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Structure and Function
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Impairment of gas exchange and structure in birch leaves (*Betula pendula*) caused by low ozone concentrations

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Summary. Injury caused by low O_3 concentrations (0, 0.05, 0.075, 0.1 $\mu l\ l^{-1}$) was analyzed in the epidermis and mesophyll of fully developed birch leaves by gas exchange experiments and low-temperature SEM: (I) after leaf formation in O_3-free and ozonated air, and (II) after transferring control plants into ozonated air. In control leaves, autumnal senescence also was studied in O_3-free air (III). As O_3 concentration increased, leaves of (I) stayed reduced in size, but showed increased specific weight and stomatal density. The declining photosynthetic capacity, quantum yield and carboxylation efficiency lowered the light saturation of CO_2 uptake and the water-use efficiency (WUE). Carbon gain was less limited by the reduced stomatal conductance than by the declining ability of CO_2 fixation in the mesophyll. The changes in gas exchange were related to the O_3 dose and were mediated by narrowed stomatal pores (overriding the increase in stomatal density) and by progressive collapse of mesophyll cells. The air space in the mesophyll increased, preceded by exudate formation on cell walls. Ozonated leaves, which had developed in O_3-free air (II), displayed a similar but more rapid decline than the leaves from (I). In senescent leaves (III), CO_2 uptake showed a similar decrease as in leaves with O_3 injury but no changes in mesophyll structure and WUE. The nitrogen concentration declined only in senescent leaves in parallel with the rate of CO_2 uptake. A thorough understanding of O_3 injury and natural senescence requires combined structural and functional analyses of leaves.

Key words: Ozone – *Betula pendula* – Leaf gas exchange – Leaf structure – Senescence

Introduction

In recent studies on forest decline in Europe, the direct impact of air pollutants on conifer foliage was not found to be the primary cause of reduced tree vigor (Koch and Lautenschlager 1988; Schulze 1989). Xerophytic conifer needles, which are characterized by low photosynthetic capacity and low stomatal conductance (Körner et al. 1979), may indeed be less prone to pollutant uptake and injury than leaves with high capacity and conductance (Reich 1987). Plants with these latter features, such as agricultural crops and broad-leaved trees, are known to be impaired by the low ozone concentrations nowadays prevailing in many natural environments (Reich and Amundsen 1985). However, most findings on broad-leaved trees derive from species native to North America, while much less is known about the response of European tree species to low ambient ozone levels. The fact that in rural sites of Central Europe the CO_2 assimilation of herbaceous crops is limited by ozone (Lehnherr et al. 1987) must direct attention to the ozone sensitivity of European trees with mesophytic leaves.

In contrast to the knowledge about the impact of SO_2 in leaves, few mechanistic studies have been carried out about developing ozone injury in the leaf epidermis and mesophyll (Mohren 1988; Mooney and Winner 1988). Moreover, some former investigations may reflect artifacts related to the way the ozone was generated (Brown and Roberts 1988).

Therefore, this study on cuttings of the mesophytic European tree species *Betula pendula* addressed processes of onsetting leaf injury with analytical gas exchange experiments (Lange et al. 1987) and 'low-temperature scanning electron microscopy' (Beckett and Read 1986; Scheidegger et al. 1991), using low ozone concentrations generated from pure oxygen. The approach also compared the decline of ozonated leaves with that of autumnal leaf senescence.

Materials and methods

Plants and ozone fumigations. From 14 April through 1 November 1989 cuttings of one birch clone (*Betula pendula* Roth) were grown in 10 l pots filled with sand and a basal layer of inert synthetic clay beads (1 plant/pot, fertilized, well-watered). When transferred into the field

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Table 1. Ozone concentrations and doses of the fumigation experiments and in the ambient air at the experimental site (in the rural surroundings of Zurich, Switzerland) from mid-May through early October 1989

Fumigation treatments: Ozone concentrations continuously applied ($\mu l\ l^{-1}$):	0 (control)	0.050	0.075	0.100
Ambient air at the experimental site: Number of hours with higher ozone concentrations than those given above		664	70	4
Ozone doses ($\mu l\ l^{-1}$ h) of (1) *Fumigation treatments*:	0	174	261	348
(2) *Ambient air* – at the experimental site (550 m a. s. l) ~ about 7 km W of Zurich:	124			
– at the field station 'Bachtel' (1115 m a. s. l) about 26 km ESE of Zurich:		206[a]		

[a] Measured by ATAL/Zurich (Mr. Waldenmeyer)

fumigation chambers (Keller 1976) on 16 May 1989, the plants were separated into four O_3 treatments (20 plants/treatment, 4 plants/chamber). The O_3 concentrations were 0 (control), 0.05, 0.075, and 0.1 $\mu l\ l^{-1}$, and monitored by a 'Monitor Labs 8810' instrument. Ozone was generated from pure oxygen (Fischer, model 502) and continuously added to charcoal-filtered air. The treatments covered the range of O_3 concentrations and doses measured in the region around the experimental site (Table 1).

Leaves selected in investigations. Structure and gas exchange of similarly aged, fully developed leaves attached to the main stem were compared between O_3-fumigated and control plants: from June through August with leaves, which had formed during O_3 exposure (investigation I). The leaves selected were either without visual symptoms or with early visual O_3 injury (little, light-green yellowish dots) or with established yellowish-bronze discoloration and the onsetting formation of tiny black necroses. Advanced O_3 injury (large necroses, wilting, premature leaf loss) was not investigated. In September, investigation II addressed control plants with leaves, which had almost completed development before being ozonated with 0.1 $\mu l\ l^{-1}$ since 21 August 1989. With similar leaf selection, tiny black necroses occurred earlier than in (I) and before the yellowish discoloration. In October (investigation III), senescent but still turgescent control leaves were compared until complete yellowing with mid-summer control leaves and with ozonated leaves.

Gas exchange experiments. These were conducted on attached complete leaves with a thermo-electrically climate-controlled cuvette system (Walz), which was installed in the field close to the fumigation chambers. The evening before the measurement, plants randomly chosen from treatments were brought to the measuring site and were shielded from direct sunlight and rain during the experiments. Transpiration rate was determined using Walz dewpoint mirrors and a by-pass loop. The mass flow of the by-pass through a water trap was controlled by a differential H_2O IR gas analyzer (BINOS 4 b, Leybold-Heraeus) to zero vapor differences between cuvette inlet and outlet. Differential and absolute CO_2 BINOS 4 b measured the net-CO_2 uptake of the leaf and the absolute CO_2 concentration, c_a, of the O_3-free system air. C_a was adjusted by a mass flow-controlled CO_2-dispensing system (Walz). The intensity, I, of the artificial light source used (8 'Multi-Mirror' halogen bulbs, General Electric) was measured with a GaAsP photodiode (Hamamatsu G1118) after calibration with a quantum sensor (LI-190, LICOR), leaf temperature, T_l, was registered with a 0.1 mm chromel/alumel thermocouple.

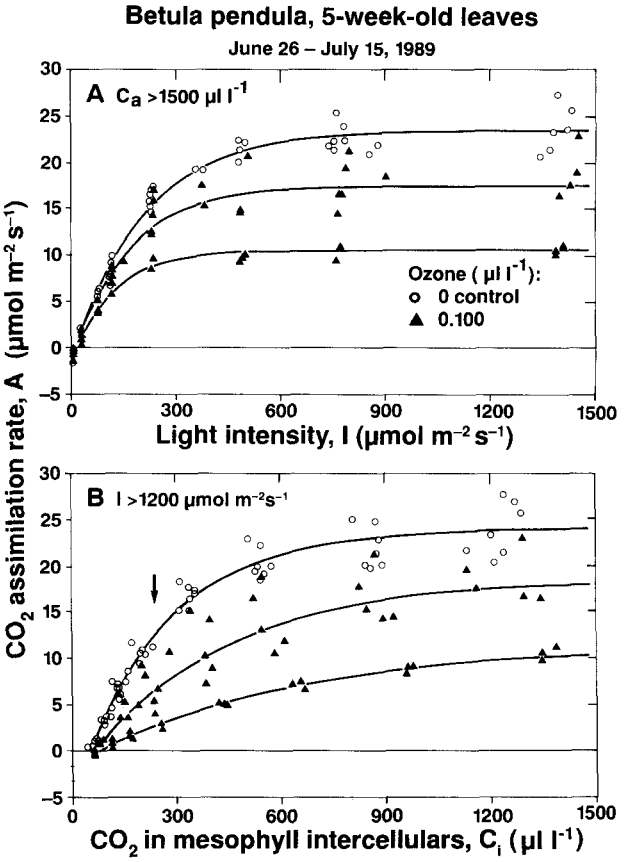

Fig. 1. Investigation I; **A**: CO_2 assimilation rate as dependent on light intensity (experiment 1) and, **B**: on the CO_2 concentration of the mesophyll intercellulars (experiment 2; the same leaves as in A). The responses of 7 control leaves are compared with those of 8 ozonated leaves, each leaf representing an individual plant; *drawn lines* represent mean functions of leaves with similar light and CO_2 responses: *upper line* = control and ozonated leaves without visual symptoms, *2nd line from top* = early visual ozone symptoms, *lowermost line* = established O_3 discoloration (see Materials and methods). Plateaus give mean photosynthetic capacity, A_{max}

Gas exchange rates were based on the one-sided leaf area (including discolored leaf parts), which was determined with the Delta-T areameter MK2. The nitrogen concentration, N, of experimental leaves was assessed with a Carlo Erba NA1500 analyzer.

Controlling I, T_l, Δ w (leaf/air mole fraction difference of water vapor), and c_a, each leaf selected in investigations (I), (II), and (III) was analyzed by four experiments. In experiment 1 the light response of gas exchange (c_a >1500 $\mu l\ l^{-1}$ to minimize stomatal limitation of CO_2 uptake; Lange et al. 1987); in experiment 2 the CO_2 response (I >1200 µmol photons $m^{-2}\ s^{-1}$); in experiment 3 the leaf response to constant cuvette conditions during 90 min (I >1200 µmol photons $m^{-2}\ s^{-1}$ and c_a = 340 $\mu l\ l^{-1}$); and in experiment 4 the dark respiration rate (cuvette tightly darkened with aluminum foil) was studied. In all four experiments T_l was 20° C and Δ w = 10 mmol mol^{-1}, while 'steady-state' rates of CO_2 uptake and, in experiment 3, of transpiration were allowed to establish. CO_2 uptake rates at I >1200 µmol photons $m^{-2}\ s^{-1}$ and c_a > 1500 $\mu l\ l^{-1}$ are denoted as A_{max}. Investigating one leaf per day, the four experiments were always run in the same order and at the same time (between 7 a. m. – 1 p. m.).

Leaf structure. Stomatal apertures and mesophyll structure of 3 leaves per treatment (1 leaf/plant) were investigated in (I), (II) and (III) by low-temperature scanning electron microscopy (SEM) in a Balzers cryo-preparation unit SCU 020 (Müller et al. 1991). For this purpose, one leaf disc (∅ = 8 mm between 2nd-order veins) from each half of the central

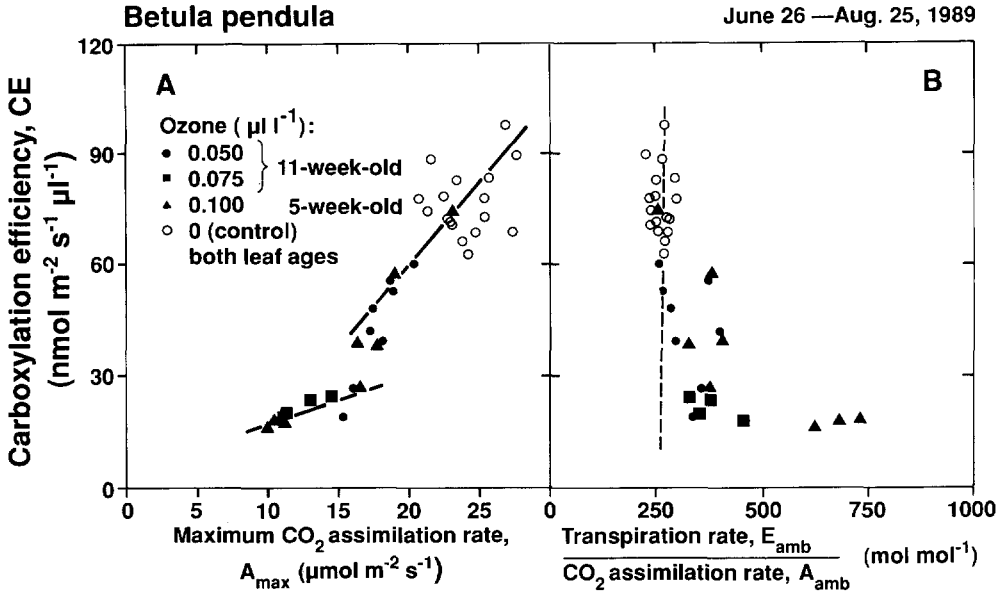

Fig. 2. Investigation I; leaves from all four ozone treatments (including the leaves of Fig. 1; each data point represents one individual leaf and plant). The O_3 doses were 84 µl l^{-1} h for 5-week-old leaves at 0.1 µl l^{-1}; 92 µl l^{-1} h for 11-week-old leaves at 0.05 µl l^{-1}; and 139 µl l^{-1} h for 11-week-old leaves at 0.075 µl l^{-1} (see text). **A**: carboxylation efficiency (CE) as related to maximum CO_2 -uptake rate, A_{max}, of individual leaves (experiment 2); the *upper line* includes data from leaves with and without early visual O_3 symptoms, the *lower line* includes leaves with established O_3 discoloration. **B**: CE as related to the ratio E_{amb}/A_{amb} (= WUE^{-1}) at c_a = 340 µl l^{-1} (experiment 3); the *dashed line* gives the mean ratio of the control

lamina had been excised at about 8 a.m. (relative humidity >70% at 20° C) into a humid chamber and frozen in liquid nitrogen immediately thereafter. Findings by this sampling procedure were consistent with those of freezing complete leaves before excision from the stem (Scheidegger et al. 1991). The stomatal density was obtained from discs excised into methanol, dyeing guard cells with JJK solution and then counting stomata under a light microscope at 10 randomly chosen disc positions of 0.3 mm^2 area each (5 leaves/treatment).

The inner air space (expressed as a percentage of the total leaf volume) of five further leaves per treatment was determined according to Koike (1988). Specific leaf weight (SLW) was calculated as dry weight by one-sided area.

Table 2. Gas exchange characteristics of control, ozonated and senescent birch leaves (absolute maxima and minima of leaves measured)

A: leaves formed under ozone during early summer (I)

	Ozone concentration (µl l^{-1})		
	0 (control)	0.05 and 0.075	0.100
Leaf age (weeks):	5 and 11 (pooled)	11	5
A_{max} (µmol m^{-2} s^{-1}; Expts. 1 and 2):	20.7 – 27.3	25.3 → 11.1	22.9 → 10.0
c_i (µl l^{-1}; at c_a = 340 µl l^{-1}; Expt. 3):	242 – 260	252 → 287	244 → 310
CE (nmol m^{-2} s^{-1} μl^{-1}; Expt. 2):	60 – 100	55 → 20	75 → 20
CO_2 compensation point (µl l^{-1}; Expt. 2):	36 – 58	45 → 76	51 → 97
QY (nmol µmol^{-1}; Expt. 1):	60 – 90	100 → 45	85 → 50
Light compensation point (µmol m^{-2} s^{-1}; Expt. 1):	5 – 20	5 – 15	5 – 25

B: 8-week-old control leaves ozonated in mid-summer (II) and autumnal senescence of control leaves formed in mid-summer (III)

	Ozone concentration (µl l^{-1})		
	0 (control, September)	0.100 (II)	0 (*) (III)
A_{max} (µmol m^{-2} s^{-1}; Expts. 1 and 2):	21.6 – 25.5	18.5 → 5.3	22.1 → 7.3
c_i (µl l^{-1}; at c_a = 340 µl l^{-1}; Expt. 3):	229 – 240	247 → 304	228 – 258
CE (nmol m^{-2} s^{-1} μl^{-1}; Expt. 2):	60 – 110	70 → 10	80 → 20
CO_2 compensation point (µl l^{-1}; Expt. 2):	36 – 58	48 → 83	45 → 70
QY (nmol µmol^{-1}; Expt. 1):	90 – 110	90 → 30	110 → 40
Light compensation point (µmol m^{-2} s^{-1}; Expt. 1):	7 – 15	5 → 35	12 → 30

(*) senescent leaves with A_{amb} > 1 µmol m^{-2} s^{-1} (Exp. 3; Fig. 6); → gives tendency of change at beginning leaf injury;
CE at c_i < 100 µl l^{-1} (I > 1200 µmol m^{-2} s^{-1}, same I also for CO_2 compensation point);
QY at I < 100 µmol m^{-2} s^{-1} (c_a > 1500 µl l^{-1}, same c_a also for light compensation point)

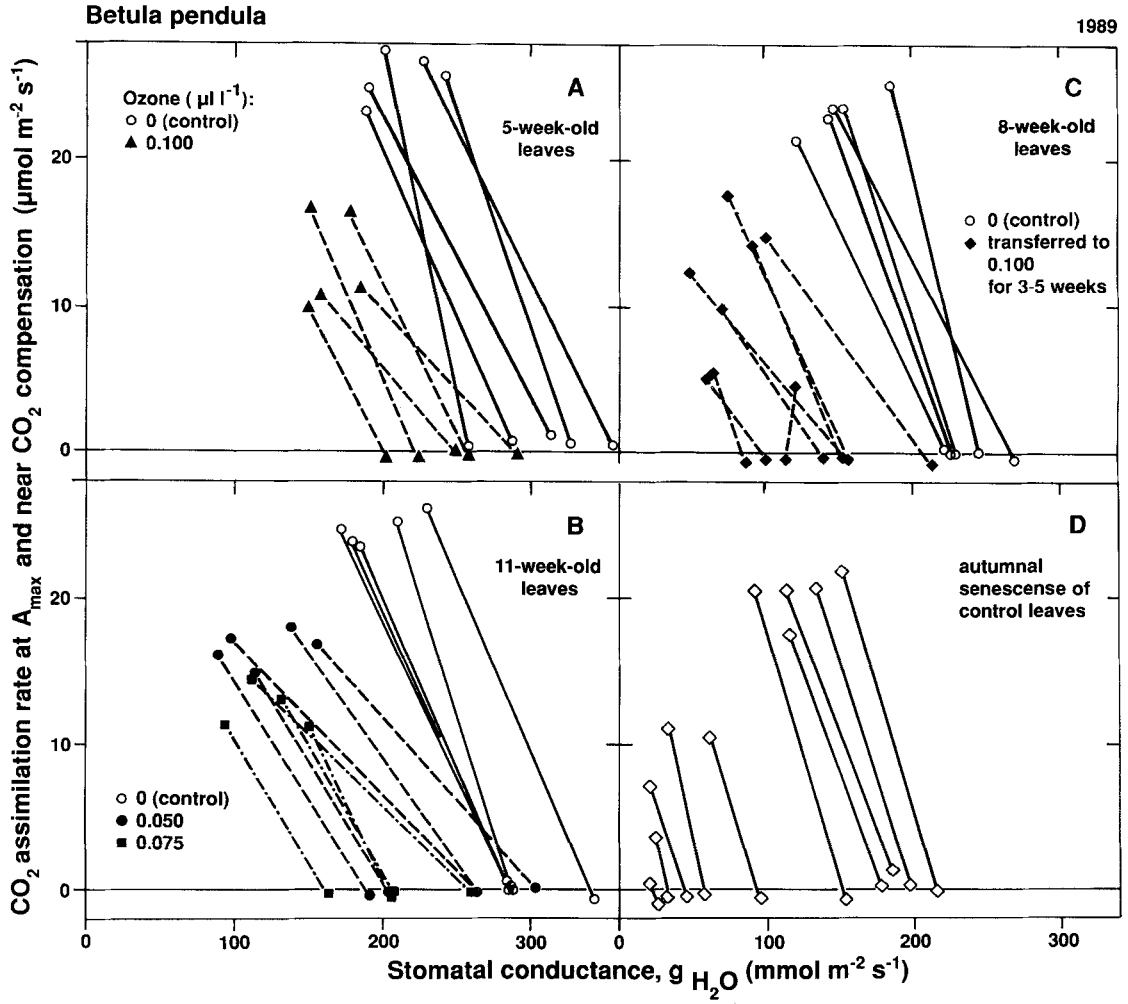

Fig. 3A–D. A_{max} (at c_a > 1500 µl l^{-1}) and CO_2 uptake rate near CO_2 compensation point (Table 2) as related to their corresponding stomatal conductances (experiment 2). For clarity, *straight lines* assign these cardinal points to individual leaves and plants, though in the given range of the driving variable c_a (which is not shown in this plot) the CO_2 uptake rate non-linearly relates to g_{H2O}. In **A** and **B** (both investigation I) only control leaves with 5 highest and ozonated leaves with 5 lowest A_{max} are shown, in **C** (investigation II) and **D** (investigation III) all leaves are shown

Results

The gas exchange of leaves formed under ozone (I)

O_3 injury gradually became apparent when the light response of the CO_2 assimilation rate, A (at high c_a), of ozonated leaves was compared with that of control leaves (Fig. 1 A). Under O_3 stress, A_{max} was more strongly depressed than the quantum yield, QY (at low irradiance). Thus, the saturating light intensity of A shifted from about 800 µmol photons m^{-2} s^{-1} in the control (and in ozonated leaves without visual symptoms) to 300 µmol photons m^{-2} s^{-1} in ozonated leaves with established discoloration. However, the CO_2 response of light-saturated A (Fig. 1 B) showed the O_3-induced reduction of A_{max} as well as a distinct decrease in the carboxylation efficiency, CE (at low CO_2 concentration). The declining CE limited the CO_2 gain from ambient air of c_a = 340 µl l^{-1} (and thus, from the corresponding CO_2 concentrations in the mesophyll intercellulars, c_i; Fig. 1 B, arrow). The lowered 'CO_2 affinity' of ozonated leaves was equally apparent when A was related to c_a (not shown; cf. Terashima et al. 1988).

The changes in Fig. 1 reflect a dose effect of ozone on gas exchange. Five-week-old leaves under the 0.1 µl l^{-1} O_3 treatment had received about the same O_3 dose as 11-week-old leaves at 0.05 µl l^{-1} (i.e. 84–92 µl l^{-1} h), while the relationship between CE and A_{max} followed a similar decline pattern under these two O_3 fumigations (Fig. 2 A). All 11-week-old leaves measured at 0.075 µl l^{-1} (O_3 dose = 139 µl l^{-1} h) displayed advanced decline. Though leaf injury progressed individually, A_{max}, CE and QY were decreased (Table 2 A), but c_i and the CO_2 compensation point were increased to similar extents at each O_3 fumigation (no clear change in light compensation at high c_a; Table 2 A).

Stomata maintained their sensitivity to CO_2 under O_3 stress, as the stomatal conductance, g_{H_2O}, was higher near the CO_2 compensation point than at A_{max} (Fig. 3 A, B). Nevertheless, the O_3-induced decline of CE (Fig. 2 A) was coupled with the progressively increasing ratio of tran-

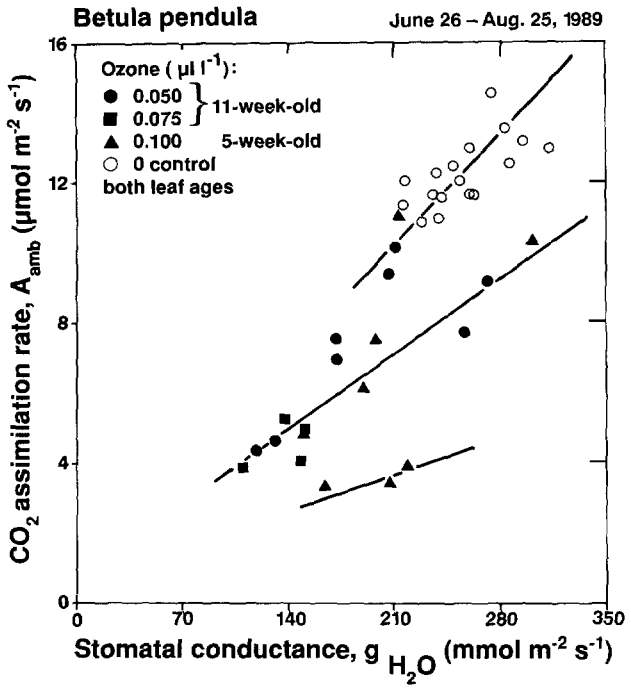

Fig. 4. Investigation I (the same leaves as in Fig. 2; each data point represents one individual leaf and plant) showing the relationship between the CO_2 uptake rate (A_{amb}) and stomatal conductance (g_{H_2O}) at A_{amb}, of individual leaves at $c_a = 340$ µl l^{-1} (experiment 3). The *upper line* describes leaves with E_{amb}/A_{amb} ratios of 225–298 mol mol^{-1} (no visual symptoms), the *2nd line from top* describes leaves of 300–455 mol mol^{-1} (with early visual O_3 symptoms up to established discoloration), and the *lowermost line* describes leaves of 626–734 mol mol^{-1} (all with established O_3 discoloration)

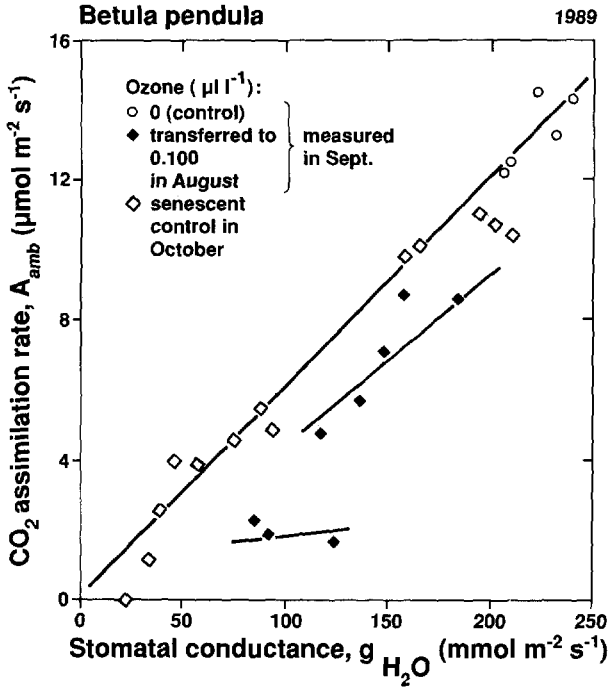

Fig. 6. Investigations II and III; showing the relationship between the CO_2 uptake rate (A_{amb}) and the stomatal conductance (g_{H_2O}) at A_{amb}, of individual leaves at $c_a = 340$ µl l^{-1} (experiment 3; each data point represents one individual leaf and plant). All leaves had formed in mid-summer, leaves measured in September were 8 weeks old; the *upper line* describes leaves with E_{amb}/A_{amb} ratios of 180–241 mol mol^{-1} (control in September and during autumnal senescence in October), the *2nd line from top* describes leaves with ratios of 252–298 mol mol^{-1} (early visual O_3 symptoms), and the *lowermost line* describes leaves with ratios of 463–897 mol mol^{-1} (established O_3 discoloration)

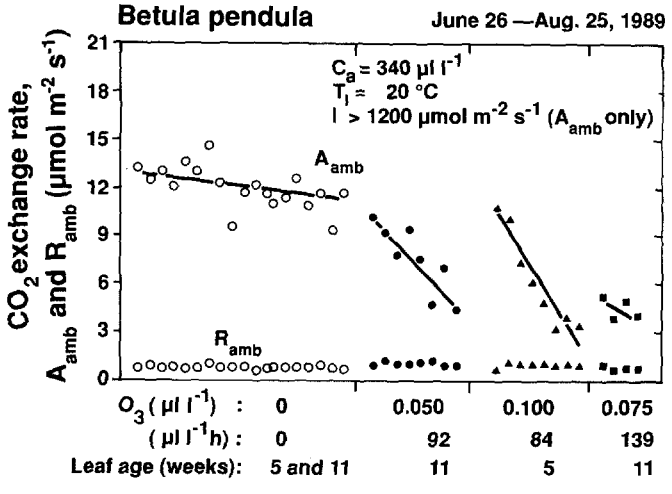

Fig. 5. Investigation I: pairs of CO_2 uptake rate (A_{amb}) and dark respiration rate (R_{amb}) at $c_a = 340$ µl l^{-1} of individual leaves and plants (experiment 4; the same leaves as in Fig. 2). Note that $R_{amb} < 0$ µmol m^{-2} s^{-1} is projected into the scale of A_{amb}; *lines* show tendency of change

spiration rate versus CO_2 assimilation rate, E_{amb}/A_{amb}, at $c_a = 340$ µl l^{-1} (Fig. 2B). Thus, the 'water-use efficiency' (WUE) declined under the impact of ozone. Figure 4 shows that both A_{amb} and g_{H_2O} were reduced as O_3 injury progressed. However, the decreasing slope in Fig. 4 reflects a stronger decline in A_{amb} than in g_{H_2O} and thus explains the decrease in WUE. In contrast to the O_3-induced break-down in carbon gain, the dark respiration rate, R_{amb} (at $c_a = 340$ µl l^{-1}), did not differ between the treatments (Fig. 5).

The gas exchange of control leaves after O_3 exposure (II) and during autumnal senescence (III)

The leaf gas exchange of control plants exposed to the 0.1 µl l^{-1} O_3 treatment in mid-summer (II) responded similarly to ozone (Table 2B, Fig. 3C, and decreasing WUE in Fig. 6) but more rapidly than in the leaves of (I). The changes in the CO_2 uptake of ozonated leaves resembled that of senescent control leaves in autumn (III, Table 2B). However, the latter leaves not only maintained the stomatal sensitivity to CO_2 (Fig. 3D) but also stable WUE until advanced yellowing (Fig. 6). Neither ozonation (II) nor senescence (III) caused R_{amb} to differ from that of mid-summer control leaves (not shown). However, while both A_{amb} and the nitrogen concentration, N, declined in senescent leaves (Fig. 7), N tended to remain stable under ozonation.

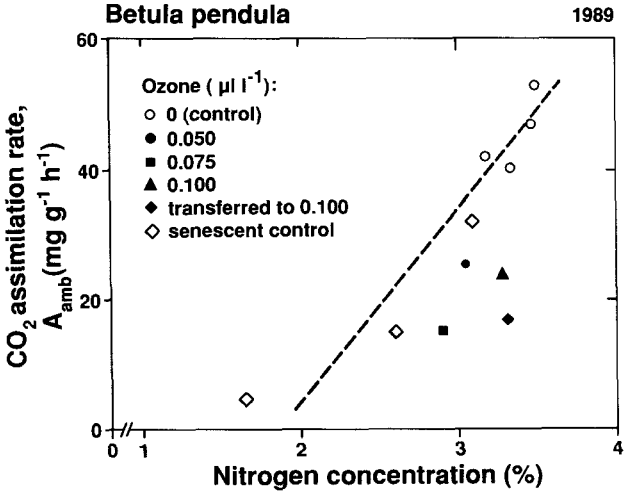

Fig. 7. CO_2 uptake rate (A_{amb}) as related to the nitrogen concentration (N) of the experimental leaves. Mean A_{amb} is assigned to N of pooled leaf samples per ozone treatment; the investigations I and II are depicted with their controls, investigation III gives three decline stages of the autumnal senescence; *dashed line* shows relation for control leaves

The structure of ozonated leaves and senescent control leaves (I–III)

Increasing O_3 concentration limited the leaf size but enhanced SLW and stomatal density (I, Table 3A), while stomatal apertures were narrowed by ozone before the occurrence of visual symptoms (Fig. 8A–D). Inside the ozonated leaves, unetchable droplet-like exudates abundantly formed on the walls of mesophyll cells (Fig. 8E). As the O_3-induced discoloration set in, the mesophyll cells began to collapse (Fig. 8F; see intact control in Fig. 8G).

Control leaves exposed to ozone before completed extension growth (II) differed only in slightly reduced size and raised SLW from the control in O_3-free air (Table 3B). Otherwise, stomatal narrowing and mesophyll degradation were similar to those in the ozonated leaves of (I). The O_3-induced mesophyll collapse was also indicated by the increased inner leaf air space (Table 3A, B). Senescent control leaves (III) displayed neither mesophyll collapse nor changes in the inner leaf air space (Table 3B).

Discussion

Before onsetting degradation, leaves formed under ozone (I) reached similar A_{max} as those in the control; however, their reduced size but enhanced SLW and stomatal density may indicate disturbed water relations during extension growth (Boyer 1970). The increased stomatal density was apparently overridden by the narrowing stomatal apertures already observed before discoloring, since g_{H_2O} was not raised above the control. As stomatal closure progressed, stomatal sensitivity to CO_2 was not lost, which underlines stomatal regulation as a rather robust mechanism of leaf function (cf. Field 1987). During the early leaf decline, guard cells and subsidiary cells appeared to be visually intact; this contrasts with findings from SO_2 and NO_x fumigations of collapsing subsidiary cells with widely opened pores in birch leaves (Wright 1988). Unless stomata are directly impaired by ozone, their closure might result from changed mesophyll metabolism (Winner et al. 1988), although the coupling between A_{amb} and g_{H_2O} (Schulze and Hall 1982) was disturbed under O_3 stress. The stomatal closure found did not limit the CO_2 uptake as reflected in decreasing WUE and CE and increasing c_i (Reich 1983; Sasek and Richardson 1989; Krupa and Kickert 1989).

Table 3. Structural features per individual birch leaf (means of 5 leaves from different plants per ozone treatment ± standard deviation. (See Materials and methods for leaf selection)

A: 8-week-old leaves in July formed under ozone fumigation (I)

Ozone concentration (µl l^{-1}):	0 (control)	0.050	0.075	0.100
Ozone dose (µl l^{-1} h):	0	68	101	137
Visual symptoms:	none	none	none	early injury
Leaf area (cm^2):	58 ± 5	53 ± 8	36 ± 12**	26 ± 8**
SLW (mg cm^{-2}):	3.2 ± 0.2	4.0 ± 4.4**	3.6 ± 0.2**	4.2 ± 0.3**
Stomatal density (mm^{-2}):	89 ± 9	89 ± 21	113 ± 23**	118 ± 11**
Leaf air space (% of total leaf volume):	37 ± 3	32 ± 4	57 ± 9**	56 ± 3**

B: 8-week-old leaves of control plants transferred to the 0.1 µl l^{-1} ozone fumigation in August when leaves were 3 weeks old (II); senescent control leaves in autumn (III)

		(II)	(III)
Ozone concentration (µl l^{-1}):	0 (control)	0.100	0
Ozone dose (µl l^{-1} h):	0	84	0
Visual symptoms:	none	early necrosis	yellowing
Leaf area (cm^2):	104 ± 11	85 ± 12*	93 ± 11
SLW (mg cm^{-2}):	4.7 ± 0.2	5.5 ± 0.2**	4.7 ± 0.5
Stomatal density (mm^{-2}):	69 ± 8	72 ± 7	67 ± 8
Leaf air space (% of total leaf volume):	34 ± 1	40 ± 4**	34 ± 4

Fumigation treatments compared with controls by Student's *t*-test: * significantly different at 5% and ** at 1%

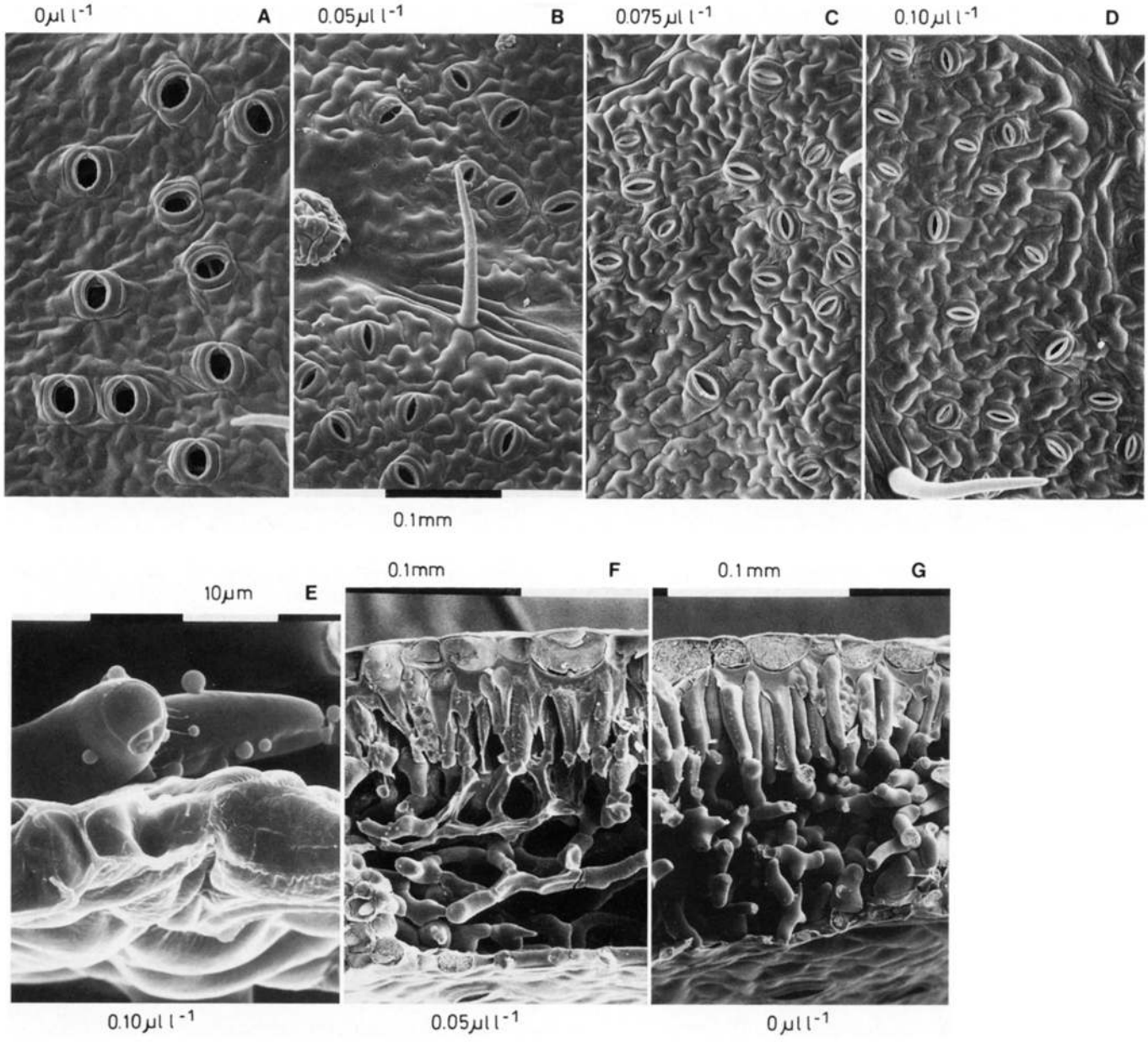

Fig. 8 A–G. Investigation I (low-temperature SEM). **A–D**: abaxial sides of birch leaves sampled at the same time and the same climatic conditions; gradual stomatal closure with increasing ozone concentration (*scale* in **A**, **C**, and **D** as shown in **B**); **E–G**: freeze-fractures through birch leaves; **E**: mesophyll cells of ozonated leaf with unetchable droplet-like exudates on the walls; **F**: onsetting collapse of mesophyll cells in ozonated leaf (O_3 dose approx. 58 µl l^{-1} h in **E** and **F**); **G**: intact mesophyll of control leaf

Non-stomatal leaf changes reduced the carbon gain. A_{max} was more strongly lowered by ozone than QY (Reich 1983; Kuno 1980) and along with CE (Sasek and Richardson 1989). Such changes may be ascribed to impaired electron transport and to the amount and activity of the rubisco enzyme in the cells (Farquhar and Sharkey 1982). However, as the gas exchange was based on the entire area of one leaf side, A_{max} declined at least as the consequence of completely collapsed mesophyll cells, this structural break-down also biasing the apparent values of QY and CE. Obviously, conclusions drawn from gas exchange about cell biochemistry might be misleading without taking into account changes in leaf structure. Nevertheless, changes in the assimilatory metabolism of the remaining mesophyll cells cannot be excluded. The early and abundantly occurring exudates on the walls of mesophyll cells may indicate disturbances in the cell metabolism. While such exudates (Read 1990) might be a general stress indication, the O_3 impact is assumed to mainly occur on the plasmalemma (Lange et al. 1989), thus very close to the cell wall. Therefore, changed light and dark reactions of photosynthesis (Urbach et al. 1989; Lehnherr et al. 1987) might be indirectly caused by responses of cell metabolism to that O_3 impact.

The increasing inner air space evidently alleviated the gas diffusion inside ozonated leaves. Thus, according to

Terashima et al. (1988), the decline found in CE represents the decreasing 'CO_2 affinity' of entire ozonated leaves. Apparently, as degradation progressed, the mesophyll collapse gradually preceded the stomatal closure, thus decreasing WUE (Greitner and Winner 1989). The unchanged dark respiration rate under O_3 stress seems to characterize fully developed leaves (Reich 1983; Hanson et al. 1988), but implies, at the given cell collapse, a raised respiration in the remaining mesophyll cells.

Compared with the leaves of (I), the more rapid but otherwise similar decay of control leaves ozonated in midsummer (II) may reflect seasonal changes in O_3 sensitivity (Reich 1983) or result from the leaf growth mainly occurring in O_3-free air. Senescent control leaves in autumn (III) resembled ozonated leaves in similarly declining CO_2 uptake and maintained stomatal regulation (Field 1987). However, the autumnal discoloring of deciduous birch (bright yellowing) differed from that caused by O_3 injury in that natural senescence requires metabolic and structural integrity (Thomas and Stoddart 1980) to assure the controlled leaf degradation as reflected in stable WUE and c_i (Schulze and Hall 1982) and in nitrogen retranslocation (Medina 1984; Matyssek 1986; Adams et al. 1990). Probably, the break-down of ozonated birch leaves was too rapid to induce processes of natural senescence to the full extent. Therefore, O_3-caused degradation of leaves should not be generally equated with senescence, as the congruence may be rather circumstantial. The mechanistic understanding of O_3 injury and senescence must obviously be achieved by combined functional and structural analyses.

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