Distribution and variations of potassium and calcium in different cross sections of Picea abies (L) Karst needles and Fagus sylvatica (L) leaves exposed to ozone and mild water stress

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Summary — Two clones of 8-year-old Norway spruce trees, and beech trees, planted directly into the soil in open-top chambers, were exposed to elevated ozone concentrations and subjected to a mild soil drought stress. The nutrient partitioning and intertreatment differences in nutrient levels were studied. In elevated ozone, both clones had increased potassium and calcium levels, whereas in beech, ozone-treated trees had decreased potassium levels. Drought caused decreases in these nutrients for all species. The effect of combining the 2 stresses was more complex, however, and the previously observed effects were not obtained in all cell tissues. Furthermore, they showed both interspecific and interclonal differences. The hypothesis that ozone affects the root nutrition and cell membrane permeability is discussed.

ozone / water stress / potassium / calcium / cell tissues

Résumé — Répartition et variations du potassium et du calcium dans différentes coupes transversales d'aiguilles de *Picea abies* (L) Karst et de feuilles de *Fagus sylvatica* (L) soumis à de l'ozone et à une sécheresse modérée. *Deux clones d'épicéas âgés de 8 ans et des hêtres ont été plantés directement dans le sol dans des chambres à ciel ouvert ; ils ont été soumis à des concentrations élevées d'ozone et à un stress hydrique modéré. Les variations et la répartition des éléments minéraux ont été étudiées. Les teneurs en potassium et calcium chez les 2 clones augmentent dans les traitements de fumigation ; en revanche chez le hêtre les teneurs en potassium diminuent. La sécheresse appliquée fait diminuer ces teneurs pour tous les arbres. L'application combinée de ces 2 stress est plus complexe et les observations faites auparavant ne se retrouvent pas dans tous les compartiments foliaires et sont différentes entre clones et espèces. L'hypothèse que l'ozone affecte la nutrition minérale racinaire et la perméabilité membranaire des cellules est discutée.*

ozone / sécheresse / potassium / calcium / compartiment cellulaire

INTRODUCTION

Atmospheric pollution (especially ozone) has often been cited as a possible contributory factor in forest decline. Furthermore, there is evidence to suggest that current levels of tropospheric ozone can cause damage to trees (Dobson *et al,* 1990) and ozone levels in Europe and North America are predicted to rise as emissions of their precursors increase in association with increased motor traffic. Much work has thus been carried out to assess the biochemical and physiological response of trees to this pollutant, but the results have often been contradictory. This may be due to interspecific differences, but is also the result of differences in the experimental conditions used (Darrall, 1989). Ozone concentrations were often unrealistically high and there has been little work done to study the interaction of ozone with environmental constraints, especially away from the laboratory.

Prinz *et al* (1987) showed that forest decline was less severe in wetter years, so studies on the effect of both drought and ozone on trees would seem to be a priority, but they are still rare.

A demonstration of the complexity of interactions between ozone and drought was made by Davidson *et al* (1992), working with *Fagus sylvatica*. In addition, ozone has been shown to affect both growth and morphology of tree root systems (Taylor *et al,* 1989). Changes to the root system or in water availability can have serious implications for nutrient uptake by plants, and changes in the total foliar nutrient concentration of trees in response to ozone fumigation have been observed. Care should be taken in interpreting these results, however, as potted plants were used and McConnaughay *et al* (1993) have shown that nutrient changes in such trees can be artifacts of the experimental conditions.

To better study the interactive effects of ozone and drought stress, an experiment was conducted on 2 tree species (*Picea abies* and *Fagus sylvatica*) planted directly into the soil, enclosed in open-top chambers and exposed to a soil drying. Studies on nutrient distribution at the cellular level are rare and have never been performed on trees exposed to both realistic ozone concentrations and drought, although some work has been done on declining spruce trees (Stelzer *et al,* 1990; Fink, 1991a,b; Garrec *et al,* 1991; Godde *et al,* 1991). The aim of our study was thus to determine foliar nutrient changes in response to ozone and drought stress and to then identify at what cell levels these changes occurred. The physiological consequences of these changes could then be assessed.

MATERIALS AND METHODS

Experimental site and plant material

The experiment was carried out at Col du Donon (Vosges, France: 48° 29' N; 7° 05' E) in a mixed spruce beech forest. This region has relatively high levels of tropospheric ozone (38 ppb yearly average). It is 727 m above sea level.

Five-year-old Norway spruce (*Picea abies* (L) Karst) and beech (*Fagus sylvatica* L) trees were planted in the soil on 19 April 1990. The Norway spruce were 2 different clones: clone 780371 from a Polish provenance, Istebna (IST) and clone 781351 from a French provenance, Gerardmer (GER). The beech trees were not clonal. The trees were supplied by AFOCEL (Association Forêt Cellulose, Charrey-sur-Saone, France). The soil was classified as "typic distrochrept".

Shortly after the trees were planted, 8 open-top chambers (see Impens, 1992 for details) were erected enclosing 9 trees in each chamber – 3 beech and 3 individuals from each of the 2 Norway spruce clones. Four plots of 9 trees without open-top chambers served as controls.

Treatments

An electric discharge generator (Trailigaz "LABO 76") supplied with pure oxygen produced the ozone. Twenty-five ppb ozone was continuously added to 2 of the chambers and 50 ppb to another 2 from 17 July 1992 to 29 October 1992. Two chambers were ventilated with nonfiltered air, while the remaining 2 were fitted with activated charcoal filters which considerably reduced the ozone levels (by 75% ± 12%). From 30 October 1992 to 30 March 1993, the added concentrations were reduced by 25 ppb. From 31 March 1993, the experimental treatments were repeated and continued until 31 October 1993. The ozone concentration in any 1 chamber was recorded for 2 5-minute periods every hour, using an "Environment SA" analyser. Photosynthetically active radiation, air temperature and air and soil humidity were also recorded (unpublished data).

In the spring of 1993, work was begun to prepare the drought stress. Wooden structures of between 4.3 and 4.5 m high were built to support sloping roofs of clear plastic (89% light transmission) that prevented rain from entering. The 4 chambers with roofs then had 70 cm deep trenches dug around them to prevent the incursion of soil water into the chambers. The drought stress was begun 1 July 1993 and then the following treatments were performed: i) filtered air: FA; ii) nonfiltered air: NFA; iii) ambient air, outside chambers: AA; iv) nonfiltered air with 25 ppb ozone added: NFA + 25; v) nonfiltered air with 50 ppb ozone added: NFA + 50.

Each of these treatments, excepting AA, had a drought stressed chamber (DS) and an unstressed (well-watered) chamber (NS).

Sampling

The material for microanalysis was sampled on 8 July and 21 August 1993 for beech leaves and 8 July, 21 August and 21 September 1993 for Norway spruce needles, always between 08.00 and 10.00 GMT. Each sample was composed of 2 beech leaves and 5 current-year spruce needles. The beech leaves were taken from 1st flush growth and the needles came from the 1st 3 whorls. The plant material was immediately plunged into liquid nitrogen to prevent movement of nutrients after sampling had occurred.

During the sampling period, the following values of microclimatic parameters were obtained:

Air temperature: 17 ± 4°C

Leaf-air vapour pressure difference (VPD): 1.3 ± 0.1 kPa

Relative humidity of the chamber air: 55 ± 12%

Photon flux density of photosynthetically active radiation: 897 ± 254 μmol m^{-2} s^{-1}.

The differences between chambers with roofs and unroofed chambers were only statistically significant for light and temperature. Even so, the temperature difference was less than 1°C for the 24-hour average.

Pre-dawn leaf water potential

The pre-dawn leaf water potential (Ψ_{wp}) was measured using a Skye pressure chamber according to the method in Scholander *et al* (1965). Measurements were made throughout the drought stress period.

X-ray microanalysis

The material to be analysed was prepared according to the method used in Le Thiec *et al* (1994). Sections of plant material were examined under a scanning electron microscope (Stereoscan 90B, Cambridge) at 15 kV, equipped with a dispersive energy microanalysis system (EDX, diode Si-Li; Analyser AN10000 10/25). To ensure that the beam of primary electrons did not penetrate other cells, 1 layer of cells on a sheet of aluminium were analysed. All measurements were made using an X-ray take-off angle of 45°, a measuring time of 100 s, a magnification of 6 000 for all examined tissues (stomata, epidermis, mesophyll, parenchyma and endodermis) and of 400 for the vascular bundle. A ZAF4 program, FLS, connected to the microanalyser gave apparent concentrations of the different elements analysed. This program takes into account any variations in volume. In order to convert the microanalysis data into real concentrations (% dry mass), powdered spruce needles and beech leaves were used as standards (CRM 100 and CRM 101 given by Community Bureau of Reference of the Commission of the European Communities).

Chemical analysis

Sampling was carried out on 20 August 1993 for beech leaves and 21 September 1993 for current-year spruce needles. The samples were cleaned (by rapid submersion in demineralised, distilled water) and then dried. Phosphorus, sulphur and cations (Mg, K, Ca, Mn) levels were mineralised (H_2O_2 and $HClO_4$; Clément, 1977) and measured by ICP (Jobin Yvon JY438 Plus). The levels of soil nutrients were also determined using these techniques (drying at 450°C and mineralisation with a $HF/HClO_4$ mixture and then placed in 2% HCl).

Statistical analyses

The statistical treatment employed was the analysis of variance ($\alpha = 0.05$) by the GLM procedure (SAS Institute Inc, 1985). Test of equality of averages using Student-Newman and Keuls was applied equally (the same letters indicate that averages are not significantly different, and the alphabetic order corresponds to decreasing values). Significance was as follows: ns = not significant; * = significant ($P < 0.05$); ** = highly significant ($P < 0.01$); *** = extremely significant ($P < 0.0001$); $\alpha = 0.05$.

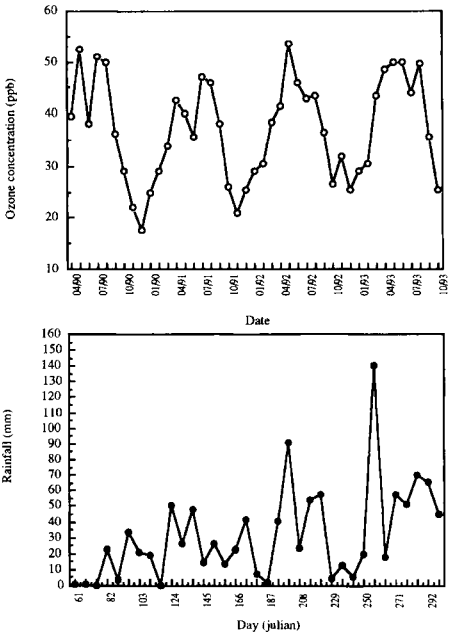

Fig 1. Mean monthly ozone concentrations at Col du Donon, since the planting of the trees and mean weekly rainfall totals for the vegetation period covered by the experiment.

RESULTS

Pollution climate and environmental factors

Figure 1 shows rainfall totals during the vegetation period and the ozone levels (monthly averages) since the trees were planted. Ozone showed the often reported fluctuations during the year with the maximum levels occurring in spring and summer. The Col du Donon is a site where summer ozone levels often exceed the maximum exposure recommended by the World Health Organisation and the EEC.

The pre-dawn leaf water potentials showed an initial decrease at the beginning of the drought period and then remained relatively constant (–0.82 ± –0.11 MPa; n = 104). There were no significant differences between species, or between ozone treatments. Because the trees were planted in the soil, there must have been incursion of soil water from outside the chambers – despite the trenches that were dug to reduce this – or the rooting system of trees was deep enough to obtain water. The relatively high rainfall during this period probably kept the water table at high levels. The Ψ_{wp} of the nonstressed trees remained between –0.05 and –0.2 MPa.

Chamber effects

The control trees outside chambers and the trees in the NFA NS chambers were

not significantly different for any measured parameter. There was a 3% difference in humidity between the chamber and outside controls and the increased temperature was never more than 2°C. Furthermore, before the drought stress was imposed, there was in effect a replication of 4 treatments (FA, NFA, NFA + 25, NFA + 50); we ensured that there were no *a priori* differences between the same chambers of any 1 treatment.

Chemical analyses

The soil analysis gave the following results:

– horizon 0–10 cm: P_2O_5 (‰): 0.195 ± 0.05; Ca ($cmol^+ kg^{-1}$): 1.16 ± 0.39; Mg ($cmol^+ kg^{-1}$): 0.54 ± 0.21; K ($cmol^+ kg^{-1}$): 0.34 ± 0.10; Mn (‰): 14.25 ± 5.93;

– horizon 20–30 cm: P_2O_5 (‰): 0.055 ± 0.02; Ca ($cmol^+ kg^{-1}$): 0.23 ± 0.11; Mg ($cmol^+ kg^{-1}$): 0.13 ± 0.03; K ($cmol^+ kg^{-1}$): 0.34 ± 0.10; Mn (‰): 8.01 ± 4.81.

The foliar analyses results are shown in table I. Ozone had a significant effect on calcium and potassium levels in both species. Drought stress significantly affected phosphorus, manganese, calcium and potassium in both spruce clones and calcium and potassium in beech only. The interaction of drought and ozone had a significant effect on calcium levels in the Istebna clone and potassium levels in Gerardmer and beech.

As the ozone dose increased, there was a tendency for a corresponding increase in calcium. This trend was also observed for potassium in both spruce clones, but the opposite effect occurred in beech. In filtered air treatments, the drought provoked a considerable decrease in both calcium and potassium. The effect of ozone changes upon imposition of the drought stress. In Istebna there is no longer an increase in Ca, but a decrease in response to increasing ozone. The potassium levels in Gerardmer are again increased by exposure to ozone, but in beech there is no observable effect on Ca or K levels.

Microanalysis

The microanalysis results for K and Ca are shown in table II for the 2 spruce clones and in table III for beech. After analysing the different cell tissues, it was found that calcium was most abundant in the epidermis. Potassium was most abundant in the epidermis of beech and the endodermis of Norway spruce.

Ozone effects

The Gerardmer clone showed increasing concentrations of potassium in all tissues, as ozone increased. Such an increase was also observed in the epidermis and guard cells and to a lesser extent the mesophyll and endodermis of Istebna. However, the vascular bundle of ozone-treated Istebna needles had a decreased K level. In ozone-treated beech, potassium was decreased in all tissues except the lower epidermis.

Calcium increases in all tissues, except the epidermis, in both clones raised in elevated ozone. Beech shows a similar increase apart from in the vascular bundle where no trend was observed.

Drought effects

The direct effects of drought can be seen from the FA treatments. In spruce, potassium showed decreases in the vascular bundle, endodermis and guard cells, but was increased in the mesophyll. Beech had reduced potassium levels in the vascular bundle, parenchyma, guard cells and lower epidermis. Calcium levels showed large decreases in the epidermis of both clones.

Table I. The total concentration of elements (± SD) (% dry weight) in beech leaves and Norway spruce needles (Gerardner and Istebna clone) ($n = 6$).

Element	*Istebna*						*Gerardmer*						*Beech*					
	S	*P*	*Mn*	*Mg*	*Ca*	*K*	*S*	*P*	*Mn*	*Mg*	*Ca*	*K*	*S*	*P*	*Mn*	*Mg*	*Ca*	*K*
Treatment																		
NS AA	0.15 (0.05)	0.20 (0.03)	0.15 (0.04)	0.09 (0.01)	0.38 (0.02)	0.70 (0.02)	0.14 (0.04)	0.22 (0.03)	0.26 (0.03)	0.08 (0.02)	0.47 (0.02)	0.62 (0.02)	0.16 (0.03)	0.17 (0.04)	0.46 (0.03)	0.04 (0.02)	0.70 (0.03)	0.65 (0.02)
NS FA	0.13 (0.06)	0.19 (0.03)	0.17 (0.03)	0.09 (0.02)	0.39 (0.02)	0.69 (0.02)	0.11 (0.02)	0.15 (0.03)	0.23 (0.05)	0.08 (0.01)	0.41 (0.02)	0.58 (0.01)	0.15 (0.03)	0.15 (0.04)	0.45 (0.06)	0.03 (0.01)	0.72 (0.05)	0.82 (0.03)
NS NFA	0.13 (0.05)	0.18 (0.02)	0.15 (0.03)	0.09 (0.03)	0.38 (0.03)	0.67 (0.02)	0.10 (0.03)	0.19 (0.02)	0.26 (0.06)	0.07 (0.01)	0.49 (0.03)	0.61 (0.02)	0.12 (0.03)	0.17 (0.05)	0.41 (0.06)	0.03 (0.01)	0.72 (0.04)	0.63 (0.02)
NS NFA + 25	0.14 (0.03)	0.21 (0.03)	0.17 (0.02)	0.12 (0.03)	0.47 (0.02)	0.73 (0.02)	0.12 (0.02)	0.19 (0.03)	0.26 (0.02)	0.09 (0.02)	0.47 (0.03)	0.61 (0.02)	0.14 (0.04)	0.16 (0.03)	0.42 (0.04)	0.05 (0.02)	0.75 (0.04)	0.65 (0.02)
NS NFA + 50	0.12 (0.05)	0.24 (0.05)	0.15 (0.04)	0.11 (0.02)	0.57 (0.03)	0.92 (0.04)	0.14 (0.03)	0.20 (0.03)	0.24 (0.03)	0.08 (0.02)	0.52 (0.03)	0.78 (0.01)	0.14 (0.04)	0.14 (0.02)	0.40 (0.04)	0.06 (0.02)	0.82 (0.05)	0.62 (0.04)
DS FA	0.09 (0.03)	0.15 (0.02)	0.08 (0.02)	0.09 (0.01)	0.30 (0.02)	0.57 (0.02)	0.08 (0.02)	0.11 (0.02)	0.11 (0.02)	0.08 (0.01)	0.21 (0.02)	0.46 (0.04)	0.12 (0.02)	0.14 (0.03)	0.32 (0.02)	0.05 (0.02)	0.36 (0.03)	0.59 (0.02)
DS NFA	0.12 (0.03)	0.13 (0.04)	0.09 (0.02)	0.08 (0.03)	0.26 (0.02)	0.71 (0.03)	0.10 (0.01)	0.12 (0.02)	0.13 (0.04)	0.09 (0.01)	0.39 (0.01)	0.71 (0.01)	0.16 (0.04)	0.12 (0.02)	0.40 (0.03)	0.04 (0.03)	0.33 (0.04)	0.68 (0.03)
DS NFA + 25	0.12 (0.03)	0.13 (0.03)	0.10 (0.01)	0.09 (0.02)	0.21 (0.03)	0.77 (0.03)	0.10 (0.02)	0.11 (0.02)	0.14 (0.02)	0.09 (0.01)	0.41 (0.03)	0.72 (0.01)	0.15 (0.03)	0.12 (0.03)	0.38 (0.04)	0.07 (0.02)	0.32 (0.04)	0.64 (0.04)
DS NFA + 50	0.12 (0.04)	0.16 (0.03)	0.08 (0.02)	0.06 (0.02)	0.22 (0.02)	0.83 (0.04)	0.11 (0.02)	0.12 (0.02)	0.13 (0.03)	0.07 (0.01)	0.41 (0.03)	0.86 (0.03)	0.15 (0.03)	0.12 (0.03)	0.43 (0.04)	0.07 (0.01)	0.33 (0.03)	0.69 (0.04)
O_3	ns	ns	ns	ns	***	***	ns	ns	ns	ns	**	***	ns	ns	ns	ns	*	***
Drought	ns	*	***	ns	***	*	ns	***	***	ns	***	**	ns	ns	ns	ns	***	**
O_3 x drought	ns	ns	ns	ns	***	ns	ns	ns	ns	ns	ns	**	ns	ns	ns	ns	ns	*

Significance of Anova was as follows: ns = not significant; * = significant ($P < 0.05$); ** = highly significant ($P < 0.01$); *** = extremely significant ($P < 0.001$); $\alpha = 0.05$.

Table II. The evolution of the elements K and Ca (± SD) (% dry weight) with ozone and water stress treatments for Norway spruce clones (n = 145).

Element	*K*					*Ca*				
Treatments	*Epidermis*	*Guard cell*	*Mesophyll*	*Endodermis*	*Vascular bundle*	*Epidermis*	*Guard cell*	*Mesophyll*	*Endodermis*	*Vascular bundle*
Gerardmer										
NS AA	0.49 ± 0.06	0.77 ± 0.06	0.85 ± 0.09	1.47 ± 0.14	0.74 ± 0.08	1.08 ± 0.10	0.15 ± 0.02	0.15 ± 0.02	0.15 ± 0.02	0.15 ± 0.03
NS FA	0.35 ± 0.03^{d}	0.63 ± 0.04^{b}	0.66 ± 0.05^{d}	1.32 ± 0.16^{c}	0.78 ± 0.07^{b}	1.05 ± 0.09^{a}	0.12 ± 0.02^{b}	0.11 ± 0.01^{c}	0.14 ± 0.02^{c}	0.12 ± 0.01^{c}
NS VFA	0.45 ± 0.04^{c}	0.81 ± 0.07^{a}	0.75 ± 0.06^{c}	1.48 ± 0.12^{b}	0.76 ± 0.08^{b}	1.02 ± 0.09^{a}	0.14 ± 0.02^{b}	0.16 ± 0.02^{b}	0.12 ± 0.02^{c}	0.16 ± 0.02^{b}
NS NFA + 25	0.51 ± 0.04^{b}	0.72 ± 0.06^{ab}	0.88 ± 0.07^{b}	2.16 ± 0.15^{a}	0.93 ± 0.08^{a}	0.87 ± 0.08^{b}	0.15 ± 0.02^{b}	0.16 ± 0.02^{b}	0.21 ± 0.03^{b}	0.19 ± 0.02^{ab}
NS + NFA + 50	0.76 ± 0.06^{a}	0.80 ± 0.07^{a}	1.11 ± 0.09^{a}	2.05 ± 0.14^{a}	0.90 ± 0.08^{a}	0.83 ± 0.08^{b}	0.20 ± 0.03^{a}	0.26 ± 0.03^{a}	0.29 ± 0.03^{a}	0.22 ± 0.03^{a}
DS FA	0.40 ± 0.04^{c}	0.56 ± 0.04^{b}	0.73 ± 0.06^{c}	0.97 ± 0.09^{c}	0.44 ± 0.04^{c}	0.56 ± 0.04^{d}	0.10 ± 0.01^{a}	0.07 ± 0.01^{c}	0.12 ± 0.02^{b}	0.14 ± 0.02^{b}
DS NFA	0.59 ± 0.05^{a}	0.68 ± 0.07^{a}	0.93 ± 0.08^{b}	2.38 ± 0.17^{a}	0.67 ± 0.09^{b}	0.79 ± 0.07^{c}	0.12 ± 0.02^{a}	0.11 ± 0.01^{b}	0.11 ± 0.01^{b}	0.16 ± 0.02^{b}
DS NFA + 25	0.49 ± 0.05^{b}	0.67 ± 0.07^{a}	1.06 ± 0.09^{a}	2.36 ± 0.16^{a}	0.82 ± 0.07^{a}	0.92 ± 0.08^{b}	0.10 ± 0.01^{a}	0.15 ± 0.02^{a}	0.15 ± 0.02^{a}	0.17 ± 0.02^{b}
DS NFA + 50	0.41 ± 0.04^{c}	0.67 ± 0.06^{a}	1.07 ± 0.09^{a}	1.57 ± 0.12^{b}	0.75 ± 0.07^{a}	1.23 ± 0.11^{a}	0.11 ± 0.01^{a}	0.16 ± 0.02^{a}	0.17 ± 0.02^{a}	0.21 ± 0.02^{a}
Istebna										
NA AA	0.68 ± 0.06	1.11 ± 0.10	0.98 ± 0.11	1.70 ± 0.20	0.90 ± 0.09	1.54 ± 0.11	0.12 ± 0.02	0.15 ± 0.02	0.18 ± 0.02	0.16 ± 0.03
NS FA	0.50 ± 0.05^{c}	0.84 ± 0.08^{b}	0.93 ± 0.09^{c}	1.60 ± 0.12^{b}	1.07 ± 0.09^{a}	1.60 ± 0.12^{a}	0.11 ± 0.01^{c}	0.11 ± 0.01^{c}	0.12 ± 0.01^{c}	0.18 ± 0.02^{b}
NS NFA	0.70 ± 0.06^{b}	1.07 ± 0.09^{a}	0.95 ± 0.10^{c}	0.67 ± 0.13^{b}	0.86 ± 0.07^{b}	1.53 ± 0.12^{a}	0.11 ± 0.01^{c}	0.15 ± 0.02^{ab}	0.17 ± 0.02^{b}	0.17 ± 0.02^{b}
NS NFA + 25	0.84 ± 0.07^{a}	1.08 ± 0.09^{a}	1.08 ± 0.10^{b}	1.73 ± 0.13^{b}	0.81 ± 0.07^{b}	1.31 ± 0.11^{b}	0.17 ± 0.02^{b}	0.14 ± 0.01^{b}	0.16 ± 0.02^{b}	0.17 ± 0.02^{b}
NS NFA + 50	0.86 ± 0.07^{a}	1.15 ± 0.10^{a}	1.26 ± 0.11^{a}	2.09 ± 0.11^{a}	0.78 ± 0.07^{b}	1.28 ± 0.07^{b}	1.28 ± 0.03^{a}	0.17 ± 0.02^{a}	0.24 ± 0.02^{a}	0.21 ± 0.02^{a}
DS FA	0.48 ± 0.03^{c}	0.71 ± 0.06^{c}	1.20 ± 0.10^{a}	1.35 ± 0.11^{c}	0.78 ± 0.07^{b}	0.72 ± 0.06^{d}	0.11 ± 0.01^{b}	0.14 ± 0.01^{b}	0.12 ± 0.01^{b}	0.17 ± 0.02^{a}
DS NFA	0.68 ± 0.06^{b}	0.96 ± 0.09^{a}	1.15 ± 0.11^{a}	1.54 ± 0.12^{b}	0.89 ± 0.08^{a}	1.28 ± 0.11^{a}	0.10 ± 0.01^{b}	0.16 ± 0.02^{a}	0.17 ± 0.02^{a}	0.14 ± 0.02^{a}
DS NFA + 25	0.83 ± 0.05^{a}	0.99 ± 0.09^{a}	1.10 ± 0.10^{ab}	2.13 ± 0.15^{a}	0.97 ± 0.09^{a}	1.17 ± 0.11^{b}	0.11 ± 0.01^{b}	0.14 ± 0.01^{b}	0.19 ± 0.03^{a}	0.14 ± 0.01^{a}
DS NFA + 50	0.89 ± 0.08^{a}	0.81 ± 0.08^{b}	1.02 ± 0.09^{b}	2.20 ± 0.15^{a}	0.82 ± 0.08^{b}	1.04 ± 0.06^{c}	0.15 ± 0.02^{a}	0.16 ± 0.02^{a}	0.16 ± 0.01^{a}	0.14 ± 0.02^{a}

Test of equality of averages of Student-Newman and Keuls has been applied: same letters indicate averages not significantly different and alphabetic order corresponds to decreasing values. α = 0.05.

Table III. The evolution of the elements K and Ca (± SD) (% dry weight) with ozone and water stress treatments for beech leaves ($n = 72$).

Element	*K*						*Ca*					
Treatments	*Lower epidermis*	*Upper epidermis*	*Guard cell*	*Palisade parenchyma*	*Spongy parenchyma*	*Vascular bundle*	*Lower epidermis*	*Upper epidermis*	*Guard cell*	*Palisade parenchyma*	*Spongy parenchyma*	*Vascular bundle*
NS AA	1.11 ± 0.12^{a}	0.86 ± 0.09	0.83 ± 0.09	0.58 ± 0.06	0.74 ± 0.06	0.60 ± 0.05	0.83 ± 0.07	0.69 ± 0.06	0.87 ± 0.08	0.48 ± 0.04	0.56 ± 0.05	0.40 ± 0.04
NS NFA	1.07 ± 0.11^{a}	0.83 ± 0.07^{b}	0.81 ± 0.07^{b}	0.55 ± 0.05^{b}	0.89 ± 0.08^{a}	0.63 ± 0.05^{a}	0.72 ± 0.06^{b}	0.61 ± 0.05^{b}	0.89 ± 0.07^{a}	0.53 ± 0.04^{b}	0.57 ± 0.05^{b}	0.35 ± 0.04^{a}
NS NFA + 25	1.06 ± 0.10^{a}	0.88 ± 0.07^{b}	0.82 ± 0.07^{b}	0.63 ± 0.05^{b}	0.72 ± 0.06^{b}	0.58 ± 0.06^{b}	0.79 ± 0.06^{a}	0.64 ± 0.05^{b}	0.86 ± 0.07^{a}	0.60 ± 0.05^{a}	0.73 ± 0.06^{a}	0.34 ± 0.03^{a}
NS NFA + 50	1.08 ± 0.11^{a}	0.76 ± 0.06^{c}	0.85 ± 0.07^{b}	0.55 ± 0.05^{b}	0.71 ± 0.06^{b}	0.59 ± 0.05^{b}	0.81 ± 0.07^{a}	1.29 ± 0.10^{a}	0.93 ± 0.08^{a}	0.63 ± 0.06^{a}	0.77 ± 0.07^{a}	0.35 ± 0.03^{a}
DS FA	0.99 ± 0.09^{a}	1.05 ± 0.09^{a}	0.88 ± 0.07^{a}	0.60 ± 0.05^{a}	0.59 ± 0.05^{a}	0.34 ± 0.03^{b}	0.42 ± 0.04^{a}	0.41 ± 0.04^{a}	0.50 ± 0.04^{b}	0.30 ± 0.02^{b}	0.30 ± 0.03^{b}	0.21 ± 0.02^{b}
DS NFA	0.73 ± 0.07^{b}	0.91 ± 0.08^{b}	0.90 ± 0.08^{a}	0.62 ± 0.05^{a}	0.53 ± 0.05^{a}	0.55 ± 0.04^{a}	0.45 ± 0.04^{a}	0.43 ± 0.04^{a}	0.58 ± 0.04^{a}	0.36 ± 0.04^{a}	0.43 ± 0.04^{a}	0.25 ± 0.03^{a}
DS NFA + 25	0.67 ± 0.05^{b}	0.73 ± 0.06^{c}	0.88 ± 0.07^{a}	0.65 ± 0.05^{a}	0.54 ± 0.05^{a}	0.53 ± 0.04^{a}	0.45 ± 0.04^{a}	0.46 ± 0.04^{a}	0.57 ± 0.05^{a}	$0,36 \pm 0.03^{a}$	0.43 ± 0.04^{a}	0.25 ± 0.22^{a}
DS NFA + 50	0.69 ± 0.05^{b}	0.73 ± 0.06^{c}	0.87 ± 0.06^{a}	0.61 ± 0.06^{a}	0.58 ± 0.05^{a}	0.53 ± 0.04^{a}	0.42 ± 0.03^{ab}	0.41 ± 0.03^{a}	0.58 ± 0.05^{a}	0.32 ± 0.03^{ab}	0.43 ± 0.04^{a}	0.24 ± 0.02^{a}

Test of equality of averages of Student-Newman and Keuls has been applied: same letters indicate averages not significantly different and alphabetic order corresponds to decreasing values. $\alpha = 0.05$.

In the mesophyll, however, Istebna had increased levels, whereas Gerardmer's calcium levels were reduced. Calcium decreased in all tissues of beech in response to drought.

Ozone–drought interactions

The combined effects of the 2 stresses are quite complicated. Ozone alone provoked an increase in potassium in the epidermis of the Gerardmer clone, but in conjunction with drought there is no longer such an increase in NFA + 50. This is also seen in the endodermis where the ozone-associated increase is restricted to the NFA and NFA + 25 treatments. Potassium levels in Istebna decrease in the mesophyll of NFA + 50, but increase in the endodermis of NFA + 25 and in the vascular bundle of all 3 ozone–drought treatments.

Calcium, which had previously shown a decrease in the epidermis in response to ozone, was increased when the 2 stresses were applied in conjunction in Gerardmer. Istebna, on the other hand, had a similar trend between ozone treatments, but the decrease in FA was less severe. The other tissues also showed less pronounced changes when ozone was applied in conjunction with a drought stress, but the direction of the effects was nevertheless the same, that is, increasing ozone concentration was associated with increasing calcium.

In beech, the previous ozone-associated potassium decrease in the lower epidermis was no longer apparent in the drought stressed trees. The guard cells had increased K for all 3 ozone treatments, but ozone effects were no longer apparent in the parenchyma and even reversed in the vascular bundle. The effects of ozone on calcium were no longer apparent in the epidermis and were diminished in the other tissues apart from the vascular bundle where there is now a decrease.

DISCUSSION

The soil analysis showed that there was a good nutrient supply at the site, with no deficiencies according to the recommended levels of Bonneau (1988). Although not deficient, magnesium was rather low in the 20–30 cm horizon.

Changes in guard cell K concentrations are important in regulating stomatal aperture (Le Thiec *et al*, 1994), which in turn can limit ozone uptake. This experiment has shown increased K concentrations in spruce needles exposed to the ozone treatments, compared to FA needles. Associated with this K increase is an increased stomatal opening. In beech, the reverse pattern was observed, so a stomatal closing in association with ozone would be expected in beech. These expectations were confirmed from stomatal conductance measurements performed on the trees on measurement days. Freer-Smith (1993) reports that other work on beech showed a similar reduction in stomatal conductance, as did Taylor and Dobson (1989) on beech exposed to ambient ozone levels.

The drought stress provoked significant decreases in guard cell K levels of Norway spruce in all treatments (this decrease was associated with a reduced stomatal conductance). In beech, however, there was no intertreatment difference in K levels and the levels even showed a relative increase compared to the well-watered treatments. These results can be explained by reference to the diurnal time course of conductance measurements. It appears that drought stressed ozone-treated plants close their stomata earlier in the day and thus receive a lower dose over a period of time as ozone levels are greater in the afternoon. In this way the trees are relatively protected from ozone damage and photosynthesis more than the well-watered trees in the morning, when vapour pressure deficits are

lower. Thus, because sampling occurred in the morning, a relatively greater stomatal conductance is found in drought stressed beech trees.

The foliar nutrient content is often used to determine the nutritional status of plants and/or to assess if latent damage has already occurred (Cape *et al,* 1990). In general, the same absolute values were not found when these analyses were compared to the microanalysis results, because the global chemical analysis encompasses several regions of the foliage (cuticle, cell wall, cellular contents, hypodermis and the resin canals), whereas the X-ray microanalysis only includes the cellular contents (vacuole and cytoplasm) and a portion of the cell wall.

There are several possibilities that could give rise to a change in foliar nutrient levels. These include soil nutrient availability, root development, foliar leaching, or redistribution of nutrients to other parts of the plant. The good nutritional quality of the soil allows us to discount the 1st of these hypotheses. Several other studies, however (reviewed by Kasana and Mansfield, 1986), have shown that ozone can affect the distribution of dry matter between the shoots and roots, leading to a relative reduction in root growth.

The potassium and calcium contents were increased in the 2 spruce clones and these changes could well lead to changes in the cell membrane permeability (Heath and Castillo, 1988; Chevone *et al,* 1990; Fink, 1991b). It seems that the intercellular derivatives of ozone are capable of inhibiting the function of membrane pumps and membrane transport mechanisms. Ca^{2+} ions, which are usually expelled from the cell by an ATP-dependent membrane transport mechanism, are now accumulated inside the cell. This can in turn interfere with the functioning of the numerous enzymes which use this ion as an activator or inhibitor (Heath and Castillo, 1988).

This potassium and calcium increase could also result from a perturbation of the mineral nutrition at root level. Barnes and Pfirrmann (1992) and Lucas *et al* (1993) explained the increase of K and Ca levels in ozone-treated trees by an increasing ion uptake at root level. Furthermore, the increase in K, which is a mobile cation and which is used as a regulator of numerous physiological processes, could be the result of an increased demand from the tree which is using it to counteract oxidative stress. An increasing metabolism needed to repair ozone damage could also cause increasing K concentrations (Schier, 1990) and Cape *et al* (1990) found that increasing K was due to a mechanism to maintain cationic balance.

In contrast to Norway spruce, beech showed decreasing K concentrations as ozone exposure increased and Ca levels were only slightly increased in NFA + 50. This could result from more K being leached by acid rain; ozone is thought to predispose foliage to such an effect (McLaughlin, 1985; Westmann and Temple, 1989; Pfirrmann *et al,* 1990). It could also just result from an ozone-associated efflux of K. Murphy and Huerta (1990) found an increase in H_2O_2 production in UV-treated plants which led to a K efflux. This could be significant as H_2O_2 is a product of ozone reactions which occur inside the plant (Runeckles and Chevone, 1992). According to Barnes *et al* (1990) and Taylor *et al* (1989), ozone can decrease beech root biomass and thus nutrient uptake, and this could also produce such a result.

As previously discussed, ozone is capable of altering cell membrane permeability and this can cause perturbations in nutrient compartimentalization (Heath and Castillo, 1988). These become apparent from the microanalysis results. Fink (1991b) concluded that there was an increase in Ca influx into the cytoplasm of mesophyll cells where normally conifers are able to export this ion out of the cell. Because, in Norway

spruce, the increase in potassium was found in all tissues and ozone attacks the mesophyll first, it would seem that this increase is due to changes in nutritional supply at the root level rather than a modification of the membrane permeability of all tissues. Furthermore, the new growth has been subjected to ozone only recently, whereas the root system has developed in an ozone environment over several growing seasons giving more time for ozone to exert its effects. In beech, the situation is different. There is perhaps a modification comparable to spruce, but as there is no apparent difference in Ca levels in the vascular bundle it seems that the effects of membrane changes are more important.

Drought acted to decrease K and Ca concentrations in the most internal tissues. The vascular bundle, by way of the xylem, supplies nutrients in solution to the various tissues and this is regulated by the endodermis (Stelzer, 1990). The drought has, by reducing water supply, also reduced nutrient supply to the plant. There was a difference between clones as regards the response of the mesophyll as only Istebna had an increasing Ca concentration. Fink (1991b) proposed that the ion cannot be evacuated from the cell because of pollution damage, but its accumulation could also be a mechanism (linked to ABA production) to protect against the effects of drought.

The drought–ozone interaction is difficult to interpret as the results are not a simple summation of the 2 stresses in isolation. In Gerardner, the effects of this interaction are essentially in the epidermis of NFA + 50 and in the endodermis. The main changes in Istebna occurred in the endodermis, mesophyll and vascular bundle and in beech, they were in the parenchyma and vascular bundle and the epidermis for Ca only. All these interactions were essentially in the NFA + 50 treatment.

To conclude, the effects of ozone and drought are antagonistic and show inter-specific differences. Both stresses were seen to effect nutrition supply from the roots, especially in Norway spruce. Membrane permeability was probably affected which led to different nutritional levels between different tissues – an effect that total foliar nutritional analyses cannot reveal.

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