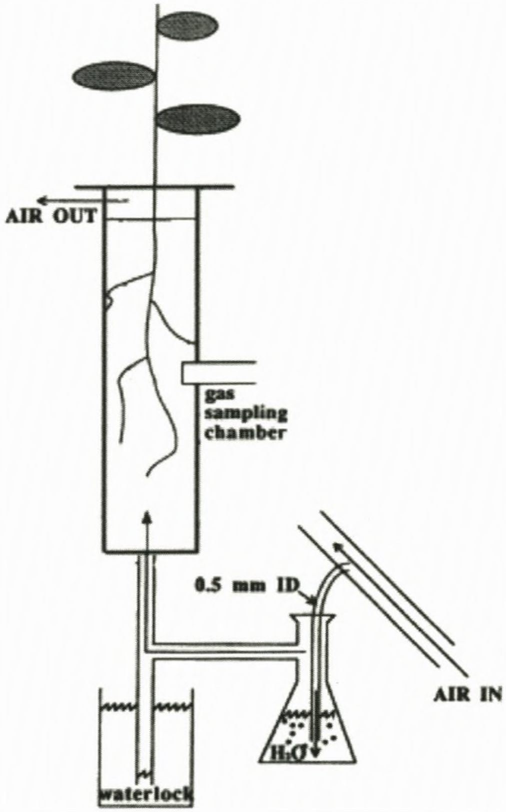


Fig. 2. Apparatus used to expose roots to different gas-mixtures. Air with a known ethylene concentration was pumped into the bottom of a container filled with sand. The incoming air was bubbled through water, which prevented desiccation of the root zone and provided a check on the flow into each pot. Equal flow rates in each pot were obtained by using 0.5 mm ID tubing from the main gas source to the Erlenmeyer flask. Irrigation solution could drain freely through the water lock. The air outlet was on the side of the pots, above the sand surface. Shoots were kept at ambient gas composition by sealing the top of each pot and pumping air from the head-space out of the greenhouse. A flexible sealant (Terostat) was used to create a gas tight seal around the stem. Gas-sampling chambers were placed at 14 cm depth.

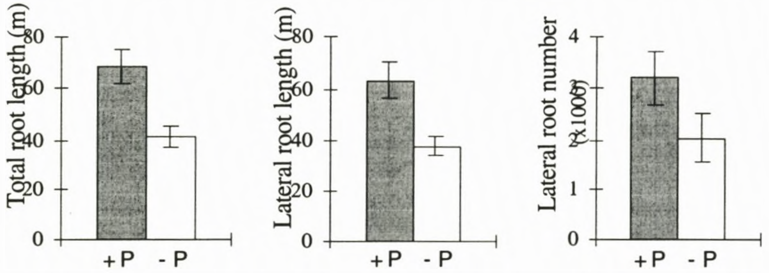


Fig. 3. Effect of sufficient (+P) and deficient (-P) phosphorus concentrations in the growth media on total root length, total lateral root length, and lateral root number (data pooled across ethylene treatments). Bars indicate standard error of mean.

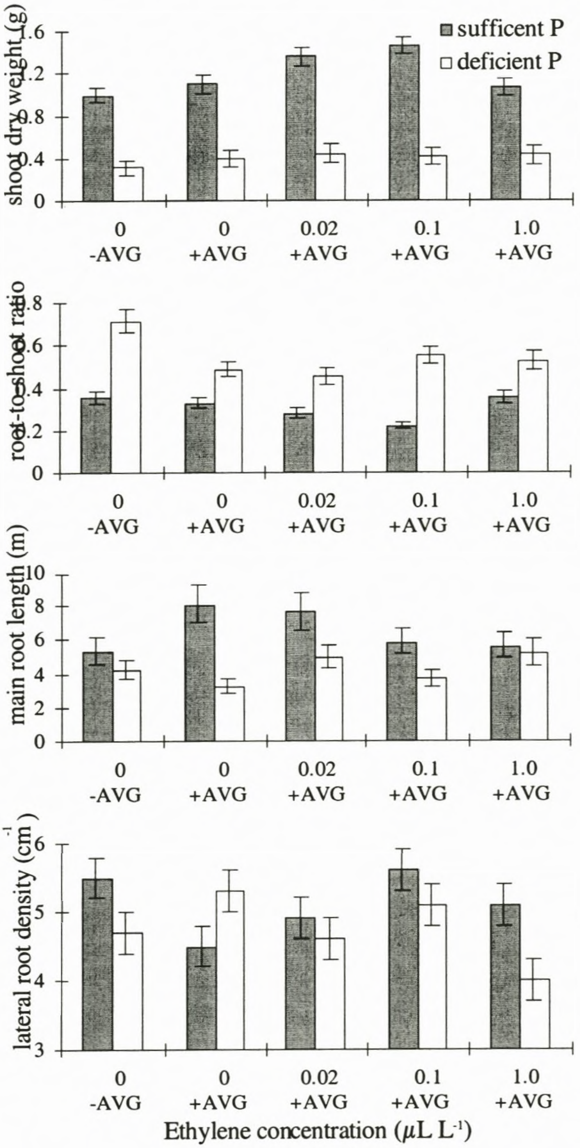


Fig. 4. The effect of AVG and ethylene treatments on shoot dry weight (DW), root-to-shoot ratio, main root length and lateral root density of phosphorus-sufficient or deficient plants. Bars indicate standard error of mean.

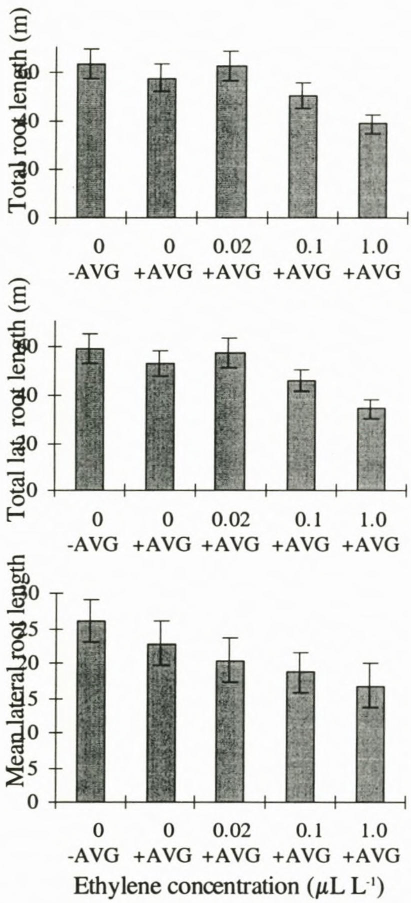


Fig. 5. Effect of AVG and various ethylene concentrations in the root zone on total root length (main plus lateral roots), total lateral root length, and average lateral root length (data pooled across phosphorus treatments). Bars indicate standard error of mean.

Table 1. ANOVA for data presented in Fig. 3, 4 and 5.

Variable	statistic	C_2H_4	Phosphorus	Phosphorus x C_2H_4	error	C total
Shoot DW (g)	df	4	1	4	90	99
	F	4.6	238	2.2		
	P	0.01	0.05	n.s.**		
root to shoot ratio	df	4	1	4	90	99
	F*	5.8	132	3.4		
	P	0.01	0.01	0.01		
total root length	df	4	1	4	90	99
	F*	6.2	49.7	1.3		
	P	0.01	0.01	n.s.		
main root length	df	4	1	4	75	84
	F*	1.3	19.8	2.5		
	P	n.s.	0.01	0.05		
total lateral root length	df	4	1	4	90	99
	F*	6.8	49.1	0.5		
	P	0.01	0.01	n.s.		
number of lateral roots	df	4	1	4	73	82
	F*	0.6	13.3	0.4		
	P	n.s.	0.01	n.s.		
lateral root density	df	4	1	4	86	95
	F*	2.2	4.2	3.5		
	P	n.s.	0,05	0.01		
mean lateral root length	df	4	1	4	72	81
	F*	2.6	0.4	0.3		
	P	0.05	n.s.	n.s.		

* data log transformed for statistical analysis

**the designation n.s. denotes no significant difference at $P<0.05$. Values are presented as mean ± standard error of mean.

Table 2. Root dry weight (DW), ethylene concentration in the root zone, and ethylene concentration per g root DW in 5 week old bean plants grown with sufficient or deficient phosphorus nutrition. Values shown are means of 10 plants ± standard error of mean. Values within columns followed by different letters indicate significant differences in a student t-test at $P<0.05$.

Phosphorus treatment	root DW (g)	root zone [C_2H_4] (μL L^{-1})	C_2H_4 gDW^{-1} root
sufficient	0.35 ±.02 a	0.027 ±.002 a	0.078 ±.007 a
deficient	0.25 ±.02 b	0.025 ±.002 a	0.124 ±.018 b

Table 3. Ethylene concentration in the root zone of ethylene and AVG-treated plants. Plants without AVG were not perfused with either ethylene or air in the root zone. Ethylene data are pooled for sufficient and deficient phosphorus plants irrigated with AVG because there were no significant differences between phosphorus treatments. Values shown are means ± standard error of mean.

Ethylene in perfused air (μL L^{-1})	Phosphorus (μM)	AVG (μM)	Ethylene (μL L^{-1})	N
0	sufficient (50)	0	0.027 ±.007	10
0	deficient (1)	0	0.025 ±.007	10
0	50 + 1	1	not detectable	20
0.02	50 + 1	1	0.026 ±.009	20
0.1	50 + 1	1	0.12 ±.036	20
1	50 + 1	1	1.12 ±.31	20

Growing pot plants with reduced phosphorus can improve root structure and drought avoidance

Kristian Borch
Danish Institute of Agricultural Sciences, Department of Ornamentals,
Kirstinebjergvej 10 DK-5792 Årslev

To increase consumer satisfaction, growers must produce high-quality plants which are compact, stress tolerant, and free from diseases. This often does not harmonise with growers' attempts to keep the production period as short as possible, by growing bedding plants with optimum light, temperature and a surplus of fertiliser to maximise growth rate. These conditions often produce plants with elongated, lush shoots, poorly developed roots, and a poor stress tolerance. Therefore, it is recommended that before shipping growers harden their plants with a short period of lower temperature and reduced fertiliser and water at the end of the production cycle (Serek, 1990). This practice encourages root growth at the expense of shoot growth. This is advantageous because plants with well-developed root systems which exploit the medium uniformly and with room for further growth are best at withstanding the fluctuations in soil moisture which occur during shipping and handling and in the hands of the consumers. Thus it might be possible to improve plant quality and stress tolerance even more by encouraging strong root growth during production by reducing the phosphorus (P) levels in the root zone.

Ornamental plants are typically grown with phosphorus levels much higher than those found in fertile soils, which may have detrimental effects. Studies using alumina-buffered phosphorus fertiliser (Al-P) show that plants can grow well at P levels as low as 10 µM P (Lynch et al., 1991, Borch et al., 1997a). We have investigated the effects of marigold (*Tagetes x hybrida 'Janie Tangarine'*) grown with reduced phosphorus on development and quality using Al-P. We specifically tested the hypothesis that low P would improve resistance or avoidance of this bedding plant to postproduction drought stress. We found that when plants grown with Al-P were exposed to drought at the marketing stage, they respired less water (Fig. 1) and therefore wilted more slowly compared to high P control plants. The low P treatment resulted in a 50% reduction in leaf area which will naturally reduce the transpiration allowing the plant to avoid drought stress for extended periods. Moreover, the roots in the Al-P buffered plants were more evenly distributed throughout the growth media whereas the roots in the high P control plants were confined to an area under the drip tube (Fig. 2). The explanation for this improved root distribution is probably the lack of a guiding P gradient and the Al-P buffered growth media. Thus, reduced leaf area together with improved root distribution in marigold plants can account for the improvement in drought avoidance in the Al-P plants.

Root structure is very important for P acquisition when P resources are scarce because root uptake of P is predominated by diffusion, due to the very low concentrations of this ion in the free soil solution (Barber, 1984). Simultaneously, root architecture, proliferation, and mass affect the rate at which root systems remove moisture from soils (Faber et al., 1991), because the water flow in soil is often the largest resistance for the soil-plant-atmosphere continuum (Nobel, 1991). Therefore we investigated if the improved drought resistance in low P plants could indeed be related to improved root structure. It was evident that the plants responded to reduced P availability by decreasing lateral root density and increasing root length at the expense of shoot growth. When lateral root density is decreased and root length is increased soil volume exploration will be enhanced (Borch et al. 1997b) increasing the possibility of finding a P rich patch in which it will branch vigorously to exploit the P (Snapp et al., 1995). This will also have a positive effect when water becomes depleted and the transport distance becomes larger, because a reduced lateral root density will give a better 'space filling' capacity and an improved ability to explore the medium for e.g. water (Fitter, 1986) improving drought avoidance. Moreover, high lateral root density is expected to be irrelevant for water uptake resistance during low water potentials in the pot because the depletion zones overlap (Fitter et al., 1991).

Thus, reduced P will improve root structure and space filling capacity enhancing water utilisation. Moreover, the decreased leaf area will also reduce momentary water uptake and thereby save water resources. Both these features can improve water stress avoidance by the plant. If we better understand the changes in root architecture and morphology that occur during low P conditions, it will enable us to prepare an ideal root zone which will allow maximum growth and development of the root system and thereby improve plant tolerance to stress factors.

References

Borch, K., Brown, K.M. and Lynch, J. 1997a. Improvement of bedding plant quality and stress resistance with low phosphorus (submitted to HortTec).

Borch, K., Bouma, T., Brown K.M. and Lynch, J. 1997b. Interactions of ethylene and phosphorus nutrition on root growth. (submitted to Plant, Cell and Environment).

Faber, B.A., D.N. Muuns, K. Schackel, and R.J. Zasoski. 1991. A method of measuring hyphal nutrient and water uptake in mycorrhizal plants. Can. J. Bot. 69:87-94.

Fitter, A.H. 1986. The topology and geometry of plant root systems: Influence of watering rate on root system topology in *Trifolium pratense.* Ann. Bot. 58:91-101.

Fitter, A.H., T.R. Stickland, M.L. Harvey, and G.W. Wilson. 1991. Architectural analysis of plant root systems 1. Architectural correlates of exploitation efficiency. New Phytol. 118:383-389.

Lynch, J., Läuchli, A. and Epstein, E. 1991. Vegetative growth of the common bean in response to P-nutrition. Crop Science 31:380-387.

Nobel, P.S. 1991. Physicochemical and environmental plant physiology. Academic Press, San Diego, CA, p. 527.

Serek, M. 1990 Effects of pre-harvest fertilization on the flower longevity of potted *Campanula carpatica* 'Karl Foerster'. Scientia Horticulturae. Vol. 44:119-126.

Snapp, S. Koide, R. and Lynch, J. 1995. Exploitation of localized phosphorus patches by common bean roots. Plant and Soil. 177:211-218.

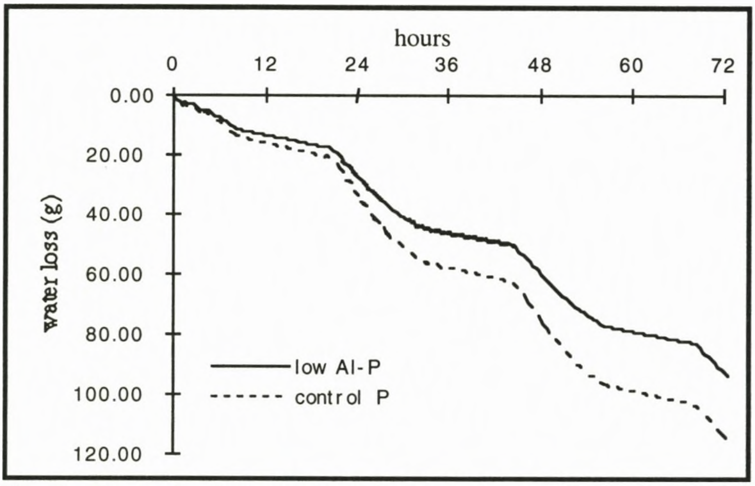


Fig. 1. Transpiration monitored as gravitrometric water loss (transpiration) from the soilless media of marigolds (*Tagetes x hybrida 'Janie Tangarine'*) in 10 cm pots at anthesis grown with two levels of P (control P and low P (Al-P)) for 72 hours. Values are means of 8 plants.

App. E: Growing pot plants with reduced P can improve root structure and drought avoidance

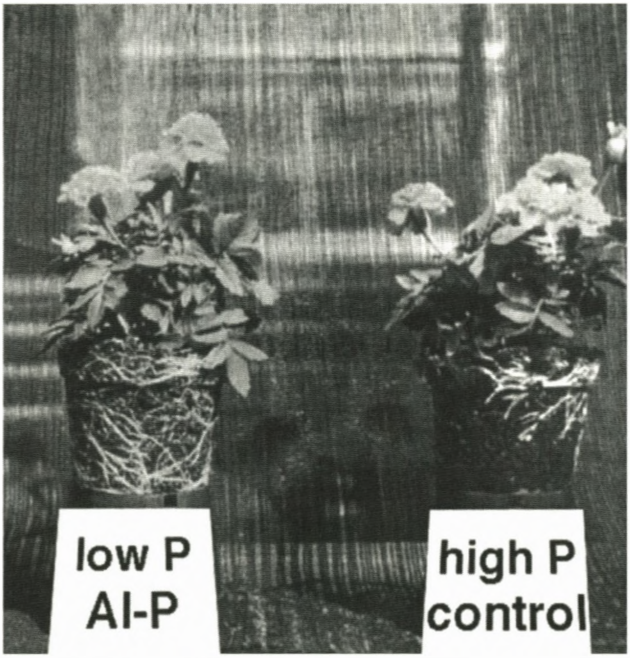


Fig. 2. Marigold (*Tagetes x hybrida 'Janie Tangarine'*) grown with high P applied through the irrigation water, and low P buffered from alumina charged with P. Note how the root of high P control plant is restricted to an area right under the drip tube.

Blad 7401 HRC 50866 689
Danmarks JordbrugsForskning, Flakkebjerg
Flakkebjerg Afd. for Plantebeskyttelse
4200 Slagelse

DIAS Foulum

P.O. Box 50, DK-8830 Tjele
Tel. +45 89 99 19 00. Fax +45 89 99 19 19

Management
Management Secretariat, Finance Secretariat

Department of Animal Product Quality
Department of Animal Breeding and Genetics
Department of Animal Nutrition and Physiology
Department of Animal Health and Welfare
Department of Agricultural Systems
Department of Crop Physiology and Soil Science

Department of Farm Management and Experimental Stations
Department of Animal Research Facilities
Central Laboratory
Public Relations
Computer Technical Services
Library
International Unit

DIAS Aarslev

Kirstinebjergvej 10, DK-5792 Aarslev
Tel. +45 63 90 43 43. Fax +45 65 99 17 22

Department of Ornamentals
Department of Fruit, Vegetable and Food Science
Department of Plant Research Services

DIAS Flakkebjerg

Flakkebjerg, DK-4200 Slagelse
Tel. +45 58 11 33 00. Fax +45 58 11 33 01

Department of Plant Biology
Department of Crop Protection
Department of Plant Research Services

DIAS Bygholm

P.O. Box 536, DK-8700 Horsens
Tel. +45 75 60 22 11. Fax +45 75 62 48 80

Department of Agricultural Engineering
Research Facilities, Bygholm

ISSN 1397-9876

Units at other locations

Department of Variety Testing
Teglværksvej 10, Tystofte, DK-4239 Skælskør
Tel. +45 58 16 06 00. Fax +45 58 16 06 06

Askov Experimental Station
Vejenvej 55, DK-6600 Vejen
Tel. +45 75 36 02 77. Fax +45 75 36 62 77

Biotechnology Group (Dept. of. Plant biology)
Thorvaldsensvej 40, 1.
DK-1871 Frederiksberg C
Tel. +45 35 28 25 88. Fax +45 35 28 25 89

Borris Experimental Station
Vestergade 46, DK-6900 Skjern
Tel. +45 97 36 62 33. Fax +45 97 36 65 43

The Ecological Experimental Station
Rugballegaard
P.O. Box 536, DK-8700 Horsens
Tel. +45 75 60 22 11. Fax +45 75 62 48 80

Foulumgaard, P.O. Box 50, DK-8830 Tjele
Tel. +45 89 99 19 00. Fax +45 89 99 19 19

Jyndevad Experimental Station
Flensborgvej 22, 6360 Tinglev
Tel. +45 74 64 83 16. Fax +45 74 64 84 89

Rønhave Experimental Station
Hestehave 20, DK-6400 Sønderborg
Tel. +45 74 42 38 97. Fax +45 74 42 38 94

Silstrup Experimental Station
Højmarken 12, DK-7700 Thisted
Tel. +45 97 92 15 88. Fax +45 97 91 16 96

Tylstrup Experimental Station
Forsøgsvej 30, DK-9382 Tylstrup
Tel. +45 98 26 13 99. Fax +45 98 26 02 11