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Response of Poplar to Ozone Alone and in Combination with NO_2 at Different Nitrogen Fertilization Levels

By

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With 7 Figures

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Summary

SCHMUTZ P., BUCHER J. B., GÜNTHARDT-GOERG M. S., TARJAN D. & LANDOLT W. 1995. Response of poplar to ozone alone and in combination with NO_2 at different nitrogen fertilization levels. – Phyton (Horn, Austria) 35 (2): 269–289, 7 figures. – English with German summary.

Cuttings of hybrid poplar (*Populus* × *euramericana* var. "Dorskamp") were exposed to ozone alone (mean concentration 60 nl l^{-1}) and in combination with NO_2 (mean concentration 100 nl l^{-1}) during 16 weeks. Three different levels of nitrogen fertilization were applied, the lowest causing growth limitation. Ozone fumigation induced premature leaf abscision, regardless of the fertilization level. Whole-plant biomass production was reduced as compared to control trees grown in filtered air. In trees under nitrogen limitation, biomass reduction by ozone occurred in the roots rather than in the above-ground woody parts. The roots, however, maintained their uptake capacity for nitrogen, leading to similar whole-plant nitrogen content in fumigated and control trees. Trees under nitrogen limitation responded to ozone with reallocation of nitrogen from the roots to the developing foliage, resulting in higher nitrogen concentrations in young leaves. As a consequence, these leaves had a higher chlorophyll concentration, elevated CO_2 assimilation rate and stomatal conductance, and a greater leaf area. In contrast, ozone-damaged older leaves exhibited chlorophyll degradation, lower CO_2 assimilation rates and a poorer water use efficiency.

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Despite nitrogen limitation, this element was not mobilized from the leaves prior to abscision, and therefore was lost for the plants. High nitrogen supply from the fertilizer suppressed nitrogen reallocation within the plants. The presence of atmospheric NO_2 did not significantly affect the plant response to ozone. The induction of foliar nitrate reductase, which is a key enzyme in nitrogen assimilation, suggested that NO_2 was used as an additional nitrogen source. Nitrogen distribution within the poplar trees is discussed in the context of acclimation strategies to ozone stress.

Zusammenfassung

SCHMUTZ P., BUCHER J. B., GÜNTHARDT-GOERG M. S., TARJAN D. & LANDOLT W. 1995. Reaktion von Pappeln auf Ozon oder Ozon kombiniert mit NO_2 bei verschiedener Stickstoffdüngung. – Phyton (Horn, Austria) 35 (2): 269–289, 7 Abbildungen. – Englisch mit deutscher Zusammenfassung.

Stecklinge einer Pappel-Hybride (*Populus* × *euramericana* var. „Dorskamp") wurden während 16 Wochen gefilterter Luft ausgesetzt, oder gefilterter Luft mit Ozon (mittlere Konzentration 60 nl l^{-1}) oder einem Gemisch von Ozon und NO_2 (mittlere Konzentrationen 60 und 100 nl l^{-1}). Es wurden drei verschieden hohe Stickstoffdüngungen verabreicht; die tiefste erzeugte bei den Pflanzen Mangelsymptome. Bei allen Düngevarianten bewirkte Ozon vorzeitigen Blattwurf. Die Biomasse war reduziert in Vergleich zu den Bäumen in filtrierter Luft (Kontrollen). Diese Biomassereduktion fand unter Stickstofflimitierung in den Wurzeln statt, und nicht in den verholzten oberirdischen Teilen. Dennoch blieb die Aufnahmefähigkeit der Wurzeln für Stickstoff erhalten, was zu vergleichbaren totalen Stickstoffgehalten bei ozonierten und Kontrollbäumen führte. Ozon bewirkte bei stickstofflimitierten Bäumen eine Umverteilung des Stickstoffs von den Wurzeln zu der sich entwickelnden Belaubung, was höhere Stickstoffkonzentrationen in jungen Blättern zur Folge hatte. In diesen Blättern erhöhte sich die Chlorophyllkonzentration, die CO_2-Assimilationsrate, die stomatäre Leitfähigkeit und die Blattfläche. Ozongeschädigte ältere Blätter zeigten hingegen Chlorophyllabbau, reduzierte CO_2-Assimilationsraten und eine schlechtere ‚water use efficiency'. Der Stickstoff dieser Blätter wurde auch unter Stickstofflimitierung vor dem Blattwurf nicht zurückgezogen und ging der Pflanze dadurch verloren. Hohe Stickstoffgabe durch die Düngung unterdrückte die Umverteilung des Stickstoffs in den Pflanzen. Die Gegenwart von atmosphärischem NO_2 hatte keinen signifikanten Einfluß auf die Reaktion der Pflanzen gegenüber Ozon. Die Induktion der Nitratreductase in den Blättern, einem Schlüsselenzym der Stickstoffassimilation, deutet jedoch darauf hin, daß NO_2 als zusätzliche Stickstoffquelle diente. Die Stickstoffverteilung in den Pappeln wird im Zusammenhang mit Anpassungsstrategien unter Ozonstreß diskutiert.

Introduction

Ozone is a widespread tropospheric air pollutant and can reach peak levels of up to 50–100 nl/l in Switzerland (BUWAL 1993). Fumigation of tree species with mean ozone concentrations between 50 and 100 nl/l produced visual symptoms, structural and biochemical changes (LANDOLT & al. 1994) and changes in biomass production and partitioning (MATYSSEK & al. 1993). However, tree response to ozone may be modified by environmental factors, such as the nutrient status. Experiments with herbaceous

species indicated higher ozone sensitivity at high nitrogen fertilization (PELL & al. 1990), but *Populus tremuloides* showed no clear dependence of ozone-induced growth reductions on the nitrogen fertilization regime (KARNOSKY & WITTER 1992).

The present study therefore was designed to analyze the response of young poplar trees to ozone at the leaf and whole-plant level. Three different levels of nitrogen fertilizer (as urea) in the soil were applied as a modifying factor. Since NO_2 has been shown to act as a nitrogen fertilizer for poplar in an earlier study (SCHMUTZ & al. 1995), NO_2 was added as an additional nitrogen source in one experiment.

Abbreviations:

LN: low nitrogen fertilization
MN: mid nitrogen fertilization
HN: high nitrogen fertilization

Materials and Methods

Potted hybrid poplars (*Populus* × *euramericana* var. 'Dorskamp') were grown in two climate chambers during 16 weeks at three different levels of nitrogen fertilization ('low', 'mid' and 'high' N, referred to as LN, MN and HN; Table 1) as described in SCHMUTZ & al. 1995. Filtered air (charcoal and Purafil) was supplied to both chambers, in one chamber with added ozone or ozone and nitrogen dioxide.

Table 1
Initial contents of macro nutrients in the pots (mg l^{-1} sand mixture).

N regime	P	K	S	Mg	Ca	Fe	NO_3^--N	NH_4^+-N	total N
LN (low N)	98	221	119	37	147	1	55	50	105
MN (mid N)	98	221	119	37	147	1	55	260	315
HN (high N)	98	221	119	37	147	1	55	575	630

Ozone was produced from pure oxygen by electrical discharge with an 'Ozone Generator 500 M' (Fischer, Germany). The concentrations of ozone were monitored with an 'Ozone Analyzer Model 8810' (Monitor Labs Inc., USA), and adjusted by a mass flow controller according to the setpoints of a computer program. Ozone concentrations in the fumigation chamber followed a sinusoidal diurnal course qualitatively similar to that in the air of rural surroundings of Zurich, Switzerland (LANDOLT & al. 1987) and reached a maximum value of 100 nl l^{-1} at 3:00 p.m. (Fig. 1). The mean ozone concentration of 60 nl l^{-1} corresponded to the air quality standard for one-hour means of the Swiss Ordinance on Air Pollution Control, which is often exceeded during summer months (BUWAL 1993). The diurnal courses of photosynthetic photon flux density, air temperature, air humidity and NO_2 concentrations were set as in SCHMUTZ & al. 1995. The ozone exposure experiment was run twice (experiment 1: 10 LN, MN and HN trees each; experiment 2: 15 LN and MN trees each per chamber). In experiment 3 (10 LN and MN trees each), ozone and NO_2 were applied simultaneously.

All analyses and measurements were performed on five trees per treatment (i.e. five replicates).

The activity of nitrate reductase (NR, E.C. 1.6.6.1 and E.C. 1.6.6.2) was determined in 2-week old leaves (one leaf per tree) as described in SCHMUTZ & al. 1995 and was based on leaf area. The measurements were performed at week 10 of experiment 2 and week 12 of experiment 3.

Gas exchange measurements were performed with a CO_2/H_2O porometer ('Kompakt', Walz, FRG). The gas analyzer used was a LI-6251 (LI-COR, USA). Photosynthetic active radiation was approx. 900 µmol photons m^{-2} s^{-1}; leaf temperature 21°C; relative humidity 65 to 75%; and CO_2 concentration 350 µmol mol^{-1}. Gas exchange of the adaxial leaf side was measured in 6 to 8 leaves per tree (leaves of same age but from different trees were treated as replicates) along the main stem leaf profile during the weeks 9, 12 and 15, referred to as 'first', 'second' and 'third' measurement period. From the gas exchange measurements the net CO_2 assimilation rate at ambient CO_2 concentration (Aamb), the stomatal conductance (g_{H_2O}) and the ratio of the transpiration rate to Aamb (E/A) were calculated, based on leaf area. The results are presented for experiment 2 only. A preliminary measurement program was performed during week 15 of experiment 1 (data not shown), the results of which were confirmed by those of experiment 2.

In week 16 of experiment 2, the concentrations of chlorophyll a and b were determined according to LICHTENTHALER & WELLBURN 1983 along a main stem profile.

After 12 weeks of fumigation, total foliage area, biomass and nutrient concentrations were determined as in SCHMUTZ & al. 1995 on a different set of five trees per treatment. The section of the main shoot which had developed posterior to week 7 was treated separately; stem and foliage of this section are referred to as 'top' stem and foliage. Stem and leaves of the main shoot formed during week 1 to 7 are referred to as 'lower' stem and leaves. In the calculated root/shoot biomass ratio the stem section of the initially planted cuttings was excluded (cf. MATYSSEK & al. 1993). In experiment 2, the roots were partitioned into coarse (> 2 mm), medium (1–2 mm) and fine (< 1 mm) root diameter classes.

The data were statistically analyzed by the nonparametric Kruskal-Wallis test and, for some parameters, by multifactor ANOVA, (STATGRAPHICS 1991) with fumigation and soil N fertilization as factors. The significance level (p) of the factorial effects are presented as $p < 0.01$ (a) and < 0.05 (b).

Results

Visual symptoms and foliage area

All trees exposed to ozone exhibited premature leaf abscision. Prior to abscision, visual symptoms such as chlorosis and necrotic spots appeared on the leaves. The degree of chlorosis varied between individual trees; occasionally leaves turned completely yellow. The time span between the emergence of the leaves and abscision due to ozone exposure was similar for all soil N treatments. The average life span of leaves produced on the main stem during week 3, 5 and 7 after planting was 34, 40 and 46 days, respectively. No leaf abscision occurred on control trees.

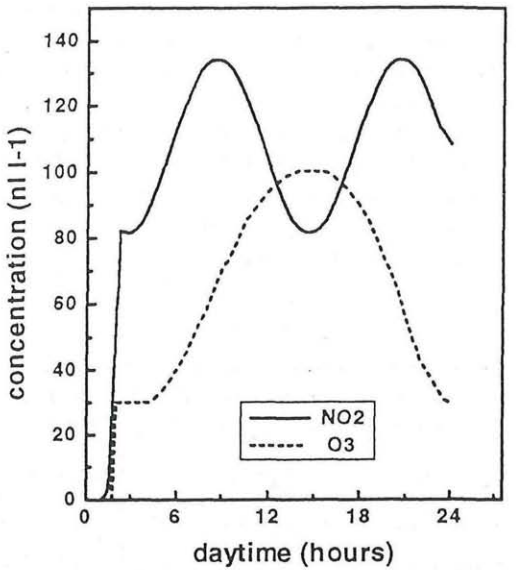

Fig. 1: Daily course of ozone and NO_2 concentrations during the fumigation experiments. Mean concentrations: 60 nl l^{-1} ozone and 100 nl l^{-1} NO_2.

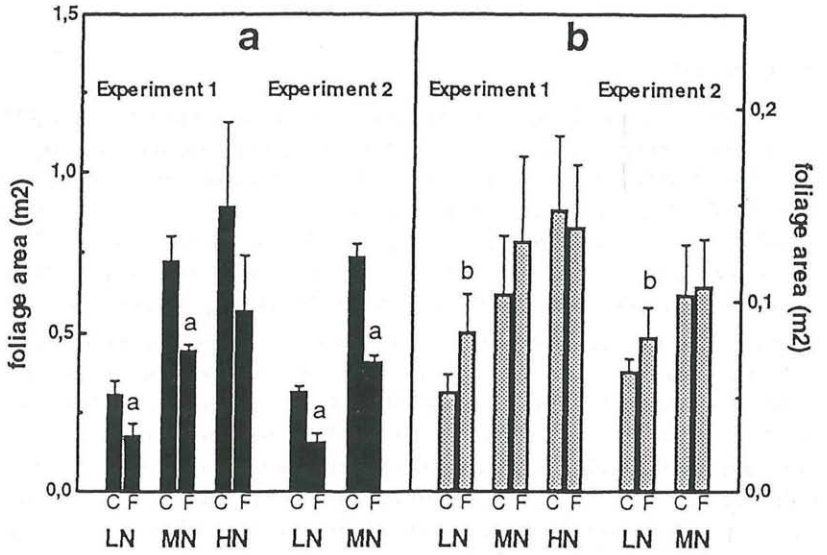

Fig. 2: Foliage area of a) foliage of the whole tree and b) foliage younger than four weeks developed on the main stem. C = control; F = fumigated with ozone. Mean values (columns) and standard deviations (error bars; n = 5). Statistical significance of fumigation effect (Kruskal-Wallis) $p < 0.01$ = a; $p < 0.05$ = b.

After 12 weeks of fumigation and considerable leaf loss, the total surface area of the remaining leaves was significantly reduced compared to control trees (Fig. 2a). Total area of the 'top leaves' (i.e. the leaves developed on the main stem after week 7), however, increased under ozone fumigation relative to control trees in the LN treatment (Fig. 2b). No significant ozone effect was found at higher fertilization levels. The area increase of the 'top leaves' under ozone fumigation was due to an increase of individual leaf area rather than to an increase of the rate of leaf production, which was not significantly altered (Table 2).

Table 2

Number and mean area of 'top' leaves, i.e. leaves produced posterior to seven weeks of ozone exposure. Significant differences between C and O_3 (Kruskal-Wallis) are labelled as a ($p < 0.01$) and b ($p < 0.05$).

Experiment	Parameter	LN C	LN O_3	MN C	MN O_3	HN C	HN O_3
1	mean leaf area (cm^2)	48	64 a	74	82	98	92
	number of leaves	11	13	14	16	15	15
2	mean leaf area (cm^2)	49	62 a	69	72	–	–
	number of leaves	13	13	15	15	–	–

CO_2 assimilation

In control trees, the CO_2 assimilation rate at ambient CO_2 concentration (Aamb) increased slightly with increasing leaf age (i.e. towards the base along the stem; Fig. 3a and 3b). In the first measuring period, leaves of the MN trees displayed considerably elevated Aamb values compared to LN trees grown in filtered air (Fig. 3a). The difference was smaller in the second measuring period (data not shown), and in the third there was almost no difference between the two N fertilization treatments (Fig. 3b).

In the first measuring period, ozone fumigation had no significant effect on leaves less than 4 weeks old. Starting at a leaf age of 4 weeks, Aamb was reduced, to a value of 20% of corresponding control leaves shortly prior to abscision (Fig. 3a). The decline in Aamb was paralleled by visual symptoms and independent of N fertilization treatment. Aamb was significantly increased in the second measuring period in 2.5- and 3-week old leaves of the ozone treatment compared to the control (data not shown).

The profile measurement in the third measuring period revealed an ozone-induced increase of Aamb by a factor of almost 2 in young leaves relative to the control treatment (Fig. 3b). Old leaves with visual symptoms had lower Aamb values than the controls; a balance between ozone-in-

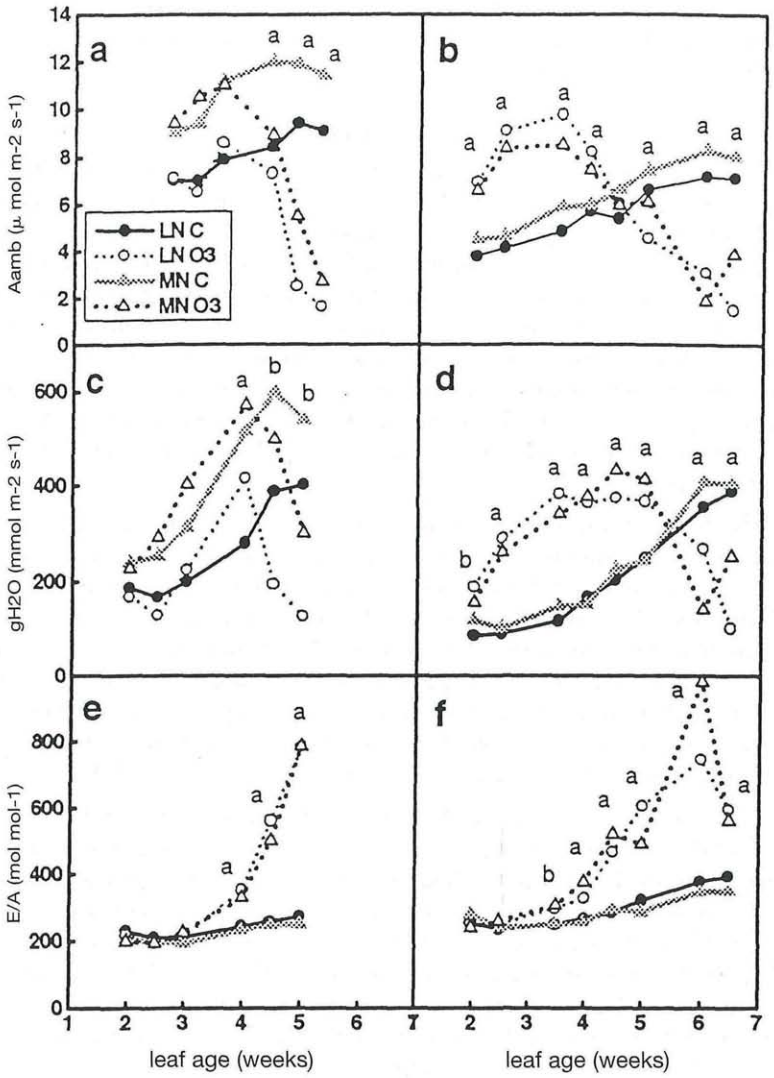

Fig. 3: a) and b) net CO_2 assimilation rate at ambient CO_2 concentrations (Aamb); c) and d) stomatal conductance (g_{H_2O}); and e) and f) transpiration rate by Aamb (E/A); of the leaves along the main stem profile, determined after 9 (a, c, e) and 15 (b, d, f) weeks of exposure. Mean values (5 replicates) for LN control (LN C), LN ozone (LN O_3) MN control (MN C) and MN ozone (MN O_3). Statistical significance of fumigation effect (multifactor ANOVA) $p < 0.01$ = a; $p < 0.05$ = b. Significant fertilization effects were found for Aamb and g_{H_2O} after 9 weeks of exposure (p values not shown). Data from experiment 2.

duced elevated Aamb and decreased Aamb due to leaf damage was established in 4.5-week old leaves. Both N fertilization treatments responded similarly to ozone exposure.

Stomatal conductance and water use efficiency

Stomatal conductance (g_{H_2O}) in green leaves increased considerably with increasing leaf age. In the first measuring period it was significantly higher in the MN than in the LN treatment (Fig. 3c). This difference had disappeared in the later measurements. (Fig. 3d). Higher stomatal conductance in fumigated leaves as compared to the corresponding control leaves was suggested in the early measurements and became significant in the third measuring period (Fig. 3c and 3d). In some leaf age classes the g_{H_2O} values had almost doubled under ozone exposure. In the oldest leaves, however, g_{H_2O} was reduced relative to the control (Fig. 3c and 3d). These leaves showed advanced injury and were abscised a few days after the measurement.

The E/A ratio indicates the number of water molecules lost by transpiration per assimilated CO_2 molecule and is the reciprocal value of the water use efficiency (WUE). In control leaves this ratio was approx. 200 mol/mol and showed little variation due to N nutrition, measuring date and the leaf position in the stem profile (Fig. 3e and 3f). Young leaves of ozone-treated trees had E/A values similar to those of the corresponding controls, but in older leaves the values were sharply elevated. The effect increased with the ozone dose (concentration × time) the leaves had received prior to the gas exchange measurements (Fig. 3e and 3f).

Since the leaves selected along the main stem profile had been produced at different phases of plant development, a true time course was determined for evaluation of the leaf age effect: Gas exchange was measured weekly from week 6 through week 9 in the leaves which had developed in week 4. The results were similar to those of the first profile measurement (Table 3): Relative to the controls, Aamb declined in fumigated leaves with increasing leaf age. Initially, the E/A ratio was approx. 200 mol/mol for control and fumigated leaves; in the latter, E/A gradually increased with ozone exposure time, up to approx. 800 mol/mol.

Chlorophyll concentrations

N fertilization (i.e. MN vs. LN) increased total chlorophyll (chlorophyll a and b) concentrations. In fumigated trees 2- to 4-weeks old leaves had elevated total chlorophyll concentrations relative to the controls (Fig. 4). For statistical analysis, the Kruskal-Wallis test was applied on the pooled data of the leaves with ages up to 5 weeks (the LN and MN treatments were analyzed separately). The ozone effect was significant for both N levels ($p < 0.001$).

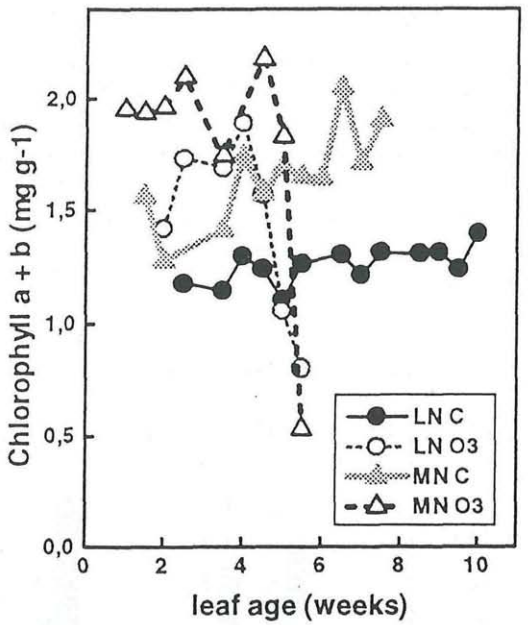

Fig. 4: Total chlorophyll concentrations (i.e. chlorophyll a and b) of the leaves along the main stem profile, determined after 16 weeks of exposure. Mean values (5 replicates) for LN control (LN C), LN ozone (LN O_3), MN control (MN C) and MN ozone (MN O_3). For statistical significance of fumigation effect refer to text. Data for experiment 2.

Table 3

Gas exchange parameters of experiment 2 measured on leaves developed during week 4; Aamb = net CO_2 assimilation rate at ambient CO_2 concentration (µmol m^{-2} s^{-1}) E/A = transpiration rate / Aamb (mol mol^{-1}). Mean values for five replicates (* = only 2 replicates left, due to leaf abscision). Significant differences between C and O_3 (Kruskal-Wallis) are labelled as a ($p < 0.01$) and b ($p < 0.05$).

Parameter	Weeks after leaf development	LN C	LN O_3	MN C	MN O_3
Aamb	2	11.2	12.3	16.1	13.5 a
	3	13.2	9.4 a	17.3	11.2 a
	4	9.4	4.2 a	12.1	7.7 b
	5	9.1		11.4	2.7 *
E/A	2	202	227	168	199
	3	217	331 a	190	282 a
	4	268	518 a	236	399 a
	5	272		249	785 *

Whole-tree biomass production

Total biomass production ('total biomass') of control trees was dependent on the N fertilization treatment (Fig. 5). MN trees produced almost twice as much biomass as LN trees. No significant fertilization effect was found between MN and HN trees in experiment 1. Ozone fumigation significantly reduced total biomass (excluding abscised leaves) in the LN and MN treatment (Fig. 5). In the HN treatment, the reduction was not significant due to high variation between replicates. The reductions ranged between 29% and 38%.

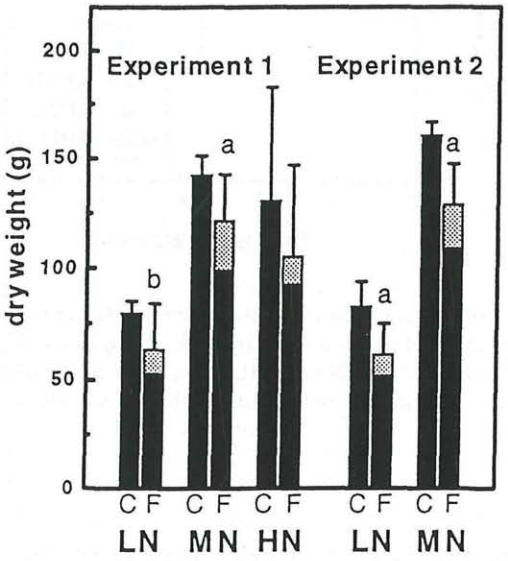

Fig. 5: Whole-plant biomass after 12 weeks of exposure (*black*) and total biomass of shed leaves (*gray*) . C = control, F = fumigated with ozone. Mean values (columns) and error bars (n = 5). Statistical significance of fumigation effect (Kruskal-Wallis) $p < 0.01$ = a; $p < 0.05$ = b (indicated values for biomass without shed leaves).

The biomass present at the harvesting date was the balance of biomass production and biomass loss due to leaf abscision. By addition of the biomass of the abscised leaves (which were collected throughout the experiments), the effective total biomass production ('total biomass 2') was calculated. In experiment 2, this value was significantly reduced by ozone exposure (Kruskal-Wallis: $p < 0.05$ for both fertilization treatments). In experiment 1 the reduction was similar (approx. 20%), but not significant due to large variations within the treatments (Fig. 5).

Biomass partitioning between plant organs

Ozone fumigation reduced the biomass of roots, cuttings (which exhibited secondary growth during the experiments) and foliage; multifactor ANOVA tests gave p values <0.01 for the fumigation effect for all three parameters in both experiments. Application of the Kruskal-Wallis test on the individual fertilization groups showed significant ozone effects for the majority of them (Table 4). However, there was no ozone effect on the biomass of the woody above-ground parts ('wood'), i.e. main stem plus lateral branches (Table 4).

Table 4

Mean biomass values for experiment 1 and 2 (ozone fumigation). Significant differences between C and O_3 (Kruskal-Wallis) are labelled as a ($p < 0.01$) and b ($p < 0.05$).

Exp.	Biomass (g)	LN C	LN O_3	MN C	MN O_3	HN C	HN O_3
1	'top' foliage	5.0	7.8 b	11.1	13.5	13.0	12.9
	total foliage	34.2	17.3 a	70.9	39.2 a	68.6	44.8
	total foliage 2	34.2	29.0	70.9	62.5	68.6	58.1
	wood	13.3	13.9	29.1	28.4	29.0	24.5
	cutting	8.9	6.4	9.9	8.1 b	8.4	7.0
	roots	23.4	14.9 b	32.5	22.8 b	24.5	16.0
	root/shoot ratio	0.49	0.46	0.33	0.34	0.24	0.23
	root/shoot 2 ratio	0.49	0.34 a	0.33	0.25 a	0.24	0.19
	total biomass/foliage area (mg/cm^2)	26	29	20	22	14	16
2	'top' foliage	6.7	8.0	13.3	11.9		
	total foliage	36.1	16.3 a	78.6	40.3 a		
	total foliage 2	36.1	26.6 b	78.6	61.8 a		
	wood	15.2	13.4	34.5	29.7		
	cutting	9.0	6.5 b	12.1	9.7 a		
	coarse roots, % of total	37	29	32	33		
	medium roots, % of total	25	28 b	22	25		
	fine roots, % of total	38	43	46	43		
	total roots	22.4	15.2 b	35.4	29.0		
	root/shoot ratio	0.44	0.51	0.31	0.42 b		
	root/shoot 2 ratio	0.44	0.38	0.31	0.32		
	total biomass/foliage area (mg/cm^2)	26	33 b	22	27 b		

No significant difference in biomass distribution between the coarse, medium and fine root fractions was found in fumigated versus control trees (with the exception of the medium root fraction of LN trees; Table 4).

The formation of lateral branches was strongly dependent on N fertilization. The ratio of branch to main stem woody biomass was approx. 0.09, 0.45 and 0.67 for LN, MN and HN control trees, respectively (experiment

1), with a high variation. Similar values were found for experiment 2 and in the ozone treatments of both experiments (data not shown).

In experiment 2, ozone fumigation reduced total foliage biomass production, including the abscised leaves ('foliar biomass 2'). In experiment 1, this was visible only as a trend (Table 4).

The 'top' foliage responded to an increase in N fertilization with a considerable increase in biomass production (Table 4). Unlike the whole-plant biomass, the biomass of the 'top' foliage was higher in HN than in MN trees, suggesting a fertilizing effect of the additional N in the later phase of the experiment. The response to ozone exposure strongly depended on the N fertilization treatment: There was no effect in MN and HN trees, but a significant increase was found in LN trees of experiment 1 (Table 4). Therefore, the biomass of the 'top' leaves showed a similar pattern as did their total surface area (Fig 2b).

The ozone effect on biomass distribution between the above-ground parts and the roots was dissimilar between the two experiments. In experiment 1, the root to shoot (R/S) biomass ratio was not altered by ozone fumigation. In contrast, fumigated MN trees in experiment 2 responded to ozone with elevated R/S ratios compared to controls (Table 4). Shoot biomass (including shed leaves) yielded a root to shoot biomass ratio (R/S 2), which was significantly reduced by ozone fumigation only in LN and MN trees of experiment 1 (Table 4). The discrepancies between the two experiments were due to different foliage biomass. The biomass ratio of roots to main stem and branches (excluding foliage) was reduced under ozone exposure in both experiments (Fig. 6a), but this reduction diminished with increasing N fertilization.

Biomass distribution between the foliage (including shed leaves) and the woody shoot parts changed under ozone in favor of the latter, as seen by the decrease of the corresponding ratio (foliar biomass 2/wood biomass; Fig. 6b). The ozone effect decreased with increasing N fertilization.

The ratio of whole-plant biomass (without abscised leaves) to total foliage area indicates the net productivity per leaf area unit. This ratio was higher in ozone-exposed than in control trees (Table 4; in experiment 1 as a trend).

Nutrients

Concentrations of N in 'top' foliage increased with increasing N fertilization level (Table 5). Ozone exposure caused a significant increase of these values in LN and MN trees, but not in HN trees. N concentrations were lower in the older leaves than in the 'top' leaves. (Table 5). With the exception of the HN treatment, the leaves shed due to ozone exposure contained N in concentrations similar to or higher than the lower leaves on the main stem of control trees (Table 5). Therefore, retranslocation of N prior to abscision was very low.

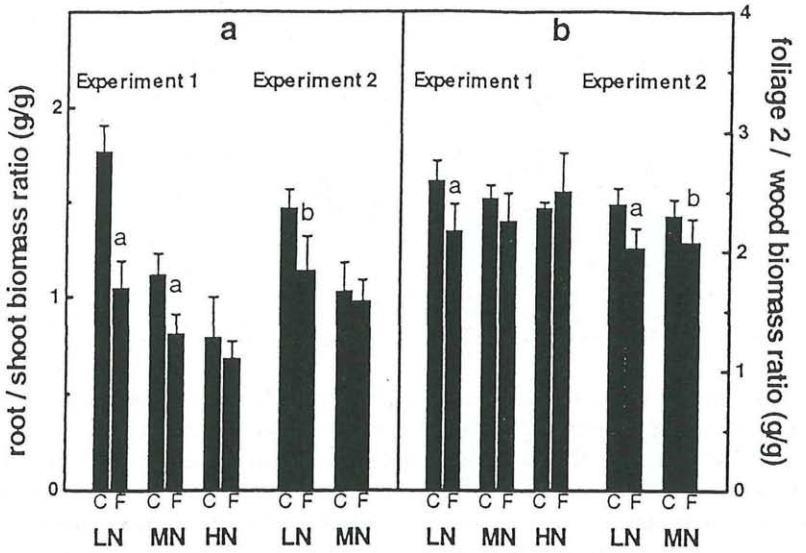

Fig. 6: Biomass ratios of a) roots to above-ground woody parts (main stem and branches); and b) foliage 2 (i.e. foliage including shed leaves) to above-ground woody parts. C = control; F = fumigated with ozone. Mean values (columns) and standard deviation (error bars; n = 5). Statistical significance of fumigation effect (Kruskal-Wallis) $p < 0.01$ = a; $p < 0.05$ = b.

Table 5

Nitrogen concentrations in % of dry matter for experiment 1 and 2. C = control; F = fumigated with ozone (exp. 1 and 2) or ozone and NO_2 (exp. 3). Significant differences between C and F (Kruskal-Wallis) are labelled as a ($p < 0.01$) and b ($p < 0.05$); shed foliage is compared to lower foliage of control trees.

Exp.	Nitrogen concentrations	LN C	LN F	MN C	MN F	HN C	HN F
1	'top' foliage	1.66	2.09 b	1.88	2.22 b	2.71	3.03
	lower foliage	0.94	1.32 a	1.36	1.58 b	2.24	2.24
	shed foliage	–	1.27 b	–	1.61 b	–	1.88
	wood	0.45	0.58 b	0.63	0.67	1.09	1.23
	roots	0.70	0.75	1.07	1.03	1.76	2.02
2	'top' foliage	1.16	1.61 a	1.33	1.66 a		
	lower foliage	0.89	0.91	1.22	1.24		
	shed foliage	–	0.99	–	1.42		
3	'top' foliage	1.39	2.08 a	1.43	2.14 a		
	lower foliage	0.86	1.45 a	1.18	1.85 a		
	shed foliage	–	1.15 a	–	1.51 a		

Table 6

Foliar concentrations of main nutrients (except N) in promilles of dry matter. Significant differences between C and O_3 (Kruskal-Wallis) are labelled as a ($p < 0.01$) and b ($p < 0.05$); values of shed foliage are compared to 'lower foliage' of control.

	'top' foliage		lower foliage (main stem)		shed foliage
Nutrient	LN C	LN O_3	LN C	LN O_3	LN O_3
phosphorus	3.2	3.2	1.6	1.8	1.4
potassium	15.5	16.9	7.6	11.0 a	10.0 b
magnesium	4.6	4.2 b	3.0	3.8 a	4.3 a
calcium	16.4	12.8 a	13.0	14.0	17.8 a

N content was calculated separately for each plant organ from N concentration and biomass. N content for leaves on branches were approximated by applying the mean concentration values for main stem leaves (nutrients in branch leaves were not measured). Since cuttings contained an unknown amount of N at the beginning of the experiments, they were not included into the calculation of whole-plant N content. The N in shed leaves was included into whole-plant N content. Increasing N fertilization had a positive effect on whole-plant N content. No significant difference was found between the control and ozone treatments, despite a trend to lower content in fumigated HN trees (Fig. 7a). However, distribution of N within the plants changed considerably in the LN and MN treatments: The 'top' foliage gained N at the expense of the root compartment (Fig. 7b). The effect was absent in HN trees. Root N content was significantly reduced in ozonated trees, but not root N concentrations (Table 5). The share of the woody above-ground parts was similar for ozonated and control trees (Fig. 7b).

In contrast to N, the other main nutrients showed no concentration rise in the 'top' foliage of the LN treatment due to ozonation; Ca and Mg concentrations even decreased (Table 6). Concentrations of K, Ca and Mg in shed leaves were elevated compared to those in the lower leaves of control trees, similar to N (Table 5 and 6). This was not the case for P. The high P concentrations in the 'top' leaves compared to those in the lower foliage indicated a transport from old to young leaves in both control and ozonated trees. 28% of the foliar P content was allocated to the 'top' foliage in the control treatment, but 45% in the ozonated trees (ozone effect: $p < 0.01$ in the Kruskal-Wallis test), indicating a promotion of acropetal transport of P under ozone exposure.

Effects of combined fumigation

Poplars exposed to a mixture of ozone and NO_2 (experiment 3) developed visual symptoms similar to those produced by ozone alone: Leaves

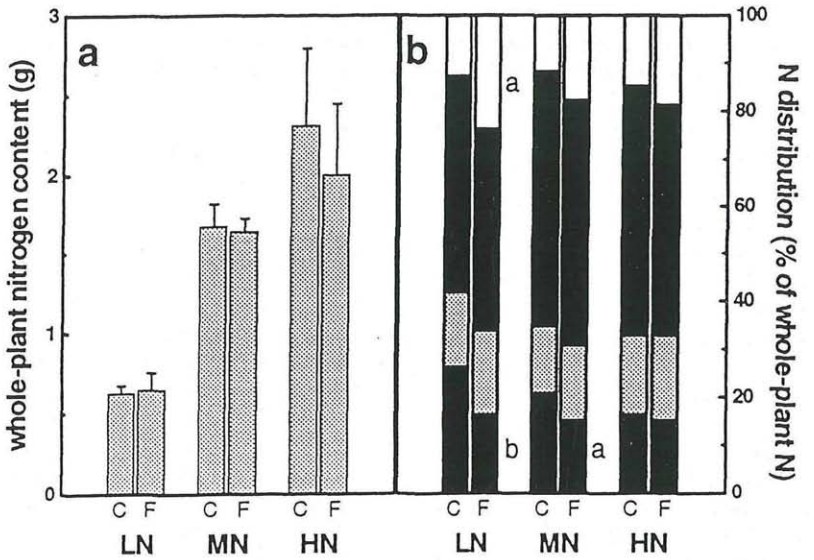

Fig. 7: a) Whole-plant nitrogen content (including shed leaves); and b) nitrogen distribution between the main organs as percents of whole-plant content . In a): mean values (columns) and standard deviation (error bars; n = 5); in b) from bottom to top: Roots (*black*); above-ground woody parts (*light gray*); leaves developed during the first seven weeks of the experiment and leaves on branches (*dark gray*); and 'top' leaves developed on the main stem after the first seven weeks (*white*). Cuttings not included. Statistical significance of fumigation effect (Kruskal-Wallis) $p < 0.01$ = a; $p < 0.05$ = b. C = control, F = fumigated with ozone. Data from experiment 1.

showed chlorosis and necrotic spots and were prematurely abscised. Leaves produced during week 3 had an average life span of 35 days, similar to those exposed to ozone alone. The total area of the remaining leaves after 12 weeks of fumigation was considerably reduced compared to control trees (Table 7). The combined fumigation caused an increase of the total area of the 'top leaves' (Table 7), as did ozone fumigation (Fig. 2b). The effect was strongest in the LN treatment.

Whole-plant biomass was significantly reduced by the combined fumigation, even if the biomass of the abscised foliage was included (Table 7). All main organs exhibited biomass reductions to various degrees, with the exception of the above-ground woody parts (i.e. stem and branches) of LN trees. The root to shoot biomass ratio (R/S) was not affected significantly by the combined fumigation. However, with inclusion of the abscised leaves to the foliage biomass, a lower ratio (R/S 2) was obtained for fumigated than for control trees (Table 7). The biomass ratio of roots to main stem and branches was reduced by

Table 7

Biomass and foliage area parameters for experiment 3 (ozone and NO_2 fumigation). Significant differences between C and F (Kruskal-Wallis) are labelled as a ($p < 0.01$) and b ($p < 0.05$).

Parameter	LN C	LN F	MN C	MN F
biomass (g):				
'top' foliage	3.9	8.2 a	8.9	11.3 b
total foliage	34.4	14.0 a	70.8	29.4 a
total foliage 2	34.4	29.9 b	70.8	49.5 a
wood	15.9	15.9	29.8	21.7 a
cutting	10.6	7.5 a	12.5	8.2 a
roots	27.0	17.1 a	38.5	21.8 a
whole plant	88.0	54.5 a	151.6	81.1 a
whole plant 2	88.0	70.4 b	151.6	101.1 a
root/shoot ratio	0.54	0.57	0.38	0.42
root/shoot 2 ratio	0.54	0.37 a	0.38	0.30 b
root/stem ratio	1.70	1.07 a	1.29	1.00 a
total biomass to foliage area ratio (mg/cm^2)	34	39	25	25
foliage area (cm^2):				
'top' foliage	358	841 a	680	1080 a
total foliage	2600	1424 a	6197	3270 a

the combined fumigation, similar to the effect of ozone fumigation. The total biomass : foliage area ratio was not changed by the fumigation (Table 7).

Nitrate reductase

The combined fumigation with ozone and NO_2 (experiment 3) resulted in elevated NR activity relative to the control leaves, independent of N nutrition from the fertilizer. Fumigation with ozone alone had no effect (Table 8).

Table 8

Induction of nitrate reductase (NR) by fumigation with ozone (exp. 2), or ozone and NO_2 (exp. 3). NR activity of fumigated trees, normalized to the corresponding control, is shown (i.e. fumigated/control). Significant differences between C and F (Kruskal-Wallis) are labelled as a ($p < 0.01$) and b ($p < 0.05$).

Experiment	LN	MN
2 (O_3)	1.5	1.1
3 ($O_3 + NO_2$)	10.5 a	12.3 b

Discussion

The most conspicious ozone effect on the fumigated poplar trees (clone 'Dorskamp') was premature leaf abscision preceded by chlorosis and the appearance of necrotic spots. Similar symptoms had been described for the same clone by MOOI 1980 and are also known for other hardwood species (e.g. birch; GÜNTHARDT-GOERG & al. 1993). Visual symptoms on the older leaves were accompanied by reduction of chlorophyll concentration and net CO_2 assimilation rate. Similar effects have been observed in many studies (cf. PYE 1988). The life span between leaf formation and abscision was similar for all fertilization treatments. The loss of foliar biomass by abscision under ozone stress was considerable. After 12 weeks of exposure, 23% (HN trees) and 35 to 40% (MN and LN trees) of total foliage biomass had been shed. As a consequence, non-green biomass was reduced as well. This reduction, however, occurred in the roots and not in the stem and branches, and the root/wood biomass ratio was therefore reduced by ozone exposure. High N supply reduced this ratio as well, the ozone effect decreasing with increasing N supply. Similar results were found for birch after ozone exposure during one vegetation period. Only trees grown under severe nutrient limitation had responded to ozone with a decrease in the root/wood biomass ratio (MAURER unpublished).

In the LN treatment, ozone changed the distribution of biomass between foliage and woody shoot parts in favor of the stem (Fig. 6b). Biomass distribution between main stem and branches (i.e. 'crown architecture') was not changed by ozone fumigation.

It is thus obvious, that the plants under ozone stress allocated their carbon gains into the various organs in a way different from those grown in filtered air, probably as a result of an acclimation.

Why did ozone exposure under N limitation stimulate growth (Fig. 2b) and performance (i.e. an almost twofold increase of Aamb with a concomitant increase in chlorophyll concentrations; Fig. 3b and 4) of the 'top' leaves? Stimulations of growth (Fig. 2a and b) and performance (Fig 3a) of the leaves were also observed with increasing N fertilization, and were paralleled by an increase in foliar N levels (Table 5). Similarly, the positive ozone effect on the 'top' leaves must be attributed to a higher N input under ozone stress. In fact, N concentration in the 'top' foliage was increased by ozone (Table 5), and total N content in this foliage fraction almost doubled. Based on a standard value for adequate N nutrition of poplar of 1.8 to 2.5% (BERGMANN 1992), the 'top' leaves of the LN control treatment were N deficient. Ozone fumigation raised the N concentrations to sufficient levels. The corresponding values for the HN treatment were already high in the control leaves and were not further elevated by ozone.

The concentrations of P and K in the 'top' leaves of LN trees ranged within the standard values given by BERGMANN 1992 and were not affected

by ozone exposure. Ca and Mg concentrations in 'top' leaves of LN trees were slightly and moderately above their corresponding standard values (1.5% and 0.3% of dry weight, respectively). This, however, appears to be unproblematic since virtually no detrimental effects of excessive Ca and Mg are known according to BERGMANN 1992.

The source of the additional N in 'top' leaves of ozonated trees was determined by the calculation of N balance in the trees. Total N content on the whole-plant level was strongly dependent on N supply from the fertilizer; however, the relationship was not linear; proportionally less N was taken up with increasing fertilization. After 12 weeks of exposure, LN trees had taken up 60%, but HN trees only 37% of the N initially present within the fertilizer granules. Ozone fumigation had no effect on total N content of the plants at any fertilization level; the increase of N content in the 'top' leaves therefore occurred at the expense of other plant parts. One obvious possibility would be the mobilization and withdrawal of N from the ozone-damaged leaves prior to abscision and subsequent reallocation into the new-forming foliage. An increase of soluble protein in young needles of ozonated *Pinus taeda* trees had been explained by this mechanism (MANDERSCHEID & al. 1992). In the present study, however, N concentrations in shed foliage of fumigated trees were still similar to those in corresponding healthy foliage of control trees. This contrasts with the N depletion generally observed during autumnal senescence (KILLINGBECK & al. 1990). Moreover, N content in total foliage (shed leaves included) increased under ozone influence. Therefore, the yellowing, ozone-damaged leaves can be ruled out as a source of additional N for the 'top' leaves.

The woody parts of the shoot were also not a net N source, as their N content was not altered by ozone exposure.

The roots of fumigated trees, however, contained significantly less N than those of control trees. This difference was due to less root biomass rather than to reduced N levels in roots under ozone exposure. It becomes clear, that under ozone influence and concomitant N deficiency, distribution of N was changed in favor of the young foliage at the expense of the roots.

Why was total N uptake equal for control and fumigated plants, despite the lower root biomass of the latter? This was surprising, since the fine root fraction was reduced as well. Elevated stomatal conductance under ozone influence (Fig. 3d) suggested additional N uptake due to a higher transpiration stream, compensating for the presumed lower root performance. However, the foliar concentrations of calcium, which is known to accumulate at the transpiration sites (BERGMANN 1992), were not elevated by ozone, which contradicts the hypothesis of a higher transpiration stream. Alternatively, we propose that N uptake by LN and MN trees was limited by the release rate of ammonium and nitrate from the fertilizer granules, rather than by root uptake capacity.

Stomatal conductance (g_{H_2O}) in response to ozone exposure may increase, decrease or remain constant, depending on environmental conditions (cf. DARRALL 1989). In our study, the increase of stomatal conductance under ozone exposure (Fig. 3d) was paralleled by an increase of the assimilation rate in young, but not in older leaves (Fig. 3b). This resulted in a lower water use efficiency in the latter, as shown by the increase of the E/A ratio with increasing duration of ozone exposure of individual leaves (see also MATYSSEK & al. 1991). Tree and leaf age exerted only small effects on the E/A ratio of the control treatments.

Effects of the combined fumigation with ozone and NO_2 were similar to those induced by fumigation with ozone alone; i.e. premature leaf loss, biomass reduction, reduced root/wood biomass ratio and increase of 'top' foliage biomass. In addition, the combined fumigation induced an increase of the activity of nitrate reductase (NR), which is the key enzyme of the assimilatory pathway for nitrate, a probable product of NO_2 after its dissolution in the mesophyll cell fluids (LEE & SCHWARTZ 1981). Earlier experiments had shown induction of NR activity by fumigation of poplar with NO_2 alone (SCHMUTZ & al. 1995), whereas ozone alone had no effect on NR activity (Table 8). An increase of NR activity by a combined ozone and NO_2 fumigation had been found in spruce needles (KLUMPP & al. 1989). Pure NO_2 had been a fertilizer for poplar (SCHMUTZ & al. 1995), and had been incorporated into the amino acid pool in spruce (NUSSBAUM & al. 1993). This was probably also the case in the combined ozone and NO_2 fumigation experiment, as the increase in NR activity indicated the incorporation of N from NO_2 into the biomass via the assimilatory pathway. A fertilizing effect of NO_2 was also indicated by the foliar N levels, which increased more under a combination of ozone and NO_2 than under ozone alone (Table 5).

The ozone effect on the exposed hybrid poplar trees can be surmised as follows: The leaves were the primary targets of ozone, as the gas entered the mesophyll via the stomata. Continual exposure to the oxidant and its degradation products damaged the mesophyll tissue (GÜNTHARDT-GOERG & al. 1993 and LANDOLT & al. 1994) leading to reduced photosynthesis and finally to premature leaf abscision. In addition, export of photoassimilates from damaged leaves probably was inhibited, as had been shown in other studies with poplar. Therefore, foliage provided less assimilates for production of non-green tissue. Since investments into the above-ground woody parts were maintained, less assimilate was allocated to the roots, resulting in poorer root growth. Nevertheless, their capacity for nutrient uptake was not reduced, as was documented by similar total N contents in fumigated and control trees. Due to the reduced root biomass, N requirement of the roots was diminished under fumigation. The economized N obviously was allocated to the developing foliage. The resulting

increase in total productivity of the 'top' foliage counteracted the effect of premature leaf loss induced by ozone. Therefore, the redistribution of N within the plant may be regarded as an adaptive response to ozone stress. The same has been reported for radish seedlings (HELD & al. 1991).

Why were the injured older leaves not taken advantage of as a nitrogen source in fumigated trees? Possibly, breakdown processes in these leaves did not proceed in the ordered manner known for natural senescence (for birch, refer to GÜNTHARDT-GOERG & al. 1993), thus preventing mobilization of foliar N compounds. Incomplete nutrient mobilization from foliage also occurred in cases of premature abscision due to unfavorable environmental conditions (e.g. drought; KILLINGBECK & al. 1990.)

This study with poplars demonstrated that ozone fumigation may lead to nutrient losses due to premature leaf abscision. The plants, however, adapted by reallocation of the limited nitrogen resources. Further studies may investigate whether similar mechanisms operate for other nutrient deficiency situations.

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