Abstracts

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1.)

Density-shifts of Membranous Organelles in Synchronous *Chlorella sorokiniana* Dependent on Nitrogen Starvation

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Summary

Membranous compounds of *Chlorella sorokiniana* were separated and identified on sucrose gradients by the method already used for higher plants. The inhibition of plasmalemma Mg^{2+}/K^{+}ATPase by Na$_3$VO$_4$ was confirmed as a tool for plasmalemma (PL) identification. Dependent on the length of N-starvation all identified membranes shifted to lower densities and the protein/lipid ratio dropped from 0.300 to 0.057. The most obvious shift was observed for mitochondria. Refeeding with NO$_3^-$ results in an increase in the density which finally reaches that of the control. These results are discussed in view of a mobilization of membrane proteins.

Key words: Chlorella – isopycnic centrifugation – nitrogen starvation – membranes.

Introduction

The method of isopycnic sucrose gradient centrifugation was used for the separation of membranes from higher plants (review by Quail, 1979). This well known technique was tranferred to Desmidiaceae by Stabenau (1978).

In this paper we report a successful use of this method to demonstrate reversible density shifts of membranes from *Chlorella* organelles caused by both nitrogen starvation and recovery from nitrogen deficiency by feeding nitrate.

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3.)

The Effect of NO$_2$-Fumigation on Aseptically Grown Spruce Seedlings

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Summary

Spruce seedlings were cultivated aseptically, in gastight containers in which the shoot and root areas were separated. Fumigation with 500 ppb NO$_2$ (11 weeks) increased the nitrate content, the metabolic activities of the nitrate assimilating enzymes and glutamine synthetase only in the shoot. In the roots the nitrate reductase activity was reduced (50%) while glutamine synthetase activity remained unchanged. No effect of the fumigation was observed on the growth of lateral roots. The data are discussed with regard to detoxification of NO$_2$ via the nitrate assimilating pathway in the shoot. It seemed as if the enzymes of nitrate reduction were under substrate limitation in the shoot of spruce seedlings.

DER EFFEKT VON SCHWEFELDIOXID-, OZON- UND STICKSTOFFDIOXID-BEGASUNG AUF DEN STICKSTOFFMETABOLISMUS STERIL KULTIVIERTER FICHTENKEIMLINGE (*PICEA ABIES* (L.) KARST.)

ANDREAS D. PEUKE

ZUSAMMENFASSUNG

1.) Die Raten der Netto-Nitrataufnahme von Fichtenkeimlingen waren bis 1 mM Nitrat abhängig von der angebotenen Nitratkonzentration. Bis 10 mM blieben sie dann konstant. Die aus diesen Daten berechneten Charakteristika des Aufnahmesystems sind K_m = 0,2 mM und V_{max} = 18 nmol mg^{-1} $24h^{-1}$.

2.) Die Netto-Nitrataufnahmeraten wurden durch niedrigen pH-Wert, Aluminium- oder Ammoniumzugaben in die Nährlösung gehemmt und die Wurzelfrischmassen vermindert.

3.) Eine Bestimmung der Nitratreduktaseaktivität (NRA) *in vitro* war nicht möglich. Es wurde ein NR-Test *in vivo* erarbeitet, mit dem Messungen in Wurzeln und Sprossen von Fichtenkeimlingen möglich sind. Die höchsten NRA wurden in den Wurzelspitzen gefunden.

4.) Durch stickstofffreie Anzucht wurde die NRA und der Nitratgehalt in den Wurzeln verringert, in den Sprossen jedoch erhöht. Mit steigender Nitratkonzentration in der Nährlösung wurde in den Sprossen die NRA erhöht und der Nitratgehalt verringert. Aluminiumzugaben in die Nährlösung hatten eine Erhöhung der NRA in den Sprossen und in der Regel auch in den Wurzeln zur Folge. Ein Bezugsgrößeneffekt konnte dabei allerdings nicht ausgeschlossen werden. Der optimale pH-Wert der Nährlösung für die NRA in Wurzeln und Sproß (ebenso wie für die Nitrataufnahme) betrug 5,5. Ammonium in der Nährlösung hemmte die NRA und verringerte den Nitratgehalt in Wurzeln und Sprossen. Selbst bei reiner Ammoniumernährung war Nitrat und Ammonium in den Pflanzen zu finden.

5.) Nach neunwöchiger Begasung der gasdicht vom Wurzelraum getrennten Sprosse war bei einer Konzentration von 35 über 70 bis 100 ppb SO_2 der Nitratmetabolismus in den Sprossen gehemmt und in den Wurzeln stimuliert. Nach Begasung mit 200 ppb SO_2 waren Nitrataufnahme und Nitratreduktion auch in den Wurzeln gehemmt, und das Wachstum war vermindert. Die Hemmung des Nitratmetabolismus in den Sprossen wurde diskutiert als Effekt - einer verminderten Verfügbarkeit von Nitrat, - einer direkten Hemmung der Enzyme durch Sulfit oder dessen Folgeprodukte - einer Kompetition um Reduktionsäquivalente mit der reduktiven Detoxifizierung von SO_2. Es konnte gezeigt werden, daß bei der Entgiftung SO_2 reduziert und metabolisiert (in Thiolen) wurde und die Steigerung der Aktivität der Superoxiddismutase von Bedeutung ist. Die Beeinflussung des Metabolismus ging einher mit ultrastrukturellen Veränderungen in den Sprossen.

6.) Durch eine 60-tägige Begasung mit Ozon (50 ppb) und einer Kombination von Ozon (50 ppb) und SO_2 (35 ppb) wurde die NRA und die Aktivität der Dehydrogenasen MDH, GDH und G6PDH in den Sprossen vermindert. In den Wurzeln dieser Pflanzen war die NRA erhöht.

7.) Eine elf-wöchige Begasung der Sprosse mit 500 ppb NO_2 stimulierte das Nitrat-reduzierende System und erhöhte die Frischmasse, den Chlorophyll-, Protein- und Nitratgehalt im Sproß. Die Wurzeln waren bis auf die Hemmung von NR und NiR unbeeinflußt. Die Wasser- und Ionenaufnahme wurde durch die Begasung nicht verändert. Die Entgiftung des NO_2 erfolgt über Reduktion, Metabolisierung und Speicherung (in der Vakuole). Ein leichter Düngeeffekt war zu verzeichnen.

Dissertation Universität Göttingen 1987 p 105.

M. L. van Beusichem (Ed.), *Plant nutrition – physiology and applications*, 53–59.

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Effects of variation in nitrogen nutrition on growth of poplar (*Populus trichocarpa*) clones

4.)

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Key words: balsam poplar, hydroponic culture, nitrogen nutrition, *Populus trichocarpa*

Abstract

Green cuttings of six balsam poplar clones were cultivated in a hydroponic medium in a growth chamber under controlled conditions. The nitrogen nutrition was varied with regard to concentration, nature of N-source and nitrate/ammonium ratio. Production of biomass, pH changes in the rhizosphere and the consumption of nitrate and ammonium were investigated. Balsam poplar is sensitive to NH_4^+. The plants grew best without or at low NH_4^+ concentrations. In NH_4^+-only nutrient solution (1.8 m*M*) the plants died within 3–6 weeks, dependent on the clone. In the nutrient solution, pH shifts were found to be correlated with variation in the use of the two N sources. We exclude acidification of the rhizosphere as sole reason for plant death.

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5.)

Nitrate Uptake and Reduction of Aseptically Cultivated Spruce Seedlings, *Picea abies* (L.) Karst.

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ABSTRACT

Spruce (*Picea abies* (L.) Karst.) seedlings were aseptically cultivated and the effects of different N-nutrition on net uptake and reduction of nitrate were investigated. The characteristics of nitrate uptake were calculated, K_s as 0·2 mol m^{-3} and V_{max} as 18 μmol g^{-1} d^{-1}.

Low pH, NH_4^+ and Al^{3+} in the medium caused a decrease in nitrate uptake rate. An *in vivo* assay was set up which allowed the measurement of NRA in both roots and needles of spruce seedlings. The *in vivo* nitrate reductase activity was repressed by ammonium and stimulated by nitrate. Nitrate reduction was similar to nitrate uptake, negatively affected by low pH and ammonium. Therefore, a limited N-supply to spruce seemed to occur when pH was low in the rhizosphere combined with the presence of Al^{3+} and NH_4^+.

Key words: Spruce, nitrate uptake, nitrate reduction.

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6.)

The Uptake and Flow of C, N and Ions between Roots and Shoots in *Ricinus communis* L.

I. GROWN WITH AMMONIUM OR NITRATE AS NITROGEN SOURCE

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ABSTRACT

Seedlings of *Ricinus communis* L. cultivated in quartz sand were supplied with a nutrient solution containing either 1 mol m^{-3} NO_3^- or 1 mol m^{-3} NH_4^+ as the nitrogen source. During the period between 41 and 51 d after sowing, the flows of N, C and inorganic ions between root and shoot were modelled and expressed on a fresh weight basis. Plant growth was clearly inhibited in the presence of NH_4^+. In the xylem sap the major nitrogenous solutes were nitrate (74%) or glutamine (78%) in nitrate or ammonium-fed plants, respectively. The pattern of amino acids was not markedly influenced by nitrogen nutrition; glutamine was the dominant compound in both cases. NH_4^+ was not transported in significant amounts in both treatments. In the phloem, nitrogen was transported almost exclusively in organic form, glutamine being the dominant nitrogenous solute, but the N-source affected the amino acids transported. Uptake of nitrogen and carbon per unit fresh weight was only slightly decreased by ammonium. The partitioning of nitrogen was independent of the form of N-nutrition, although the flow of nitrogen and carbon in the phloem was enhanced in ammonium-fed plants. Cation uptake rates were halved in the presence of ammonium and lower quantities of K^+, Na^+ and Ca^{2+} but not of Mg^{2+} were transported to the shoot.

As NH_4^+ was balanced by a 30-fold increase in chloride in the solution, chloride uptake was increased 6-fold under ammonium nutrition.

We concluded that ammonium was predominantly assimilated in the root. Nitrate reduction and assimilation occurred in both shoot and root. The assimilation of ammonium in roots of ammonium-fed plants was associated with a higher respiration rate.

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7.)

THE EFFECTS OF SO_2 FUMIGATION ON THE NITROGEN METABOLISM OF ASEPTICALLY GROWN SPRUCE SEEDLINGS

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((Received 18 May 1991; accepted 28 October 1992)

Abstract

Aseptically grown spruce seedlings were cultivated in a hydroponic system, where the roots were separated from the shoots by a gastight, silicone material. The plants were fumigated with four SO_2 *concentrations (93, 190, 270 and 530* $\mu g\ m^{-3}$*) for nine weeks. Up to 270* $\mu g\ m^{-3}$ *of* SO_2*, an inhibition of nitrogen metabolism (enzyme activities of nitrate reductase (NR) and glutamine sythetase (GS) and nitrate content) in the shoot was compensated by a stimulation in the root, while nitrogen uptake was unaffected. Only the treatment with 530* $\mu g\ m^{-3}$ *of* SO_2 *decreased enzyme activities, nitrate content in both roots and shoots as well as nitrate uptake, and inhibited the growth of plants. Increases in the content of thiols and superoxidismutase activity are discussed in terms of* SO_2 *detoxification.*

8.)

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The uptake and flow of C, N and ions between roots and shoots in *Ricinus communis* L.

II. Grown with low or high nitrate supply

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Abstract

Seedlings of *Ricinus communis* L. cultivated in quartz sand were supplied with a nutrient solution containing either 0.2 mol m^{-3} NO_3^- or 4.0 mol m^{-3} NO_3^- as the nitrogen source to obtain insufficiently (low supply, nitrogen-limited) or well-fed plants (high supply, control). During the period between 41 and 51 d after sowing, the flows of C, N and inorganic ions between root and shoot were modelled on the basis of empirical observation and expressed on a fresh weight basis. With a low nitrate supply the biomass production was decreased while the root/shoot ratio was drastically increased and the water content in the shoot was slightly reduced. Nitrogen was transported in the xylem mainly in the form of nitrate in both treatments. However, in nitrate-limited plants the ratio of nitrate to total nitrogen was lower, indicating a higher fraction of whole-plant nitrate reduction occurring in the root. The spectrum of amino acids in phloem sap was changed due to N-limitation. Nitrate and cation uptake, as well as photosynthesis was strongly decreased in nitrate-limited plants. The partitioning of C, N and ions was shifted in favour of the root compared to well-fed plants. Transport of C, N, and cations in the xylem was decreased. Flows of ions and elements in the phloem were increased relative to uptake and xylem transport. In contrast, the chloride flows were nearly the same in low- and well-fed plants, pointing to a role of chloride as a compensating ion for nitrate.

Key words: N-deficiency, *Ricinus communis*, transport, xylem, phloem, nitrogen, carbon, cations, chloride.

Introduction

The level of nitrogen supply is one of the major factors which can determine the growth of plants. In natural habitats the concentration of nitrate in the soil solution is around 1 mol m^{-3} or lower and can rise in agriculturally influenced regions up to 20 mol m^{-3} and more (Andrews, 1986). However, nitrate is not only a nitrogen source. In higher plants environmental nitrate affects several processes including the induction of a high affinity nitrate transport system, changes in root morphology and increased root respiration (Redinbaugh and Campbell, 1991). Intracellularly nitrate may act as an important osmoticum (Smirnoff and Stewart, 1985) together with malate which is generated to prevent intracellular alkalization during nitrate assimilation. The internal nitrogen percentage vary only slightly (2–5% on a dry weight basis; Marschner, 1986) in well-grown plants. Limited nitrogen supply causes reduced growth and morphological changes (Clarkson and Hanson, 1980; Marschner, 1986).

The level of nitrogen supply also affects the translocation, allocation and distribution of assimilates and nutrients in the whole plant. In *Triticum aestivum*, growth and the translocation of C and N was influenced by different levels of nitrate (Lambers *et al.*, 1982). Fetene *et al.* (1993) followed the distribution of labelled assimilates after a $^{14}CO_2$-pulse in *Urtica dioica* fed with three levels of nitrate. Duarte and Larsson (1993) induced two distinct relative growth rates in *Pisum sativum* by applying different relative nitrate addition rates, which influenced the translocation of nutrients, including nitrogen. The level of nitrate also changed the partitioning of nitrate reduction between shoot and root in *Prunus persica* (Gojon *et al.*, 1991) and in *Nicotiana tabacum* (Rufty *et al.*, 1990).

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The uptake and flow of C, N and ions between roots and shoots in *Ricinus communis* L.

III. Long-distance transport of abscisic acid depending on nitrogen nutrition and salt stress

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Abstract

Seedlings of *Ricinus communis* L. were cultivated in quartz sand and supplied with media which contained either different concentrations of nitrate or ammonium nitrogen and were treated with a low salt stress. The concentration of ABA was determined in tissues and in xylem and phloem saps. Between 41 and 51 day after sowing, abscisic acid (ABA) flows between roots and shoots were modelled. Long-distance transport of ABA was not stimulated under conditions of nitrate deficiency (0.2 mol m^{-3}). However, when ammonium was given as the only N source (1.0 mol m^{-3}), ABA transport in both xylem and phloem was increased significantly. Mild salt stress (40 mol m^{-3} NaCl) increased ABA transport in nitrate-fed plants, but not in ammonium-fed plants. The leaf conductance was lowered by salt treatment with both nitrogen sources, but it was always lower in ammonium-fed compared to nitrate-fed plants. A negative correlation of leaf conductance to ABA levels in leaves or flow in xylem was found only in comparison of ammonium-fed to nitrate-fed plants.

Key words: Abscisic acid, ammonium, *Ricinus communis*, phloem, xylem, transport, nitrate, nitrogen nutrition.

Introduction

It is known that the growth, morphology and physiology of higher plants are affected by cultural conditions. Nitrogen is arguably the most important nutrient (Clarkson and Hanson, 1980; Runge, 1983; Marschner, 1986), and nitrogen deficiency or an unfavourable nitrogen source may result in the transmission of a stress signal from root to shoot. Plants growing in nitrogen-deficient situations often show symptoms which resemble the xeromorphic responses of droughted plants (Stålfelt, 1956; Fahn and Cutler, 1992; Mothes, 1932; Simonis, 1948; Lundkvist, 1955, 1956). Some workers have also demonstrated an accumulation of ABA in the leaves of nitrogen-deficient plants, but in most cases the effect was small (tobacco: Mizrahi and Richmond, 1972; sunflower: Goldbach *et al.*, 1975; potato: Krauss, 1978; Marschner and Krauss, 1981; Krauss and Marschner, 1982; tomato: Chapin, 1990; Daie *et al.*, 1979; grapevine: Scienza and Düring, 1980; cotton: Radin and Ackerson, 1981; Radin *et al.*, 1982; barley and tomato: Chapin *et al.*, 1988). This may be due to analysis of total tissue extracts. Davies and Zhang (1991) and Zhang and Davies (1990) have demonstrated that drought-dependent ABA increases become much clearer when distinct compartmental fractions such as xylem or apoplastic saps were analysed. We have investigated the influence of N-nutrition on ABA concentrations in the transport fluids and organs of *Ricinus communis* and have used these data to describe quantitatively ABA flows in relation to N-nutrition. Similar work was done before by Wolf *et al.* (1990) with salt-stressed lupins. In the present paper, ABA flows were modelled for plants receiving different nitrate concentrations (0.2, 1.0 and 4.0 mol m^{-3}), ammonium (1.0 mol m^{-3}) and salt stress (40 mol m^{-3} NaCl with 1.0 mol m^{-3}

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Abbreviations: ABA: abscisic acid; dwt: dry weight; fwt: fresh weight.

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10.)

Effects of nitrogen source, nitrate concentration and salt stress on element and ion concentrations in transport fluids and on C and N flows in *Ricinus communis* L.

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Key words: N-nutrition, phloem, *Ricinus communis*, salt stress, transport, xylem

Abstract

Ricinus communis L. was supplied with 0.2, 1.0, 4.0 m*M* nitrate or 1.0 m*M* ammonium and treated with a mild salt stress 40 m*M* NaCl (1.0 m*M* nitrate or ammonium). Between 41 and 51 days after sowing, element and ion concentrations in xylem and phloem sap were determined, and flows of C and N were modelled. Nutritional conditions particularly affected anion concentrations in the root-pressure xylem sap. Nitrate was the major N-compound in xylem sap of nitrate-fed, and amino acids in that of ammonium-fed plants. Lower nitrate was compensated mainly by chloride as an anion and by amino acids as a N-solute. Under salt treatment, Na^+ and Cl^- levels increased, but a high selectivity of ion uptake into the xylem was observed. The phloem sap was less affected by nutritional conditions; only under stress conditions higher ion concentrations in the xylem, i.e. mainly of Na^+ and Cl^-, reflected in the phloem sap. Most of the N taken up was first transported to the shoot. In plants provided with adequate N, 70 – 77% of the N was incorporated into the shoot. This partitioning was shifted in favour of the shoot in salt-stressed, and in favour of the root, in N-limited plants, in which a net export of N from the shoot occurred. Salt stress and N-limitation decreased the photosynthetic and respiratory rates in *Ricinus* shoots, root respiration was stimulated by ammonium assimilation. Higher N assimilation in the root increased the proportion of C transported to the root, which was used there for respiration. Concomitantly more amino acids were translocated and led to higher recycling of carbon to the shoot via the xylem.

11.)

VI. Nitrate or Ammonium Uptake and Transport, and Rapid Regulation of Nitrate Reduction in Higher Plants

By Andreas D. Peuke and Werner M. Kaiser

1. General Introduction

Higher plants acquire nitrogen from the soil mainly in the form of nitrate and/or ammonium. The two N sources are taken up by the roots, where part of the nitorgen can be utilized directly or stored (mainly as nitrate). If nitrate or ammonium uptake exceed storage and utilization by roots, part of the inorganic nitrogen will be transported to the shoot, where it can be reduced and metabolized or stored as before. The first two sections of this chapter review physiological aspects of nitrate and ammonium uptake by the roots and their transport to the shoot. The third section focuses on the aspect of a rapid regulation of nitrate reduction in roots and shoots by environmental factors such as light, CO_2, or oxygen availability.

Progress in Botany, Vol. 57

12.)

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The uptake and flow of C, N and ions between roots and shoots in *Ricinus communis* L.

IV. Flow and metabolism of inorganic nitrogen and malate depending on nitrogen nutrition and salt treatment

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Received 28 March 1995; Accepted 23 November 1995

Abstract

***Ricinus* plants were supplied with nutrient solutions containing different N-sources or different nitrate concentrations and were also exposed to mild salinity. Between 41 and 51 d after sowing, the ratio of inorganic to total nitrogen in xylem and phloem saps, the content of inorganic nitrogen and malate in tissues, and nitrate reductase activities were determined. The flows of nitrate, ammonium, and malate between root and shoot were modelled to identify the site(s) of inorganic nitrogen assimilation and to show the possible role of malate in a pH-stat mechanism. Only in the xylem of nitrate-fed plants did inorganic nitrogen, in the form of nitrate, play a role as the transport solute. The nitrate percentage of total nitrogen in the xylem sap generally increased in parallel with the external nitrate concentration. The contribution of the shoot to nitrate reduction increased with higher nitrate supply. Under salt treatment relatively more nitrate was reduced in the root as compared with non-treated plants. Ammonium was almost totally assimilated in the root, with only a minor recycling via the phloem. Nitrate reductase activities measured *in vitro* roughly matched, or were somewhat lower than, calculated rates of nitrate reduction. From the rates of nitrate reduction (OH^--production) and rates of malate synthesis ($2H^+$-production) it was calculated that malate accumulation contributed 76, 45, or 39% to the pH-stat system during nitrate reduction in plants fed with 0.2, 1.0 or 4.0 mM nitrate, malate flow in the phloem played no role. In tissues of ammonium-fed plants no malate accumulation was found and malate flows in xylem and phloem were also relative low.**

Key words: Ammonium, *Ricinus communis*, phloem, xylem, transport, nitrate, nitrate reductase, nitrogen assimilation, malate.

Introduction

The contribution of roots and shoots to assimilation of inorganic nitrogen in higher plants may vary considerably, depending on species and nutritional conditions (Andrews, 1986). The site of nitrogen assimilation is likely to affect strongly phloem translocation of photosynthates for delivery of carbon skeletons for amino acid synthesis. When inorganic nitrogen is assimilated in the roots, an adequate supply of carbohydrates must be provided for organic nitrogen to be transported to the shoot via the xylem. The form of N is also of importance for charge balance in transport fluids and in tissues (Raven and Smith, 1976). With nitrate as the N-source, nitrate is the major inorganic anion in the xylem of *Ricinus* (Peuke and Jeschke, 1995) and this requires cotransport of cations. In addition to its functions as N-source and negative charge, nitrate may also represent an important intracellular osmoticum as pointed out by Smirnoff and Stewart (1985). If the root is the major site of N-assimilation more organic nitrogen than nitrate will be transported in the xylem. The last can be the case if

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Abbreviations: *NRA* nitrate reductase activity, NR nitrate reduction, FW fresh weight.

13.)

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Effects of P deficiency on the uptake, flows and utilization of C, N and H_2O within intact plants of *Ricinus communis* L.

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Abstract

The influence of P deficiency on the uptake, flow and utilization of C, N and H_2O by intact NO_3-fed castor bean plants (*Ricinus communis* L.) was studied over a 9 d period in the middle of their vegetative growth. The modelling techniques incorporated data on net increments or losses of C, N and H_2O in plant parts, photosynthetic gains in and respiratory losses of C, molar C:N ratios of solutes in phloem and xylem sap and transpirational losses of H_2O. Plant growth was inhibited within 3 d of withholding P supply and dry matter production was less than one-third of the controls. Leaf growth was particularly depressed, while root growth was much less affected than that of the shoot. Shoot:root ratio of low-P plants was 1.5 compared with 2.6 under P supply. Over the 9 d study period total plant C and N increased by 560 and 47 mmol, respectively, in the controls, but by only 113 and 6.9 mmol in the low-P treatment. The particularly low increment of N in P-deficient plants was due principally to decreased NO_3^- uptake. Flows of C and N during the study period were markedly different between control and P-deficient plants. The partitioning profile for C in P-deficient plants showed a dramatic inhibition of net photosynthesis and attendant photoassimilate flow. Proportional downward to upward allocation of carbon increased with increase in sink size of the root relative to shoot. This was reflected in greater relative allocation of C to root dry matter and root respiration than in P-sufficient plants, and suppressed cycling of C from root to shoot via xylem. Nitrogen intake and xylem transport to the shoot of P-deficient plants were only 15% of the control and, as in the case of C, downward allocation of N predominated over upward phloem translocation. Apart from these severe changes, however, the basic patterns of N flows including xylem-to-phloem and xylem-to-xylem transfer of N were not changed, a feature highlighting the vital nature of these transfer processes even under deficiency conditions. The alterations in flows and partitioning of C, N and H_2O in response to low-P conditions are discussed in relation to the corresponding effects of moderate salt stress in *Ricinus* and the conclusion is reached that changes in nutrient flows under P deficiency were more highly co-ordinated than when plants experience salt stress. Flow profiles under P deficiency which favour root growth and activity are viewed as a means for increasing the potential capability of the plant to acquire P from the nutrient medium.

Key words: *Ricinus communis* L., P deficiency, carbon, nitrogen, water, partitioning, xylem transport, phloem transport.

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Abbreviations: DAS: days after sowing, DM: dry matter, C_{min}: minimal concentration down to which the plants can reduce external ion concentration in the aqueous phase of the rooting medium.

14.)

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

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Abstract

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

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

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

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

Introduction

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

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

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15.)

Transport, synthesis and catabolism of abscisic acid (ABA) in intact plants of castor bean (*Ricinus communis* L.) under phosphate deficiency and moderate salinity

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Abstract

Flows of abscisic acid (ABA) were investigated in whole plants of castor bean (*Ricinus communis*) grown in sand culture under either phosphate deficiency or moderate salinity. Xylem transport of ABA in P-deficient plants was stimulated by a factor of 6 whereas phloem transport was affected only very slightly. ABA deposition into leaves of P-deficient plants was not appreciably different from the controls because of strong net degradation in leaves. Since conjugation of ABA was strongly reduced in all organs of P-deficient plants ABA was presumably metabolized mainly to phaseic acid and dihydrophaseic acid. The increased import of ABA occurred predominantly into fully differentiated but not senescent leaves and showed a good correlation with the inhibition of leaf conductance under P deficiency.

As with low-P-plants salt stress increased ABA synthesis in roots and associated transport in the xylem. However, salinity caused a distinctly greater accumulation of ABA in the leaves, stem segments and the apex than in P-deficient plants. As opposed to P deficiency, ABA export in the phloem from the leaves was stimulated by salinity. Modelling of ABA flows within an individual leaf over its life cycle showed that young growing leaves imported ABA from both phloem and xylem, whereas the adult non-senescent leaves were a source of ABA and thus provided a potential shoot-to-root stress signal as well as an acceptor for reciprocal signals from root to shoot. In senescing leaves ABA flows and accumulation were somewhat retarded and ABA was lost in net terms by export from the leaf.

Key words: Abscisic acid, phosphorus deficiency, salt stress, phloem and xylem transport.

Introduction

It now seems well established that roots of plants growing in drying soil have some capacity to sense decreasing soil water content and in response send some form of stress signal to the shoot where stomatal reactions and leaf growth can be affected, even when leaf water potentials have not changed appreciably. There is a large body of evidence that it is the plant hormone abscisic acid (ABA) which acts as the root-to-shoot stress signal, although there seem to be some situations in which other as yet unidentified hormonal factors might also be involved (Munns and King, 1988). Low moisture of soils from extreme habitats is often accompanied by high salt concentrations in the soil solution. Additionally saline soils are often very alkaline (up to pH 11, Wild, 1988) and may cause nutrient deficiencies especially of phosphate and iron by readily forming insoluble precipitates which plants are not able to utilize unless their roots acidify the rhizosphere sufficiently.

The effect of plant nitrogen supply on ABA relations has been investigated in the past by several authors. Recently Peuke *et al.* (1994, and literature on ABA and nitrogen relations cited therein) have shown that nitrate nutrition has only weak effects on long distance ABA transport, but if replaced by ammonium roots increase both their ABA biosynthesis and release of ABA to the xylem stream. Increased ABA concentration in the xylem is then well correlated with reduced leaf conductance and a general inhibition of leaf growth.

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16.)

Foliar application of nitrate or ammonium as sole nitrogen supply in *Ricinus communis*

I. Carbon and nitrogen uptake and inflows

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(*Received 21 August 1997; accepted 8 January 1998*)

SUMMARY

Following a precultivation with pedospheric nitrogen nutrition, nitrate or ammonium solutions were supplied to the shoots of *Ricinus* plants by spraying (during the experimental period) resulting in an increase of biotic/organic and abiotic/inorganic particles on the surface, which significantly increased wetting of the leaf surfaces. The distribution of particles on the surface of sprayed leaves, in particular crystals around and in stomata, indicated the possible entry of nutrients via thin water films through the stomatal pores in addition to diffusion through the cuticle. Ammonium was taken up more readily than nitrate by the foliage, but both at relatively low rates which caused N limitation. Interestingly, the inorganic N, both in the form of nitrate and even ammonium, was entirely assimilated in the shoots; phloem transport of inorganic N to the root was negligible. The flows of malate, and the acidification of the apoplastic washing solution of leaves in ammonium-sprayed plants pointed to the role of metabolism of malate and excretion of protons in maintaining pH during ammonium assimilation in the shoot. Ammonium-sprayed plants incorporated the N in the same amounts in shoots and roots, only 38% of the shoot-borne N being recycled in the xylem. In nitrate-sprayed plants the root was not only favoured in N partitioning, but even a net export of previously incorporated N from the shoots occurred which reflected the N limitation. The N limitation also affected carbon metabolism, in particular the flows of C, incorporation in the shoot and photosynthesis, which were decreased when compared with data from recent experiments with pedospheric well fed *Ricinus*. However, there was little difference in C flows between nitrate and ammonium-sprayed plants with respect to respiration, C partitioning and, most interestingly, in relative stimulation of root growth. The loss of C from dark respiration of the shoots was high on a f. wt basis as well as in relative terms, owing to exclusive N assimilation in the shoot. In general the plants invested untargeted increases in root growth as a result of N limitation irrespective of the imposed artificial treatment which made the shoot the site of mineral N uptake.

Key words: *Ricinus communis* L., foliar application, carbon, nitrogen, nitrate, ammonium, phloem transport, xylem transport.

INTRODUCTION

In higher plants, inorganic nitrogen is usually taken up by the roots as nitrate and/or ammonium. Shoot organs, however, can take up mineral nutrients as well (Clarkson, Kuiper & Lüttge, 1986; Raven, 1988; Marschner, 1995). In agriculture, a common method of fertilization is the application of nutrients by spraying a solution onto the leaves (foliar nutrition). Gaseous air pollutants like NO_2, NH_3 and SO_2 can also diffuse into shoot tissue and can be taken up into the cells. Uptake of the nutrients into the leaves could occur either by penetration through the stomata, or by diffusion through the cuticle. Once in the leaves, the nutrients must be dissolved in the aqueous phase of the cell wall before uptake into

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17.)

The effects of light on induction, time courses, and kinetic patterns of net nitrate uptake in barley

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ABSTRACT

Barley seedlings (*Hordeum vulgare* L.) were grown hydroponically with (induced) or without (uninduced) nitrate in a light/dark cycle with high photon flux density to determine the effects of light on time courses, induction and kinetics of net nitrate uptake. Nitrate uptake was induced by external nitrate in both light and dark and was prevented by 1 mol m^{-3} p-fluorophenylalanine. In high light, nitrate uptake was about 2-fold higher than in low light. During time course experiments the uptake rates oscillated due to daily light–dark changes. Rates of nitrate uptake also increased at about 2200 h during continuous darkness. This increase coincided approximately with the time at which the dark period started during the previous culture of the plants, indicating that it was due to a mechanism associated with an endogenous diurnal rhythm. When calculating the kinetics of nitrate uptake, a model with two saturable systems, including a high-affinity system (HATS) and a low-affinity system (LATS), gave the best fit to data in all treatments. The apparent affinity of the HATS ranged from 7·7 to 12·2 mmol m^{-3} in induced plants in all light conditions. The effect of light on the HATS was mainly an increase of apparent V_{max} in the step from low to high light. In uninduced plants the HATS operated at a very low activity which was strongly enhanced during induction. Interpretation of the calculated kinetics of the LATS was much more difficult on the basis of net uptake data. The apparent affinity of the LATS increased from 24·3 mol m^{-3} in low light up to 0·17 mol m^{-3} after acceleration in high light. These extreme changes in apparent affinity of the LATS could not be explained satisfactorily, and the nature of this system is also discussed with respect to the method used.

Key-words: *Hordeum vulgare* L.; net nitrate uptake; light (intensity); kinetic parameters; induction time courses; Poaceae.

Abbreviations: FPA, p-fluorophenylalanine; FW$_R$, fresh weight of the roots; HATS, high-affinity transport system; K_d, rate constant; K_m, Michaelis-Menten constant; LATS, low-affinity transport system; V_c, constant uptake rate; V_{max}, maximal uptake rate.

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INTRODUCTION

The uptake of nitrate by roots of plants not previously exposed to nitrate is usually at a low, constitutive level, but exposure to external nitrate can increase the rate by 2- to 5-fold above this constitutive level. Nitrate uptake occurs thermodynamically uphill and can be inhibited by processes which depress synthesis of ATP and proteins (for a review, see Clarkson 1986). The energy dependence of nitrate uptake is consistent with a NO_3^-: $2H^+$ symport (Glass, Shaff & Kochian 1992). Subsequent steps in nitrate metabolism, nitrate reduction and ammonium assimilation, are also inducible and energy dependent since reducing power, ATP and C-skeletons are needed. The uptake and assimilation of ammonium and nitrate require a significant amount of root respiration (Bloom, Sukrapanna & Warner 1992). Hence in an intact plant, nitrate uptake, N metabolism, C metabolism and ultimately photosynthesis are all linked. These steps in nitrogen metabolism are all life-dependent processes which are influenced by environmental factors like light, temperature or stress conditions as well as variations during ontogeny (Imsande & Touraine 1994). So, if light conditions change, a signal from the shoot, the site of light perception, to the root, the site of nitrate uptake, would be not surprising. Rideout *et al.* (1993) concluded from their experiments with soybean that carbohydrate flows from the shoot to the root may regulate nitrate uptake more than the level of nitrate in the root itself. However, the mechanistic effects of light on nitrate uptake in higher plants have not been studied as extensively as other factors such as concentrations of nitrate, temperature and inhibitors. These results were more descriptive. Rao & Rains (1976b) reported that illumination stimulated nitrate absorption in barley, and Aslam *et al.* (1979) showed that nitrate uptake was 20% faster in the light than in the dark. Clement *et al.* (1978) found that the nitrate uptake in simulated swards of ryegrass was related to diurnal, day-to-day, and seasonal changes in radiation. Delhon *et al.* (1995) recently showed that nitrate influx was down-regulated in the dark. No information is available about the effects of light on the kinetic parameters of nitrate uptake.

A ‘dual uptake system’ for the kinetics of nitrate uptake was first described in maize (Neyra & Hageman 1975), in barley (Rao & Rains 1976a), and in Arabidopsis (Doddema & Telkamp 1979). These and subsequent observations led

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18.)

Foliar application of nitrate or ammonium as sole nitrogen supply in *Ricinus communis*

II. The flows of cations, chloride and abscisic acid

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SUMMARY

Following a precultivation with pedospheric nitrogen nutrition, *Ricinus* plants were supplied with nitrogen solely by spraying nitrate or ammonium solution onto the leaves during the experimental period. The chemical composition of tissues, xylem and phloem exudates was determined and on the basis of the previously determined nitrogen flows (Peuke *et al.*, *New Phytologist* (1998), **138**, 657–687) the flows of potassium, sodium, magnesium, calcium, chloride and ABA were modelled. These data, which permit quantification of net-uptake, transport in xylem and phloem, and utilization in shoot and root, were compared with results obtained in plants with pedospherically-supplied nitrate or ammonium and data in the literature. Although the overall effects on the chemical composition of supplying ammonium to the leaves were not as pronounced as in pedospherically supplied plants, there were some typical responses of plants fed with ammonium (ammonium syndrome). In particular, in ammonium-sprayed plants uptake and transport of magnesium decreased and chloride uptake was increased compared with nitrate-sprayed plants. Furthermore, acropetal ABA transport in the xylem in ammonium-sprayed *Ricinus* was threefold higher than in nitrate-sprayed plants. Additionally, concentrations of anions were more or less increased in tissues, particularly in the roots, and transport fluids. The overall signal from ammonium-sprayed leaves without a direct effect of ammonium ions on uptake and transport systems in the root is discussed.

Key words: *Ricinus communis* L., foliar N application, cations, anions, ABA, phloem transport, xylem transport.

INTRODUCTION

Nitrogen is the most important mineral nutrient for plants. On a dry matter basis the principal elements in higher plants are carbon, hydrogen, oxygen, nitrogen, potassium, sulphur and phosphorus. In autotrophic higher plants carbon is taken up from the atmosphere, whilst mineral nutrients are usually taken up by the roots. Shoot organs, however, can take up mineral nutrients as well (Raven, 1988; Pearson & Stewart, 1993; Marschner, 1995). In contrast to the above-mentioned elements, nitrogen, which is taken up at the greatest rates of all minerals, is available for plants as either the anion nitrate or the cation ammonium. The use of nitrate or ammonium as an N-source may have fundamental consequences for the growth, development and metabolism of the plants (Haynes & Goh, 1978; Runge, 1983; Pearson & Stewart, 1993; Marschner, 1995). One such consequence is the charge balance in the plants. If ammonium is taken up there is a deficiency in negative charge. Van Beusichem, Kirkby & Baas (1988) calculated an excess of cation over anion uptake in ammonium-fed *Ricinus* and the opposite effect under nitrate supply. Indeed, when ammonium was taken up, lower concentrations of cations but higher concentrations of anions were found in tissues (Allen & Smith, 1986; Arnozis & Findenegg, 1986; van Beusichem *et al.*, 1988; Pearson & Stewart, 1993; Lang & Kaiser, 1994).

A direct effect of ammonium on cation uptake systems has been assumed (Haynes & Goh, 1978; Lee & Ayling, 1993). Of particular interest are interactions between ammonium and potassium uptake (Scherer, MacKown & Leggett, 1984; Wang, Siddiqi & Glass, 1996; Smart & Bloom, 1998) and between ammonium and nitrate uptake (Lee & Drew, 1989; Jackson & Volk, 1995; Smart & Bloom, 1998). Furthermore, as a result of different ion uptake, the nitrogen source also has a profound influence on the composition of the transport fluids

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19.)

Abscisic Acid—A Hormonal Long-Distance Stress Signal in Plants Under Drought and Salt Stress

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ABSCISIC ACID AS A STRESS HORMONE IN PLANTS

When abscisic acid (ABA) is applied externally to plants, their water relations are improved. ABA reduces water loss by promoting stomatal closure and can increase water uptake into roots. ABA application also promotes characteristic developmental changes that can help the plant cope with a range of environmental stresses. Examples of such changes are the restricted growth of shoots, the reduction in leaf surface area, a stimulation of root extension, lateral root growth, and root hair development. All these effects of ABA application, together with the observation that environmental stress stimulates ABA biosynthesis and ABA release from sites of synthesis to the sites of action, suggest a role for ABA as a stress hormone in plants.

MECHANISMS TO INCREASE THE ABA CONCENTRATION AT THE PRIMARY SITE OF ACTION AT THE STOMATA

It was shown previously [1] that the biosynthesis and metabolism of ABA are stimulated by the plant water deficit when the bulk leaf turgor is reduced close to zero. Stomatal reactions, however,

20.)

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The characterization of inhibition of net nitrate uptake by salt in salt-tolerant barley (*Hordeum vulgare* L. cv. California Mariout)

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Abstract

Barley seedlings (*Hordeum vulgare* L. cv. California Mariout) grown hydroponically for 14–19 d without addition of NaCl were used for describing the effects of salt application on net nitrate uptake and for the calculation of kinetic parameters. The addition of NaCl, KCl, $CaCl_2$, and Na_2SO_4 to the uptake solution in the experiments led to similar inhibition of nitrate uptake, only at low and very high salt concentrations were ion-specific effects found. The same decrease in nitrate uptake can also be achieved by sorbitol or betaine at corresponding osmolalities. Thus, it was concluded that the inhibition of uptake was caused mainly by the osmotic effects of salts. Differences in the mechanisms of inhibition were detected between the two systems of nitrate uptake (high affinity system: HATS, and low affinity system: LATS). The HATS was inhibited non-competitively by NaCl, an apparent K_i of 60 mol m^{-3} was calculated using a Dixon-plot. Fitting an equation assuming a non-competitively inhibited HATS by computer program to the raw data resulted in an apparent K_i of about 37 mol m^{-3}. In contrast, the LATS was affected in a complex way: up to 60 mol m^{-3} NaCl the affinity was increased, which led to a stimulation of nitrate uptake at low nitrate concentrations (<2 mol m^{-3}). An inhibition of the LATS became obvious at concentrations above 3 mol m^{-3} nitrate (for all applied salt concentrations) or with 100 mol m^{-3} NaCl (throughout the whole nitrate range). Related plots of the data pointed to a competitive effect.

Key words: Barley, *Hordeum vulgare* L., net nitrate uptake, high affinity transport system (HATS), low affinity transport system (LATS), salt, inhibition, apparent kinetic parameters.

Introduction

The growth and physiological reactions of higher plants are affected by salinity in many ways. One effect is that in a salinized rhizosphere the uptake of mineral nutrients is inhibited, including uptake of nitrogen which is quantitatively the most important mineral for plants.

The rate of nitrate uptake was shown to be lowered by both NaCl and Na_2SO_4, but nitrate reduction was affected less in barley (Aslam *et al.*, 1984). The inhibitory effect of salinity was diminished by adding calcium to the solution (Ward *et al.*, 1986). The different effects of osmotic stress caused by NaCl or polyethylene glycol on nitrate metabolism in ryegrass has been investigated (Ourry *et al.*, 1992). The kinetics of nitrate uptake in non-salinized and salinized wheat seedlings have been compared (Hawkins and Lewis, 1993; Botella *et al.*, 1994). In these papers, however, nitrate uptake was treated as a single mechanism over a wide range of nitrate concentration (up to 10 or 1 mol m^{-3} nitrate, respectively). However, the uptake of nitrate into the roots of plants is mediated by at least a biphasic system (Rao and Rains, 1976; Doddema and Telkamp, 1979; Siddiqi *et al.*, 1990; Aslam *et al.*, 1992; Peuke and Jeschke, 1998; for a review see Peuke and Kaiser, 1996). In the low nitrate concentration range one system with a high affinity following Michaelis–Menten characteristics operates. With increasing external nitrate concentrations a second system contributes to uptake, but the nature of this system is

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Abbreviations: HATS, high affinity transport system; LATS, low affinity transport system; K_i, inhibitor constant; V_{max}, maximal activity; K_m, Michaelis-Menten constant; FW_R, fresh weight of the root.

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21.)

PROTOPLASMA

Dynamic studies of phloem and xylem flow in fully differentiated plants by fast nuclear-magnetic-resonance microimaging

Rapid communication

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Summary. A fast nuclear-magnetic-resonance imaging method was developed in order to measure simultaneously and quantitatively the water flow velocities in the xylem and the phloem of intact and transpiring plants. Due to technical improvements a temporal resolution of 7 min could be reached and flow measurements could be performed over a time course of 12–30 h. The novel method was applied to the hypocotyl of 35- to 40-day-old, leafy plants of *Ricinus communis* which were subjected to different light-dark regimes. The results showed that the xylem flow velocities and the xylem volume flow responded immediately to light on–off changes. Upon illumination the flow velocity and the volume flow increased as expected in respect to literature. In contrast, the phloem flow velocity did not change in response to the light-dark regimes. Interestingly, though, the volume flow in the phloem increased during darkness. These findings can be explained by assuming that the conducting area of the phloem becomes enlarged during the dark period due to opening of sieve pores.

Keywords: Phloem flow; Xylem flow; Water transport; Nuclear-magnetic-resonance imaging; *Ricinus communis* L.

Introduction

Nuclear magnetic resonance (NMR) imaging is a noninvasive and nondestructive technique and provides a great variety of contrast mechanisms such as chemical shift, relaxation times, diffusion or flow velocities. Recently, flow-weighted NMR imaging was used to obtain quantitative patterns of the flow velocity in the xylem of (intact) plants (Xia et al. 1993, Kuchenbrod et al. 1996). Due to the small diameter of the vessels (up to 50 μm) and flow velocities of less than 1 mm/s sophisticated equipment and pulse sequences are required to achieve a reasonable signal-to-noise ratio in the NMR images. Phloem flow is even more difficult to monitor because the diameter of the sieve tubes is significantly smaller than that of the xylem vessels and, thus, the total volume flow in the phloem is much less than that in the xylem.

Despite these difficulties, Köckenberger et al. (1997) recently reported measurements of the phloem flow in *Ricinus communis* seedlings by NMR flow-imaging techniques. However, due to the dimensions of the magnet bore, only 6-day-old (nontranspiring) seedlings could be investigated. Moreover, the total time for measuring a single flow data set was 4.5 h which made it impossible to detect dynamic flow changes in response to a light-dark regime.

In this communication, we will present measurements of phloem flow together with xylem flow in leafy, transpiring *R. communis* plants. Due to technical improvements the temporal resolution was only 7 min which was sufficient for dynamic (functional) studies. This could be shown by measurements of the response of the xylem and phloem flow upon light-dark regimes.

Material and methods

The experiments were performed with 35- to 40-day-old *R. communis* L. plants. The cultivation of the plants was similar to the

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XYLEM AND PHLOEM TRANSPORT, ASSIMILATION AND PARTITIONING OF NITROGEN IN *RICINUS COMMUNIS* UNDER SEVERAL NUTRITIONAL CONDITIONS

22.)

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INTRODUCTION

On land, higher plants face the problem of having the site of photosynthesis, which is responsible for capturing light energy to fix CO_2, displaced from the site where water and mineral nutrients are taken up. The problem is solved by the existence of the two long distance transport systems: xylem and phloem. The xylem transports water, mineral nutrients, products and signals from the root to the shoot. In contrast, the phloem transports assimilation products from shoot to root, and, from photosynthetically active – 'source' tissues, to growing areas within the shoot – the so called 'sinks'.

Of the mineral nutrients taken up by the plants, nitrogen is not only taken up at greatest rates, but, is also metabolised and transported to a large extent between organs in higher plants. Since most N is taken up by roots, the shoots must be supplied with N via the xylem. Furthermore, a considerable amount of N is recycled via the phloem. Nitrogen must be assimilated before it can be used in plant metabolism (disregarding some other functions of inorganic N, such as osmotic, charge effects, or signals) and this requires energy and C-skeletons for the synthesis of amino acids.

According to the main site of N-assimilation, plants can be classified in two groups: (1) N-assimilation takes place in the root, and (2) it takes place in the shoot. If inorganic N is assimilated in the root, the necessary energy and C-skeletons must be transported to the root. After assimilation, amino acids are transported in the xylem to the shoot. If inorganic N is mostly assimilated in the shoot, inorganic N must be loaded into the xylem and transported to the shoot after the uptake into the root cortex. In this case, counterions are necessary for N-transport in the xylem. Amino acids are then synthesised in the shoot, and consequently the root must be supplied with organic N by basipetal transport in the phloem. Naturally, the reduction of nitrate and assimilation of resulting ammonium takes place in both root and shoot. The partitioning of nitrate reduction depends on a number of factors.

C is needed to provide energy for reduction of nitrate, assimilation of ammonium and also to provide C-skeletons. Therefore, C is transported within the plant between different organs to varying degrees. Primarily, C and N must be transported from sites of acquisition to sites which are likely to be growing i.e. N moves from the root to leaves or C from the leaves to the roots.

The long distance transport, assimilation and incorporation of N (and other elements or ions) in a whole plant can be investigated by modelling of ion and element flows (Pate *et al.*, 1979a; Jeschke *et al.*, 1985; and Jeschke and Pate, 1991a). For this method the proportion of ions or elements in the transport fluids and their incremental increases in those ions or elements within different organs are necessary. These models allow us to make positive statements about uptake, flow in xylem and phloem, and incorporation of elements and ions in the whole plant. For compounds which can be metabolised it is also possible to make statements about the site and extent of net-metabolism (synthesis or degradation). The models reported

Nitrogen in a sustainable ecosystem: from the cell to the plant
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SUMMARY *Ricinus communis* plants were grown with nitrate or ammonium as N-source, salt stress, high or low nitrate supply, deficiency of nitrogen, phosphorus or potassium, and with foliar application of nitrogen. During the experimental period (41 to 51 days after sowing) the flows of elements and ions were modelled according to the method of Pate et al. (1979). These models permit quantification of uptake, transport in xylem and phloem, and incorporation in organs. For ions, which can be metabolised like inorganic nitrogen, the sites of assimilation, synthesis or degradation can be also shown. Most of newly taken up nitrogen was transported in the xylem to the shoot (100% of uptake including recycled N), where only a part of it was used for growth. The rest was recycled back to the root, about 23% of the xylem born N. In well-fed plants, 70-80% of N was incorporated in the shoot. Nutrient deficiency shifted this portion in favour of the root, whereas under mild salt stress more N was used in the shoot. Inorganic N was not mobile in the phloem, and ammonium was not transported in the xylem, but was always assimilated at the site of uptake. When supplied to the leaves, ammonium was assimilated in the shoot. Nitrate can be reduced in roots as well as in shoots. The contribution of the shoot in reduction increased with increasing supply. Deficiency of N and K as well as mild salt stress shifted the reduction more to the root, P deficiency enhanced the reduction in the shoot. Foliar supplied nitrate was almost exclusively reduced in the leaves. For the distribution of nitrate reduction between root and shoot effects of xylem transport might be responsible. In well-fed plants about 50% of photosynthetically gained C was used for shoot growth and 25% for respiration. In the case of nutritional limitation the cost for root growth and respiration (50%) increased relatively. Additionally, the loss of C by phloem export was increased, if the root was the major site of N assimilation, as was the case under conditions of NO_3^- and K-deficiency, salt stress and ammonium nutrition. This demonstrated the role of N assimilation as a major sink for C, since carbohydrates were needed as energy sources and C-skeletons for amino acid synthesis. Beneath the interactions of N and C, other ions affected or were affected by nutritional conditions of N. For example the uptake and flows of chloride were increased, if nitrate was limiting. The xylem transport of nitrate was influenced by salinity or potassium. In ammonium- vs. nitrate-fed plants and when ammonium was applied to the shoots only, cation flows were decreased.

23.)

The Chemical Composition of Xylem Sap in *Vitis vinifera* L. cv. Riesling During Vegetative Growth on Three Different Franconian Vineyard Soils and as Influenced by Nitrogen Fertilizer

ANDREAS D. PEUKE*

Cuttings of grapevine (*Vitis vinifera* L. cv. Riesling clone B 68) grafted on SO4 (Selection Oppenheim No. 4) rootstocks were grown in pots with three different soils from Franconian vineyards derived from different geological formations (namely, Loess, Muschelkalk (shell lime), or Keuper). Additionally, the influence of N-fertilizer treatment was investigated. From the midrib of leaves six to eight of the sole shoot, xylem sap was collected simultaneously by pressurizing the rhizosphere during the vegetative growth phase. The chemical composition of xylem sap was determined and compared with that of the aqueous soil extract. In Muschelkalk soil, carbon, nitrogen, and calcium were present in the greatest concentrations. Sulfur, boron, magnesium, sodium, and potassium were greatest in Keuper, and the concentrations in Loess soil were intermediate. Aqueous extraction of the soils resulted in a two-fold greater concentration of total solutes in Keuper extract compared with Muschelkalk, and more than threefold than in Loess. The apparent volume flow was greatest in the middle leaves along the shoot and in plants grown on Keuper; additionally there was a tendency for fertilizer treatment to increase flow. The concentrations of mineral ions in xylem sap were the same in all the leaves of a shoot of grapevine. An important exception was the supply to the leaves of amino acids, which increased in concentration along the transpiration stream and were greatest in the youngest leaves (particularly in non-fertilized plants). Potassium was the dominant cation in xylem sap and was greatest in plants grown on Keuper. Concentrations of sodium and calcium were increased in non-fertilized plants, but not significantly in vines grown on Muschelkalk. In xylem sap, nitrate was the major anion, followed by malate. Nitrate concentration was greatest in plants grown on Muschelkalk, while malate was greater in plants grown on Keuper. Chloride, sulfate, and phosphate concentration in sap were increased by fertilizer treatment. Abscisic acid was markedly increased in xylem sap of non-fertilized plants grown on Loess and Muschelkalk and was discussed as a signal for nutrient limitation. If Keuper was the substrate it was also increased by fertilizer treatment. Of the organic N-compounds, glutamine was the largest fraction. On the basis of the relation of nitrate to total N in xylem sap, it could be assumed that about 40% to 75% of nitrate reduction took place in the shoots. In general, soil type had only a moderate effect on the chemical composition of the xylem sap compared with the effect of N-fertilizer.

KEY WORDS: grapevine (*Vitis vinifera* L. cv. Riesling), cations, anions, abscisic acid, xylem sap, soil (geological formation)

Leaves and other shoot parts are supplied with mineral nutrients and organic products of root uptake and metabolism (organic forms of N, organic acids, phytohormones) via the xylem. One of the first steps in this process is the secretion/loading of ions into the xylem by the stellar parenchyma [32]. The composition of the soil solution has a marked influence on the composition of the xylem sap. For example, the relative concentrations of nitrate or ammonium generally have large effects on the composition of the transport fluids in plants [1,4,5,22,23,29,30] as have concentrations of other ions such as K^+, Mg^{++}, and Ca^{++}.

Grapevine is an old agricultural plant and the quality of wine is the object of much debate [31]. While many factors including cultivar, climate, agriculture methods, *etc.* play roles in determining quality, soil type (including fertilizer) plays a major role [31]. The chemical and physical properties of soils influence the wine in numerous ways, but mostly indirectly since grapes are supplied largely by the phloem during ripening [15]. Recent studies have highlighted the circulatory nature of xylem and phloem [8,10,12,13,22,25,26]. In general, the composition of xylem sap reflects both mineral and water uptake and also the general nutrition status of the plant. In addition, xylem sap is the major means of transport of abscisic acid (ABA), a major hormonal signal of drought and salinity, from roots to shoots [7]. Therefore, the xylem is not only responsible for solute exchange, but also for transport of root to shoot signals. ABA is considered to be an

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24.)

Simultaneous measurement of water flow velocity and solute transport in xylem and phloem of adult plants of *Ricinus communis* over a daily time course by nuclear magnetic resonance spectrometry

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ABSTRACT

A new method for simultaneously quantifying rates of flow in xylem and phloem using the FLASH imaging capabilities of nuclear magnetic resonance (NMR) spectrometry was applied in this study. The method has a time resolution of up to 4 min (for the xylem) and was used to measure the velocity of flows in phloem and xylem for periods of several hours to days. For the first time, diurnal time course measurements of flow velocities and apparent volume flows in phloem and xylem in the hypocotyl of 40-d-old *Ricinus communis* L were obtained. Additional data on gas exchange and the chemical composition of leaves, xylem and phloem sap were used to assess the role of leaves as sinks for xylem sap and sources for phloem. The velocity in the phloem (0·250 ± 0·004 mm s^{-1}) was constant over a full day and not notably affected by the light/dark cycle. Sucrose was loaded into the phloem and transported at night, owing to degradation of starch accumulated during the day. Concentrations of solutes in the phloem were generally less during the night than during the day but varied little within either the day or night. In contrast to the phloem, flow velocities in the xylem were about 1·6-fold higher in the light (0·401 ± 0·004 mm s^{-1}) than in the dark (0·255 ± 0·003 mm s^{-1}) and volume flow varied commensurately. Larger delays were observed in changes to xylem flow velocity with variation in light than in gas exchange. The relative rates of solute transport during day and night were estimated on the basis of relative flow and solute concentrations in xylem and phloem. In general, changes in relative flow rates were compensated for by changes in solute concentration during the daily light/dark cycle. However, the major solutes (K^+, NO_3^-) varied appreciably in relative concentrations. Hence the regulation of loading into transport systems seems to be more important to the overall process of solute transport than do changes in mass flow. Due to transport behaviour, the chemical composition of leaves varied during the day only with regard to starch and soluble carbohydrates.

Key-words: chemical composition; fast nuclear magnetic resonance imaging., long-distance transport; phloem flow; xylem flow

INTRODUCTION

Characterization of long-distance transport systems is fundamental to understanding the physiology of higher plants. In the xylem and phloem, water and solutes are transported by a mass flow. Transported solutes vary widely in composition (e.g. inorganic ions taken up from the environment by the roots, organic compounds produced by plant metabolism) and, in the phloem in particular, photo-assimilates are transported as energy in chemical form. For a number of physiological questions, knowledge of transport velocities and rates of transport are of basic interest. Additionally, the rate of response of transport in xylem and phloem to rapid changes in environmental conditions (such as light, temperature, humidity) is integral to many aspects of plant metabolism but poorly understood owing to lack of quantitative methods. Current knowledge of the 'driving forces' for xylem and phloem transport of water and solutes is still based on gradients in hydrostatic pressure and water potential for the xylem (but see Benkert *et al*. 1995 and Thürmer *et al*. 1999), and chemical potential (gradients in concentrations of photo-assimilates, the pressure-flow theory of translocation, Münch 1930) for the phloem. Gas exchange of water vapour, CO_2 and O_2 and associated processes are central to the regulation of long-distance transport in plants. Both phloem and xylem systems are reasonably complex and susceptible to manipulation. For these reasons, it has remained difficult to investigate the long-distance transport systems of plants *in situ*.

Heat-based methods have been used to trace movement of xylem sap and can yield reasonably accurate measures

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New Phytologist

Research

25.)

Drought affects the competitive interactions between *Fagus sylvatica* seedlings and an early successional species, *Rubus fruticosus*: responses of growth, water status and $\delta^{13}C$ composition

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Summary

- Competitive interactions between European beech (*Fagus sylvatica* L.) seedlings and the early successional species *Rubus fruticosus*, and the role of water availability, are reported and discussed in relation to management practices and climatic changes in beech ecosystems of Central Europe.
- Responses of growth, water status, gas exchange and carbon isotope composition ($\delta^{13}C$) to two competition and three irrigation treatments were examined in a factorial-design glasshouse study.
- Under regular irrigation, coexistence with *R. fruticosus* did not significantly affect growth, water potential and gas exchange of beech seedlings. However, moderate water shortage caused a twofold reduction in beech biomass and changes in root : shoot ratios. Drought lowered transpiration rates and predawn water potentials (below the xylem embolism threshold) for *F. sylvatica*; $\delta^{13}C$ of leaves and fine roots increased (discrimination was reduced). By contrast, significantly lower $\delta^{13}C$ of *R. fruticosus* foliage indicated an improved water status. Competitive interference intensified the effects of reduced irrigation.
- Water availability regulates the competitive interactions between beech seedlings and *R. fruticosus*. Natural regeneration of beech seedlings may be inhibited by interference from a species such as *R. fruticosus*, especially during summer drought, as predicted by actual climate models.

Key words: *Fagus sylvatica*, competition, water availability, growth, water potential, carbon isotope composition, transpiration.

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Introduction

Throughout their life-cycle, trees interact with neighbouring plants composed of different life forms, physiology and resource requirements. For trees, coexistence with other species may have negative effects, such as soil water and nutrient depletion and competition for radiation, or positive effects such as nitrogen fixation (Flint & Childs, 1987; Messier & Kimmins, 1990; Neary *et al.*, 1990; Chang *et al.*, 1996b). Information on the ecophysiological basis of interactions between trees and neighbouring plants is required to understand, predict and manipulate regeneration and successional processes in forests. Moreover, knowledge of the indirect effects of understorey vegetation on seedling recruitment patterns may have important silvicultural implications and economic benefits. Several cases have been reported of low productivity or even failure of plantations and of regeneration as a result of competition from understorey species (Brand, 1991; Morris *et al.*, 1993; Marino & Gross, 1998).

A number of management practices, including thinning, have focused on improving ecosystem productivity and are routinely used in forests, both to minimize competition between trees and to increase growth rates. Thinning frequently increases irradiance at the forest floor and may improve conditions for

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26.)

Flows of elements, ions and abscisic acid in *Ricinus communis* and site of nitrate reduction under potassium limitation

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Abstract

In a pot experiment *Ricinus communis* plants were cultivated in quartz sand and supplied daily with a nutrient solution which contained 4 mol m^{-3} nitrate as the nitrogen source and either full strength potassium (1.3 mol m^{-3}, control) or 8% potassium (0.1 mol m^{-3}, K^+-limitation). Although the final fresh weight of the whole plant was not affected by K^+-limitation, the root–shoot ratio was increased due to a relatively increased root growth and inhibited development of younger shoot parts. Owing to K^+-limitation, photosynthesis was slightly decreased, while dark respiration of the shoot markedly decreased and root respiration was nearly doubled. The transport of carbon in the phloem, and to some extent in the xylem, was greater and the root was favoured in the partitioning of carbon. This was also true for nitrogen and potassium which were both taken up at lower rates, particularly potassium. In these two cases a high remobilization and recycling from the old part of the shoot was observed. By contrast, uptake of sodium was 2.4-fold higher under K^+-limitation and this resulted in increased flows in the plants, which was discussed generally as a means for charge balance (in combination with a slight increase in uptake of magnesium and calcium). Nitrate reduction took place in the same portion in the root and shoot. This was a shift to the root compared to the control and points to an inhibition of xylem transport caused by limitation of K^+ as an easily permeating countercation. Low K^+ supply also resulted in an increased biosynthesis of ABA in the roots (265%). This caused a slightly increased deposition of ABA in the roots (193%) and a 4.6-fold higher root-to-shoot and a doubled shoot-to-root ABA signal in the xylem or phloem, respectively. The high degradation of ABA in the shoots prevented ABA accumulation there.

Key words: *Ricinus communis* L., potassium limitation, cations, anions, ABA, phloem transport, xylem transport.

Introduction

Within the last decade a series of papers has been published dealing with the effects of nutrient deficiency and nutritional disorder in long-distance solute transport and partitioning, including the stress signal abscisic acid in castor bean plants. The nutritional conditions included the type of nitrogen source (Peuke and Jeschke, 1993), salt stress (Peuke and Jeschke, 1995; Peuke *et al.*, 1996), foliar application of nitrogen (Peuke *et al.*, 1998) as well as the limitation or deficiency of nitrogen (Peuke *et al.*, 1994*a*) or phosphorus (Jeschke *et al.*, 1996, 1997*a*, *b*). However, the influence of potassium, the third most important nutrient, has not yet been investigated in the long-distance transport of solute and stress signals.

The importance of potassium for plant life is well documented. Potassium, a macronutrient for plants, is present in plant dry matter next to carbon, hydrogen, oxygen, and nitrogen and before sulphur and phosphorus. In commercial NPK fertilizers in agriculture, potassium is used to improve the yield. Potassium plays a role in a wide range of functions in plants: photosynthesis, enzyme activation, protein synthesis, osmotic

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New
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Research

27.)

Identification of drought-sensitive beech ecotypes by physiological parameters

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Summary

- The effects of drought on European beech (*Fagus sylvatica*) were assessed in a pot experiment under controlled conditions.
- Plants from 11 autochthonous provenances originating from regions in Germany, which differed in annual precipitation, were exposed to a 3-wk drought period in a glasshouse after the first stage of shoot growth had been completed.
- Drought reduced the water content to 97% of control in leaves and axes and to 92% in the roots. A strong reduction of predawn water potential in roots and shoots, as well as on transpiration rate, was found. In the roots, the effect on water potential was the same for all provenances, but differences were observed in the shoot water potential. Leaf concentrations of abscisic acid (ABA), proline and sucrose increased in the drought-treated plants compared with the controls.
- Two extreme clusters from opposite climatic sites were identified by cluster analysis. A drought-sensitive cluster, originating from regions with high annual precipitation, had low water potential and transpiration rates, as well as high concentrations of fructose, ABA and proline after drought. Water potential and transpiration rates were less affected by drought in the other cluster, which comprised two provenances of relatively dry habitats, and concentrations of hexose, ABA and proline were low.

Key words: beech (*Fagus sylvatica*), provenances, drought stress, water potential, transpiration, osmoprotectants, abscisic acid.

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Introduction

Global environmental conditions have changed rapidly over the last century as a result of human activities. One of these changes is the increase of atmospheric CO_2 by fossil fuel combustion, deforestation and biomass burning contributing to the glasshouse effect (Enquete-Komission, 1994; Saxe *et al.*, 1998; UNEP/IUC, 1999). Based on current trends, it is expected that within this century CO_2 concentrations will double and global temperature will rise by about 1–3.5°C (UNEP/IUC, 1999). As a consequence, precipitation and evaporation patterns will change and forests and other ecosystems will be exposed to drought and flooding events.

The increased frequency and severity of drought caused by climatic changes will affect plants directly through water depletion and indirectly by reduced nutrient uptake (Saxe *et al.*, 1998). Plants in a CO_2-enriched climate of the future might tolerate drought better through stomatal closure and/or decline in stomatal density (Bowes, 1993), but elevated CO_2 may also increase leaf area and thereby counteract the favourable effects of reduced stomatal aperture/density under water limitation (Saxe *et al.*, 1998).

For long-living plants, such as forest trees, the expected climatic changes will become relevant within the lifespan of an individual within the community. European beech (*Fagus sylvatica*) is one of the most important forest trees in central Europe and is known to be relatively drought sensitive (Ellenberg, 1992). Beech forests have mainly developed by natural regeneration and ecotypes have developed that are adapted to the local climatic conditions (Müller-Stark, 1997).

28.)

Spatially and temporally resolved measurement of xylem and phloem flow by NMR micro-imaging

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Abstract

An introduction to nuclear magnetic resonance (NMR) flow measurement on plants is given in this article. Practical considerations due to the need for special equipment are discussed as an introduction for the reader not yet familiar with NMR techniques. Examples demonstrate the current achievements and limitations with respect to spatial and temporal resolution. The outlook points out current developments for *in-situ* experiments.

Introduction

Most readers might have heard about nuclear magnetic resonance (NMR) in a medical context. Its non-invasive character makes this technique particularly popular for application on humans. Another less known but equally important advantage of NMR is its sensitivity to a vast number of contrast mechanisms. Investigation of chemical properties was the first application even before imaging techniques were introduced. Mapping of NMR relaxation times (which can be an indicator for mobility of water (van As et al., 1986; Veres et al., 1991), diffusion constants or even pH are just a few other examples. Most applications use the proton NMR signal, but there are other NMR active nuclei like sodium, which can be used for mapping sodium uptake in a plant

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29.)

ORIGINAL ARTICLE

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Carbon, nitrogen, phosphorus, and sulphur concentration and partitioning in beech ecotypes (*Fagus sylvatica* L.): phosphorus most affected by drought

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Abstract Beech seedlings of different drought sensitivity originating from 11 German provenances were grown in pots and cultivated in a greenhouse. The present paper aims to give insights on uptake, transport and use of macronutrients, since the knowledge of drought effects on the nutrition of trees is low compared to water relations. Therefore, the elemental composition, the ratio of inorganic to total content, and the partitioning between roots and shoots of carbon, nitrogen, phosphorus, and sulphur were investigated as affected by provenance and drought treatment. Phosphorus and phosphate concentrations decreased in all tissues after a 3 week drought treatment simulating a summer drought period. In roots carbon increased and nitrate decreased, in stems nitrogen decreased but nitrate increased following drought. The observed effects on phosphorus and phosphate are discussed in terms of lower phosphate mobility in the substrate due to lower water availability. The decrease in the ratio of phosphate to phosphorus in the tissues suggests the use of vacuolar phosphate pools for maintaining organic phosphorus homeostasis. The partitioning of all macronutrients was not affected by drought, although phosphorus and phosphate were significantly lowered in tissues. In most of the parameters studied significant differences between provenances were found. The recently observed drought sensitivity of provenances was not reflected in the strength of concentration changes or partitioning of macronutrients by drought over provenances.

Keywords European beech (*Fagus sylvatica* L.) · Drought · Nutrition · Partitioning · Phosphorus

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Introduction

Actual predictions of climate changes due to anthropogenic atmospheric CO_2 increase project an average warming of 1–3.5°C for mid-latitude regions within the next 100 years (Saxe et al. 1998; UNEP/IUC 1999). For Europe, changes in the frequency of extreme precipitation events are expected, resulting in more drought and flooding (IPCC 1997; UNEP/IUC 1999). Longer periods of reduced rainfall are expected, particularly in summer, when a number of crops and fruit ripen and trees like European beech undergo a second phase of growth. The pace of climate change will be fast compared to the speed at which forest trees grow. Therefore, present forest ecosystems will face new environmental conditions during their lifetime. Alteration in species composition of forest ecosystems are likely to occur (IPCC 1997; Saxe et al. 2001).

Changes in atmospheric CO_2 concentrations are known to influence plant physiology and morphology in past (Pataki 2002) as well as today's and future climate. A considerable biomass increment for trees caused by elevated CO_2 has been predicted as long as other environmental parameters are not growth limiting (Saxe et al. 1998; Ceulemans et al. 1998). The multiple interacting changes due to global warming will therefore result in an orchestrated response of a number of physiological processes, the outcomes of which are difficult to predict. The benefit of enhanced drought tolerance due to reduced stomatal conductance at elevated atmospheric CO_2 may be more than compensated by higher leaf biomass (Saxe et al. 1998; Wullschleger et al. 2002).

The effects of predicted climate changes on nutrient availability for ecosystems are still under debate with contrasting statements (Saxe et al. 1998, 2001; Ceulemans et al. 1998). In general, plants increase their nutrient capacity under elevated CO_2 (Ceulemans et al. 1998).

Drought is a form of stress which affects gas exchange, cell growth and division, phytohormones, metabolic and transport processes of plants (Hsiao 1973). The effects of

30.)

Phytoremediation with Transgenic Trees

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In the present paper actual trends in the use of transgenic trees for phytoremediation of contaminated soils are reviewed. In this context a current field trial in which transgenic poplars with enhanced GSH synthesis and hence elevated capacity for phytochelatin production are compared with wildtype plants for the removal of heavy metals at different levels of contamination and under different climatic conditions. The studies are carried out with grey poplar (*Populus tremula* x *P. alba*), wildtype plants and plants overexpressing the gene for γ-glutamylcysteine synthetase (*gshI*) from *E. coli* in the cytosol. The expression of this gene in poplar leads to two- to four-fold enhanced GSH concentrations in the leaves. In greenhouse experiments under controlled conditions these transgenic poplars showed a high potential for uptake and detoxification of heavy metals and pesticides. This capacity is evaluated in field experiments. Further aims of the project are to elucidate (a) the stability of the transgene under field conditions and (b) the possibility of horizontal gene transfer to microorganisms in the rhizosphere. The results will help to assess the biosafety risk of the use of transgenic poplar for phytoremediation of soils.

Key words: Phytoremediation, Transgenic Trees, Poplar

Introduction

After decades or even centuries of human activities in industry, mining, or military a huge amount of area in developed countries shows high contamination with heavy metals or organic pollutants. Therefore, phytoremediation, *i.e.*, bioremediation with plants, of polluted soils has received significant attention (Cunningham and Ow, 1996; Gleba *et al.*, 1999; Meagher, 2000; Dietz and Schnoor, 2001). Phytoremediation is an emerging new technology that uses plants to remove or degrade various pollutants from soils. A number of plant species are able to accumulate high amounts of heavy metals in their above-ground tissues or to degrade various organic soil pollutants (Salt *et al.*, 1998; Kömives and Gullner, 2000).

Exposure of plants to heavy metals induces synthesis of compounds that chelate these metals and thus contribute to their detoxification (Rauser, 1999). Among the chelators, sulfur-rich peptides, *i.e.* metallothioneins and phytochelatins, are of particular importance. Metallothioneins are sulfur-rich proteins of 60–80 amino acids containing 9–16 cysteine residues (Hamer, 1986; Robinson *et al.*, 1993; Rauser, 1999). Phytochelatins (PC) are a family of γ-glutamylcysteine oligopeptides with glycine or other amino acids as the C-terminal constituent (Grill *et al.*, 1985; Rauser, 1999). The γ-Glu-Cys units are repeated 2–7 times. The C-terminal amino acids of phytochelatins include β-Ala, Cys, Ser, or Glu (Grill *et al.*, 1986; Klapheck *et al.*, 1994; Rauser, 1999). Phytochelatins are synthesised from glutathione (GSH) and its derivates by phytochelatine synthase in the presence of heavy metal ions (Vatamaniuk *et al.*, 1999; Ha *et al.*, 1999) (see Fig. 1). The gene encoding phytochelatine synthase was recently cloned from *Arabidopsis* and yeast (Vatamaniuk *et al.*, 1999; Ha *et al.*, 1999; Clemens *et al.*, 1999). Following Cd or Cu exposure, PCs were found in yeast, algae, lower and higher plants (Kondo *et al.*, 1984; Gekeler *et al.*, 1988, 1989). Cadmium is the most effective inducer of PCs, but Cu, Pb, Zn, Sb, Ag, Zn, or Hg also induce their formation (Grill *et al.*, 1987; Maitani *et al.*, 1996). PCs form ligand complexes with these metals which are further sequestered into the vacuoles. Mutants in PC synthesis are hypersensitive to Cd and other metals (Howden *et al.*, 1995).

Abbreviations: γ-EC, γ-L-glutamyl-L-cysteine; γ-ECS, γ-glutamylcysteine synthetase; GSH, glutathione; GSSG, oxidized glutathione; GST, glutathione *S*-transferase.

EMBO *reports*

31.)

Phytoremediation

Molecular biology, requirements for application, environmental protection, public attention and feasibility

Andreas D. Peuke & Heinz Rennenberg

Over centuries, human industrial, mining and military activities as well as farming and waste practices have contaminated large areas of developed countries with high concentrations of heavy metals and organic pollutants. In addition to their negative effects on ecosystems and other natural resources, these sites pose a great danger to public health, because pollutants can enter food through agricultural products or leach into drinking water (EC, 2002; EEA, 2003). In the EU alone, an estimated 52 million hectares—more than 16% of the total land area—are affected by some level of soil degradation. The largest and probably most heavily contaminated areas are found near industrialized regions in northwestern Europe, but many contaminated areas exist around most major European cities (EEA, 2003). There could be between 300,000 and 1.5 million of these sites in the EU (EC, 2002)—the uncertainty in this estimate is due to the lack of common definitions and a scarcity of accurate data on the size and the level of contamination of affected sites.

> In the EU alone, an estimated 52 million hectares—more than 16% of the total land area—are affected by some level of soil degradation

Cleaning up contaminated soil is a costly enterprise—the overall cost to remediate affected sites in the EU is estimated to be between €59 and €109 billion (EC, 2002). Furthermore, current methods of soil remediation do not really solve the problem. In Germany, for instance, only 30% of soils from contaminated sites are cleaned up in soil remediation facilities (SRU, 2004); the remaining soil must be stored in waste disposal facilities. This does not solve the problem, it merely transfers it to future generations. Obviously, there is an urgent need for alternative, cheap and efficient methods to clean up heavily contaminated industrial areas.

This could be achieved by a relatively new technology known as phytoremediation, which uses plants to remove pollutants from the environment. Due to its elegance and the extent of contaminated areas, it has already received significant scientific and commercial attention (Salt et al, 1998; Gleba et al, 1999; Meagher, 2000; Dietz & Schnoor, 2001; Guerinot & Salt, 2001; Krämer & Chardonnens, 2001; McGrath & Zhao, 2003; Peuke & Rennenberg, 2005). Phytoremediation uses wild or genetically modified plants (GMPs) to extract a wide range of heavy metals and organic pollutants from the soil. Initial experiments with transgenic plants have shown that they are indeed efficient in drawing metals from heavily contaminated soils. However, despite this and other advantages, the progress and application of this technology to tackle widespread environmental problems is being hampered by ideology-driven, restrictive legislation over the use and release of GMPs in Europe, and particularly in Germany.

Phytoremediation comes in several forms. Phytoextraction removes metals or organics from soils by accumulating them in the biomass of plants. Phytodegradation, or phytotransformation, is the use of plants to uptake, store and degrade organic pollutants; rhizofiltration involves the removal of pollutants from aqueous sources by plant roots. Phytostabilization reduces the bioavailability of pollutants by immobilizing or binding them to the soil matrix, and phytovolatilization uses plants to take pollutants from the growth matrix, transform them and release them into the atmosphere.

> …there is an urgent need for alternative, cheap and efficient methods to clean up heavily contaminated industrial areas

Most scientific and commercial interest in phytoremediation now focuses on phytoextraction and phytodegradation, which use selected plant species grown on contaminated soils. These are then harvested to remove the plants together with the pollutants that have accumulated in their tissues. Depending on the type of contamination, the plants can either be disposed of or used in alternative processes, such as burning for energy production. In essence, phytoextraction removes pollutants from contaminated soils, concentrates them in biomass and further concentrates the pollutants by combustion.

It is also possible to recover some metals from plant tissue (phytomining), which humans have done for centuries in the case of potassium (potash), and which may even become economically valuable (Meagher, 2000). In addition to accumulating toxic minerals in their tissues, plants are also able to take up a range of harmful organic compounds, including some of the most abundant environmental pollutants such as polychlorinated biphenyl (PCB), halogenated hydrocarbons (trichloroethylene, TCE) and ammunition wastes (nitroaromatics such as trinitrotoluene (TNT) and glycerol trinitrate (GTN)). Subsequent metabolism in plant

32.)

IMPROVED PHYTOREMEDIATION OF CONTAMINATED SOILS BY CHANGES IN SULFUR METABOLISM

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Introduction

In developed countries, large areas have been contaminated over centuries with heavy metals and organic pollutants by industrial, mining and military activities as well as farming and waste disposal (Pilon-Smits and Pilon 2002; Peuke and Rennenberg 2005a). These contaminations do not only pose a thread to the environment and to natural resources, they are also a danger to public health, because they can enter the food chain and can be leached out into the drinking water (Dietz and Schnoor 2001). Obviously, there is already a large requirement for cleanup of contaminated soils that will further expand with the industrialization of developing countries. The current costs for cleanup of contaminated soils are staggering (Peuke and Rennenberg 2005a; Pilon-Smits 2005) and the methods applied often do not solve the problem. A high amount of contaminated soils is stored in waste disposals, thereby transferring the problem to future generations. Thus, there is a high demand for low-cost, efficient methods of soil decontamination.

Phytoremediation is a relatively new, low-cost approach for cleaning up not only contaminated soils, but also aquatic environments and the atmosphere. For cleanup of soils, it uses plants and their associated microorganisms in the rhizosphere (Pilon-Smits 2005). Phytoremediation has been proven to effectively remove from soils a whole set of organic pollutants such as trichlorethane, pesticides, explosives, hydrocarbons, fuel additives, or polychlorinated biphenyls, as well as inorganic pollutants such as plant macronutrients, plant trace elements, non-essential elements for plants, or radioactive isotopes (Pilon-Smits 2005). During the last decade phytoremediation has not only received scientific but also commercial attention, but a considerable phytoremediation market currently exists in the USA only (Meagher 2000; McGrath and Zhao 2003; Pilon-Smits 2005). Recently, fast growing trees have been identified as particularly useful for phytoremediation, because they can produce large biomass that can be used for other purposes (wood products, bioenergy, *etc.*) subsequent to phytoremediation, can be cultivated in short rotation forestry plantations similarly to agricultural systems, produce an extensive root system that intensively explores and stabilizes the soil, are in many cases not part of food-chains and can be harvested before flowering. The latter is of particular advantage for the use of genetically modified plants (GMPs) in phytoremediation with respect to biological safety (Peuke and Rennenberg 2005a; b).

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33.)

Effects of cold-girdling on flows in the transport phloem in *Ricinus communis:* is mass flow inhibited?

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ABSTRACT

The effects of cold girdling of the transport phloem at the hypocotyl of *Ricinus communis* on solute and water transport were investigated. Effects on the chemical composition of saps of phloem and xylem as well as of stem tissue were studied by conventional techniques and the water flow in the phloem was investigated by NMR imaging. Cold girdling reduced the concentration of sucrose but not that of inorganic solutes or amino acids in phloem saps. The possibility that cold treatment inhibited the retrieval of sucrose into the phloem, following leaching from the sieve tubes along a chemical gradient is discussed. Leaching of other solutes did not occur, as a result of missing promoting gradients in stem tissue. Following 3 d of cold girdling, sugar concentration increased and starch was synthesized and accumulated in stem tissue above the cold girdling region and along the cold-treated phloem pathway due to leaching of sugars from the phloem. Only in the very first period of cold girdling (< 15–30 min) was mass flow inhibited, but recovered in the rest of cold treatment period to values similar to the control period before and the recovery period after the cold treatment. It is concluded that cold treatment affected phloem transport through two independent and reversible processes: (1) a permanent leaching of sucrose from the phloem stem without normal retrieval during cold treatment, and (2) a short-term inhibition of mass flow at the beginning of cold treatment, possibly involving P proteins. Possible further mechanisms for reversible inhibition of water flow are discussed.

Key-words: *Ricinus communis*; cold girdling; NMR-imaging; phloem; water flow.

INTRODUCTION

One of the most important features that have allowed higher plants to conquer dry land are the phloem and xylem which transport water, photo-assimilates, nutrients and signals over long distances within the plant. The phloem is responsible for the transport of metabolic products and for the recycling of mineral nutrients from the shoot to the root or within the shoot from mature leaves to younger growing parts. The phloem can be divided into three functional areas: loading, transport and unloading phloem (van Bel 1993, 2003a, b). While the loading phloem is responsible for active loading of photoassimilates into the phloem, the unloading phloem delivers assimilates to sink tissues, and the transport phloem translocates water and solutes from source to sink. In the transport phloem, which generally can be found from the main leaf veins downwards, release as well as loading (retrieval) of sugars takes place (Patrick 1997; van Bel 1998, 2003a, b; Komor 2000; Lalonde *et al*. 2003; Gould, Minchin & Thorpe 2004). Along the way the transport phloem provides the surrounding tissue with assimilates for processes such as maintenance and growth.

The forces that drive phloem transport and dictate the direction of flow originate in the source–sink relations between the phloem and the tissue surrounding it (van Bel 1998, 2003a, b; Bancal & Soltani 2002; Henton *et al*. 2002; Thompson & Holbrook 2003; Lalonde *et al*. 2003; Gould *et al*. 2004). It has been shown that in the phloem, but also in the xylem, complex and fragile gradients in pressure and osmotic potential exist that are easily disturbed by invasive experimentation (van Bel 1998, 2003a, b;Bancal & Soltani 2002; Lalonde *et al*. 2003). It is widely accepted that these potential gradients drive long-distance transport in the phloem as well as the xylem, even though the current debate around the validity of the cohesion tension theory (Zimmermann *et al*. 2004) has demonstrated that not everybody agrees on the question of how large the water potential gradients in xylem vessels can be.

Long-distance phloem transport within intact plant systems is especially difficult to measure because only few techniques are suitable and because of the extreme sensitivity of the phloem to wounding. In a number of studies dyes or radioactive tracers were applied or injected to investigate transport in the phloem (see *inter alia* Patrick 1997; Knoblauch & van Bel 1998; Oparka & Turgeon 1999; Komor 2000). Additionally, the small vessel sizes, the slow flow velocities and the small flowing volume per unit of

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34.)

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The effect of drought on C and N stable isotopes in different fractions of leaves, stems and roots of sensitive and tolerant beech ecotypes

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ABSTRACT

Beech seedlings from 11 German climatic provenances were exposed to a realistically timed drought treatment in a greenhouse experiment. The stable isotope composition of carbon (C) and nitrogen (N) was analysed in pooled bulk material of roots, stems and leaves, as well as in the aqueous extracts and starch fractions. The δ^{13}C values increased in bulk samples (BS) of roots, stems and leaves by drought, although no leaf growth occurred during the experimental period. A clear drought effect on δ^{13}C in aqueous extracts was detected in leaves. In aqueous extracts of stems and roots as well as in starch fractions of all organs, abundance of δ^{13}C also tended to be increased by drought, but this effect was not statistically significant. For both δ^{13}C and δ^{15}N, enrichment was observed from the site of uptake/source to the site of use/sink. A gradient for δ^{13}C in all fractions from leaves (–29.49, –28.89 and –27.85‰) to stems (–28.81, –27.48 and –26.98‰) and to roots (–27.60, –26.37 and –26.48‰) was detected in BS, aqueous extracts and starch, respectively. An opposite gradient for δ^{15}N was found in BS: 1.59‰, 1.84‰ and 3.05‰ in roots, stems and leaves, respectively. δ^{15}N was neither affected by drought in the BS nor in aqueous extracts, but an effect of provenance was observed. Particularly in roots and stems, drought-sensitive provenances showed the strongest shifts in δ^{13}C induced by drought and the lowest δ^{15}N values. In the present experiment, δ^{13}C values were more affected by the environmental factor drought, while δ^{15}N values were more affected by the genetic factor provenance.

Key-words: drought stress, *Fagus sylvatica*, isotope discrimination, provenances.

INTRODUCTION

Climate change models predict that increasing atmospheric CO_2 partial pressure will cause average surface temperatures to increase by 1–3.5 °C in mid-latitude regions during the next 100 years (Saxe, Ellsworth & Heath 1998; UNEP 1999). As a consequence, precipitation and evaporation patterns will change in Europe, and ecosystems will be exposed to more intense drought and heavy rain events in summer (IPCC 1997; UNEP 1999). Changing climatic conditions will become of particular importance for trees because of their long lifespan. Forests will face altered environmental conditions during their lifetime, with likely consequences for species composition and forest management (IPCC 1997; Saxe *et al*. 2001). Natural regeneration of the drought-sensitive European beech (*Fagus sylvatica* L.) – one of the most important deciduous tree species in Central Europe – may be significantly affected by such climate alterations, specially because the area of distribution includes sites with shallow limestone-derived soils with low water storage capacity (e.g. Schwäbische Alb, Fränkische Alb, Schweizer Jura and French Jura). Therefore, projected short summer drought periods caused by global warming may inhibit natural regeneration in critical habitats.

Owing to its sensitivity towards environmental constraints, δ^{13}C and δ^{15}N are now widely used to assess the effects of changing climatic condition on plant ecophysiology. However, a question that requires further research is which parts, tissues, chemical fractions or compounds of plants reflect the best particular biotic or abiotic conditions. Bulk materials have been isotopically analysed to provide physiological or environmental insights over the lifespan of the plant part sampled (see in review Adams & Grierson 2001; Evans 2001; Dawson *et al*. 2002). Specific individual compounds or groups of compounds such as water extractable C, cellulose, lipids, sugars and starch have been isotopically analysed to provide information about metabolism integrated over particular time integrals (Picon, Ferhi & Guehl 1997; Gleixner *et al*. 1998; Ghashghaie *et al*. 2001; Ponton *et al*. 2001; Terwilliger *et al*. 2001; Arndt & Wanek 2002; Barbour, Walcroft & Farquhar 2002; Damesin & Lelarge 2003). Leaf material is most commonly studied, but axial tissue has also drawn attention (Picon *et al*. 1997; Ponton *et al*. 2001; Arndt & Wanek 2002; Barbour *et al*. 2002; Damesin & Lelarge 2003; Fotelli *et al*. 2003). Not much is known about environmental effects on stable isotope abundance in the roots (Robinson *et al*. 2000; Emmerton *et al*. 2001; Arndt & Wanek 2002; Fotelli *et al*. 2003). Variations

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7 Heavy Metal Resistance and Phytoremediation with Transgenic Trees

ANDREAS D PEUKE AND HEINZ RENNENBERG

7.1 Introduction

Phytoremediation is the technology that uses plants to remove or degrade various pollutants from the environment. It has received significant scientific and commercial attention during the last decades (Salt et al. 1998; Gleba et al. 1999; Meagher 2000; Dietz and Schnoor 2001; Guerinot and Salt 2001; Krämer and Chardonnens 2001; van der Lelie et al. 2001; Schwitzguébel et al. 2002; Hannink et al. 2002; McGrath and Zhao 2003; Vassilev et al. 2004; Krämer 2005; Peuke and Rennenberg 2005a,b; Pilon-Smits 2005). Salt et al. (1998) and Dietz and Schnoor (2001) distinguish between different types of phytoremediation: (1) phytoextraction, (2) phytodegradation/-transformation, (3) rhizofiltration (removal of pollutants from aqueous phases by plant roots), (4) phytostabilization, (5) phytovolatilization (using plants to volatilise pollutants), and (6) removal of pollutants from the air by plants. Most attention is focussed on phytoextraction, phytodegradation and phytostabilization (Fig. 7.1).

For phytoextraction, plants are grow on contaminated soil and harvested from time to time while the biomass can be used in different ways depending on the type of contamination. As an example, plant material can be burned for energy gain (Fig. 7.1a). The aims are to remove pollutants from the soil and to concentrate them in biomass; final combustion of plant material will concentrate contamination further by a factor of around 10 in dry matter. The resulting ashes must be deposited in conventional dumps or added to a smelter. Recovery of metals from plant tissue ("phytomining"), which was done in the case of potassium ("potash") for centuries by humans, may be economical (Meagher 2000; van der Lelie et al. 2001). Phytomining may constitute a "green" alternative to existing, environmentally destructive, opencast mining practice or to exploitation of ore bodies which are uneconomic by conventional methods (Brooks et al. 1998). Plants are also able to take up radioisotopes like ^{134}Cs and ^{137}Cs which are of environmental concern after discharges from nuclear installations (White and Broadley 2000; Schwitzguébel et al. 2002).

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Tree Transgenesis: Recent Developments

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Oxygen isotope enrichment of organic matter in *Ricinus communis* during the diel course and as affected by assimilate transport

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Summary

- The oxygen isotope composition in leaf water and organic compounds in different plant tissues is useful for assessing the physiological performance of plants in their environment, but more information is needed on $\Delta^{18}O$ variation during a diel course.
- Here, we assessed $\Delta^{18}O$ of the organic matter in leaves, phloem and xylem in stem segments, and fine roots of *Ricinus communis* during a full diel cycle. Enrichment of newly assimilated organic matter in equilibrium with leaf water was calculated by applying a nonsteady-state evaporative enrichment model.
- During the light period, $\Delta^{18}O$ of the water soluble organic matter pool in leaves and phloem could be explained by a 27‰ enrichment compared with leaf water enrichment. Leaf water enrichment influenced $\Delta^{18}O$ of phloem organic matter during the night via daytime starch synthesis and night-time starch remobilization. Phloem transport did not affect $\Delta^{18}O$ of phloem organic matter.
- Diel variation in $\Delta^{18}O$ in organic matter pools can be modeled, and oxygen isotopic information is not biased during transport through the plant. These findings will aid field studies that characterize environmental influences on plant water balance using $\Delta^{18}O$ in phloem organic matter or tree rings.

Key words: evaporative enrichment, oxygen isotopes, phloem-to-xylem exchange, phloem transport, transitory starch, xylem transport.

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Introduction

The determination of the oxygen isotope composition in leaf water and organic compounds is a promising tool for assessing the physiological performance of plants in their environment (Wang & Yakir, 1995; Barbour *et al.*, 2001, 2002; Cernusak *et al.*, 2005). In particular, the isotopic information in organic matter pools with different turn-over times (e.g. short-lived phloem sugars representing recent assimilates; long-lived structural compounds such as tree ring cellulose) is now widely used – often together with $\delta^{13}C$ signatures – to integrate the influence of a range of environmental factors on the water balance of plants (Saurer *et al.*, 1997; Scheidegger *et al.*, 2000; Keitel *et al.*, 2003; Brandes *et al.*, 2006). The oxygen isotope composition of organic matter is mainly influenced by two factors: the $\delta^{18}O$ signature of source water and the evaporative enrichment of mean lamina leaf water, which is the reaction water for the newly produced assimilates (Fehri & Letolle, 1977; Cernusak *et al.*, 2003b). Mean lamina leaf water enrichment depends in turn on the diffusion of ^{18}O enriched water from the sites of evaporation back into the mesophyll cells and the convection of unenriched xylem water via the transpiration stream in the opposite direction (Farquhar & Lloyd, 1993; Barbour *et al.*, 2000a). As evaporative enrichment

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Research article

Transpiration, CO_2 assimilation, WUE, and stomatal aperture in leaves of *Viscum album* (L.): Effect of abscisic acid (ABA) in the xylem sap of its host (*Populus x euamericana*)

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Abstract

Leaves of the mistletoe *Viscum album* (L.) show a high rate of transpiration, even when the host is under severe drought stress. The hypothesis that a strong control of ABA influx from the xylem sap of the host into the mistletoe prevents stomatal closure in mistletoe leaves was tested under the following conditions: sections of poplar twigs carrying a mistletoe were perfused with artificial xylem sap that contained different ABA concentrations and both transpiration and ABA levels were analysed in mistletoe leaves. Despite variation by a factor of 10^4, the ABA content of the host xylem did not affect ABA levels, leaf transpiration, CO_2 assimilation, WUE, or the degree of stomatal aperture in mistletoe leaves. These observations support the hypothesis of a strong control of ABA influx from the host of the xylem into the mistletoe, although degradation of ABA before it enters the mistletoe leaves cannot be excluded. This mechanism may ensure a water and nutritional status favourable for the mistletoe, even if the water status of the host is impaired.

Despite the lack of short-term sensitivity of ABA levels in mistletoe leaves to even strong changes of ABA levels in the xylem sap of the host, ABA levels in mistletoe leaves were relatively high compared to ABA levels in the leaves of several tree species including poplar. Since significant transpiration of the mistletoe leaves was observed despite high ABA levels, a diminished sensitivity of the stomata of mistletoe leaves to ABA has to be concluded. The stomatal density of adaxial *Viscum* leaves of 89 ± 23 stomata per mm is lower than those reported in a study performed at the end of the 19th century.

Keywords: ABA; Abscisic acid; Assimilation; Stomatal aperture; Transpiration; Xylem sap

1. Introduction

One of the most striking features of the mistletoe *Viscum album* (L.) is its high rate of transpiration, even when the host is under severe drought stress [23,54]. As a consequence, twigs of the host that are distal to the insertion of a mistletoe often dry out completely and die [53]. For several reasons, this observation is surprising. Excessive water loss of leaves under drought stress is usually prevented to some extent by regulating the degree of stomatal aperture [40,52] and abscisic acid (ABA) produced in the roots and allocated to the leaves with the transpiration stream is a central component of the

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Experimental evidence for diel variations of the carbon isotope composition in leaf, stem and phloem sap organic matter in *Ricinus communis*

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ABSTRACT

Carbon isotope fractionation in metabolic processes following carboxylation of ribulose-1,5-bisphosphate (RuBP) is not as well described as the discrimination during photosynthetic CO_2 fixation. However, post-carboxylation fractionation can influence the diel variation of $\delta^{13}C$ of leaf-exported organic matter and can cause inter-organ differences in $\delta^{13}C$. To obtain a more mechanistic understanding of post-carboxylation modification of the isotopic signal as governed by physiological and environmental controls, we combined the modelling approach of Tcherkez *et al.*, which describes the isotopic fractionation in primary metabolism with the experimental determination of $\delta^{13}C$ in leaf and phloem sap and root carbon pools during a full diel course. There was a strong diel variation of leaf water-soluble organic matter and phloem sap sugars with relatively ^{13}C depleted carbon produced and exported during the day and enriched carbon during the night. The isotopic modelling approach reproduces the experimentally determined day–night differences in $\delta^{13}C$ of leaf-exported carbon in *Ricinus communis*. These findings support the idea that patterns of transitory starch accumulation and remobilization govern the diel rhythm of $\delta^{13}C$ in organic matter exported by leaves. Integrated over the whole 24 h day, leaf-exported carbon was enriched in ^{13}C as compared with the primary assimilates. This may contribute to the well-known – yet poorly explained – relative ^{13}C depletion of autotrophic organs compared with other plant parts. We thus emphasize the need to consider post-carboxylation fractionations for studies that use $\delta^{13}C$ for assessing environmental effects like water availability on ratio of mole fractions of CO_2 inside and outside the leaf (e.g. tree ring studies), or for partitioning of CO_2 fluxes at the ecosystem level.

Key-words: isotope modelling; post-carboxylation fractionation; starch; transport.

INTRODUCTION

Whereas carbon isotope discrimination during photosynthetic CO_2 fixation is a comparatively well-described and understood phenomenon (Farquhar, O'Leary & Berry 1982; Farquhar, Ehleringer & Hubick 1989), much less is known about the isotopic fractionation associated with the metabolic processes following carboxylation in leaf tissues (Hobbie & Werner 2004; Badeck *et al.* 2005; Brandes *et al.* 2006). However, fractionations because of equilibrium, kinetic and fragmentation (Tcherkez *et al.* 2004) isotope effects beyond CO_2 diffusion and fixation by ribulose 1·5-bisphosphate carboxylase/oxygenase (Rubisco) are of importance because they result in differences in isotopic signatures among metabolites and in non-statistical intramolecular isotope distributions (Schmidt & Gleixner 1998; Schmidt 2003; Tcherkez & Farquhar 2005).

Among the most obvious consequences of these effects is that the carbon isotope composition of organic matter may differ between plant organs depending on the $\delta^{13}C$ of exported and non-exported compounds. Badeck *et al.* (2005) reviewed more than 80 publications for differences in $\delta^{13}C$ between organs and showed that heterotrophic tissues are generally enriched in ^{13}C compared wirht autotrophic organs. As temporal variations in photosynthetic discrimination were excluded as an explanation of inter-organ differences, there must be either post-carboxylation fractionation in autotrophic tissues and export of ^{13}C-enriched metabolites across organ boundaries (Hobbie & Werner 2004) or fractionation during heterotrophic metabolism (Helle & Schleser 2004), or both (Brandes *et al.* 2006).

Post-carboxylation carbon isotope fractionation might account for diel variations in the isotopic composition of carbon exported from the leaves to heterotrophic tissues

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RESEARCH PAPER

Chloroplast parameters differ in wild type and transgenic poplars overexpressing *gsh1* in the cytosol

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Keywords
Chloroplast shape; chloroplast size; glutathione; heavy metal; transgenic poplar; γ-glutamylcysteine synthetase.

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ABSTRACT

Poplar mutants overexpressing the bacterial genes *gsh1* or *gsh2* encoding the enzymes of glutathione biosynthesis are among the best-characterised transgenic plants. However, this characterisation originates exclusively from laboratory studies, and the performance of these mutants under field conditions is largely unknown. Here, we report a field experiment in which the wild-type poplar hybrid *Populus tremula* × *P. alba* and a transgenic line overexpressing the bacterial gene *gsh1* encoding γ-glutamylcysteine synthetase in the cytosol were grown for 3 years at a relatively clean (control) field site and a field site contaminated with heavy metals. Aboveground biomass accumulation was slightly smaller in transgenic compared to wild-type plants; soil contamination significantly decreased biomass accumulation in both wild-type and transgenic plants by more than 40%. Chloroplasts parameters, *i.e.*, maximal diameter, projection area and perimeter, surface area and volume, surface/volume ratio and a two-dimensional form coefficient, were found to depend on plant type, leaf tissue and soil contamination. The greatest differences between wild and transgenic poplars were observed at the control site. Under these conditions, chloroplast sizes in palisade tissue of transgenic poplar significantly exceeded those of the wild type. In contrast to the wild type, palisade chloroplast volume exceeded that of spongy chloroplasts in transgenic poplars at both field sites. Chlorophyll content per chloroplast was the same in wild and transgenic poplars. Apparently, the increase in chloroplast volume was not connected to changes in the photosynthetic centres. Chloroplasts of transgenic poplar at the control site were more elongated in palisade cells and close to spherical in spongy mesophyll chloroplasts. At the contaminated site, palisade and spongy cell chloroplasts of leaves from transgenic trees and the wild type were the same shape. Transgenic poplars also had a smaller chloroplast surface/volume ratio, both at the control and the contaminated site. Chloroplast number per cell did not differ between wild and transgenic poplars at the control site. Soil contamination led to suppression of chloroplast replication in wild-type plants. From these results, we assume that overexpressing the bacterial *gsh1* gene in the cytosol interacts with processes in the chloroplast and that sequestration of heavy metal phytochelatin complexes into the vacuole may partially counteract this interaction in plants grown at heavy metal-contaminated field sites. Further experiments are required to test these assumptions.

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Nutrient composition of leaves and fruit juice of grapevine as affected by soil and nitrogen fertilization

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Abstract

Effects of soil type and nitrogen (N) fertilizer–application rates on the nutrient composition of grapevine (*Vitis vinifera* L. cv. Riesling) leaves during a growing cycle were compared with the composition of the resulting grape juice. Grapevines were planted in 75 L containers that had been installed in a vineyard and filled with three different vineyard soils (loess, shell lime, and Keuper). Four typical levels of N fertilizer (40, 80, 120, and 160 kg N ha^{-1}) were applied. Elemental composition of mature leaves sampled seven times during the growing cycle as well as of the extracted grape juice was analyzed. The time of sampling affected all measured elements (C, N, Ca, K, P, Mg, S, Fe, Zn, Mn, and B) in the leaves. Nitrogen-fertilizer rate affected the concentrations of all elements except Ca and Mg, while the soil type had significant effects on elemental composition of the leaves with the exception of N, B, and Ca. Soil type had a significant effect on K, S, Mn, and B in the grape juice. Increasing rates of N fertilizer increased C concentration in the grape juice significantly and affected its elemental composition similar to the effects in leaves. This may be explained with the role of leaves as the source for supplying the grapes during ripening *via* phloem transport. Cluster analysis for the elemental composition of soils, leaves, and grape juice revealed no consistent relationships indicating that other soil characteristics in addition to the mineral concentration influence the elemental composition of grapevine leaves and grape juice.

Key words: elemental composition / grape juice / grapevine / leaves / nitrogen-fertilizer rate / soil type

Accepted March 19, 2009

1 Introduction

Along with climatic conditions, soil type and nutrient supply are important determinants of quantity and quality of yield of agricultural plants. An integrated view of agricultural management aims to decrease inputs while maintaining or increasing yield quantity and quality (*Vance*, 2001; *Jeuffroy* et al., 2002). Knowledge of the minimum fertilizer input that will result in the desired yield quantity and/or quality is of special interest (*Gastal* and *Lemaire,* 2002). Aside from economic considerations, fertilizer use has ecological implications, too. For example, the leaching of nitrate from vineyards drastically increased as nitrogen (N)-fertilizer inputs increased (*Müller*, 1993), which subsequently led to high levels of nitrate in groundwater. In the case of grapevine, the impact of climate, soil, and crop management on the quality of the end product—wine—is the subject of extensive discussion (*Jackson* and *Lombard*, 1993; *Keller*, 2005). The effects of N supply on grapevine N metabolism, N transport, and accumulation, as well as growth and yield has been the subject of a number of investigations (*Bell* et al., 1979; *Kannenberg*, 1990; *Müller*, 1991, 1993; *Keller* et al., 1995; *Keller*, 2005).

In viticulture, analysis of the chemical composition of leaves is an important management tool (*Kannenberg*, 1990). The nutrient status of leaves will directly affect total biomass production including the allocation of mineral nutrients to the fruits. *Pate* (1980) concluded that for C, N, and K, the supply of fruits by phloem is more important than by xylem. In grapes, it was shown by *Lang* and *Düring* (1991) that during ripening, at the time of breakdown of cellular compartmentation, import *via* phloem increased drastically. However, during these processes there are a number of transporter-mediated steps that will influence the quantity and the distribution of nutrients in the plant. Therefore, it is unclear if the soil's "mineral signature" will be reflected in the leaves or fruits.

In the present study, the effect of mineral composition of soils, derived from different geological formations, and of N fertilizer–application rates on the elemental composition of grapevine leaves was investigated during an entire vegetation period and compared with the elemental composition of the grape juice. To avoid climatic effects, soils were collected and filled into pots in which grapes were grown in a vineyard under the same environmental conditions and management. The aim of this study was to identify correlations between the elemental composition of soils, the grapevine leaves, and the corresponding juice.

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REVIEW PAPER

Correlations in concentrations, xylem and phloem flows, and partitioning of elements and ions in intact plants. A summary and statistical re-evaluation of modelling experiments in *Ricinus communis*

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Abstract

Within the last two decades, a series of papers have dealt with the effects of nutrition and nutrient deficiency, as well as salt stress, on the long-distance transport and partitioning of nutrients in castor bean. Flows in xylem and phloem were modelled according to an empirically-based modelling technique that permits additional quantification of the uptake and incorporation into plant organs. In the present paper these data were statistically re-evaluated, and new correlations are presented. Numerous relationships between different compartments and transport processes for single elements, but also between elements, were detected. These correlations revealed different selectivities for ions in bulk net transport. Generally, increasing chemical concentration gradients for mineral nutrients from the rhizosphere to the root and from the xylem to leaf tissue were observed, while such gradients decreased from root tissue to the xylem and from leaves to the phloem. These studies showed that, for the partitioning of nutrients within a plant, the correlated interactions of uptake, xylem and phloem flow, as well as loading and unloading of solutes from transport systems, are of central importance. For essential nutrients, tight correlations between uptake, xylem and phloem flow, and the resulting partitioning of elements, were observed, which allows the stating of general models. For non-essential ions like Na^+ or Cl^-, a statistically significant dependence of xylem transport on uptake was not detected. The central role of the phloem for adjusting, but also signalling, of nutrition status is discussed, since strong correlations between leaf nutrient concentrations and those in phloem saps were observed. In addition, negative correlations between phloem sap sugar concentration and net-photosynthesis, growth, and uptake of nutrients were demonstrated. The question remains whether this is only a consequence of an insufficient use of carbohydrates in plants or a ubiquitous signal for stress in plants. In general, high sugar concentrations in phloem saps indicate (nutritional) stress, and high nutrient concentrations in phloem saps indicate nutritional sufficiency of leaf tissues.

Key words: Castor bean, flow model, long distance transport, nutrient deficiency, nutrients, phloem transport, signalling, uptake, xylem transport.

Introduction

On land, Higher Plants face the problem of having photosynthesis, i.e. the site for the capturing of light energy and CO_2, displaced from the site where water and mineral nutrients are taken up. Therefore, one of the chief requirements for land plants is the presence of long-distance transport systems. In cormophytes, these demands are fulfilled by the actions of phloem and xylem. The xylem transports water, mineral nutrients, metabolic products, and signals from the root to the shoot. By contrast, the phloem transports assimilation products from photosynthetically active or remobilizing 'source' tissues, to growing areas within the shoot and the root, the so-called 'sinks', via

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RESEARCH PAPER

Phloem sap and leaf δ^{13}C, carbohydrates, and amino acid concentrations in *Eucalyptus globulus* change systematically according to flooding and water deficit treatment

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Abstract

Phloem is a central conduit for the distribution of photoassimilate, nutrients, and signals among plant organs. A revised technique was used to collect phloem sap from small woody plants in order to assess changes in composition induced by water deficit and flooding. Bled phloem sap δ^{13}C and sugar concentrations were compared to δ^{13}C of bulk material, soluble carbon extracts, and the neutral sugar fraction from leaves. Amino acid composition and inorganic ions of the phloem sap was also analysed. Quantitative, systematic changes were detected in phloem sap composition and δ^{13}C in response to altered water availability. Phloem sap δ^{13}C was more sensitive to changes of water availability than the δ^{13}C of bulk leaf, the soluble carbon fraction, and the neutral soluble fraction of leaves. Changes in water availability also resulted in significant changes in phloem sugar (sucrose and raffinose), inorganic nutrient (potassium), and amino acid (phenylalanine) concentrations with important implications for the maintenance of phloem function and biomass partitioning. The differences in carbohydrate and amino acid composition as well as the δ^{13}C in the phloem, along with a new model system for phloem research, offer an improved understanding of the phloem-mediated signal, nutrient, and photoassimilate transduction in relation to water availability.

Key words: Amino acids, *Eucalyptus*, flooding, phloem sap, raffinose, sucrose, water deficit.

Introduction

Phloem is the major conduit for the transport of solutes and signalling among tissues of higher plants. The composition of phloem sap therefore offers considerable promise for use in diagnostic assessments of plant health. The development of such assessments is limited by our ability to sample phloem easily, with the characterization of phloem contents limited to a handful of studies of mainly herbaceous species (a for a review see Turgeon and Wolf, 2009).

Recently, several authors have begun investigating the nature and composition of phloem sap in tree species (Gessler *et al.*, 2004, 2007; Scartazza *et al.*, 2004; Tausz *et al.*, 2008) using a phloem collection technique originally developed by Hartig (1860) and further developed for *E. globulus* by Pate *et al.* (1974, 1984). Using a razor blade, a small incision is made in the stem to the depth of the cambium and phloem 'bleeds' from the surface of the cut. This method has considerable advantages over more traditional techniques of phloem sap collection (highlighted by Turgeon, 2006) in that it is fast and it avoids the need for chelating reagents and extraction procedures, thus reducing contamination from companion cell contents (for review see Turgeon and Wolf, 2009).

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43.)

Over-expression of bacterial γ-glutamylcysteine synthetase (*GSH1*) in plastids affects photosynthesis, growth and sulphur metabolism in poplar (*Populus tremula* × *Populus alba*) dependent on the resulting γ-glutamylcysteine and glutathione levels

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ABSTRACT

We compared three transgenic poplar lines over-expressing the bacterial γ-glutamylcysteine synthetase (*GSH1*) targeted to plastids. Lines Lggs6 and Lggs12 have two copies, while line Lggs20 has three copies of the transgene. The three lines differ in their expression levels of the transgene and in the accumulation of γ-glutamylcysteine (γ-EC) and glutathione (GSH) in leaves, roots and phloem exudates. The lowest transgene expression level was observed in line Lggs6 which showed an increased growth, an enhanced rate of photosynthesis and a decreased excitation pressure (1-qP). The latter typically represents a lower reduction state of the plastoquinone pool, and thereby facilitates electron flow along the electron transport chain. Line Lggs12 showed the highest transgene expression level, highest γ-EC accumulation in leaves and highest GSH enrichment in phloem exudates and roots. This line also exhibited a reduced growth, and after a prolonged growth of 4.5 months, symptoms of leaf injury. Decreased maximum quantum yield (F_v/F_m) indicated down-regulation of photosystem II reaction centre (PSII RC), which correlates with decreased PSII RC protein D1 (PsbA) and diminished light-harvesting complex (Lhcb1). Potential effects of changes in chloroplastic and cytosolic GSH contents on photosynthesis, growth and the whole-plant sulphur nutrition are discussed for each line.

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Key-words: APS reductase; ATP sulphurylase; chlorophyll fluorescence; glutathione; long-distance transport; maximum quantum yield; phloem; photosystem II; sulphate assimilation; transgenic poplar.

Abbreviations: γ-EC, γ-glutamylcysteine; γ-ECS, γ-glutamylcysteine synthetase; GSH, glutathione (reduced state); *GSH1*, γ-glutamylcysteine synthetase gene; *GSH2*, glutathione synthetase gene; GSSG, glutathione (oxidized state).

INTRODUCTION

Glutathione (GSH) is an important component of the primary metabolism of plants. GSH is involved in various processes including storage and transport of reduced sulphur (Rennenberg, Schmitz & Bergmann 1979; Rennenberg 1984; Herschbach 2003), stress response to reactive oxygen (Polle & Rennenberg 1993; Foyer & Noctor 2005a,b) and heavy metals (Rauser 1995, 1999; Cobbett 2000a,b; Peuke & Rennenberg 2005a,b), as well as detoxification of xenobiotics (Rennenberg & Lamoureux 1990; Edwards & Dixon 2005). In addition, GSH is a key component to maintain the cellular redox state (Meyer & Hell 2005; Mullineaux & Rausch 2005). For example, under oxidative stress (Rouhier, Lemaire & Jacquot 2008) or during flower development (Xing, Lauri & Zachgo 2006), synthesis of various cellular proteins is under GSH-mediated redox control. The GSH-mediated redox control can occur via glutathionylation of proteins, and thus at the transcriptional and/or post-transcriptional level (Dietz 2008; Meyer 2008). GSH is synthesized in two ATP-dependent steps catalysed by consecutive action of γ-glutamylcysteine synthetase (γ-ECS), which forms γ-glutamylcysteine (γ-EC) from cysteine (Cys) and glutamate, and glutathione synthetase (GSHS), which adds glycine to the γ-EC.

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Research paper

Impacts of drought on mineral macro- and microelements in provenances of beech (*Fagus sylvatica* L.) seedlings

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Beech seedlings originating from 11 German provenances with different climatic conditions were grown in pots and cultivated in a greenhouse. The composition of macro- and microelements in roots, axes and leaves was measured after half of the seedlings were subjected to a simulated summer drought. The recently described sensitivity of these provenances to drought was compared with drought-mediated changes in the elemental and ionic composition in organs of the seedlings; in addition, partitioning between roots and shoots was evaluated. A number of element concentrations were decreased in roots due to drought (K 94% of control, Mg 94%, Mn 75% and Zn 85%). However, chloride concentration increased in all organs (115–125%) and was the only element affected in leaves. Some changes in ionome can be related to sensitivity of provenances, but it is difficult to decide whether these changes are a result of, or a reason for, drought tolerance or sensitivity. Observed increases in chloride concentration in all plant parts of drought-treated beech seedlings can be explained by its function in charge balance, in particular since the level of phosphate was reduced. As a result of chloride accumulation, the sum of added charges of anions (and cations) in water extracts of leaf and root material was similar between drought and control plants. Since only the partitioning of Ca and Al (both only in axis) as well as Mn was affected and other elements (together with previously observed effects on C, N, S and P) remained unaffected by drought in all provenances, it can be concluded that direct effects by means of mass flow inhibition in xylem and phloem are unlikely. Secondary effects, for example on the pH of transport sap and the apoplastic space, cannot be excluded from the present study. These effects may affect partitioning between the apoplast and symplast and therefore may be significant for drought sensitivity.

Keywords: anions, beech, cations, plant nutrition, water stress.

Introduction

European beech (*Fagus sylvatica* L.) is one of the dominant tree species in Central Europe that is known to be drought sensitive (Backes and Leuschner 2000, Gessler et al. 2004). Climate models predict an increase in air temperature and changing precipitation patterns, which in combination can increase the risk of severe drought periods in the current range of distribution of beech (IPCC 2007). During the 2003 summer drought, beech forests in Central Europe were among those forests that expressed the largest reductions in net ecosystem productivity (Ciais et al. 2005). Drought affects tree function in many ways, including gas exchange, cell growth and division, phytohormone levels, metabolism and transport processes (Hsiao 1973). Owing to drought, gradually decreasing stomatal conductance, predawn leaf water potential, assimilation and growth are commonly observed in trees, accompanied by a stimulation of fine root growth (Leuzinger et al. 2005). In addition, both greater temperatures and periods with limited water supply are thought to alter the ability of terrestrial ecosystems to take up elements. Leaves, roots and stems generally differ in their sensitivity to drought. Therefore, a whole-tree approach rather than restricted studies at the leaf level is required to properly address the consequences of drought on tree function (Leuschner et al. 2001).

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45.)

RESEARCH PAPER

Over-expression of *gsh1* in the cytosol affects the photosynthetic apparatus and improves the performance of transgenic poplars on heavy metal-contaminated soil

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Keywords
Cell number; cell size; chloroplast; γ-glutamylcysteine synthetase; glutathione; mesophyll structure.

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ABSTRACT

Recent studies of transgenic poplars over-expressing the genes *gsh1* and *gsh2* encoding γ-glutamylcysteine synthetase (γ-ECS) and glutathione synthetase, respectively, provided detailed information on regulation of GSH synthesis, enzymes activities and mRNA expression. In this experiment, we studied quantitative parameters of leaves, assimilating tissues, cells and chloroplasts, mesophyll resistance for CO_2 diffusion, chlorophyll and carbohydrate content in wild-type poplar and transgenic plants over-expressing *gsh1* in the cytosol after 3 years of growth in relatively clean (control) or heavy metal-contaminated soil in the field. Over-expression of *gsh1* in the cytosol led to a twofold increase of intrafoliar GSH concentration and influenced the photosynthetic apparatus at different levels of organisation, *i.e.*, leaves, photosynthetic cells and chloroplasts. At the control site, transgenic poplars had a twofold smaller total leaf area per plant and a 1.6-fold leaf area per leaf compared to wild-type controls. Annual aboveground biomass gain was reduced by 50% in the transgenic plants. The reduction of leaf area of the transformants was accompanied by a significant decline in total cell number per leaf, indicating suppression of cell division. Over-expression of γ-ECS in the cytosol also caused changes in mesophyll structure, *i.e.*, a 20% decrease in cell and chloroplast number per leaf area, but also an enhanced volume share of chloroplasts and intercellular airspaces in the leaves. Transgenic and wild poplars did not exhibit differences in chlorophyll and carotenoid content of leaves, but transformants had 1.3-fold fewer soluble carbohydrates. Cultivation on contaminated soil caused a reduction of palisade cell volume and chloroplast number, both per cell and leaf area, in wild-type plants but not in transformants. Biomass accumulation of wild-type poplars decreased in contaminated soil by more than 30-fold, whereas transformants showed a twofold decrease compared to the control site. Thus, poplars over-expressing γ-ECS in the cytosol were more tolerant to heavy metal stress under field conditions than wild-type plants according to the parameters analysed. Correlation analysis revealed strong dependence of cell number per leaf area unit, chloroplast parameters and mesophyll resistance with the GSH level in poplar leaves.

INTRODUCTION

The sulphur-containing tripeptide glutathione (GSH) is the major low molecular mass peptide in plants and is present at millimolar concentrations within cells. It is involved in many cellular processes through its influence on intracellular redox state (Noctor & Foyer 1998), its function as a transport form and reservoir of reduced sulphur (Rennenberg 2001) and its significance in the cross-talk between sulphur, nitrogen and carbon metabolism (Kopriva & Rennenberg 2004). In addition, GSH plays an important role in the defence of plant cells against reactive oxygen species (Noctor & Foyer 1998), xenobiotics (Edwards & Dixon 2005) and heavy metals (Cobbett & Goldsbrough 2002).

Lines of the poplar hybrid, *Populus tremula* × *P. alba*, over-expressing the bacterial genes *gsh1* or *gsh2* encoding γ-glutamylcysteine synthetase (γ-ECS) or glutathione synthetase, respectively, are widely known as good models for studies of the effects of enhanced glutathione (GSH) biosynthesis in plants (Noctor *et al.* 1998; Kopriva & Rennenberg 2004; Rennenberg & Peuke 2005). Therefore, these poplar transformants are among the best-characterised transgenic plants (Noctor *et al.* 1998). In laboratory experiments, transgenic poplars over-expressing γ-ECS in the cytosol did not differ from wild-type lines morphologically during initial growth and development (Noctor *et al.* 1996; Gullner *et al.* 2001) but had distinct functional features. Transformants were more tolerant towards chloroacetanilide herbicides than

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Chapter 13

Methods for Xylem Sap Collection

M. Alexou and A.D. Peuke

Abstract

Xylem and phloem are essential for the exchange of solutes and signals among organs of land plants. The synergy of both enables the transport and ultimately the partitioning of water, nutrients, metabolic products and signals among the organs of plants. The collection and analysis of xylem sap allow at least qualitative assumptions about bulk transport in the transpiration stream. For quantification of element-, ion-, and compound-flow, the additional estimation of volume flow is necessary. In this chapter we describe methods for collecting xylem sap by (1) root pressure exudate, (2) Scholander-Hammel pressure vessel, (3) root pressurizing method according to Passioura, and (4) (hand/battery) vacuum pump.

Key words: Xylem sap, Root pressure, Scholander-Hammel pressure vessel, "Passioura vessel"

1. Introduction

One of the most important and characteristic features of higher plants is their adaptation to life on land via the evolution of long distance transport systems. The requirement of long distance transport is fulfilled by phloem and xylem in cormophytes, which enable the transport of water, nutrients, and signals among the organs of plants (1). In the xylem water, minerals, products from root metabolism, and signals are transported from the root to transpiring parts of the shoot, particularly the photosynthetically active leaves. Large and especially tall plants must exhibit special features in their xylem. For example, tall trees (up to 100 m in height) must overcome significant gravitational forces in order to lift transport saps to the top of the tree.

The current view of the driving forces of long distance transport is based on gradients in the transport systems, i.e., gradients in hydrostatic pressure, water potential, and chemical potential. Gas exchange (water vapor, CO_2, O_2) and associated processes are central factors in regulating the long distance transport.

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47.)

Original Article

Experimental evidence for diel δ^{15}N-patterns in different tissues, xylem and phloem saps of castor bean (*Ricinus communis* L.)

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ABSTRACT

Nitrogen isotope signatures in plants might give insights in the metabolism and allocation of nitrogen. To obtain a deeper understanding of the modifications of the nitrogen isotope signatures, we determined δ^{15}N in transport saps and in different fractions of leaves, axes and roots during a diel course along the plant axis. The most significant diel variations were observed in xylem and phloem saps where δ^{15}N was significantly higher during the day compared with during the night. However in xylem saps, this was observed only in the canopy, but not at the hypocotyl positions. In the canopy, δ^{15}N was correlated fairly well between phloem and xylem saps. These variations in δ^{15}N in transport saps can be attributed to nitrate reduction in leaves during the photoperiod as well as to ^{15}N-enriched glutamine acting as transport form of N. δ^{15}N of the water soluble fraction of roots and leaves partially affected δ^{15}N of phloem and xylems saps. δ^{15}N patterns are likely the result of a complex set of interactions and N-fluxes between plant organs. Furthermore, the natural nitrogen isotope abundance in plant tissue is not constant during the diel course – a fact that needs to be taken into account when sampling for isotopic studies.

Key-words: day/night cycle; isotope fractionation; nitrogen; transport.

INTRODUCTION

Nitrogen isotopes are well-recognized tools in plant physiology and eco(physio)logy (Högberg 1997; Robinson 2001; Dawson *et al.* 2002). The natural nitrogen isotope composition (δ^{15}N) is now being widely used in research on N cycling in organisms and ecosystems. ^{15}N natural abundances are used in fundamentally different ways, from traditional ^{15}N tracers by integrating N cycle processes via N isotope fractionations and the mixing of various N-containing pools (Robinson 2001). Nitrogen isotope composition of plant material is determined by the isotope ratio of the external nitrogen source (nitrate, ammonium, amino acids and/or N_2) and physiological mechanisms within the plant like assimilation events, loss of nitrogen, resorption and reallocation of nitrogen (Högberg 1997; Robinson, Handley & Scrimgeour 1998; Comstock 2001; Evans 2001; Robinson 2001; Dawson *et al.* 2002; Werner & Schmidt 2002; Craine *et al.* 2009). The bulk nitrogen pool of plant organs contains multiple N species such as inorganic nitrogen, amino acids, proteins and chlorophylls. Variations in δ^{15}N can thus also be attributed to different mixing ratios of different N species, each of which could potentially have a distinct δ^{15}N (Werner & Schmidt 2002; Tcherkez 2011; Gauthier *et al.* 2012). Consequently, there is substantial variation in δ^{15}N values between ecosystems, plant species, plant individuals or plant parts and biochemical fractions. Handley *et al.* (1999) and Craine *et al.* (2009) reported that foliar δ^{15}N increased with decreasing mean annual precipitation and with increasing mean annual temperature. The variation range in plant δ^{15}N is generally –10 to +10‰ (Evans 2001) with quite large differences between plants with nitrate as the sole nitrogen source (Högberg 1997). It is believed that nitrate availability contributes to this variability, plant cultivated under low nitrate concentration being less depleted in ^{15}N than those cultivated under higher nitrate supply (Evans 2001). In case of low nitrate concentration in the soil, the efflux of (^{15}N-enriched) nitrate from the root to the soil is limited, and as a result, all nitrate taken up will be assimilated. Additionally, at higher nitrate supply, more nitrate is transported to the shoots for assimilation (Peuke *et al.* 1996), which may affect the δ^{15}N of the shoots. Furthermore, there are differences in δ^{15}N between plant organs, reported both in the lab and in the field (Högberg 1997), with shoots being generally ^{15}N-enriched compared with roots (Yoneyama *et al.* 1997; Peuke, Gessler & Rennenberg 2006). It is believed that this difference in δ^{15}N value is caused by the isotope fractionation against ^{15}N during nitrate reduction: nitrate molecules left behind after reduction in roots are ^{15}N-enriched and translocated to shoots (for a review, see Tcherkez & Hodges 2008). As a matter of fact, the allocation of nitrogenous compounds of contrasted δ^{15}N is thus certainly the cornerstone causing differences in δ^{15}N between plant parts.

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48.)

Original Article

Phloem flow and sugar transport in *Ricinus communis* L. is inhibited under anoxic conditions of shoot or roots

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ABSTRACT

Anoxic conditions should hamper the transport of sugar in the phloem, as this is an active process. The canopy is a carbohydrate source and the roots are carbohydrate sinks. By fumigating the shoot with N_2 or flooding the rhizosphere, anoxic conditions in the source or sink, respectively, were induced. Volume flow, velocity, conducting area and stationary water of the phloem were assessed by non-invasive magnetic resonance imaging (MRI) flowmetry. Carbohydrates and $\delta^{13}C$ in leaves, roots and phloem saps were determined.

Following flooding, volume flow and conducting area of the phloem declined and sugar concentrations in leaves and in phloem saps slightly increased. Oligosaccharides appeared in phloem saps and after 3 d, carbon transport was reduced to 77%. Additionally, the xylem flow declined and showed finally no daily rhythm. Anoxia of the shoot resulted within minutes in a reduction of volume flow, conductive area and sucrose in the phloem sap decreased. Sugar transport dropped to below 40% by the end of the N_2 treatment. However, volume flow and phloem sap sugar tended to recover during the N_2 treatment.

Both anoxia treatments hampered sugar transport. The flow velocity remained about constant, although phloem sap sugar concentration changed during treatments. Apparently, stored starch was remobilized under anoxia.

Key-words: *Ricinus*; carbohydrates; flooding; isotopic signature; phloem; xylem.

INTRODUCTION

The primary products of photosynthesis – sugars – are translocated within plants mostly by the phloem system. The classic 'Druckstrom-' (pressure flow) theory (Münch 1930) divided the phloem system in three parts: loading/collection, transport and unloading/release phloem. Sugars are loaded in the sieve tubes of the collection phloem in source tissues and unloaded/escape from the sieve tubes of the release phloem in sinks. The resulting turgor difference across the phloem drives mass flow in the source-to-sink direction (e.g. van Bel 2003; Lalonde *et al*. 2003; Gould *et al*. 2005; Pickard & Abraham-Shrauner 2009; Knoblauch & Oparka 2012; De Schepper *et al*. 2013). Along the translocation pathway – the transport phloem – sugars and water are continuously lost from and retrieved into the sieve tubes (van Bel 1993; De Schepper *et al*. 2013). Additionally, the phloem may be a pathway for signal transport (De Schepper *et al*. 2013; Turnbull & Lopez-Cobollo 2013).

In most plants, sucrose is the major osmotically active solute in the phloem and thus also provides the driving force for mass flow in the phloem (Lalonde *et al*. 2004). All plant species transport sucrose in the phloem. Some species transport detectable amounts of raffinose, stachyose and/or sugar alcohols, which can exist in even higher concentrations than sucrose (Lalonde *et al*. 2004). Next to sugars, amino N compounds and potassium largely account for phloem sap osmotic concentrations and hence pressure differences between source and sink.

In general, phloem loading and unloading can occur symplastic or apoplastic (Lalonde *et al*. 2003; De Schepper *et al*. 2013). Sucrose, and probably certain amino acids, is loaded via the apoplastic pathway into minor veins by proton symporters localized in plasma membranes of sieve element/companion cell complexes. The transport systems across the membranes are energized by proton-motive force (van Bel 2003; Lalonde *et al*. 2003; Turgeon 2010). In contrast, oligosaccharides seem to be loaded symplastically. The so-called polymer trapping involved in this loading pathway is also active from a thermodynamical point of view, although it does not involve active transport of ions or molecules across a membrane (Turgeon 2010). In the initial step, sucrose diffuses via plasmodesmata from the mesophyll into the companion cells, where raffinose and stachyose, which cannot diffuse back, are synthesized (Turgeon 2010; De Schepper *et al*. 2013). In summary, most phloem loading processes are energy dependent and therefore sensitive to conditions which hamper the synthesis of ATP, such as low oxygen concentrations. In addition to the energy demand for

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Growth and photosynthetic responses in *Jatropha curcas* L. seedlings of different provenances to watering regimes

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Abstract

Seedlings from four provenances of *Jatropha curcas* were subjected to 80, 50, and 30% of soil field capacity in potted experiments in order to study their responses to water availability. Our results showed that with the decline of soil water availability, plant growth, biomass accumulation, net photosynthetic rate, stomatal conductance (g_s), and transpiration rate (E) decreased, whereas leaf carbon isotope composition ($\delta^{13}C$), leaf pigment contents, and stomatal limitation value increased, while maximal quantum yield of PSII photochemistry was not affected. Our findings proved that stomatal limitation to photosynthesis dominated in *J. curcas* under low water availability. The increase of $\delta^{13}C$ should be attributed to the decrease in g_s and E under the lowest water supply. *J. curcas* could adapt to low water availability by adjusting its plant size, stomata closure, reduction of E, increasing $\delta^{13}C$, and leaf pigment contents. Moreover, effects of provenance and the interaction with the watering regime were detected in growth and many physiological parameters. The provenance from xeric habitats showed stronger plasticity in the plant size than that from other provenances under drought. The variations may be used as criteria for variety/provenance selection and improvement of *J. curcas* performance.

Additional key words: carotenoids; chlorophyll fluorescence; gas exchange; water-use efficiency.

Introduction

It is well known that either excessive or insufficient soil water content leads to stress (waterlogging or drought) for plants. Accordingly, plants possess genetically controlled mechanisms that allow them to live and grow under stress (Boyer 1982) including changes in structure, photosynthesis, osmotic adjustment, antioxidative protection, organ, whole-plant hydraulics (Dichio *et al.* 2013), and water-use efficiency (WUE) (Kheira and Atta 2009). Plant establishment and productivity are tightly related to leaf carbon gain and its response to drought. Under water stress, a reduction in photosynthesis was attributed to both stomatal and/or nonstomatal limitations (Varone *et al.* 2012). Generally, stomatal limitations are invoked by stomatal closure and nonstomatal limitations include both diffusive (reduced mesophyll conductance) and metabolic (photochemical and enzymatic limitations) processes (Galmés *et al.* 2007, Varone *et al.* 2012). As a photochemical parameter, maximal quantum yield of PSII photochemistry (F_v/F_m) of dark-adapted leaves by chlorophyll (Chl) fluorescence measurements is often used to indicate plant photosynthetic

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Abbreviations: C_a – ambient chamber CO_2 concentration; Car – carotenoids; Chl – chlorophyll; C_i – intercellular CO_2 concentration; E – transpiration rate; FC – soil field capacity; FM – fresh mass; F_v/F_m – maximal quantum yield of PSII photochemistry; g_s – stomatal conductance; L_s – stomatal limitation value; P_N – net photosynthetic rate; R_s – root/shoot ratio; WUE – water-use efficiency; $\delta^{13}C$ – carbon isotope composition.

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RESEARCH PAPER

ABA flow modelling in *Ricinus communis* exposed to salt stress and variable nutrition

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Abstract

In a series of experiments with *Ricinus communis*, abscisic acid (ABA) concentrations in tissues and transport saps, its *de novo* biosynthesis, long-distance transport, and metabolism (degradation) were affected by nutritional conditions, nitrogen (N) source, and nutrient limitation, or salt stress. In the present study these data were statistically re-evaluated, and new correlations presented that underpin the importance of this universal phytohormone. The biggest differences in ABA concentration were observed in xylem sap. N source had the strongest effect; however, nutrient limitation (particularly phosphorus limitation) and salt also had significant effects. ABA was found in greater concentration in phloem sap compared with xylem sap; however, the effect of treatment on ABA concentration in phloem was lower. In the leaves, ABA concentration was most variable compared with the other tissues. This variation was only affected by the N source. In roots, ABA was significantly decreased by nutrient limitation. Of the compartments in which ABA was quantified, xylem sap ABA concentration was most significantly correlated with leaf stomatal conductance and leaf growth. Additionally, ABA concentration in xylem was significantly correlated to that in phloem, indicating a 6-fold concentration increase from xylem to phloem. The ABA flow model showed that biosynthesis of ABA in roots affected the xylem flow of ABA. Moreover, ABA concentration in xylem affected the degradation of the phytohormone in shoots and also its export from shoots via phloem. The role of phloem transport is discussed since it stimulates ABA metabolism in roots.

Key words: ABA, castor bean, flow models, nutrition, phloem transport, signalling, salt stress, stomatal conductance, xylem transport.

Introduction

Abscisic acid (ABA) is a major plant signal related to abiotic and biotic stress. The most prominent role of ABA is the effect on stomata due to drought and salt stress (Schachtman and Goodger, 2008; Osakabe *et al.*, 2014). ABA is also paramount for regulation of plant growth and development (Wilkinson and Davies, 2002; Cutler *et al.*, 2010; Antoni *et al.*, 2011). In particular, the impact of ABA on plant growth is well documented for seed development, and root and leaf growth (Wilkinson and Davies, 2002; Hartung *et al.*, 2002; Dodd, 2005; Schachtman and Goodger, 2008; Cutler *et al.*, 2010; Boursiac *et al.*, 2013; Chater *et al.*, 2014).

The definition of a phytohormone—as for all hormones—is a biologically active substance where the site of synthesis is spatially separated from the site of signal perception and effect. Therefore, the transport via the long-distance transport system xylem is pivotal for the effects of ABA on stomata (Sauter *et al.*, 2001; Hartung *et al.*, 2002; Dodd, 2005; Jiang and Hartung 2008; Schachtman and Goodger, 2008;

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51.)

ORIGINAL ARTICLE

Diagnostic tools for nutrition status in *Eucalyptus globulus*: changes in leaves, xylem and phloem sap compounds according to N-, P-, and K-withdrawal or salt application

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Abstract

Key message **The results presented here demonstrate the capacity for phloem sap as a diagnostic tool for monitoring the nutritional status of plants and highlight the processes of remobilisation and source–sink dynamics.**

Abstract It is essential to develop methods that accurately reflect plant nutritional status for growth. In a greenhouse experiment with potted eucalyptus, we studied the effects of withdrawing single macronutrients N, P, or K, or salt application on the abundance of components in phloem, xylem and leaves to identify and refine methods to rapidly and effectively assess the nutrient status of *Eucalyptus globulus* trees. Clear effects on growth (and photosynthesis) were found for "−N" treatment and NaCl application. Effects of nutrient withdrawal were detected indicating our methodologies are useful for nutrient availabilities below the threshold of growth responses. While "−P" resulted in significantly lower P in xylem sap, the corresponding effect for "−K" on K in xylem sap was not found, although Ca and Na increased. Salt application increased Na in xylem sap sixfold. In leaf material "−N" reduced N. Surprisingly, "+NaCl", "−P" and "−K" did not change the corresponding elements in leaves. Additionally, "−P"-treatment had the tendency to affect C-, N-, K- and particularly P-increment (n.s.). In phloem sap, "+NaCl" resulted in several effects, most prominently doubling Na concentration. Importantly, "−N" increased amino acids in phloem sap (n.s.). Statistically significant relationships between concentrations in leaves and phloem sap were found for δ^{13}C and amino acids. Within the results obtained from phloem sap, sugars and bivalent cations (Ca and Mg) are correlated with that obtained from the leaves as well as Na and P. The role and comparative advantages of phloem sap as a diagnostic tool for nutritional status are discussed.

Keywords Tasmanian blue gum (*Eucalyptus globulus*) · Nutrition · Salt stress · Leaves · Xylem · Phloem

Abbreviations

"−N"	N-withdrawal
"−P"	P-withdrawal
"−K"	K-withdrawal
"+NaCl"	Salt application

Introduction

Developing efficient tools for the rapid and reliable assessment of plant nutritional status is beneficial for both monitoring plant health and to provide diagnostic information to inform management of nutrient supplements. The availability of water, light and mineral nutrients is essential for plant biomass production as CO_2 is sequestered into photoassimilates to the extent that nutrients, temperature or water availability permits (Körner 2015). Thus, understanding plant nutrient status is a vital component of managing plant nutrition.

Nutritional supplements (fertiliser) are used to correct for nutrient deficiencies, primarily consisting of N, P and

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Research article

Effects of simulated atmospheric nitrogen deposition on foliar chemistry and physiology of hybrid poplar seedlings

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ABSTRACT

During recent decades, the southern and eastern regions of Asia have experienced high levels of atmospheric N deposition. Excess N deposition is predicted to influence tree growth and species composition in the regions, but visual or physiological assessments alone are not sufficient to determine the real effects of atmospheric N deposition. In this study, we simulated atmospheric wet deposition of inorganic N by spraying a NO_3^- solution ($20\,mmol{\cdot}L^{-1}$) or a mixture of NO_3^- ($20\,mmol{\cdot}L^{-1}$) plus NO_2^- (100 or $300\,\mu mol{\cdot}L^{-1}$) on leaves of hybrid poplar (*Populus alba* × *Populus berolinensis*) seedlings and examined morphoanatomical traits and physiological processes. Leaves of seedlings sprayed with single or mixed N solutions developed marginal necrosis, curling, and small cracks on the adaxial surface. The silicon (Si)-rich crystals were larger (about 100% increase in crystal diameter compared to untreated seedlings) on the adaxial leaf surface, with a significant positive correlation between the atomic percentage of N and Si on the crystal areas of the surface. Leaves were sensitive to NO_2^- compared with NO_3^- even at a low concentration; water content, dry mass, and photochemical variables significantly declined and dark respiration increased only in leaves treated with mixed N form. Mixed N foliar applications significantly increased leaf concentrations of the free amino acids Glu, Gln, and Asn and organic acids oxaloacetic acid and citric acid. Besides, mixed N treatment stimulated leaf transamination, as indicated by significant increases in Ala and Asp concentrations and activities of glutamic oxalacetic transaminase and glutamic pyruvic transaminase. However, mixed N applications led to declines in leaf concentrations of putrescine (by 65%, $p = 0.01$) and spermine (by 53%, $p = 0.01$). A higher proportion of NO_2^- ($300\,\mu mol{\cdot}L^{-1}$) in mixed N solution was inhibitory to key N-metabolic enzymes and N translocation via the phloem. Our results showed that wet deposition of airborne N pollutants modified surface properties and induced additional detrimental effects related to N-compound foliar absorption. Furthermore, our findings indicate that detoxification of reactive N is apparently related to N assimilation and export from the treated leaves via the phloem.

1. Introduction

During the past two decades (1995–2015), atmospheric reactive N species and their deposition have increased continuously at a global scale (Stevens et al., 2018). In some regions of Asia, atmospheric inorganic N is mainly wet-deposited in mixed N forms (Liu et al., 2011). A dominant deposition N form in the regions of Japan and China was NO_3^--N, and NO_2^--N accounted for a small fraction of total N deposition (Hayashi et al., 2007; Chen et al., 2019). In previous simulations of inorganic N wet deposition, NO_3^- or NH_4NO_3 solutions of different concentrations have been often used (Liao et al., 2010; Mao et al., 2018); however, less emphasis has been placed on NO_2^- or a mixture of NO_3^- and NO_2^-. A mixed N species may be more realistic for simulating atmospheric inorganic N deposition.

Atmospheric N deposition can influence plant growth either via leaves or roots (Bourgeois et al., 2019). Root-originating impacts are indirectly through N deposition-mediated soil acidification and changes in soil microbial diversity (Liu et al., 2011). Foliar N deposition directly

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OPINION

Why is phloem sap nitrate kept low?

1 | INTRODUCTION

It has now been 61 years since phloem sap composition has first been described, using sap extracted from willow (Peel & Weatherley, 1959). In that study, using colorimetric methods, nitrate was found to be undetectable. Several subsequent studies also reported the absence of nitrate in other species such as castor bean (*Ricinus communis*) (Hall & Baker, 1972) and overviews of phloem sap composition established the generally very low, or undetectable, concentration in phloem sap nitrate (Ziegler, 1975). Furthermore, experiments with ^{15}N-nitrate labelling in legumes have shown that nitrate could not be transported from xylem to phloem at detectable levels (Pate, Sharkey, & Lewis, 1975). There are more recent reports where nitrate was found to be undetectable, for example in maize (Lohaus et al., 2000). It is thus widely accepted that phloem sap nitrate is of negligible importance and in particular, that nitrate circulation from shoots to roots does not occur. For example, in a recent review, the backflow of nitrate via the phloem to regulate root development is not mentioned (figure 3 in Tegeder & Masclaux-Daubresse, 2018). Furthermore, in textbooks and university lectures, it is often reported that nitrate is absent from phloem sap and thus does not flow back from shoots to roots, see for example (Taiz, Zeiger, Moller, & Murphy, 2015). However, many pieces of recent evidence suggest the contrary and provide possible reasons explaining why phloem nitrate concentration is usually low. They are presented in this Opinion.

2 | PHLOEM SAP MAY CONTAIN NITRATE

In fact, there are noticeable exceptions where nitrate is not absent from phloem sap composition (Figure 1a–f). It is the case of cereals (wheat, rice), with phloem sap nitrate of up to 8 mM (Hayashi & Chino, 1985, 1986). In addition, in palm trees, nitrate has been found in phloem exudates for either trunk and inflorescence peduncles (van Die & Tammes, 1975). Extensive analysis of castor bean phloem sap has shown that the average nitrate phloem concentration is 0.59 mM, ranging from no detectability to 2.4 mM (Peuke, 2010). This is of course much less than in xylem, which contains up to 25 mM nitrate. Interestingly, nitrate is much more abundant in phloem (0.29 mM) than xylem sap (3 μM) in Western candle tree (*Banksia prionotes*) (Jeschke & Pate, 1995). These findings cannot be explained by the technique of sap collection (i.e., potential adulteration by other tissues upon sampling). The above-cited studies used very different techniques (aphid-stylet and pure phloem sap exudation) and therefore a systematic contamination is unlikely. In effect, nitrate can be found in phloem sap using the aphid stylet method (i.e., involving stylectomy), which gives access to very high purity phloem sap (van Helden, Tjallingh, & van Beek, 1994).

3 | NITRATE REDISTRIBUTION VIA PHLOEM SAP CIRCULATION

Nitrate redistribution via phloem circulation has been recently suggested to occur in three physiological contexts (Figure 1g), supported by the characterization of nitrate transporters in *Arabidopsis*: remobilization from leaves, development of reproductive structures and nitrate backflow from shoots to roots.

3.1 | Nitrate transfer and remobilization in leaves

Three nitrate transporters are involved in nitrate transfer and/or redistribution to the phloem from xylem or source nitrate-containing tissues: NPF2.13/NRT1.7, NPF1.1/NRT1.12, and NRT1.2/NRT1.11 (Iqbal et al., 2020). In particular, it has been recently shown that NPF2.13/NRT1.7 is essential for remobilization of nitrate from old leaves to deliver nitrate into the phloem, making it available for new, developing leaves and enhancing nitrogen use efficiency (Chen, Chen, Tseng, & Tsay, 2020). NPF1.1/NRT1.12 and NPF1.2/NRT1.11 are involved in the transfer of xylem nitrate to phloem in the petiole thereby facilitating nitrate remobilization from mature (old) leaves to young leaves (Hsu & Tsay, 2013).

3.2 | Development of reproductive structures

Up to now, two nitrate transporters have been shown to be of importance in silique and seeds: NPF2.12/NRT1.6, and NPF5.5. NPF2.12/NRT1.6 is capable of transporting both nitrate and gibberellin $GA_{1/3}$, and is expressed intensively in funiculus vascular bundles, which are mostly made of phloem tissue in *Arabidopsis* (Almagro, Lin, & Tsay, 2008). In *NPF2.12/NRT1.6* mutants, mature seeds contain effectively less nitrate, suggesting that NPF2.12/NRT1.6 is involved in transporting nitrate from maternal tissue to the developing embryo via the phloem (Almagro et al., 2008). NPF5.5, which has been shown to transport nitrate, is involved in controlling embryo N content: in knock-out mutants, embryos at the bent cotyledon stage have a