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Performance of two *Picea abies* (L.) Karst. stands at different stages of decline

IV. Xylem sap concentrations of magnesium, calcium, potassium and nitrogen

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Summary. The nutrient relations (nitrogen, magnesium, calcium, potassium, and manganese) of the xylem sap of spruce trees, *Picea abies* (L.) Karst., growing at a healthy and a declining site in Northern Bavaria, were followed on a diurnal and seasonal basis between April and October 1985. There were significant differences between the two sites in the xylem sap concentrations of all elements investigated except nitrogen. Nutrient concentrations remained constant diurnally despite changes in transpiration and xylem water potential. However, during periods between precipitation events, concentrations of elements in xylem sap decreased with decreasing xylem water potential. Apparent differences in needle chlorosis of spruce trees at the two sites were associated with consistent differences in nutrient contents of their xylem sap and needles.

Key words: Forest decline – Spruce – Nutrients – Xylem sap

During the last decade, the needles of *Picea abies* (L.) Karst. in the Fichtelgebirge have been observed to have various and increasing degrees of chlorosis, which has been associated with magnesium deficiency (Zech et al. 1985; Oren et al. 1988a). Among other factors, magnesium deficiency has been attributed to either leaching of magnesium from the soils due to acid deposition, thus reducing the supply to the plant (Mollitor and Raynal 1982; Zöttl and Mies 1983), or to leaching of ions from needles receiving adequate supply from the soil (Evans 1982).

In order to separate the effects of nutrient supply via the xylem from the effects of nutrient loss due to leaching, we investigated differences in xylem sap concentrations of magnesium, potassium, calcium, and nitrogen at a healthy and at a declining site. Only at the declining site was there a high proportion and variation of trees with needle chlorosis. Nutrient concentrations in the xylem sap were related to the soil water status and the element concentrations in the needles.

Materials and methods

Study site

The two sites were located 15 km apart near the villages of Oberwarmensteinach (declining) and Wülfersreuth (healthy) in Northern Bavaria, FRG. Trees at the declining site were growing on a podzol and showed a large variation in needle yellowing and needle loss, whereas trees at the healthy site were growing on a podzolised cambisol and lacked symptoms of needle yellowing. Five circular plots of 80 m^2 were randomly positioned in each stand, including 25- to 35-year-old trees. More details about the sites, their growth and nutrient status can be found in Oren et al. (1988a, b).

Collection of xylem sap

All samples were taken from many trees on each plot including declining ones if present. Twigs composed of current and last year's growth were randomly collected from the bottom of the sun crown (7 m above ground). They were sealed in double polyethylene bags and stored on ice until sap extraction. Maximum error in determining xylem water potential (Ψ_x) resulting from storage of sample shoots for up to 6 h was not more than 0.05 MPa.

Shoots were stripped of their bark from the cut end to about 1.5 cm in order to avoid contamination by phloem sap, and sealed in a pressure chamber with the cut end protruding. The xylem water potential was determined. The projected end of the shoot was then fitted with a 5 cm long piece of silicone tube with an internal diameter slightly smaller than the exposed end of the shoot. The pressure was then increased by 0.6 MPa over the xylem water potential. Exuding sap was collected with a glass pipette. After the sap had almost stopped exuding, the pressure was increased by another 0.6 MPa over the last balance pressure. This procedure was repeated so that three categories of expressed sap ($\Psi + 0.6$, $\Psi + 1.2$, and $\Psi + 1.8$ MPa) were collected from each twig. In most cases, 2 twigs in each pressure step were adequate to collect the 500 µl xylem sap required for the nutrient analysis. All the samples of xylem sap were stored at -20 °C until nutrient determinations were made.

The xylem sap was collected on (1) a diurnal basis (6 a.m. to 6 p.m.), (2) a daily basis (once at noon every day) and (3) a seasonal basis (predawn). The diurnal collection was made twice per site at 3-h intervals using balance pressure plus 1 or 1.2 MPa overpressure. The daily collection was made on 10 days and 5 days in September and October respectively, with gaps not exceeding 1 day. The seasonal collection was made at four distinct phenological stages: in April when ground was frozen and little transpiration occurred, in May at time of bud break, in July after full needle expansion, and in October when weather condi-

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tions were similar to those in April. The seasonal comparison concentrated on magnesium, potassium, calcium, and manganese, which were found to be different between the sites from needle analysis (Oren et al. 1988b).

Nutrient analysis

The Kjeldahl nitrogen in a 100-µl sample of the xylem sap was determined, after digestion at 320 °C, by Nessler reagent (Strauch 1965) with a spectrophotometer (Gilford, Model 250). Magnesium, potassium, and calcium concentrations in all but the seasonal comparison were determined by atomic absorption spectrophotometry (AAS, Perkin Elmer 420). Except for nitrogen, nutrients in sap extracted for the seasonal comparison and in the needles were determined with Inductively Coupled Plasma Atomic Emission spectrophotometer (Model JY 48, Jobin-Yvon). Needles were analysed after wet pressure ashing in 65% nitric acid. Total nitrogen in needles was determined with a C/N analyser (Model 1500, Carlo Erba Strumenta Zione, Italy). In order to describe the general nutrient status of trees in the plots, needle element analysis was done on 1 to 5-year-old needles and weighted by the proportion of needles in each age class (Oren et al. 1988a).

Statistical analysis

Differences in ion concentrations between levels of overpressure, hour of day, dates, and phenological stages were tested for significance by analysis of variance with sites as blocks and xylem water potential (Ψ_x) as a covariable. A least-significant-difference test was used to separate the means. Differences in concentration between sites were tested using a *t*-test.

Table 1. Mean (±SE) element concentrations in the xylem sap of *Picea abies* decreased significantly with incremental increase in the pressure applied over leaf water potential (overpressure), ($n=50$/overpressure)

Element (mmol l^{-1})	Overpressure (MPa)			Significance (*F*-test)
	+0.6	+1.2	+1.8	
Nitrogen	3.15±0.12^a	2.91±0.12^a	1.73±0.11^b	0.001
Magnesium	0.27±0.01^a	0.25±0.01^a	0.20±0.01^b	0.001
Potassium	1.88±0.07^a	1.81±0.09^a	1.34±0.07^b	0.001

^{a,b} Any two means on the same line with the same superscript are not significantly different. Two means on the same line with different superscripts are significantly different ($P \leq 0.05$)

Results

It was thought necessary to identify at which balance pressure or over-pressure above Ψ_x the concentration would be affected by the extraction. Both $\Psi_x+0.6$ and $\Psi_x+1.2$ MPa yielded similarly high concentrations of all nutrients compared to $\Psi_x+1.8$ MPa (Table 1). This decline at high over-pressures was probably not observed by Stark et al. (1985) because of their method of xylem water extraction. They pressurized twigs to various levels over the xylem water potential, by using one pressure applied to each twig. Since the sap quantity extracted decreases with each incremental increase in over-pressure, the amount of sap collected at high over-pressure in their study included a proportionally low volume of water. The most obvious reason for a decline at high overpressure is dilution by water squeezed out of cells. We decided to eliminate this source of variation by collecting sap at 1.0 MPa over-pressure in all subsequent analyses.

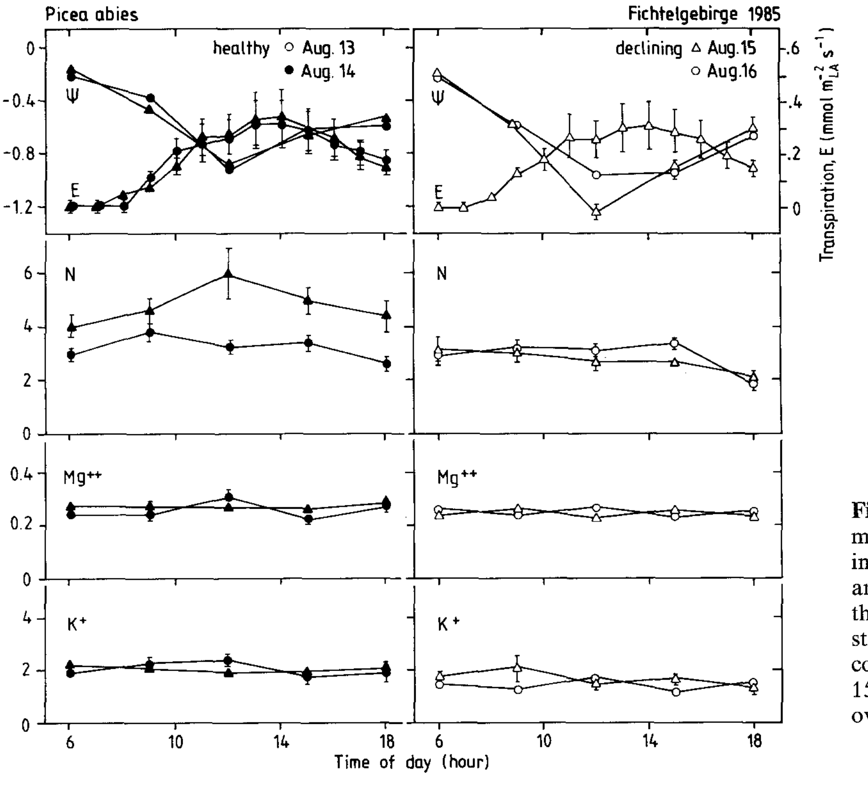

Fig. 1. Diurnal changes in nitrogen, magnesium, and potassium concentrations in the xylem sap, and leaf water potential and transpiration as measured by mass flow through the stem, in healthy and declining stands of *Picea abies*. Xylem sap was collected at a pressure of 1.0 MPa (13 and 15 August) and 1.2 MPa (14 and 16 August) over the balancing water potential

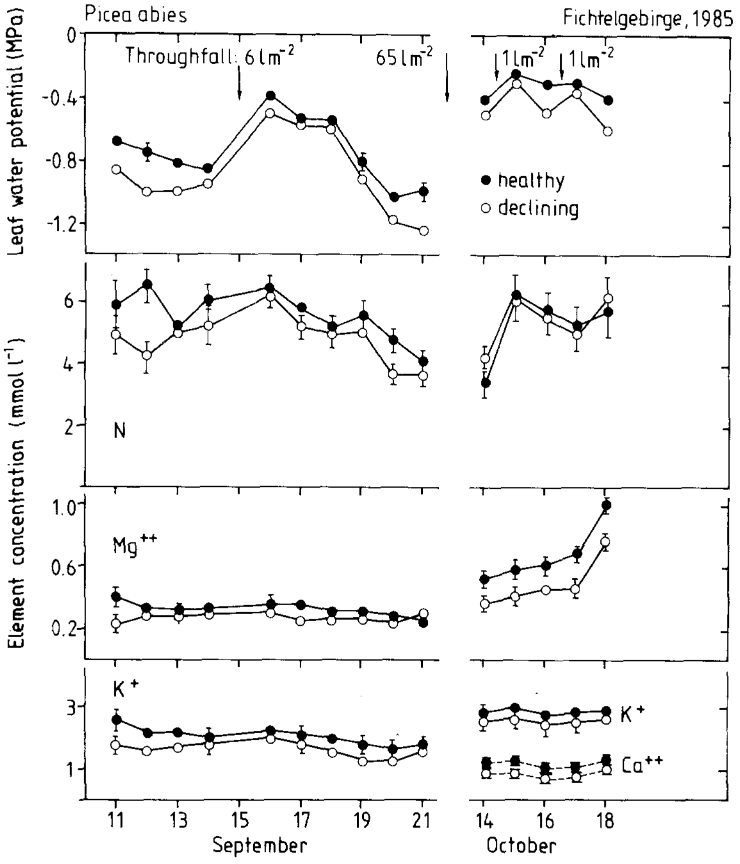

Fig. 2. Daily changes in nitrogen, magnesium, potassium, and calcium concentrations in the xylem sap and in leaf water potentials for measuring periods in September and October. The plant material was harvested between 10 and 12 a.m. Two pairs of data points are enclosed by *dashed lines* in the nitrogen figure. The *lower pair* represent values immediately after a rain event while the *upper pair* represent values for a day later. In the magnesium figure, the two data points clearly above the rest were collected on the same day and the reason for their high values is unknown

Table 2. Mean (±SE) concentration of xylem sap of *Picea abies* collected daily before noon. Magnesium and potassium concentrations increased significantly from September to October and, including that of calcium, were higher in the healthy compared with the declining site. Nitrogen was not different between months or sites ($n = 100$ in September, 50 in October, and 75/site). ANOVA showed no interaction of months and site ($P \geq 0.05$)

Element (mmol l^{-1})	Month		Site	
	September	October	Healthy	Declining
Nitrogen	5.23±0.13	5.36±0.22	5.44±0.16	5.10±0.16
Magnesium	0.29±0.01***	0.58±0.03	0.44±0.02**	0.35±0.02
Potassium	1.81±0.05***	2.65±0.08	2.26±0.07**	1.92±0.08
Calcium[a]	–	1.03±0.04	1.20±0.04***	0.89±0.05

Note: ** Significantly different at $P \leq 0.01$; *** Significantly different at $P \leq 0.001$

[a] Calcium was not analysed in September

The diurnal changes (Fig. 1) in leaf water potential from 6 a.m. to 6 p.m. had no effect on the diurnal concentrations of the nutrients ($P \leq 0.05$). This differs from the findings of Stark et al. (1985) who observed large hourly fluctuations in concentrations of all ions, including nitrogen, magnesium and potassium, with minimum levels at 6 a.m. and maximum levels, or peaks, at 3 p.m. The differences between the two studies may be related to differences in the environments, but in order to eliminate a possible source of variation, the daily xylem sap collection was made only in the late morning (10 to 12 a.m.).

In the daily comparison of the two sites (Figs. 2, 3), both stands showed similar trends in xylem sap nutrient content. Magnesium, calcium, and potassium but not nitrogen, values differed significantly between sites (Table 2). Light rain only slightly affected the nutrient concentrations, but a major rain storm in the middle of the daily measuring sequence strongly affected the concentration of elements in the xylem sap. The declining site had consistently lower concentrations than the healthy site, irrespective of whether the soil was dry or wet.

The noon leaf water potentials were consistently lower at the declining than at the healthy site during a dry period in September and a rainy period in October which together with the higher daily variation indicate that this site was more sensitive to changes in soil moisture. Although on any given day there was no correlation between leaf water potential and the concentration of elements in xylem sap

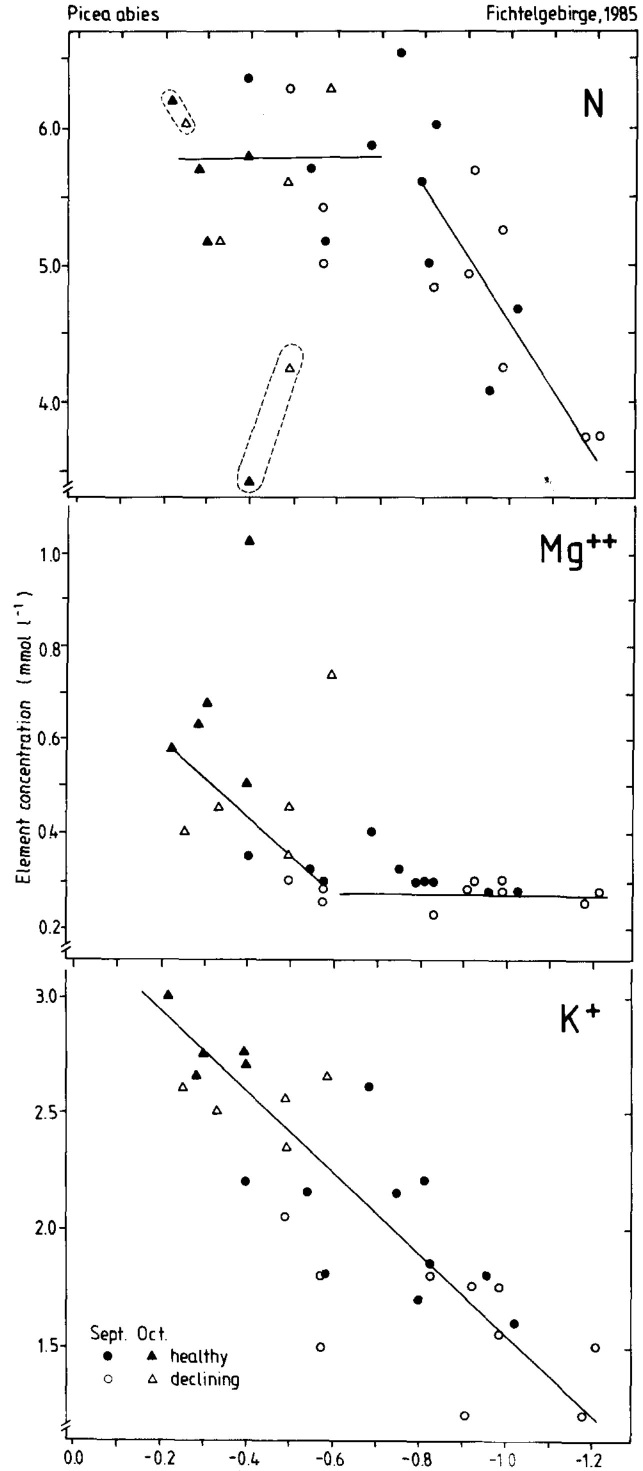

Fig. 3. Nitrogen, magnesium, and potassium concentrations in the xylem sap related to the leaf water potential. Measurements and sap collection were made between 10 and 12 a.m.

(Fig. 1), such an association emerged for potassium at both sites, and for nitrogen at the declining site ($P \leq 0.001$) and magnesium at the healthy site ($P = 0.01$) when these relationships were investigated over a period of several days (Fig. 3). The magnesium values in the last measurement days in October were very high at both sites for as yet unknown reasons. When the values from that day were not included in the correlation between magnesium and water potential, the correlations improved at both sites (Table 1). The concentrations of total nitrogen, magnesium, and potassium in xylem sap decreased as leaf water potential decreased. Since calcium was determined only in October, the lack of correlation between calcium and leaf water potential in Table 3 is probably due to the small variation in the data. Nevertheless, the high correlation between magnesium and calcium ($r = 0.80$, $P \leq 0.001$) suggests that, given a wider range, calcium would also correlate with leaf water potential. A smaller range in water potential at the healthy site may be responsible for the lack of correlation between nitrogen concentration and leaf water potential. However, the complete overlap in values from both sites may indicate that the relations were general. After one rain event, at both sites an increase in nitrogen concentration lagged a day behind that of leaf water potential (Fig. 3).

When the xylem sap concentrations of magnesium, potassium, calcium, and manganese were compared among four phenological stages, maximum levels were revealed for magnesium in April before bud break and for potassium thereafter, in May (Table 4). A maximum in xylem sap concentrations at this time was also reported by Stark and Spitzer (1985) for *Pinus ponderosa*. The pattern of xylem sap concentrations between the sites was similar to that found in the September/October measurements, but only magnesium was significantly higher at the healthy site. The reason for this may be the difference in the harvest time during the day. Early in the morning, the concentrations of most elements can be very low (Stark et al. 1985). In October element concentrations in sap collected at dawn (Table 4) were lower than those collected in parallel but at mid-morning (Table 2).

There were no differences in the nitrogen concentration of needles, and large differences in magnesium and calcium concentration, between green trees growing at the two sites (Table 5). Potassium concentration in needles (Table 5) was significantly higher at the healthy site ($P \leq 0.01$) only in the youngest needle age class (Oren et al. 1986). The concentration of manganese was higher in needles from the declining site, but the difference was not significant due to high variation in needle manganese at that site.

Comparing site differences in element concentration of needles with differences in concentrations of the xylem sap on a daily basis (compare Tables 2 and 5), calcium, magnesium, and nitrogen were similar in both needles and xylem sap. Magnesium and calcium were significantly higher at the healthy than at the declining site, but nitrogen was the same. There was also an agreement in the trend for manganese and potassium when needle concentration was compared with the seasonal trend of elements in the xylem sap (compare Tables 1 and 5).

Discussion

Magnesium concentrations in needles from the healthy site were just below the optimal range, but those from the declining site just above the level where strong deficiency symptoms occur (8 µmol g^{-1}dw) and within the range where some symptoms (yellowing) are apparent (Ingestad 1959; Oren 1988c). It follows that the apparent damage to the needles at the declining site can probably be attributed to insufficient supply of magnesium to the needles, as indicated by the lower concentration of magnesium in xylem sap of trees from that site. Low magnesium concen-

Table 3. Correlation coefficients among mean site element concentrations and mean site leaf water potential of *Picea abies* during 15 days in September and October only ($n = 14$/correlation for all but calcium where $n = 5$/correlation). Data from Fig. 2

	Nitrogen		Magnesium		Potassium	
	Healthy	Declining	Healthy	Declining	Healthy	Declining
Leaf water potential (10–12 a.m.)	-0.46	-0.73***	-0.84***	-0.66**	-0.84***	-0.80***
Calcium	0.40	0.73**	0.86***	0.80***		
Magnesium	-0.19	0.41				

Note: Correlations are significant at $P \leq 0.01$ (**) at 0.001 (***)

Table 4. Mean ($\pm$SE) element concentration in the xylem sap of *Picea abies* varied considerably throughout the growing season. The concentrations of magnesium, calcium, and potassium were higher at the healthy than at the declining site, but only differences in magnesium concentration were significant ($n = 10$/month, and 20/site except for calcium $n = 15$/site)

Element (mmol l^{-1})	Month				Significance (F-test)	Site	
	April	May	July	October		Healthy	Declining
Magnesium	0.32 ± 0.07^{a}	0.19 ± 0.02^{b}	0.15 ± 0.02^{b}	0.17 ± 0.04^{b}	0.05	0.25 ± 0.02*	0.17 ± 0.04
Potassium	0.86 ± 0.16^{b}	2.63 ± 0.48^{a}	1.38 ± 0.08^{b}	1.20 ± 0.09^{b}	0.001	1.55 ± 0.22	1.50 ± 0.27
Calcium^{c}	–	0.86 ± 0.10^{a}	0.64 ± 0.10^{a}	0.70 ± 0.09^{a}	NS	0.82 ± 0.08	0.66 ± 0.07
Manganese	0.03 ± 0.01^{b}	0.08 ± 0.02^{a}	$0.07 \pm 0.02^{a,b}$	$0.05 \pm 0.01^{a,b}$	0.05	0.03 ± 0.01***	0.08 ± 0.01

^{a,b} Any two means on the same line with the same superscript are not significantly different. Two means on the same line with a different superscript are significantly different ($P \leq 0.05$). * Significantly different at $P \leq 0.05$; *** Significantly different at $P \leq 0.001$
^{c} Calcium was not analysed in April

Table 5. Mean ($\pm$SE) element concentration in 1 to 5-year-old needles of *Picea abies* (weighted by the proportion of needles in each age class). Samples were taken in April from trees in the plots where xylem sap was collected. Notice no significant differences in N concentration between the sites while the concentrations of magnesium and calcium were significantly higher at the healthy than at the declining site ($n = 5$/site)

Element (µmol gDw^{-1})	Site	
	Healthy	Declining
Nitrogen	1551.1 ± 71.0	1607.6 ± 131.0
Magnesium	38.5 ± 3.0***	16.8 ± 1.2
Potassium	122.0 ± 7.3	119.4 ± 15.8
Calcium	212.2 ± 22.0*	105.6 ± 30.4
Manganese	10.9 ± 1.3	16.2 ± 4.3

Note: * Significantly different at $P \leq 0.05$; *** Significantly different at $P \leq 0.001$

trations in needles at the declining site were also correlated with lower wood production than at the healthy site (Oren et al. 1988d). A primary reason for the lower concentration of magnesium and calcium in xylem sap of trees from the healthy compared with the declining site was the lower concentration of these elements in the soil solution (Meyer et al. 1988). It is also notable that no differences were found in potassium and nitrogen concentrations between the sites, and that manganese concentration was higher at the declining site, in both xylem sap and soil solution.

It is evident from our investigations that concentrations of total nitrogen, magnesium, and potassium in the xylem sap of trees from the two sites was insensitive to diurnal water movement. This is similar to the findings of Schulze and Bloom (1984) and unlike those of Stark et al. (1985). However, as leaf water potential decreased as a result of drought, the nutrient concentrations in the xylem sap also decreased (Fig. 3). This indicates that when there was drought in the soil the transport of mineral nutrients from the root to the xylem was affected either by soil ion availability, or by the ability of roots to absorb and transport ions to the shoot, or by both (Parrondo et al. 1975; Shaner and Boyer 1976). Indeed, Meyer et al. (1988) found that roots at the declining site were more unevenly distributed than roots at the healthy site, concentrating more in the organic layers and thus, probably, experiencing short but numerous drought periods during which uptake of water and nutrients from these layers was impaired. From Fig. 3 it appears that nitrogen remained at a relatively lower water potential than magnesium. Magnesium reached a minimum value while nitrogen was still at its high value at a leaf water potential of -0.6 MPa. We are unable to decide if the difference in behaviour of magnesium and nitrogen reflects differences in uptake or in recycling within the plant. The significance of the different behaviour of magnesium versus nitrogen needs further attention.

We conclude that the differences in the foliar nutrient concentrations in trees from the two sites were consistent with their concentration differences in the xylem sap. Figure 4 shows that magnesium and calcium concentrations in needles were closely related to the concentrations of these elements in the xylem sap and may explain the variation among trees. Such relationships did not emerge for potassium and nitrogen, perhaps because of the relatively small range in concentrations of these elements and due to their phloem mobility. The slope of the relations shown in Fig. 4 reflects primarily the combined effects on the nutrient concentration in the xylem of the element concentrations in

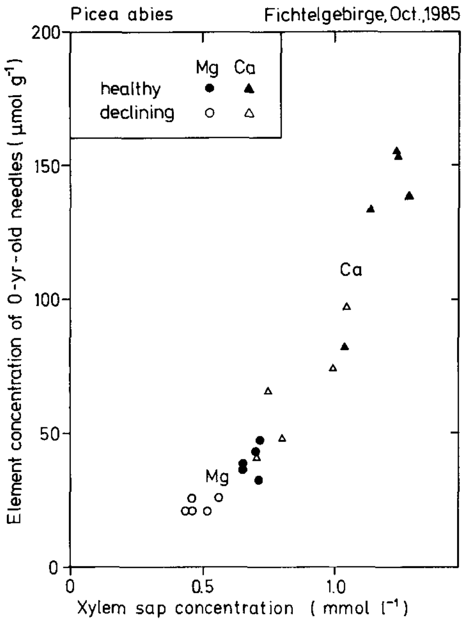

Fig. 4. Relations between element contents of 1-year-old needles and the average xylem concentration for the measuring period in October

the soil, the properties of the root system (Meyer et al. 1988), and the moisture content of different soil horizons. If leaching of elements from needles did contribute strongly to differences in element concentrations of needles, the two stands would have parallel slopes in Fig. 4, with the slope of the declining stand below that of the healthy stand because of higher proton input in the former. Thus, at a given level of xylem sap concentration of any cation, the trees of the declining stand would have lower concentrations of that cation in their needles. However, the data in Fig. 4 do not support such relationships. Therefore an impaired supply of elements to the canopy seems to dominate other processes in creating differences in needle nutrient status.

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