Europ.J.Hort.Sci., **70** (5). S. 209–216, 2005, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

N and K Translocation in the Xylem Sap of *Ligustrum ovalifolium* L. during Spring Growth

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Summary

Stored organic and mineral nutrients are essential for spring growth of ligneous woody plants. The nitrogen (N) and potassium (K) dynamics were studied on *Ligustrum ovalifolium* L. grown in container for two years with or without fertilisation during the second spring. Allocation of nutrient elements towards new shoots was determined. Nutrient transport in the xylem sap was qualified and quantified by a mathematical coupling between sap flow velocity and sap composition. Sap nutrient concentrations increased after bud break, corresponding to an accumulation of mobilised elements before their use for shoot growth. Glutamine and K were identified as the main compounds translocated in the xylem sap and are a consequence of both mobilisation and uptake by roots of fertilised plants. They were mobilised from storage throughout spring although uptake by roots started just after the beginning of shoot growth. In unfertilised plants, N amounts in shoots were close to that of total N which circulated in xylem sap. On the other hand, total N and K circulating in the xylem sap of fertilised plants, and K of unfertilised plants, were higher than the amounts accumulated in shoots. The differences were attributed to the nutrient replenishment of perennial organs and to the internal N and K re-circulating between shoots and roots. The roles of the internal N and K re-circulating are discussed.

Zusammenfassung

N und K Verlagerung im Xylemsaft von *Ligustrum ovalifolium* L. während des Frühjahrswachstums. Eingelagerte organische und mineralische Nährstoffe sind für den Austrieb von verholzten Pflanzen lebenswichtig. Die Stickstoff- (N-) und Kalium- (K-)Dynamik wurde während des zweiten Frühlings an zwei Jahre mit und ohne Düngung in Containern kultivierten Pflanzen von *Ligustrum ovalifolium* L. untersucht. Es wurde die Verlagerung von Nährstoffen hin zu neuen Trieben bestimmt. Der Nährstofftransport im Xylemsaft wurde qualitativ und quantitativ durch eine mathematische Formel aus Geschwindigkeit und Zusammensetzung des Saftflusses analysiert. Mit dem Aufbrechen der Knospen erhöhte sich die Nährstoffkonzentration im Xylemsaft entsprechend der Anhäufung von moblisierten Nährelementen vor ihrer Verwendung für das Sprosswachstum. Glutamin und K wurden als Hauptkomponenten der Verlagerung im Xylemsaft identifiziert was sowohl auf eine Mobilisierung als auch auf die Aufnahme durch die Wurzeln von gedüngten Pflanzen zurückzuführen ist. Sie werden das ganze Frühjahr über aus den Einlagerungen mobilisiert, obwohl ihre Aufnahme durch die Wurzeln schon direkt nach dem Beginn des Sprosswachstums startet. In ungedüngten Pflanzen entspricht die N-Menge in den Sprossen annähernd dem im Xylemsaft zirkulierenden Gesamt-N. Andererseits waren die im Xylemsaft zirkulierenden Mengen an Gesamt-N und K in gedüngten und an K in ungedüngten Pflanzen höher als die in den Sprossen angehäufte Menge. Die Unterschiede wurden auf die Einlagerung von Nährstoffen in mehrjährigen Organen und auf die interne Verschiebung von N und K zwischen Sprossen und Wurzeln zurückgeführt. Die Bedeutung dieser internen Verschiebung von N und K wird diskutiert.

Key words. absorption – cations – mobilisation – N speciation – nutrition – ornamental shrub

Introduction

Soilless container cultures are characterised by the use of a filtering substrate and a small container volume, inducing rapid variations of substrate nutrient status. In order to optimise shrub growth cultivated in containers, non-limiting and frequent supplies are recommended (COÏC and LESAINT 1983), which generate a high leaching, particularly in spring when fertilisation is applied on rainy days. Improvements were proposed to reduce leaching (LEMAIRE et al. 1995; GUÉRIN et al. 1997; CHARPENTIER et al. 2001) but these fertilisation managements do not take into account the perennial plant capacity to store and mobilise nutrients. These nutrients are stored in perennial organs during autumn, principally in the roots and in older parts of the trunk

for N (MILLARD 1996). Nitrogen is stored as free amino acids (TROMP 1970; SCHNEIDER et al. 1996) and specific storage proteins (WETZEL et al. 1989). During spring growth, N is mobilised from the storage organs towards new organs *via* the xylem sap (MILLARD et al. 1998; MALAGUTI et al. 2001). Mobilisation can occur before or at the same time as N uptake by roots (NEILSEN et al. 2001; MILLARD et al. 2001). Mobilised N can account for up to 90 % of total N in new organs in *Prunus persica* (L.) Batsch (MUNOZ et al. 1993), allowing plant growth to be, to a great extent, independent of spring fertiliser application. Quantification of the internal nutrient cycling could enable improved fertilisation management. The measurement of internal dynamics in *Picea abies* (L.) Karst has been already reported by DAMBRINE et al. (1995), who coupled xylem sap analysis with xylem sap flow velocity. Mineral fluxes were of the same level or higher than root uptake for Mg, K and P. Several authors have pointed out that the spring growth pattern is associated with an increase in xylem sap amino acid concentrations immediately following bud break (SAUTER 1981; FERGUSON et al. 1983; MILLARD et al. 1998; MALAGUTI et al. 2001). MILLARD et al. (1998) proposed the same coupling method as an alternative to using ^{15}N to quantify spring mobilisation. More recently, FRAK et al. (2002) showed that this method was well-suited to estimate N mobilisation for spring growth in *Juglans regia* L.. All these studies were conducted on trees in soil or soilless systems but notwithstanding, no result has been published on shrubs. Shrub behaviour could differ due to the relatively small proportion of perennial organs on total biomass, compared to trees. In other respects, shrubs cultivated in containers are associated with higher spring substrate temperatures than those in soil cultures. This could allow a more precocious xylem sap flow recovery and change mobilisation and absorption rates during spring growth. This study was therefore conducted on a container-grown ornamental shrub species with the objectives of:

(1) identifying the transported compounds in the xylem sap,
(2) determining the pattern of N and K mobilisation during spring,
(3) evaluating the role of N and K re-circulating in xylem sap and
(4) studying the interactions between mobilisation and absorption in order to propose a delay for fertilisation return.

Material and Methods

Experimental design

One-year-old privet rooted cuttings (*Ligustrum ovalifolium* L.) were cultivated from March 2000 to June 2001 in Angers, France (Lat. 47 °30 ' N, long. 0 °35 ' W, and alt. 56 m, mean annual rainfall 850 mm). The plants were grown outdoors in containers (4 L the first year and 7 L the second year) in a perlite/sphagnum peat mixture (50/50, v/v; pH 6). Plants (density of 6 plants per m^2) were supplied with drip irrigation (4 L h^{-1}). The water supply was controlled by tensiometers and irrigation was triggered when water potential values were lower than –8 kPa. In 2000, plants were fertilised with nutrient solution in spring (May 16th–July 19th) and in autumn (September 7th–October 26th). During the summer, they were irrigated only with tap water. The composition of the nutrient solution was, in mg L^{-1}: 125 N-NO_3, 203 K, 111 Ca, 34 P, 20 Na, 18 Mg and minor elements. The composition of tap water was, in mg L^{-1}: 2.7 N-NO_3, 4.5 K, 46.4 Ca, 0.1 P, 18.4 Na and 5.1 Mg. In November 2000, plants were re-potted in 7 L containers with the same substrate and placed outdoors, receiving only rainwater during the winter. In 2001, the experiment was separated in two plots (108 containers on 18 m^2 each): one receiving only tap water and the other one receiving nutrient solution from February 26th to June 5th.

Plant harvesting

In 2001, plant harvests were performed twenty times from the closed bud stage to the beginning of flowering (*i.e.* from January 8th to May 29th). Bud break occurred on February 19th, and this date was expressed as the reference day (day 0) (Table 1). At each harvest, three plants per treatment were randomly collected and separated into roots, trunk (from year 1999), stems and old leaves (2000) and new shoots (2001). The samples for the analysis were dried at 60°C and ground. Total soluble N, was determined by Kjeldhal digestion and K by spectrophotometry after mineralisation by hydrochloric acid.

Xylem sap collection

At each plant harvest, sap was extracted by applying suction on the trunk and thickest stems (4–5 per plant) as described by BOLLARD (1953). All the xylem sap collected from one plant was pooled and frozen immediately at –20 °C. Samples were centrifuged for 5 minutes at 21,500 g prior to cation and anion analysis. FRAK et al. (2002) showed diurnal variations in sap composition in *Juglans regia* L., with a xylem sap composition close to daily mean composition at mid morning. Therefore, sap collections were done always at the same time of the day and at mid morning (between 9 and 11 a.m.). To verify, on *Ligustrum ovalifolium* L., that the collected sap had N and K contents closed to the daily mean ones, we followed the diurnal content of xylem sap in April 2003, in a similar experiment on storage remobil-

Table 1. Stages of development and corresponding sampling dates for spring growth (2001) of *Ligustrum ovalifolium* L.. All stages were reached at the same date regardless of fertilisation treatment.

Development stages	Dates (2001)	Days from bud break
Closed bud	January 8th	–42
Bud break	February 19th	0
Elongation	March 27th	36
End of elongation	May 9th	76
Beginning of flowering	May 29th	99

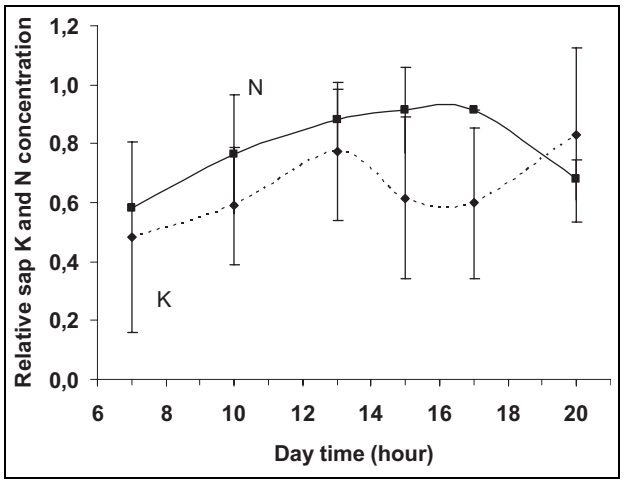


Fig. 1. Diurnal changes in relative concentration of K (dotted line) and total N (full line) in xylem sap of unfertilised *Ligustrum ovalifolium* L., measured on 15 April 2003. Relative concentration refers to the ratio of actual to maximum diurnal value. Vertical bars indicate ±SE for n=3.

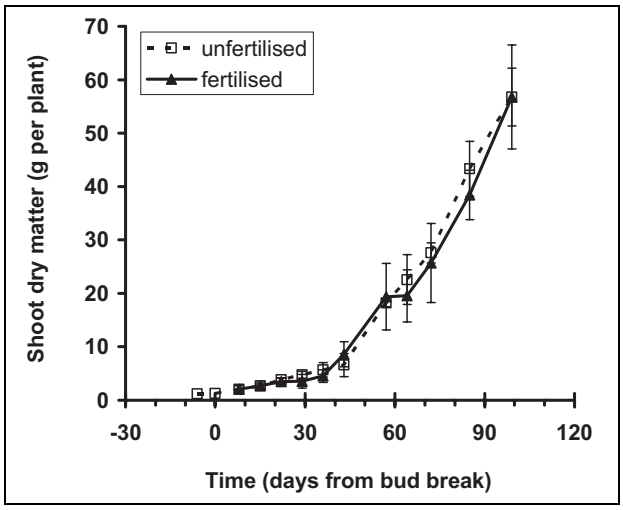


Fig. 2. Changes in shoot dry matter in spring unfertilised (dotted line) and fertilised (full line) Bud break occurred at February 19th (day 0) and fertilisation began at day 7. Vertical bars indicate ±SE for n=3.

isation. We noted diurnal variations lower than those obtained in *Juglans regia* L. (FRAK et al. 2002) and at mid -morning N and K concentrations were closed to the daily mean composition (Fig. 1).

Xylem sap analysis

Five cations (NH_4, Ca, Mg, Na and K) and six anionic forms (nitrate and amino acids: glutamate (Glu), aspartate (Asp), glutamine (Gln), asparagine (Asn) and arginine (Arg)) were analysed by a Capillary Electrophoresis instrument (Waters, Milford, MA, USA). Separations of amino acids and nitrate were performed using a method described by SALAÜN and CHARPENTIER (2001). For the separation of cations, we used an electrolyte consisting of 0.5 g L^{-1} tropolone (2 hydroxy-2,4,6-cycloheptatrieone), 0.5 g L^{-1} UVCat2 (Waters, Milford, MA, USA) and 0.5 g L^{-1} 18-Crown-6 (1,4,7,10,13,16-heptaoxacyto-ocyodecan). The final pH was about 4.5. Capillary electrophoresis accuracy was comprised between 0.05 mg L^{-1} for N-NO_3^- and 0.06 mg L^{-1} for N-Arg (SALAÜN and CHARPENTIER 2001).

Data from xylem sap composition were subjected to analyses of variance with a test of Newman-Keuls to determine the significance of difference between stages or between fertilisation treatments at P=0.05.

Xylem sap flow

Xylem sap flow was measured with Dynagage (Dynamax, Houston, Texas) sensors (SGA 10) fixed on the shrub trunk. Measurements were based on the heat balance method (STEINBERG et al. 1990), without intrusion into the plant. Thermocouples of the sensors were connected to a data logger, which scanned data every minute. In 2001, sap flow meters were applied to the trunk of three spring unfertilised plants from bud break to flowering (i.e from February 19th to May 29th). They provided a continuous measure of xylem sap flow velocity. Xylem sap flow results are expressed in grams per time unit, after verification that the xylem sap density is close to 1 g mL^{-1}.

Quantification of translocation in xylem sap

The sap flow was measured continuously and the N (amino acids, nitrate and ammonium) and potassium (K) compounds were analysed weekly. The N or K flux was calculated by multiplication of the measured sap flow with the N or K xylem sap content measured at the closest harvest.

Results

Spring plant growth and N and K contents

The evolution of shoot dry mass production during the second spring of the experiment was similar for spring fertilised and for unfertilised plants (Fig. 2) and reached 64.6±3.6 and 62.8±2.8 g dry mass per plant respectively, when flowering started (day 99). During a first period (day 0 – day 36 after bud break), dry mass production was low (Fig. 2) and N and K contents in perennial parts did not decrease significantly (Table 2); no significant difference was measured between fertilised and unfertilised plants (Table 2). A second phase, with more rapid dry mass production was observed until day 99 when flowering started (Fig. 2). At this stage, nutrient compositions differed with fertilisation treatment (Table 2), fertilisation increased significantly the N and K contents in trunk but did not affect the N content in roots and K content in shoots.

Spring translocation in the xylem sap of unfertilised plants

All elements circulating in the xylem sap of unfertilised plants during spring were mobilised from storage organs. Total nitrogen concentration in xylem sap in-

Table 2. N and K contents (mg per plant) of *Ligustrum ovalifolium* L. organs at bud break, during elongation and at the beginning of flowering for spring fertilised and unfertilised plants. Means of three plants. At bud stage, the three replicates of shoots (leaves and stems of the year) were pooled (*). Letters a and b indicate significant differences, at a same stage (day 36 or 99), between unfertilised and fertilised plants at P=0.05 (Test Newman-Keuls). Letters x and y indicate significant differences between stages (days 0, 36 and 99) at P=0.05 (Test Newman-Keuls), in perennial organs of unfertilised plants.

Stage		Bud break	Elongation		Beginning of flowering	
Day from bud break		0	36		99	
Nutrient	Organs		unfertilised	fertilised	unfertilised	fertilised
N	roots	552.5 x	426.0 a xy	509.3 a	313.2 a y	460.4 a
	trunk	317.7 x	223.1 a x	275.8 a	81.5 a y	210.9 b
	stems	301.4 x	201.4 a x	235.2 a	123.5 a y	263.7 b
	shoots	43.8*	223.1 a	196.6 a	830.3 a	1448.9 b
K	roots	652.6 x	545.2 a x	466.8 a	384.9 a x	619.0 b
	trunk	109.0 x	99.9 a x	96.7 a	118.0 a x	200.4 b
	stems	162.2 y	127.2 a y	131.3 a	220.2 a x	268.7 a
	shoots	16.2*	100.7 a	91.6 a	816.9 a	1514.5 b

Table 3. Comparison of amino acid and mineral element concentrations (mmol L^{-1} sap) in the xylem sap of unfertilised and fertilised *Ligustrum ovalifolium* L. harvested at closed bud, during elongation and at the beginning of flowering. Means of three plants. ND for not detected. Letters a and b indicate significant differences, at a same stage (day 36 or 99), between unfertilised and fertilised plants at P=0.05 (Test Newman-Keuls). Letters x and y indicate significant differences between stages (days –26, 36 and 99 for unfertilised plants, days 36 and 99 for fertilised ones) at P=0.05 (Test Newman-Keuls).

Stage	Closed bud	Elongation		Beginning of flowering	
Day from bud break	–26	36		99	
Compound		unfertilised	fertilised	unfertilised	fertilised
N-Gln	2.16 y	39.07 a x	42.29 a x	2.91 a y	10.50 a y
N-Arg	8.21 x	6.31 a x	4.51 a x	0.21 a y	1.26 a y
N-Asp	0.26 y	2.29 a x	0.34 b x	ND	0.41 x
N-Glu	0.19	0.68 a	0.20 a x	ND	0.30 x
N-Asn	ND	0.48 a	0.69 a x	ND	0.86 x
$N\text{-}NO_3$	2.02 x	1.61 a x	5.51 b x	0.56 a x	1.97 a x
$N\text{-}NH_4$	ND	ND	12.6	ND	ND
Total N	12.84 y	50.44 a x	54.44 a x	3.68 a y	14.52 a y
K	7.95*	54.44 a x	24.57 a x	16.89 a x	21.34 a x
Ca	1.58*	7.36 a x	4.62 a x	1.50 a x	1.81 a x
Mg	ND	1.62 a x	1.25 a	0.70 x	0.65 *
Na	ND	4.28 a x	1.61 a x	0.67 a x	0.88 a x

*: only one chemical analysis, no statistical analysis

creased about 4-fold between closed bud and elongation stages and then fell to very low levels at the beginning of flowering (Table 3). Nitrogen was mainly translocated in the form of amino acids, mainly Gln, Arg and Asp. Arginine was the principal xylem sap component at closed bud stage, representing 64 % of total N. At elongation stage, N-Gln was predominant and represented 77 % of total N (Table 3) whereas N-Arg quantity was reduced and was only one-sixth of the concentration of N-Gln.

The main cations in xylem sap were K (83 % of total cation mass) and Ca to a lesser extent (12 %). K content followed a close pattern as that of Gln throughout spring growth (Fig. 3), with a peak observed 36 days after bud break (54.4 for K and 19.5 for Gln in mmol L^{-1}). Then, Gln and K contents decreased, this decrease is less regular for K content than for Gln content, which fell to a very low value at the beginning of flowering.

The standard errors of xylem sap component values were sometimes high. As the analytic inaccuracies were

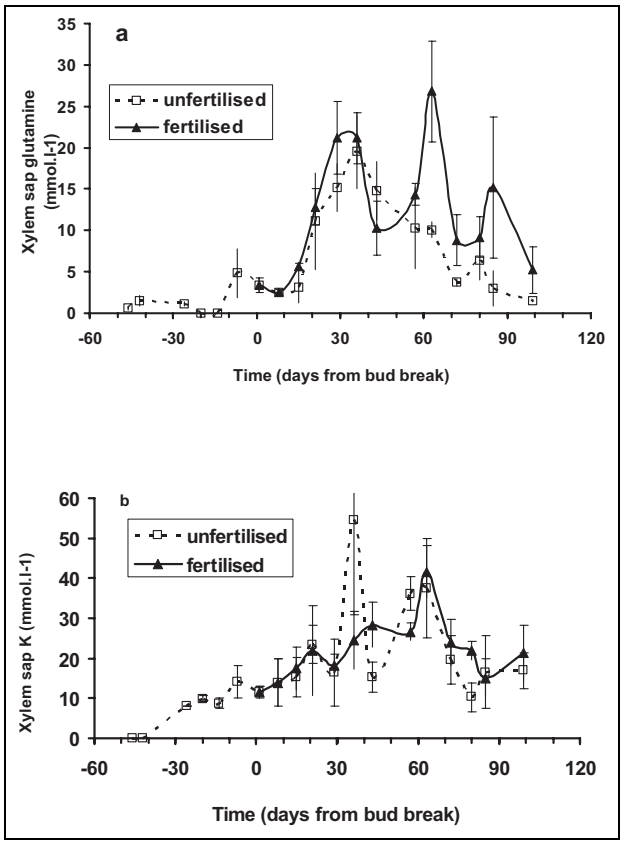


Fig. 3. Changes in glutamine (2a) and in potassium (2b) concentrations in the xylem sap of unfertilised (dotted line) and fertilised (full line) *Ligustrum ovalifolium* L.. Bud break occurred at February 19th (day 0) and fertilisation began at day 7. Vertical bars indicate ±SE for n=3.

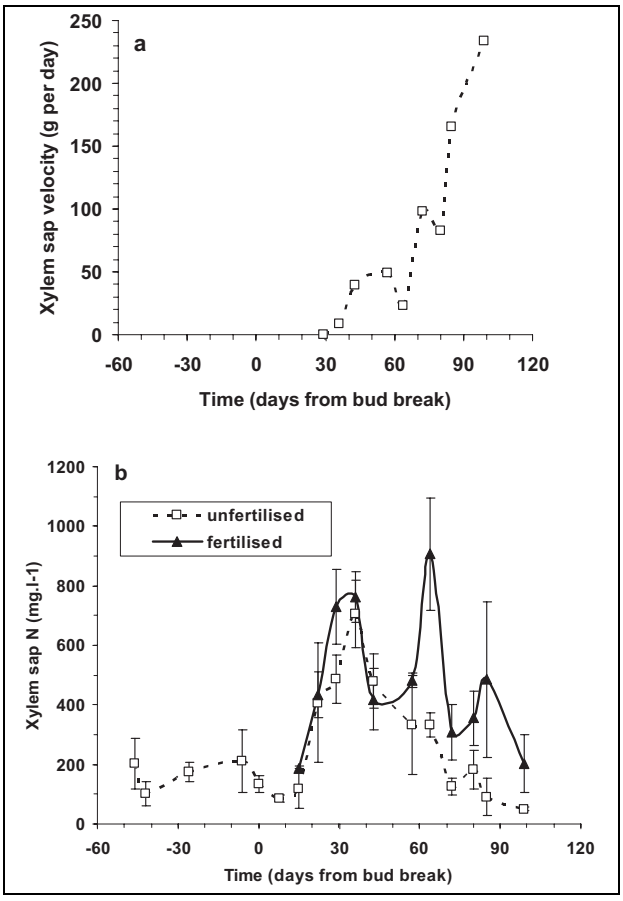


Fig. 4. Changes in xylem sap flow velocity on unfertilised plants (4a) and in xylem sap N concentrations on unfertilised (dotted line) and fertilised (full line) *Ligustrum ovalifolium* L. (4b). Bud break occurred at February 19th (day 0) and fertilisation began at day 7. Vertical bars indicate ±SE for n=3.

low (see material and methods), standard errors were essentially due to the variability of growth stage between harvested plants.

The xylem sap flow velocity became detectable about 36 days after bud break (Fig. 4a). From this time, the sap flow increased as shoot biomass increased (Fig. 2). This velocity increase was concomitant with a reduction in xylem sap N concentration, due to higher transpiration level and use in shoots (Fig. 4b). The limited flow observed between 56 and 63 days after bud break (Fig. 4a) was associated with low diurnal temperatures (5 to 10 °C), which reduced metabolism.

The amount of N transported in the xylem sap was calculated from xylem sap flow velocity multiplied by the xylem sap N concentration. The total N transported by xylem was compared with the N content in shoots during spring (Fig. 5). The pattern of total N flux was in agreement with the temporal course of N recovered in new shoots of unfertilised plants. Before elongation, when the xylem sap flow was not detectable, buds contained 19 % (156 mg per plant) of the total N content present at the beginning of flowering. Then, during elongation and before flowering, the amount of N transported (x) in the xylem sap was strongly correlated to the N accumulation in shoots (y) for unfertilised plants ($y=0.66x+275.13$, $r^2=0.89$). At the beginning of flowering (day 99), N xylem sap transport (1058 mg per plant) followed the same order as that of the N found in shoots (830 mg per plant) (Fig. 5).

Translocation in the xylem sap of spring fertilised plants

On fertilised and unfertilised plants, Gln concentrations increased to a maximum obtained during elongation (19.5±4.5 mmol L^{-1} in unfertilised plants and 21.1±3.4 mmol L^{-1} in fertilised ones) (Table 3). Spring fertilisation had no effect on the xylem sap Gln concentration until the middle of the elongation (Fig. 3, day 57), then the internal recirculated N between shoots and roots resulted in significantly higher sap Gln concentrations in fertilised plants (Fig. 5).

The cation concentrations in the xylem sap were not significantly modified by spring fertilisation. In fertilised plants, K was always the main transported cation (Table 3) and the pattern of Gln and K contents were similar all over spring (Fig. 3).

During elongation we noted a rise in Gln and K concentrations in xylem sap (day 63), on both treatments for K and only on fertilised plants for N-Gln (Fig. 3). This concentration increase was due to unfavourable climatic conditions, which temporarily restricted plant activity.

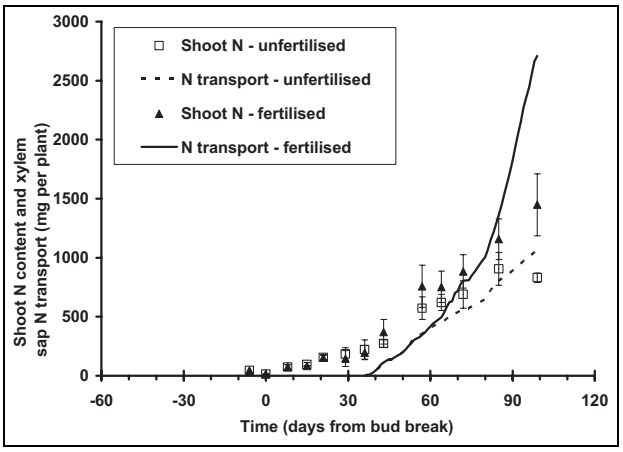


Fig. 5. Changes in shoot N content (symbols) and xylem sap N transport (lines) on unfertilised (square, dotted line) and fertilised (triangle, full line) *Ligustrum ovalifolium* L. plants. Bud break occurred at February 19th (day 0) and fertilisation began at day 7. Vertical bars indicate ±SE for n=3.

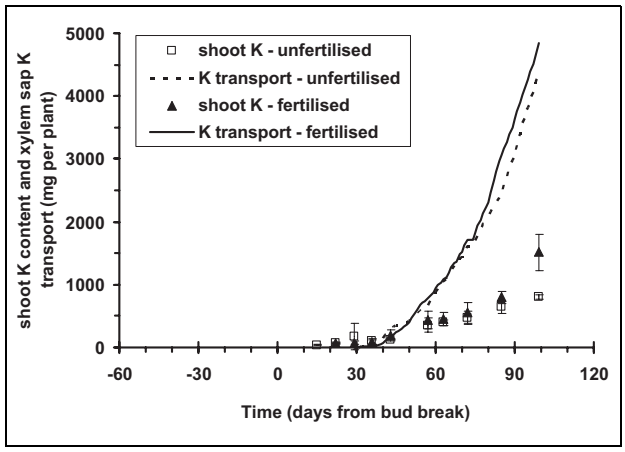


Fig. 6. Changes in shoot K content (symbols) and xylem sap K transport (lines) on unfertilised (square, dotted line) and fertilised (triangle, full line) *Ligustrum ovalifolium* L.. Bud break occurred at February 19th (day 0) and fertilisation began at day 7. Vertical bars indicate ±SE for n=3.

In spring-fertilised plants, the calculated N xylem sap transport was higher than N mobilisation in the shoots at the beginning of flowering (Fig. 5). Thus, at this stage, the N deposited in the shoots was 1449 mg per plant, as opposed to 2709 mg N transported in the xylem sap. In the case of K, the xylem sap transport was always clearly higher than the new shoot K content, 5 fold for spring unfertilised plants and 3 fold for spring-fertilised plants, respectively (Fig. 6).

Discussion

Translocated forms in the xylem sap of Ligustrum ovalifolium *L.*

We assessed that throughout the spring, N was translocated in the xylem sap of *Ligustrum ovalifolium* L. predominantly in the form of amino acids (Gln, Arg, Asp, Asn and Glu). Prior to bud break Arg and later Gln accounted for the principal components of N mobilisation. Likewise, FRAK et al. (2002) demonstrated an amino acid succession in *Juglans regia* L., with Arg occurring at the very beginning of bud break and Gln, Cit and Asp one month later. Arginine and Gln are well suited to N transport because of their solubility, mobility and N richness. Glutamine had been reported as the principal amino acid in the xylem sap, at bud break, in *Salix* (SAUTER 1981), *Populus deltoïdes* Bartr. ex Marsh (SCHNEIDER et al. 1994), *Vitis vinifera* L. (GLAD et al. 1992) and *Actinidia deliciosa* A.Chev. (CLARK et al. 1986). In *Ligustrum ovalifolium* L., we demonstrated that Gln was the predominant N form for both mobilisation and transport after absorption. The same pattern has been observed in *Malus domestica* Borkh., where Asn played the same role as Gln in *Ligustrum* (MALAGUTI et al. 2001).

Potassium was the principal cation identified in *Ligustrum ovalifolium* L. xylem sap, as in *Vitis vinifera* L. (PEUKE 2000) and *Populus deltoïdes* Bartr. ex Marsh (BABIN et al. 2000), where the concentrations reported were 10 and 5 fold lower, respectively. Calcium was identified as the second frequent cation, but at much lower levels. Conversely, Ca represented the principal cation in *Actinidia deliciosa* A.Chev. (CLARK et al. 1986).

It is apparent that in *Ligustrum ovalifolium* L. potassium is transported in the same way than Gln, as both elements followed similar translocation patterns. The concomitant accumulation of K and Gln had also been observed at bud break in *Populus deltoïdes* Bartr. ex Marsh (BABIN et al. 2000). Both concentrations were of the same order in fertilised *Ligustrum*, while in unfertilised plants K concentrations were higher than Gln concentrations. Potassium could be considered as accompanying Gln or other anionic forms (malate, NO_3, etc.), as suggested by ENGELS and KIRKBY (2001) in maize.

Interaction between N mobilisation and N absorption at spring growth

We determined that, at the beginning of elongation, 19 % of the final shoot N content in unfertilised plants and 11 % of fertilised ones was already deposited in the buds (Table 2). This deposition could be linked to N transport *via* xylem sap, which was not yet detectable with our flow sensor, or *via* phloem sap. In *Fagus sylvatica* L. leaves, most of the N assimilated, before or at bud break, arised from phloem (GEßLER et al. 1998a), perhaps by mass flow.

In unfertilised *Ligustrum ovalifolium* L., N mobilisation was continuous during the spring growth. During elongation, xylem sap N-NO_3^- concentration was higher in fertilised than in unfertilised plants. Thus, during early shoot elongation, NO_3^- was absorbed and transported to aerial parts without reduction in roots, even when reduction was generally observed in roots of ligneous plants (GOJON et al. 1992). The concomitance of N absorption and remobilisation in *Ligustrum* was also observed in *Betula pendula* Roth., and *Pinus sylvestris* L. cultivated in pots, where root N uptake con-

Table 4. Allocation of cumulated N and K circulating in xylem sap between shoots, perennial organs and a re-circulating pool (in mg per plant), in fertilised *Ligustrum ovalifolium* L.. Allocation to perennial organs was obtained by difference of contents at the beginning of flowering between fertilised and unfertilised plants (data in Table 2). Bracketed values represent percentages of nutrient circulated in xylem sap.

Nutrient	Circulated in xylem sap	Shoots	Perennial organs	Re-circulating pool
N	2709 (100)	1449 (54)	417 (15)	843 (31)
K	4837 (100)	1515 (31)	365 (8)	2957 (61)

tributed N to new leaves immediately following bud break (MILLARD et al. 2001). The Gln patterns were identical on fertilised and unfertilised plants up to the beginning of elongation. From middle of elongation, Gln concentration was higher in fertilised plants. Glutamine came from both mobilisation of stored N and reduction of absorbed N, so no distinction could be made between the two processes by coupling xylem sap flow velocity and N analysis, as had been shown by MALAGUTI et al. (2001), on *Malus domestica* Borkh..

We observed that plant spring growth was minimally affected by the absence of spring fertilisation. Nevertheless, in the same experiment, SALAÜN (2002) showed that nutrient reserves were exhausted at flowering and could be replenished during summer and autumn to ensure plant perenniality.

N and K distributions in aerial organs

At flowering, the amounts of N and K transported in the xylem sap, measured at collar, in fertilised *Ligustrum ovalifolium* L. plants, and K in unfertilised ones, were higher than the deposition in the shoots (Fig. 5 and 6). There may be several reasons for this:

(1) Allocation of nutrients to perennial organs.
Newly absorbed N and K were not exclusively directed towards new tissues but also to old organs (trunk, stems and roots), where storage pools were replenished. To determine this pool, as perennial organs are both nutrient sources and sinks, we calculated the difference of nutrient content at the end of growth between fertilised and unfertilised plants. So 15 % of the N and 8 % of K transported in the xylem sap of fertilised *Ligustrum ovalifolium* L. was allocated towards perennial organs (Table 4).

(2) Nutrients re-circulating.
Coupling xylem sap flow velocity and xylem sap analysis allowed the quantification of a total flux, but not a net flux. Indeed, some of the nutrients circulating in the xylem sap were retranslocated from shoots to roots *via* the phloem sap, before being redirected to shoots again (MARSCHNER et al. 1996). The part of nutrient re-circulating could be quantified by difference between cumulated xylem sap quantities and the quantities allocated to sinks (shoot and perennial organs). So, in fertilised *Ligustrum ovalifolium* L., re-circulated reached 31 % for N and 61 % for K (Table 4); values comparable to the 33 % and 51 % respectively, obtained on maize (ENGELS and KIRKBY 2001). So, the higher amounts of N and K transported in the xylem sap, compared to shoot nutrient contents, were affected in a major part to nutrient re-circulating and in lesser part to perennial organs replenishment. The re-circulating N regulates the nitrate net uptake by the roots in order to adapt absorption to N demand of the whole plant (GEßLER et al. 1998b; YOUSSEFI et al. 2000).

Our results pointed out that K was transported in close amounts in the xylem sap of unfertilised and fertilised plants but, in unfertilised plants, the root K concentration decreased during spring growth. Thus, the role of K as accompanying cation on the transport of amino acids in xylem sap and organic acids (especially malate) in the phloem (MARSCHNER et al. 1996) appeared to be a priority compared to the filling up of perennial organs, especially roots in *Ligustrum*. The K re-circulating percentage was higher than the N one, in relation with its high mobility and its non-integration in plant structure.

The study of the circulating pattern of N and K in the xylem sap of *Ligustrum ovalifolium* demonstrated the plant nutritive autonomy at spring growth, which could be valorised by delaying fertilisation return in spring.

Acknowledgements

The authors would like to thank Y. Barraud, E. Billaud, M. Sigogne and G. Sintès (UMR SAGAH, Angers) for analysis and plant harvesting, and A. Lacombe (INRA UCDIST, Jouy-en-Josas) for revising the English version of the manuscript. This work was supported by a PhD grant (M. Salaün) from the Regional Council of Pays de la Loire.

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Received July 01, 2004 / Accepted April 25, 2005

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