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Effect of foliar application with acid mist on the photosynthesis of potassium-deficient Scots pine seedlings

Pekka Nygren & Pertti Hari

TIIVISTELMÄ: HAPPAMAN SUMUTUKSEN VAIKUTUS KALIUMIN PUUTTEESTA KÄRSIVIEN MÄNNYN TAIMIEN YHTEYTTÄMISEEN

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The interactive effects of potassium deficit and foliar application with acid water (pH 5.5, 4.5, 4.0, 3.5, 3.0 given consecutively) on the CO₂ exchange rate of *Pinus sylvestris* L. seedlings was investigated in field conditions. No reduction of the CO₂ exchange rate was observed in the seedlings supplied with sufficient potassium. Only the seedling having the lowest needle K concentration (2.4 mgg⁻¹) had an apparently low CO₂ exchange rate before the applications with acid water. The CO₂ exchange rate of most of the seedlings with low needle K concentration (3.9–6.0 mgg⁻¹) decreased after the acid water application. The threshold acidity for the reduction varied between pH 4.0 and 3.0 depending on the needle K concentration. The reduction was more apparent at high irradiance. It was concluded that acid precipitation disturbs the CO₂ exchange only in conditions of mineral nutrient deficit.

Kaliumin puutteesta kärsivien männyn (*Pinus sylvestris* L.) taimien hiilidioksidin vaihtonopeuden vastetta neulasien sumutukseen happamalla vedellä (pH 5,5, 4,5, 4,0, 3,5, 3,0 annettuna alenevassa sarjassa) tutkittiin kenttäkokeissa. Kastelu ei vaikuttanut CO₂-vaihtonopeuteen taimissa, joiden neulasten kaliumpitoisuus oli korkea. Ennen sumutusta CO₂-vaihtonopeus oli selvästi alhainen vain taimella, jonka neulasten kaliumkonsentraatio oli pienin (2,4 mgg⁻¹). Happamalla vedellä sumuttamisen jälkeen CO₂-vaihtonopeus laski suurimmalla osalla taimia, joiden neulasten kaliumpitoisuus oli alhainen (3,9–6,0 mgg⁻¹). Kynnyshappamuus vaihteli välillä pH 4,0 ja 3,0, riippuen neulasten kaliumkonsentraatiosta. Lasku oli selvintä voimakkaan irradianssin oloissa. Tulosten perusteella pääteltiin, että hapan sade vaikuttaa CO₂-vaihtoon ainoastaan, jos puu kärsii samalla mineraaliravinteiden puutteesta.

Keywords: potassium concentration, acid deposition, *Pinus sylvestris*, seedlings, photosynthesis.
FDC 161 + 425

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1 Introduction

Many photosynthetic reactions are dependent on the acidity of mesophyll cell sap. These include the enzymatic reactions in Ribulose-1,5-bisphosphate carboxylation, which have a rather low H^+ concentration optima (Wraight 1982) and the functioning of the photosynthetic electron transport chain (Wild 1987). It therefore seems obvious that if the hydrogen ion concentration of the mesophyll cell sap increases, e.g. as a possible result of acid rain, then disturbances would occur in photosynthesis.

In greenhouse conditions, only very acid water has caused changes in photosynthesis. Simulated acid rain of pH 2.0 stimulated oxygen evolution in the bean (*Phaseolus vulgaris* L.) (Ferenbaugh 1976), but reduced the CO_2 exchange rate in *Platanus occidentalis* L. (Neufeld et al. 1985). In the latter study, the CO_2 exchange rate of three other North American deciduous tree species remained unaffected. In field conditions, the three months of foliar applications with dilute sulphuric acid of pH 3.0 reduced the photosynthetic rate in Scots pine (*Pinus sylvestris* L.) seedlings by 10–30 % (Katainen and Kellomäki 1981).

No changes of CO_2 exchange have been observed in the Norway spruce (*Picea abies* (L.) Karst.) devoid of visible injuries in the damaged forest areas of Germany, while visibly damaged trees showed clearly reduced CO_2 exchange rates (Beyschlag et al. 1987, Schulze et al. 1987, Wild 1987, Lange et al. 1989). The same pattern also applies to the photosynthetic capacity (Lange et al. 1987, Oren and Zimmermann 1989). Generally, the trees with a reduced CO_2 exchange rate suffered from magnesium deficit (Beyschlag et al. 1987, Lange et al. 1987, Lange et al. 1989). In the Eastern United States the calcium deficient Red Spruce (*Picea rubens* Sarg.) saplings were found to have elevated dark respiration rate and, following that, a reduced net CO_2 exchange rate (McLaughlin et al. 1991). In the case of visibly injured trees, the reduction of CO_2 exchange rate may be simply caused by

the loss of photosynthetic tissue (cf. Neufeld et al. 1985), or the excessive leaching of enzymes and enzyme activating ions (cf. Tukey 1970, Klemm 1989).

In an earlier paper (Nygren et al. 1987), we postulated the hypothesis that the cation exchange reactions between hydrogen and potassium ions, commonly observed in the forest canopies (Parker 1983, Ulrich 1983, Miller 1984), regulate the acidity of the mesophyll cell sap. The exchange reactions are supposed to take place on the leaf surfaces, or in the stomata prior to H^+ ions entering into the cell sap. The H^+ ions can then be transferred through the membranes by cation exchange to the vascular tissue to be transported to the roots where they may be used for nutrient uptake by cation exchange and the K^+ ions transferred to the leaf surface may then be lost via leaching (cf. Miller 1985, Klemm 1989). Thus, there would be a continuous K^+ flow from the roots to the leaves and a H^+ flow from the leaves to the roots.

In natural conditions these flows may form part of an internal cation circulation in the forest (Miller 1985). However, if the rain water H^+ load is excessive the balance between the H^+ and K^+ ions may be disturbed, and if the tree suffers simultaneously of potassium deficit, the H^+ cations may penetrate to the mesophyll cell sap because of the lack of the stabilizing mechanism. So, the trees well supplied with potassium would tolerate better the external hydrogen ion load caused by the acid precipitation, while the metabolism of the trees having a poor potassium status would be disturbed.

The aim of the present article is to present the results of a series of experiments on the interactive effects of potassium deficit and foliar application with acid water on photosynthesis of Scots pine seedlings and to discuss the results in the light of the hypothesis presented above. References will be made also to the study about the needle ultrastructure carried out with the same plant material (Holopainen and Nygren 1989).

2 Material and methods

2.1 Material

Two experiments were planned to test the presented hypothesis. In the summer of 1986, a preliminary experiment was carried out, and in summer 1987 a more complete experiment was realized. Both of the experiments were carried out in Southern Finland at the Forestry Field Station of the University of Helsinki, (61° 51' N, 24° 17' E, 160 m asl.).

The plant material used in both years was two-year-old nursery grown plus seed stand Scots pine seedlings, which were transplanted to 8 l plastic containers filled with 0.5–1.5 mm grain quartz sand. The nutrients were supplied for the seedlings by means of the watering solution, which potassium concentration was varied to create different needle K concentrations. The needle K concentrations were then treated as a continuous variable, and no attempt was made to classify according to the K level of the watering solution.

The basic nutrient solution used contained the main nutrients in optimal ratios for Scots pine, as presented by Ingestad (1979). The concentrations were 100 mg l⁻¹ for nitrogen, 14 mg l⁻¹ for phosphorus, 6 mg l⁻¹ for calcium, 6 mg l⁻¹ for magnesium and 45 mg l⁻¹ for potassium, with the necessary micro nutrients added by means of Hoegland's micro nutrient solution. The acidity of the nutrient solution was regulated to pH 4.7–4.9 by the addition of sulphuric acid.

2.2 Experiments

On 31 May 1986, four seedlings were transplanted to the quartz sand culture. The potassium concentrations of the four different watering solutions were 45 mg l⁻¹, 18 mg l⁻¹, 9 mg l⁻¹ and 0 mg l⁻¹. The seedlings were watered daily with 250 ml of nutrient solution. The nutrient solution was allowed to drain through holes in the bottom of the containers.

The seedlings were grown in the field until 25 August, when the carbon dioxide exchange measurements began. During the growing period, the quartz sand was protected from rainfall, but the shoots were subjected to the normal H^+ load of the rain. The rainfall during the growing period was 226 mm. The rainfall weighted mean

H^+ concentration of the rain water was $3.56 \cdot 10^{-5} \text{ mol l}^{-1}$, corresponding to pH 4.4. The pH range of the rain water was 3.9–5.3.

The CO_2 exchange measurements in the field were carried out between 25 August and 17 September. The current-year shoots were inserted into trap-type plexiglass chambers, which closed automatically when the chamber came up for measurement. Each chamber was closed for 100 sec. The measuring system contained five chambers, which were measured in sequence. Four chambers were used for measuring the CO_2 exchange rate of the test seedlings and one was used to measure the reference CO_2 concentration of the ambient air. The interval between CO_2 measurements was 16 min. 40 sec. for each seedling.

From the closed chamber the gas was fed to an infrared gas analyzer (Hartman & Braun, URAS 1) for CO_2 concentration analysis. The CO_2 concentration of the chamber air was recorded prior to the opening of the chamber by a data logger (Nokia Ltd., PP 6404), which served as the central unit of the data acquisition system. The irradiance and ambient air temperature were recorded simultaneously. A pyranometer sensor (Li-Cor Inc., LI-200SB) placed above the seedlings was used for irradiance measurements and a copper-constantan thermocouple was used for temperature measurements. The data logger measurements were stored on the minicomputer (Digital Equipment Corp., PDP 11/34) of the Forestry Field Station. A detailed description of the measuring system has been presented by Korpilahti (1988).

During the measuring period, the seedlings were sprayed ten times with distilled water acidified by the addition of sulphuric acid. A manual atomizer was used for the sprayings. Each application consisted of 100 ml of water with pH values of 5.5 (26 and 27 Aug.), 4.5 (29 and 30 Aug.), 4.0 (6 and 7 Sep.), 3.5 (9 and 10 Sep.) and 3.0 (12 and 13 Sep.). During the measuring period, also the shoots of the seedlings were protected from natural rainfall.

After the experiment, the needle area of the shoot inserted to the chamber was determined using the modified Tiren's equation. The main nutrient contents of the needles were analyzed using Kjeldahl's method for nitrogen, the mo-

lybdenum blue method for phosphorus, an atom absorption spectrophotometer for calcium and magnesium and a flame photometer for potassium.

In 1987, forty seedlings were transplanted to quartz sand on 7 May. The four potassium concentrations of watering solutions were 45 mg l⁻¹, 20 mg l⁻¹, 10 mg l⁻¹ and 5 mg l⁻¹. The watering followed the same procedure as in 1986.

The seedlings were grown in the greenhouse of the Forestry Field Station until 17 June, when they were transferred outside. The seedlings were protected from the rainfall during the growing period, and dry surface depositions were washed off weekly by spraying each seedling with 100 ml of deionized water. The field measurements of CO₂ exchange began on 26 July. A seedling from each treatment was randomly selected for these measurements. The rest of the seedlings were used for the study of the needle ultrastructure which was published separately (Holopainen and Nygren 1989).

The seedlings used for CO₂ exchange measurements were typical representatives of the group of forty plants, in which no differences in the seedling quality indicators were found between the K levels of watering solution, except in the height of current-year main shoot which was significantly smaller in the seedlings which received 5 mg l⁻¹ K compared to those which received 45 mg l⁻¹ (P = 0.05 in Tukey's studentized range test; Holopainen and Nygren 1989).

The field measurements of CO₂ exchange were carried out between 26 July and 25 August using the same measuring apparatus as in 1986. In 1987, the measuring interval for each seedling was 13 min. 20 sec. The seedlings were sprayed with distilled water, acidified as in 1986. Each application consisted of 100 ml of water with pH values of 4.5 (28–31 July), 4.0 (3–7 Aug.), 3.5 (10–13 Aug.) and 3.0 (17–20 Aug.). After the experiment, the needle area of the shoot inserted to the chamber was measured and the concentrations of the main nutrients were analyzed as in 1986.

2.3 Data analysis

To separate the treatment effects from the natural fluctuation of the CO₂ exchange rate caused by environmental factors, a modelling technique

was applied for the analysis of the field data. A dynamic model describing the irradiance and temperature response of the CO₂ exchange rate was derived from the data measured at the beginning of the spraying period. Since the dependence of the CO₂ exchange rate on environmental factors remains quite constant during a growing season (Hari et al. 1981, Korpilahti 1988), it was assumed that the same model should fit during the whole measuring period, and that any systematic deviations from the model reflect the disturbances caused by the acid spray.

The model structure presented by Korpilahti (1988) was applied for the modelling. Let I(x,t) denote the irradiance at place x at the moment of time t and T(t) the ambient air temperature at the moment of time t, f₁(I(x,t)) the response of the photosynthetic rate to irradiance, f₂(T(t)) the response of the photosynthetic rate to temperature, and r(T(t)) the response of the respiration rate to temperature. The CO₂ exchange rate at place x at moment t, p(x,t), is the difference between the photosynthetic rate and the respiration rate (Korpilahti 1988):

$$p(x,t) = f_1(I(x,t)) \cdot f_2(T(t)) - r(T(t)) \quad (1)$$

A Michaelis-Menten type function was applied for the irradiance response of photosynthetic rate, a saturating function for the effect of temperature on photosynthetic rate and an exponential function for the effect of temperature on respiration rate (Korpilahti 1988). Thus:

$$f_1(I(x,t)) = (P_m \cdot I(x,t)) / (I(x,t) + a) \quad (2)$$

$$f_2(T(t)) = 1 - \exp(-a_1 \cdot (T(t) - a_2)) \quad (3)$$

$$r(T(t)) = c_1 \cdot \exp(c_2 \cdot (T(t) - c_3)) - 1 \quad (4)$$

where the parameter values were a₁ = 0.32 °C⁻¹, a₂ (minimum temperature of gas exchange) = -5 °C, c₂ = 0.036 °C⁻¹ and c₃ (equal to a₂) = -5 °C. The parameters P_m (rate of light saturated photosynthesis), a (irradiance in which half of the maximum photosynthetic rate is reached) and c₁ (specific dark respiration rate at 15 °C) were specific to each seedling (Korpilahti 1988). The parameter estimation was carried out using a modified steepest descent iteration procedure to minimize the residual sum of squares.

3 Results

3.1 Nutrient concentrations of the needles and the CO₂ exchange model

Table 1 presents the concentrations of the main nutrients in the needles of the seedlings used in the field experiments of CO₂ exchange after the spraying period in 1986 and 1987. The nutrient concentrations were slightly lower in 1986, when the seedlings had been exposed to rainfall during the growing period, than in 1987. The needles with low K concentration had also apparently low Ca concentration. However, the correlation was weak and statistically insignificant (Pearson r = 0.61, P = 0.11). The potassium treatment did not affect the concentrations of the other main nutrients except in the seedling without K in the watering solution, which had rather low nitrogen and phosphorus concentrations compared to the other seedlings. None of the seedlings presented visible symptoms of any nutrient deficiencies.

The K concentrations of the seedlings watered with the solutions of 45 and 20 mg l⁻¹ of K in 1987 were quite high, close to the concentrations presented as optimal nutrient supply for Scots pine by Ingstad (1979). The needle K concentrations of the 1986 seedlings subjected to the K treatments of 45 and 18 mg l⁻¹ and the 1987 seedling watered with 10 mg l⁻¹ K solution were about the order observed in natural conditions (van Goor 1978, Lehtonen et al. 1976) whereas the concentrations of the seedlings wa-

tered with the solutions of 9 and 5 mg l⁻¹ of K were somewhat low and the K concentration of the seedling watered without K addition was remarkably low. The seedlings will subsequently be referred to with the letter K followed by the needle K concentration and, if necessary, the experiment year.

In 1986, the parameters of the CO₂ exchange model (eqs. 1–4) were estimated from the data measured on 26 August, which was the first day of spraying with pH 5.5 water. In 1987, the parameters were estimated from the data measured on 31 July, which was the last day of spraying with pH 4.5 water. This day was selected because of a leakage in the control seedling (K11.3) chamber noticed and repaired on 30 July, five days after the beginning of the study. The parameter values are presented in Table 2 with the proportion of variance explained by the model on the parameter estimation day.

The values of the parameter P_m, the rate of the light saturated photosynthesis, were of the same order in all seedlings, except in seedling K2.4 which had a very low P_m value, and the P_m value for seedling K4.8 was quite high. Most of the P_m values were lower than those reported by Korpilahti (1988) for natural conditions, the latter being 3.7–3.9 μmol m⁻² s⁻¹. However, since there were no systematic changes following the K treatment, it seems justifiable to conclude that the K deficit itself did not affect photosyn-

Table 1. Concentrations of the main nutrients (mg g⁻¹ of dry mass of needles) in the needles of the seedlings used in the CO₂ exchange measurements.

	Potassium concentration of watering solution (mg l ⁻¹)	Nutrient concentration (mg g ⁻¹)				
		N	P	Ca	Mg	K
1986	45	19.29	1.67	0.73	0.88	6.9
	18	22.60	1.56	0.70	0.94	6.0
	9	19.07	1.66	0.41	0.81	3.9
	0	13.15	1.16	0.31	0.72	2.4
1987	45	23.04	2.57	0.72	1.60	11.3
	20	22.54	2.06	0.44	1.22	9.4
	10	26.03	2.20	0.44	1.31	6.0
	5	26.40	2.06	0.46	1.48	4.8

Table 2. Values of the parameters of the CO₂ exchange model (eqs. 2–4) specific to each seedling in the field experiments and the proportion of explained variance R² for the day of parameter estimation.

Needle K concentration (mgg ⁻¹)	P _m	Parameter value a	c ₁	R ²
11.3	3.3	195	0.32	0.89
9.4	4.2	175	0.38	0.86
6.9	2.6	190	0.21	0.94
6.0/86	3.1	200	0.23	0.94
6.0/87	3.8	170	0.32	0.91
4.8	5.0	165	0.44	0.90
3.9	2.8	170	0.23	0.94
2.4	1.6	205	0.14	0.95

P_m = rate of light saturated photosynthesis (μmolm⁻²s⁻¹)
a = irradiance in which half of the light saturated photosynthetic rate is reached (Wm⁻²)
c₁ = dark respiration rate at 15 °C (μmolm⁻²s⁻¹)

thesis. The low P_m value for seedling K2.4 is an exception, but since the concentrations of the other main nutrients in the needles were also lower than in other seedlings, the disturbances in the CO₂ exchange may reflect a more complex set of nutrient imbalances than only the K deficit. The values of parameter c₁, dark respiration rate at 15 °C, approximately followed the changes of the value of P_m. The values were about the same order as those reported by Korpilahti (1988) for natural conditions.

The fit of the model to the data is presented in Fig. 1 by showing the daily course of the measured CO₂ exchange rate and the prediction calculated using the presented model for seedlings K6.9, K3.9 and K6.0/87 during the day when the parameter estimation was carried out. The proportion of the variance explained by the model was quite high for each seedling, varying between 86 and 95 %.

3.2 The effect of acid water

The compatibility between the measured and predicted CO₂ exchange rate during the measuring period was examined using the daily means of the model residual. The daily mean residuals are presented in Fig. 2 as a function of the acidity of the spraying water for each seedling. Typical variance of the residuals can be observed in Figs. 1, 3 and 4. The mean residual for the seedlings watered with 45 mg l⁻¹ K solution

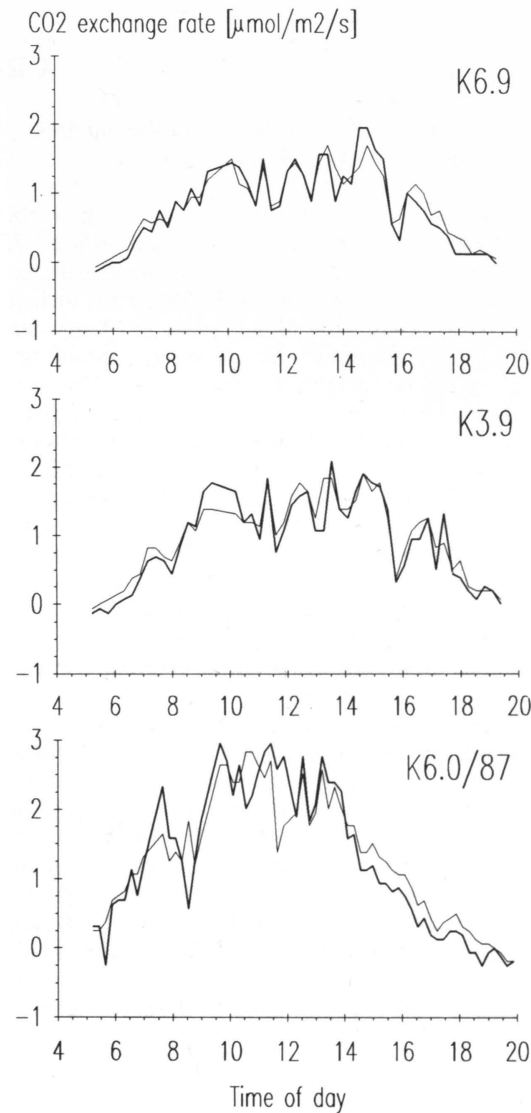


Fig. 1. Daily course of measured (thick line) and predicted (thin line) CO₂ exchange rates of the seedlings K6.9 (top) and K3.9 (middle) on 26 August 1986, and of seedling K6.0/87 (bottom) on 31 July 1987, the day used for parameter estimation.

in both years (K6.9 and K11.3) remained quite close to zero during the whole measuring periods.

The mean residuals of seedlings K6.0/86 and K3.9 were negative almost throughout the measuring period. The deviation from the prediction

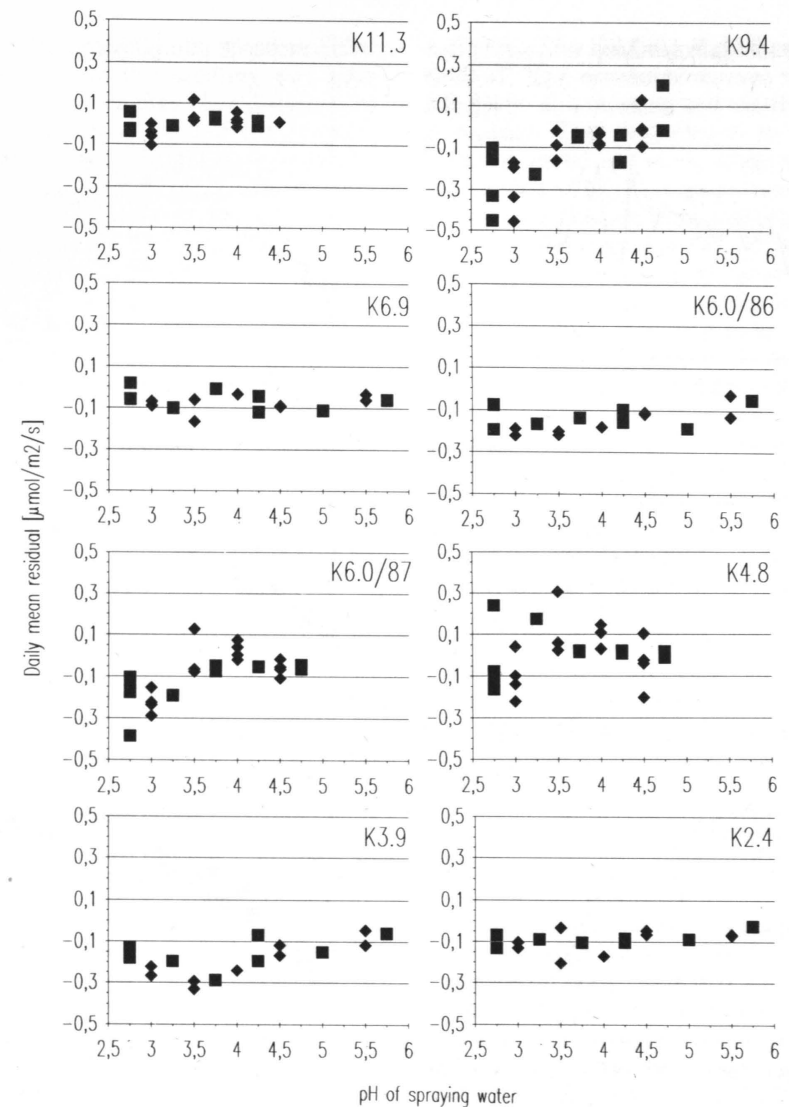


Fig. 2. The daily mean residual (μmolm⁻²s⁻¹) of the CO₂ exchange model (eqs. 1–4) as a function of the acidity of the spray water applied on the same day (◆). The values for the rest days (■) have been marked between the pH values of the spray water applied before and after the rest period.

was a little larger in seedling K3.9 than in K6.0/86 beginning with pH 4.0. The mean residuals of seedling K6.0/87 were initially close to zero but became clearly negative at pH 3.0. The absolute mean residuals of seedling K2.4 were quite small but negative throughout the measuring period. In seedling K2.4, the residual values relative to the measured CO₂ exchange rate were about the same order as in seedling K6.0/86.

The CO₂ exchange rates did not recover during the rest days between the sprayings (Fig. 2).

The interpretation of the data is complicated by the behaviour of the mean residual of seedlings K9.4 and K4.8 (1987). The mean residuals of the former show a clear decrease as function of increasing acidity of the spray water, especially at pH 3.0, although the K concentration in the needles of this seedling was very high. How-

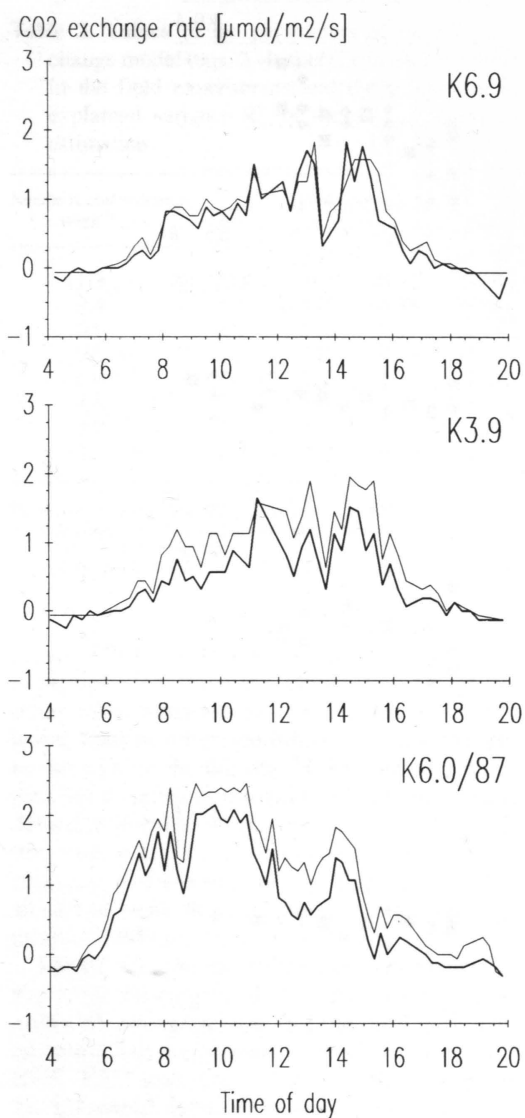


Fig. 3. The daily course of measured (thick line) and predicted (thin line) CO₂ exchange rate of the seedlings K6.9 (top) and K3.9 (middle) on 13 September 1986, and of the seedling K6.0/87 on 19 August 1987. The spray water applied for all the seedlings was pH 3.0.

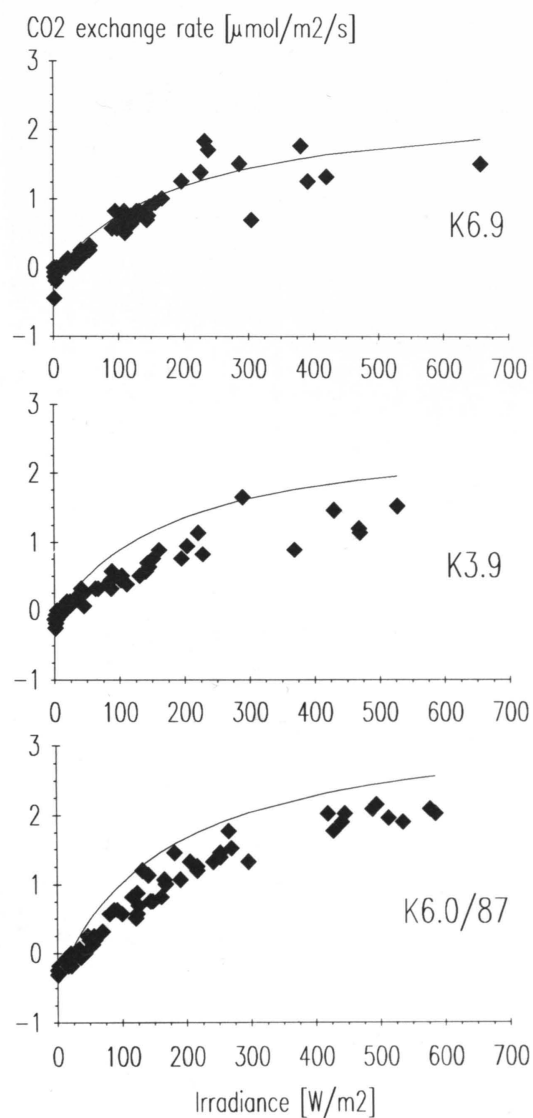


Fig. 4. The response of the CO₂ exchange rate to irradiance of seedlings K6.9 (top) and K3.9 (middle) on 13 September 1986, and of the seedling K6.0/87 (bottom) on 19 August 1987. The spray water applied for all the seedlings was pH 3.0. The respective predicted (eqs. 1–4) response curves are also presented.

ever, it should be noted that the Ca concentration of the needles of this seedling was quite low (0.44 mg g⁻¹), which may have disturbed its metabolism or mesophyll sap buffering capacity. On the other hand, the mean residuals of seedling K4.8 were strongly positive throughout the measuring period, although the K concentration in its needles fell between that of seedlings K6.0/86 and K6.0/87, and seedlings K3.9 and K2.4. Also the variance of the mean residuals of this seedling is quite large compared to the variance of the other seedlings, which may reflect an undetected measuring error.

The daily course of measured and predicted CO₂ exchange rate of seedlings K6.9, K3.9 and K6.0/87 near to the termination of the spraying period is shown in Fig. 3. The model derived at the beginning of the measuring period still fitted the measured data for seedling K6.9. The pattern of the predicted daily course of the CO₂ exchange rate was quite similar to the measured one for seedlings K3.9 and K6.0/87, but there was a difference in magnitude. The deviation

was greater for seedling K3.9 than for seedling K6.0/87. The deviation seemed to be smaller during the dim morning and evening hours than at midday. This dependence on irradiance is better observed in Fig. 4, where the measured and predicted CO₂ exchange rates are plotted against the irradiance measured at the same time.

The reduction in the CO₂ exchange rate of seedlings K3.9 and K6.0/87 most likely reflects the disturbance of photosynthesis caused by the acid water. These disturbances are also the most likely explanation for the behaviour of the mean residuals as a function of the acidity of the spray water in seedlings K6.0/86 and K2.4, and may also explain the reduction of the residuals in the seedling K9.4 at pH 3.0. However, the results of this seedling reflect more likely the effect of Ca deficit together with acid mist application, rather than the initially expected K deficit/acid water interaction. If not biased by measuring errors, the results of the seedling K4.8 may reflect genetic differences in the resistance to the H⁺ load of the spraying water and/or potassium deficit.

4 Discussion

The few studies about the effects of acid precipitation on photosynthesis (e.g. Ferenbaugh 1976, Katainen and Kellomäki 1981, Neufeld et al. 1985) suggest that photosynthesis is very resistant to acid precipitation. This is in accordance with the field observations, that atmospheric pollution does not directly affect the photosynthesis of Norway spruce in the damaged forest areas in Germany, but indirect effects, via nutrient deficiencies, cause the observed disturbances (Beyschlag et al. 1987, Lange et al. 1987, Lange et al. 1989). The experiments presented in this paper are also in accordance with the German observations, since the CO₂ exchange rate was clearly affected by the acid spraying water only in the potassium deficient seedlings.

A phenomenon commonly observed in the studies in which disturbances in the photosynthetic rate have been noted is that the transpiration rate has remained unaffected (e.g. Beyschlag et al. 1987, Schulze et al. 1987, Lange et al. 1989). This indicates that the stomata operate normally, and that the disturbances in photosynthesis are most likely of metabolic nature.

Such a metabolic response is supported by field observations in the forest decline areas of Germany. Wild (1987) reported a heavy reduction in the photosynthetic electron transport rate in damaged Norway spruces. The chlorophyll content of the needles of visibly injured Norway spruces has been observed to be low when compared to the healthy looking trees (Beyschlag et al. 1987, Lange et al. 1987, Wild 1987). This low chlorophyll concentration correlated with the magnesium deficit in the needles (Lange et al. 1989).

In the present experiments, the photosynthetic response to irradiance in the potassium deficient seedlings was affected after spraying with acid water. This phenomenon is most observable at high irradiance (e.g. Fig. 4), which may reflect disturbances in one or more of the photosynthetic light reactions. This reaction may be the electron transport chain as observed by Wild (1987), or the functioning of proton or electron translocating membranes as suggested by Evans (1984).

The potassium deficit has been observed to

cause a decrease of the Ribulose-1,5-bisphosphate carboxylate activity in alfalfa (*Medicago sativa* L.) (Peoples and Koch 1979). If the same kind of effect also exists in Scots pine, the potassium deficit itself might have disturbed the photosynthesis. For the seedlings used in this study disturbances caused by the K deficit seem quite unlikely, since the differences in the CO₂ exchange rate observed before the spraying period did not follow the needle K concentrations. Only in the seedling K2.4 a highly reduced CO₂ exchange rate was observed even at the beginning of the spraying period, but this seedling had also quite low needle nitrogen, phosphorus and calcium concentrations.

The potassium deficit alone did not cause ultrastructural disturbances in the chloroplasts of Scots pine seedlings treated as in the 1987 field experiment, but induced vacuole deformations, which indicate osmotic imbalances of the cells. The acid water treatment caused protrusions in the chloroplasts facing the cell wall in the seedlings with high needle K concentration, but the thylakoids appeared undisturbed. In the seedlings with low needle K concentration the application with acid water caused lipid structures inside the chloroplasts, increased density of the chloroplast stroma and disintegration of chloroplast envelope accompanied with deterioration of cytoplasm. (Holopainen and Nygren 1989.)

These findings about the needle ultrastructure are in accordance with the results of the measurements of the CO₂ exchange rate presented here. The first appearance of the ultrastructural damages adjacent to the cell wall, especially at the plasma membrane, also partly supports our hypothesis about the regulation mechanism of the external H⁺ ion load by cation exchange with K⁺ ions: the cell walls form the most likely route for the transfer of the protons from the stomata to the vascular tissue, since the resistance to flow in the cell walls is much lower than across the protoplasts (Kramer and Kozlowski 1979). If the tree is well supplied with potassium, the excessive H⁺ load slightly damages the cell organelles next to the transfer route, but the protons do not enter to the mesophyll cell sap in high quantities. If the tree suffers from potassium deficit, there is not enough K⁺ ions to keep the proton flow in the cell wall and the protons penetrate to the cell sap damaging its ultrastructure and disturbing the normal biochemical activity, including photosynthesis.

The data about the well K supplied but slightly Ca deficient seedling K9.4 suggest that also

the needle Ca concentration plays some role in the regulation of the CO₂ exchange, but the effective mechanism remains unknown. No increased dark respiration, as observed by McLaughlin et al. (1991) in Red Spruce, was observed in this study, since the respiration rates were close to those observed in natural conditions in Scots pine (Korpilahti 1988). While the role Ca cannot be further discussed in the light of our data, these observations leave open the possibility that all main base cations, K⁺, Ca²⁺ and Mg²⁺ (e.g. Lange et al. 1989) are needed for a complete regulation of the photosynthetic and/or respiratory metabolism in acid deposition conditions.

It should be noted that our results as such are not extrapolable to the natural conditions. Since the intention of the experiments was to test the existence of the interaction of potassium deficit and external proton load on the photosynthesis, the factors disturbing the phenomenon were tried to eliminate. Thus, the rainfall chemistry was not simulated, but pure sulphuric acid diluted to distilled water was used. However, the cations in the rain water may smooth the effects of the acids. Furthermore, the spray was quite misty forming a water film on the needle surface which stimulates the penetration of the protons into the mesophyll (Miller 1984). On the other hand, the amount of water applied was quite small, the total spraying being the order of 10–15 % of the summer rainfall at our study site.

Taking into account the restrictions presented, it can be concluded that the present experiments support the hypothesis that the acid precipitation of measured field proton concentrations alone has only a minor effect on photosynthesis, but the interactive effect with mineral nutrient deficits may be severe. This interactive effect has been found earlier in connection with the magnesium deficit, which most likely affects photosynthesis via reduced needle chlorophyll concentrations (Beyschlag et al. 1987, Lange et al. 1987, Lange et al. 1989), and in connection with calcium deficit, which increased the dark respiration rate (McLaughlin et al. 1991). As far as we know, the present experiments were the first in which the potassium deficit was investigated from this point of view. These disturbances occurred in healthy looking seedling, but obviously the needle ultrastructure was already injured (cf. Holopainen and Nygren 1989).

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