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The response of spring wheat (*Triticum aestivum* L.) to ozone at higher elevations

II. Changes in yield, yield components and grain quality in response to ozone flux

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SUMMARY

The effect of ozone on the yield, yield components and grain composition of spring wheat (*Triticum aestivum* L., cv. Albis) was investigated in 1989 and 1990 at 900 m above sea level. Plants were grown from the three-leaf stage until harvest in open-top chambers ventilated with charcoal-filtered air (CF), unfiltered air (UF) or unfiltered air with one of two levels of O_3 added when global radiation exceeded 400 J m^{-2} s^{-1} (O_3-1, O_3-2). Ambient plots (AA) without chambers were used as a reference. Mean 7-h d^{-1} (09.00–16.00 h) O_3 concentrations in the CF, O_3-1 and O_3-2 treatment, respectively, was approximately 0·5, 1·4 and 1·8 times the concentration in the UF treatment, which was 70 $\mu g\ m^{-3}$ in 1989 and 76 $\mu g\ m^{-3}$ in 1990. The OTC environment was characterized by a warmer, more humid microclimate and reduced soil moisture, compared with the open field. In 1989, this effect of the chambers was more pronounced than in 1990. When compared with AA plots, chamber enclosure significantly reduced grain and straw yield in 1989, while in 1990 no difference was observed. Grain yield decreased with increasing seasonal mean O_3 concentration as a result of reductions in the weight of individual grains and in the number of grains per head. A small decrease in the ratio between grain weight and total above-ground biomass (harvest index) indicated a shift in biomass allocation. With increasing O_3, small or no changes were observed in protein, starch and K concentrations, and in protein quality (Zeleny value) of the grain. Relationships between different measures of O_3 exposure and relative grain yield were constructed with a quadratic model. With mean concentrations (7-h d^{-1} mean or radiation-weighted mean) or with a cumulative index (SUMO6 = sum of concentrations above 60 $\mu l\ l^{-1}$) to describe exposure, the response of grain yield to increasing O_3 was smaller in 1990 than in 1989. Nearly identical exposure-response functions were obtained for both seasons, with the mean O_3 flux used to estimate the absorbed dose of O_3. Quadratic exposure-response functions suggest that ambient levels of O_3 reduced grain yield by 9·5% and 11·6% in 1989 and 1990, respectively. Using Weibull exposure–response functions, a comparison with results from an earlier study at 485 m above sea level indicates reduced sensitivity at the higher elevation.

Key words: Ozone, ozone flux, *Triticum aestivum* (wheat), yield.

INTRODUCTION

The relationship between ozone (O_3) and crop yield has been the subject of numerous studies in recent years (see review by Heagle 1989). Quantitative exposure-response relationships have been established for a series of species grown under a range of environmental conditions. It has been concluded that ambient O_3 concentrations reduce the yield of some major crops in the Unites States (Heck *et al.*, 1983), in Switzerland (Fuhrer *et al.*, 1989), Sweden (Pleijel *et al.*, 1991) and Germany (Adaros, Weigel and Jäger, 1990). There are large differences in O_3 sensitivity between species and cultivars (Guderian, Tingey & Rabe, 1985). Wheat is among the O_3-sensitive species, while other crops like barley (Pleijel *et al.*, 1992) or *Vicia faba* (Sanders *et al.*, 1990) are less sensitive to O_3.

The majority of O_3 exposure-response studies with agricultural crops have been carried out at low elevations. These sites are often characterized by a typical diurnal profile of O_3, with peak concen-

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trations during the afternoon and low concentrations at night when other pollutants are present (Fuhrer, 1985). The similarity in the relative response of wheat yield to increasing O_3 observed during several seasons at low altitudes in Switzerland (Fuhrer *et al.*, 1989) and in Sweden (Pleijel *et al.*, 1991) suggests that the effect of O_3 is similar under a wide range of climatic and soil conditions. So far, little attention has been paid to the response of vegetation grown at higher elevations, where the diurnal characteristics of exposure to O_3 are different from those at lower sites (Fuhrer, 1985; Lefohn, Shadwick & Mohnen, 1990), and plants grow under reduced atmospheric pressure and lower mean temperature. Under these conditions, the concentration of O_3 in units of mass per unit volume at equivalent volume fractions is reduced and the deposition velocity of O_3 over a given surface is higher (Larsen & Vong, 1990). These differences between sites at different elevations could affect the relationship between O_3 and crop responses. In order to evaluate the effect of altitude on crop responses, the present two-year experiment was carried out on a hilltop (at 900 m above sea level) with a diurnal O_3 profile typical for higher sites, but still within the range of altitudes where agricultural crop production is intensive and O_3 effects may be of economic importance. In order to enable the comparison of the results with those of a previous study at a lower (485 m) site (Fuhrer *et al.*, 1989; Fuhrer *et al.*, 1990), the same species and cultivar, and basically the same exposure technique was used.

To characterize O_3 exposure, daily means (7-h d^{-1}, 12-h d^{-1}, 24-h d^{-1}) of O_3 concentrations (commonly expressed as volume fraction) averaged over the experimental period were used in most studies. Some authors have suggested the use of cumulative exposures indices in order to take into account the length of the exposure period (Tingey, Hogsett & Lee, 1989), or means of weighted concentrations (Tingey *et al.*, 1989; Lefohn & Runeckles, 1987). Earlier, we used a radiation-weighted mean concentration proposed by Rawlings *et al.* (1988) to account for temporal variations in O_3 uptake via the stomata, and to obtain a closer link between measured exposure concentrations and the physiologically active O_3 dose inside the leaves (Fuhrer *et al.*, 1989). In the present study, an estimated O_3 flux (Grandjean Grimm & Fuhrer, 1992) calculated from radiation-weighted concentrations is used. The aim was to study the effect of O_3 on the yield and grain quality of spring wheat, and to relate grain yield to the mean O_3 flux.

MATERIALS AND METHODS

Experimental site

The experiment was conducted at Zimmerwald (46° 53′ N, 7° 26′ E). The site is located about 10 km south of Bern on top of a foothill of the Swiss prealps at 900 m above sea level. The soil is brown earth with a pH of approximately 6·5. Two separate parts of the field were used in 1989 and 1990. Prior to the experiment, the soil was tested for spatial homogeneity with respect to pH, nutrient content, and soil structure. Soil cores (0–20 cm depth) were taken across the field and analyzed as described earlier (Fuhrer *et al.*, 1990).

Crop cultivation, experimental design and treatments

Spring wheat (*Triticum aestivum* L., cv. Albis) was cultivated by using standard local agronomic practices. Seeds (167 kg ha^{-1} in 1989 and 188 kg ha^{-1} in 1990) were mechanically sown on 28 March 1989 (19 March 1990) with 16·7-cm row spacing. Open-top chambers (OTC) were established on 16 May (14 May) over 24 plots (2·25 m^2) at the 4-leaf stage and remained in place until harvest. The OTCs were described in detail in the first paper of this series (Grandjean Grimm & Fuhrer, 1992). Treatments started on 19 May (16 May). The treatments used were: charcoal-filtered air (Camcarb 2000) (CF), unfiltered air (UF), unfiltered air with one of two levels of O_3 added (O_3-1 and O_3-2). Each treatment consisted of 6 OTCs with two chambers per fan box (i.e 3 true replicates per treatment), with 6 unenclosed circular plots (AA) for comparison. Experimental plots wre arranged in 3 completely randomized blocks. Plant protection and fertilization were the same for OTCs and AA plots. No supplementary irrigation was used. For the O_3-1 and O_3-2 treatment, O_3 was generated by electrical discharge (Fischer 500 M O_3 generator) in pure oxygen supplied from a tank. The amount of O_3 added to unfiltered air was kept constant. The timing of the generation of O_3 was controlled by a radiation sensor. Ozone addition in the O_3-1 and O_3-2 treatments was restricted to time periods with solar radiation exceeding 400 J m^{-2} s^{-1}, ensuring that active fumigation occurred only when plants were physiologically most active and not during the night or on cloudy and rainy days. Figure 1 shows the average diurnal O_3 profiles in the different treatments for both years.

Analysis of environmental variables and pollutant concentrations

Temperature and relative humidity (Rotronic MP-100 hygrometer/thermometer) and global radiation (Häni pyranometer Solar 130) were measured 10 cm above the canopy in the open field and inside one OTC of each treatment. Rainfall was collected with funnels at 5 places inside one OTC (in the centre and 20 cm from the chamber wall in N, S, W and E direction) and at one place in an AA plot. Soil water potential was measured with tensiometers (Tensior

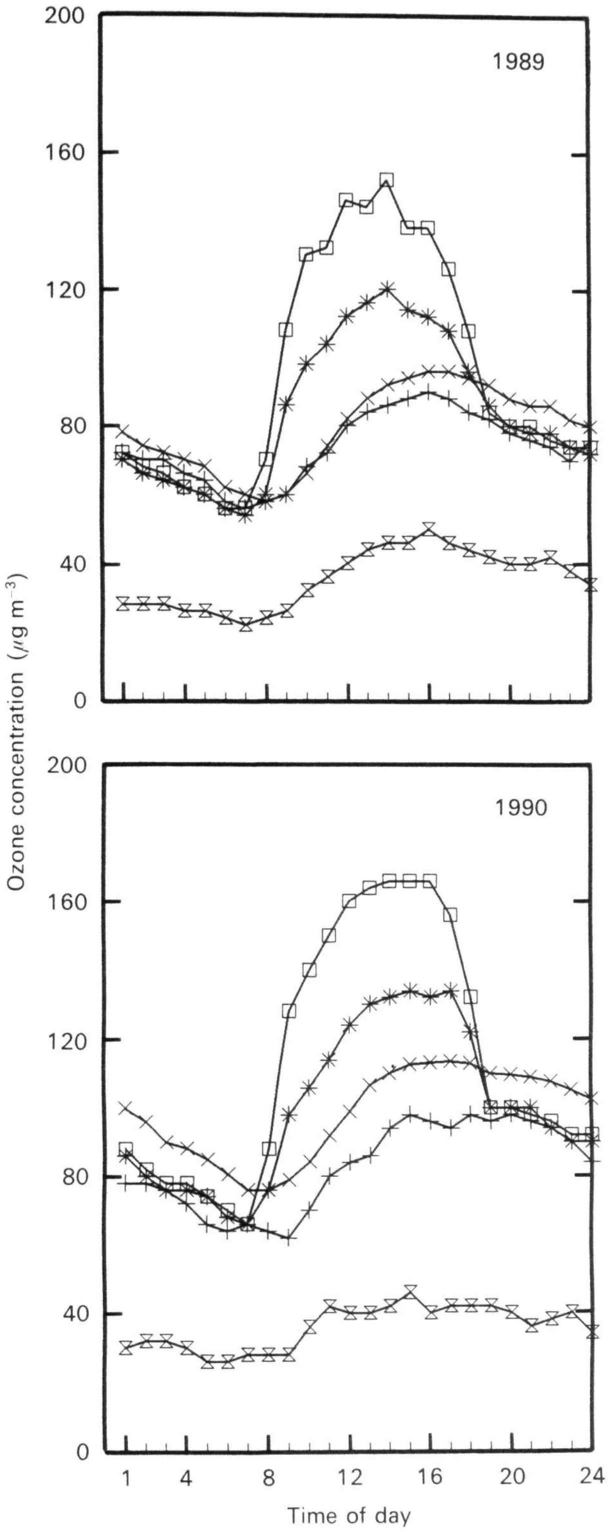

Figure 1. Mean diurnal pattern of O_3 concentration in the different treatments during the experiments with spring wheat in 1989 and 1990. ×—×, ambient air (AA); —, charcoal-filtered air (CF); +—+, unfiltered air (UF); *—*, unfiltered air with addition of O_3 (O_3-1); □—□, unfiltered air with O_3 addition (O_3-2).

2, Umweltanalytische Messgeräte, Goldach, Germany) in the centre of one OTC and in an AA plot. Soil water content was determined gravimetrically once a week at two places in the open field. Soil samples were taken between depths of 0–30, 30–60 and 60–90 cm. Bulk density at each depth was determined once and used to calculate volumetric soil water contents.

Ozone was measured in air samples taken about 10 cm above the canopy in ambient air (AA), in one OTC of the CF and UF treatment, and in 3 OTCs of each of the 2 treatments with O_3 additions (one OTC of each pair). The data from the 3 chambers were averaged to obtain the treatment mean. Air samples were pumped continuously through insulated sampling lines (PTFE Teflon, 10 mm diameter). Losses of O_3 in the sampling lines were determined before and after the experiment and concentrations were corrected accordingly. Ozone was analyzed by u.v. absorption (Dasibi model 1008 RS O_3 monitor). Concentrations displayed as volume fractions were converted to mass concentrations (μg m^{-3}) by taking into account local atmospheric pressure and temperature. One monitor was used for the continuous analysis of ambient air. A second analyzer was used for air samples from OTCs. Computer-controlled valves switched at 5-min intervals between sampling lines from the different OTCs. During the 1989 experiment, nitrogen oxides (NO, NO_X) were analyzed continuously in air samples from OTCs by chemiluminescence (Tecan CLD 502 NO/NO_X monitor). Concentrations were usually close to or below the detection limit of the analyzer (i.e. < 10 μg m^{-3}). Because of the low concentrations, nitrogen oxides were not analyzed routinely during the 1990 experiment.

Gas sampling and data logging were controlled by an intelligent multi-function board (Sorcus ML1 Multi-LAB/1 working in conjunction with a personal computer. Raw data were collected at 5-min intervals and stored on disks.

Harvest and plant analysis

OTCs were harvested on 14 August 1989 (9 August 1990) and AA plots on 21 August (16 August). Four 50-cm row sections were cut by hand. Samples used for the determination of yield, yield components and grain composition were not taken closer than 10 cm from the chamber wall. Remaining plants were used for grain quality tests.

Main shoots were measured and the number of ears per sample counted before being separated from the rest of the material, hand-threshed and cleaned. Prior to the determination of the dry weight, all material was oven-dried at 80 °C to < 1 % moisture content. The number of grains in each sample was counted and used to calculate the weight of individual grains from the total grain weight. The harvest index was calculated as the relative fraction (in %) of grain weight from the total dry weight. Values of the 4 sub-samples of each OTC were averaged.

Grain samples were milled to a flour-like powder and stored in sealed containers in the dark at 4 °C. The powder was oven-dried at 105 °C before analysis. Analytical procedures for protein, starch and K were given before (Fuhrer *et al.*, 1990). Grain samples were sent to the Swiss Federal Research

Station for Agronomy (Zurich-Reckenholz) for standard quality testing (Saurer *et al.*, 1991). Zeleny values as a measure of protein quality are reported.

Statistical analysis

Homogeneity of variances was tested with Bartlett's Test (Sachs, 1974). Differences between treatment means were analyzed statistically by one-way analysis of variance with pairs of chambers as replicates ($N = 3$). Least significant differences (LSD) were calculated when the F-test was significant ($P = 0{\cdot}05$) (Sachs, 1974). The effect of chamber enclosure was analyzed by comparing the means from the UF treatment with those from AA plots. Ozone exposure was characterized by means (7-h d^{-1}, 24-h d^{-1} or means for treatment periods only), radiation-weighted means according to Rawlings *et al.* (1988) and Fuhrer *et al.* (1989), the cumulative dose SUMO6 according to Tingey *et al.* (1989), and mean O_3 flux calculated on the basis of the linear relationships for radiation-weighted concentrations (O_3*) and O_3 flux given by Grandjean Grimm & Fuhrer (1992). Neglecting O_3 deposition to external surfaces (as estimated from the intercept), these relationships were:

1989: mean O_3 flux $= 0{\cdot}46\ O_3$*;

1990: mean O_3 flux $= 0{\cdot}33\ O_3$*

Quantitative exposure-response functions were constructed by fitting the data with a quadratic or with the Weibull model (Rawlings & Cure, 1985).

RESULTS

Environmental conditions

In 1989 a dry period occurred in late May and early June (weeks 8–10). In 1990, no extremely dry or wet periods occurred during flag leaf development and flowering (weeks 8–15) (Fig. 2). Table 1 contains means of environmental parameters. The data reveal generally higher temperatures and reduced water availability in 1989.

Precipitation inside the OTCs was reduced to about half the amount collected in the open field, and in 1989 this led to reduced soil water potentials. The OTC environment was thus characterized by a warmer and more humid microclimate with less soil moisture than in the open field.

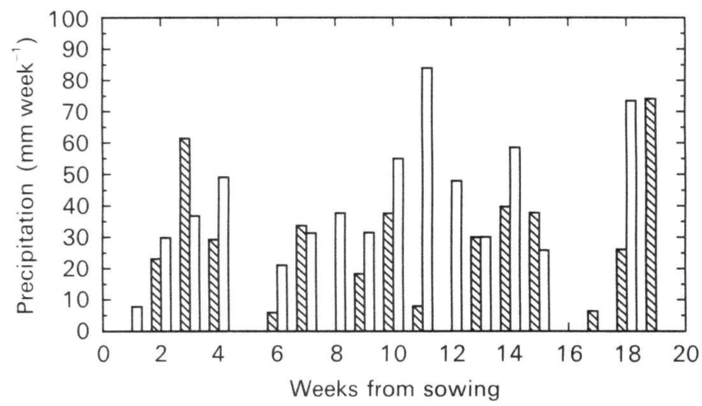

Figure 2. Weekly amount of precipitation from April to September in 1989 (dashed bars) and 1990 (open bars).

Table 1. *Environmental conditions in OTCs and ambient plots during the exposure periods*

	1989	1990
Exposure in open-top chambers	16 May – 14 August 91	14 May – 9 August 88
Exposure duration (days)		
Mean air temperature (°C)[a]		
Ambient plots	17·5 (23·5/12·4)	16·2 (21·8/11·9)
OTC	18·8 (27·0/12·2)	17·7 (25·6/12·6)
Integrated total solar radiation (MJ m^{-2})		
Ambient plots	2105	1912
OTC	1738	1607
Saturation vapour pressure deficit (kPa)		
Ambient plots	0·43	0·55
OTC	0·38	0·47
Precipitation (mm)		
Ambient plots	305	447
OTC	156	195
Volumetric soil water content (%)		
Ambient plots		
0–30 cm	22·2	31·5
30–60 cm	19·0	26·2
60–90 cm	18·4	23·8
OTC	n.d.[b]	n.d.[b]
Soil water potential (MPa) at −10 cm		
Ambient plots	−0·045	−0·012
OTC	−0·070	−0·035

[a] In parentheses: mean maximum temperature/mean minimum temperature.
[b] n.d., not determined.

Table 2. *Ozone exposure characteristics*

	1989					1990				
	AA	CF	UF	O_3-1	O_3-2	AA	CF	UF	O_3-1	O_3-2
Mean concentrations ($\mu g\ m^{-3}$):										
7-h d^{-1} (09.00–16.00 h)	74	36	70	97	122	90	34	76	109	140
24-h d^{-1}	72	32	67	76	86	86	32	74	90	103
Radiation-weighted mean	79	38	74	99	126	96	37	81	118	151
Treatment mean[a]		49	79	104	131		38	83	128	164
Cumulative dose ($mg\ m^{-3}\ h$)										
SUMO6		0	10	39	75		0	20	69	110
Uptake ($\mu g\ m^{-2}\ min^{-1}$)										
O_3 Flux		31	54	71	100		21	46	69	99

[a] Mean during treatment periods for treatment days only (1989: 560 h; 1989: 291 h).

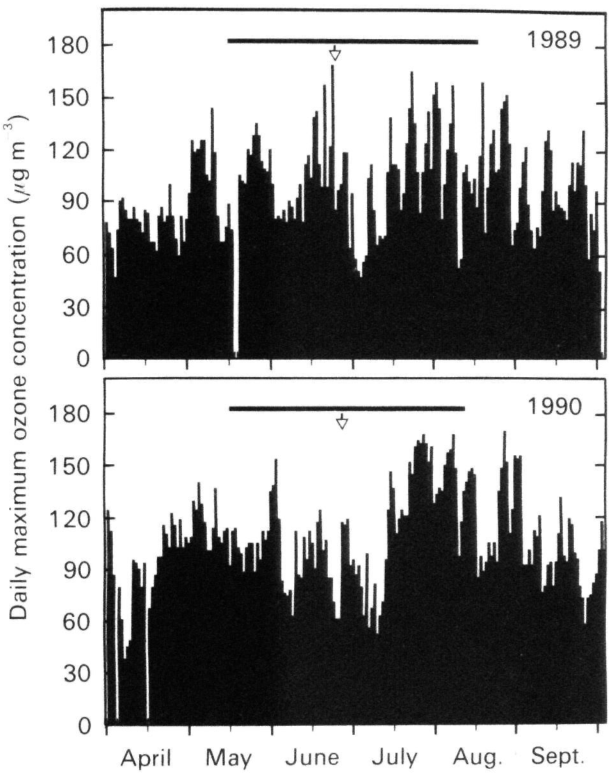

Figure 3. Daily 1-h maximum O_3 concentration at Zimmerwald (Switzerland) between April and September of 1989 and 1990. Horizontal bars indicate periods of exposure of spring wheat in OTCs. The arrow indicates the beginning of anthesis inside OTCs (22 June 1989; 26 June 1990).

Ozone concentrations

In Table 2, different O_3 exposure indices are summarized. Ozone concentrations in ambient air were higher in 1990 than in 1989. This was expressed by higher mean concentrations, but also by higher peak values recorded during specific episodes (Fig. 3). Between 1 April and 30 September, the 1-h mean of 120 $\mu g\ m^{-3}$ was exceeded 231 times in 1989 and 401 times in 1990. An episode with concentration maxima of approximately 170 $\mu g\ m^{-3}$ (equivalent to 95 $\mu l\ l^{-1}$) occurred during grain maturation in 1990. Shortly before and during anthesis, O_3 levels were relatively moderate in both seasons. The 7-h d^{-1} and the 24-h d^{-1} means were similar because O_3 concentrations did not decline much during the night. Similar to mean AA concentrations, treatment means were substantially higher in 1990. This was in spite of the fact that the number of days with active fumigation was lower in 1990. Because of the more frequent occurrence of high concentrations, the cumulative exposure index SUMO6 was much higher in 1990. The calculated mean O_3 flux in CF and UF chambers was higher in 1989, and about the same in both years for the O_3-1 and O_3-2 treatments.

Yield and yield components

The various yield parameters analyzed responded differently to increasing O_3 (Table 3). In both years, no difference between the 4 treatments was observed for the number of heads m^{-2}. A significant treatment effect was found in only one experiment (1989) for the number of grains per head. All other parameters showed significant treatment effects in both years. Grain yield was sensitive to increasing O_3. The decrease in grain yield with increasing O_3 was related primarily to the decreasing weight of individual grains, and secondarily to decreasing numbers of grains per head. The difference between grain yield in CF and UF was significant in 1989, but not in 1990. The decline in harvest index with increasing O_3 indicated a change in the allocation of biomass to different parts of the plant.

The comparison between the data from AA plots with those from the UF treatment showed that chamber enclosure significantly affected yield and yield components in 1989. Only the length of main shoots was the same inside and outside OTCs. Grain yield in AA plots was much higher than in UF chambers because of an increase in the number of heads m^{-2}. Plants in AA plots had larger numbers of grains per head, but individual grains were smaller than in OTCs. In 1990, chamber enclosure had a negative effect on straw yield, but others parameters remained unaffected.

Table 3. *Effect of treatment and chamber enclosure on yield and yield components of spring wheat**

Treatment	Main shoot (cm)	Straw yield (t ha^{-1})	Grain yield (t ha^{-1})	Grains head^{-1}	Heads m^{-2}	Grain weight (mg grain^{-1})	Harvest index (%)
			1989				
CF	99·5 a	5·77 a	6·61 a	38·4 a	456 a	37·7 a	48·8 ab
UF	98·2 ab	5·37 b	6·30 a	36·2 ab	462 a	38·4 a	49·7 a
O_3-1	96·2 bc	4·98 c	5·36 b	36·1 ab	451 a	34·0 b	47·1 b
O_3-2	95·6 c	5·08 bc	4·67 c	34·7 b	438 a	29·6 c	42·9 c
F value	11·0	5·5	16·5	3·3	0·8 NS	42·6	21·4
LSD (0·05)	1·4	0·44	0·63	2·4	—	1·8	1·8
AA	97·3	7·50	8·02	48·8	588	28·5	46·6
F value†	1·0 NS	57·0	28·6	49·0	22·1	177·6	63·3
LSD (0·05)	—	0·54	0·61	3·6	52·7	1·5	0·8
			1990				
CF	108·4 a	7·29 a	7·39 a	37·2 a	515 a	39·1 a	50·3 a
UF	104·1 bc	6·62 b	6·88 b	35·2 a	508 a	38·6 a	50·9 a
O_3-1	105·1 b	6·40 bc	6·30 c	36·1 a	481 a	36·4 b	49·5 a
O_3-2	101·8 c	5·82 c	5·02 d	33·6 b	485 a	31·0 c	46·3 b
F value	12·5	9·5	52·4	3·7 NS	1 NS	55·3	16·7
LSD (0·05)	2·5	0·65	0·46	—	—	1	0·1
AA	101·2	7·83	6·92	34·9	566	34·9	46·8
F value	16·0 NS	18·9	0·1 NS	41·5 NS	9·9 NS	41·3 NS	222·5 NS
LSD (0·05)	—	0·77	—	—	—	—	—

* Treatment means followed by the same letter are not significantly different ($P = 0{\cdot}05$).
† The difference between means from AA and NF was tested.

Table 4. *Effect of treatment and chamber enclosure on the composition and quality of grain from spring wheat*

Treatment	Protein (% d. wt)	Starch (% d. wt)	K (% d. wt)	Zeleny (ml)
		1989		
CF	12·8 a*	64·5 a	0·44 a	48·5 c
UF	13·2 a	63·9 a	0·41 b	52·0 b
O_3-1	13·1 a	64·2 a	0·42 ab	53·2 ab
O_3-2	13·4 a	62·9 b	0·44 a	56·0 a
F value	0·5 NS	4·6	3·05 NS	8·1
LSD (0·05)	—	1·0	—	3·2
AA	13·0	63·2	0·43	50·0
F value†	0·8 NS	0·1 NS	14·5 NS	3·7 NS
LSD (0·05)	—	—	—	—
		1990		
CF	13·1 b	61·3 a	0·49 ab	50·2 b
UF	13·1 b	61·1 a	0·45 b	49·8 b
O_3-1	13·7 b	62·1 a	0·46 ab	56·3 ab
O_3-2	14·4 a	60·9 a	0·51 a	61·0 a
F value	8·2	1·5 NS	2·87 NS	4·9
LSD (0·05)	0·6	—	—	7·1
AA	13·0	63·3	0·44	53·3
F value	0·4 NS	10·2 NS	0·10 NS	0·9 NS
LSD (0·05)	—	—	—	—

* Means followed by the same letter are not significantly different ($P = 0{\cdot}05$).
† The difference between means of AA and NF was tested.

Grain composition and quality

Differences in grain composition between the different treatments were small (Table 4). While protein concentration tended to increase, starch concentrations decreased with increasing O_3. Each of these trends was significant in only one of the experiments. No treatment effect on the K concentration was detected. A small but consistent increase in Zeleny values with increasing O_3 was observed in both years indicating a trend towards better protein quality. Chamber enclosure did not affect the composition of quality of grain.

Exposure-response relationships

Different measures of O_3 exposure (Table 1) and data for relative grain yield (yield in the UF

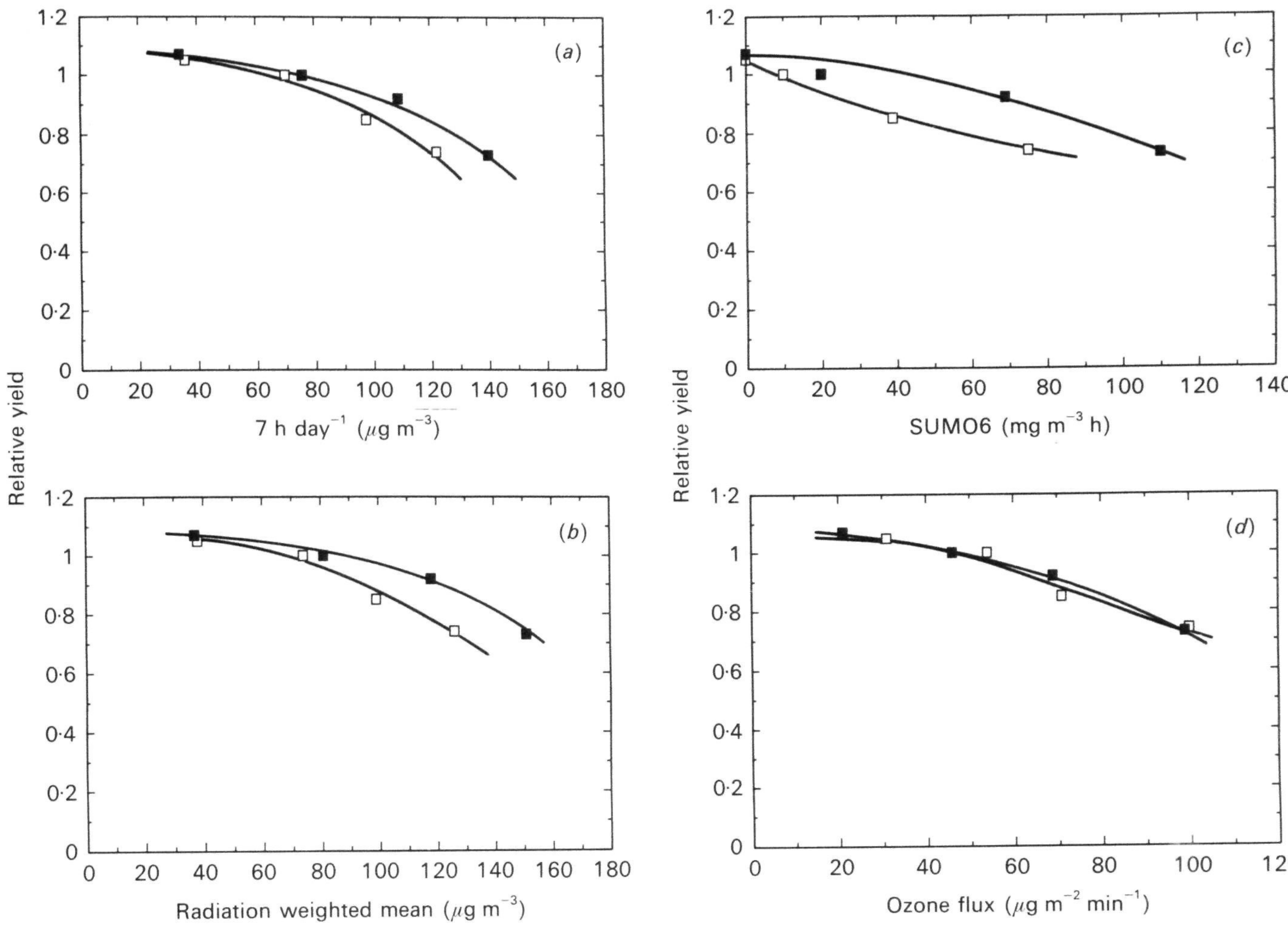

Figure 4. Exposure-response curves for the effect of O_3 on the relative grain yield of spring wheat in 1989 (□—□) and 1990 (■—■) using different O_3 exposure indices (a–d). Grain yield in the UF treatment is used as a reference. Curves were produced with a quadratic function.

treatment was used as a reference) calculated from Table 3 were used to construct exposure-response curves for the two experiments. A quadratic model was used for the plots in Figure 4. With a 7-h d^{-1} mean, the relationship was different for the two years, with a weaker response of grain yield to increasing O_3 in 1990 (Fig. 4*a*). Similar relationships were obtained with radiation-weighted concentrations (Fig. 4 *b*). With the cumulative exposure index SUMO6, the relationships differed strongly between years, but the response was again weaker in 1990 (Fig. 4*c*). Virtually no difference between the two years was obtained when O_3 flux was used to describe exposure (Fig. 4*d*). Hence, by using a mean O_3 flux, which is corrected for the deposition of O_3 to external surfaces, year-to-year differences in the yield response to O_3 became negligible.

With the use of a quadratic function for the relationship between radiation-weighted O_3 concentration (μg m^{-3}) and absolute grain yield (t ha^{-1}), a reduction in yield at ambient of O_3 relative to O_3 levels in the CF treatment (see Table 2) by 9·5 % and 11·6 % was calculated for 1989 and 1990, respectively.

In order to compare the data from this study with those from an earlier one at a lower site, Weibull functions were determined for the relationship between radiation-weighted mean O_3 concentration (in μg m^{-3}) and grain yield (in t ha^{-1}) for both years (in parentheses: coefficient of determination, r^2):

$$1989: \quad \text{yield} = 6{\cdot}67 \exp[-(O_3/178)^{2{\cdot}74}] \quad (r^2 = 0{\cdot}56)$$

$$1990: \quad \text{yield} = 7{\cdot}44 \exp[-(O_3/216)^{2{\cdot}72}] \quad (r^2 = 0{\cdot}84)$$

With these functions it could be calculated that a reduction in grain yield by 25 % occurs at radiation-weighted mean O_3 concentrations of 113 μg m^{-3} and 137 μg m^{-3} in 1989 and 1990, respectively.

DISCUSSION

The results of this study confirm that spring wheat is sensitive to O_3, and that there is a quantitative relationship between grain yield and mean seasonal O_3 exposure. This relationship varies between seasons, with mean concentrations (7-h d^{-1} or radiation-weighted mean) or the cumulative dose (SUMO6) used as O_3 exposure indices. Year-to-year variation in the exposure-response relationship is diminished when the mean O_3 flux is used, which corrects for differences in O_3 deposition to external surfaces. A number of environmental factors, canopy structure, and physiological processes affect the transfer of O_3 from the air to the leaf interior. Depending on these factors, the quantitative relationship between the degree of exposure to O_3 and the absorbed dose may be different in different seasons. Grandjean Grimm & Fuhrer (1992) have shown that under the drier conditions prevailing in 1989 a lower leaf area index could be measured. It is suggested that reduced shading inside the canopy compensated for a reduction in leaf conductance caused by reduced water availability. Consequently, the canopy conductance to O_3 during the drier season (1989) was similar to that in 1990. By using the corrected mean O_3 flux, this influence of canopy structure on O_3 flux is accounted for. Radiation-weighted concentrations alone neglect differences in transfer rates caused by factors other than stomatal conductance. Hence, the results of this study show that the 'pollutant absorbed dose' (Fowler & Cape, 1982) is the relevant parameter with respect to the plant's response, but a large enough number of direct measurements are necessary for the calculation of the mean O_3 flux.

Grain yield reductions in response to increasing O_3 stress are caused by a reduction in the weight of individual grains. As shown before, this can be related to accelerated senescence of the flag leaves (Grandjean & Fuhrer, 1989) and reduced photosynthesis (Lehnherr *et al.*, 1987). In addition, the priority of biomass allocation to different plant parts is changed to the disadvantage of the grain, as indicated by the change in harvest index. A similar change in biomass allocation was observed in a number of other studies (see review by Miller, 1988). The shift in allocation of carbohydrates leads to a decrease in the concentration of starch in grains, while other constituents (protein, minerals) increase with increasing O_3. The trend in grain composition observed here was the same as observed in comparable studies (Slaughter *et al.*, 1989; Fuhrer *et al.*, 1990; Pleijel *et al.*, 1991). The increase in Zeleny values at above-ambient O_3 levels indicates a small increase in grain quality. In both experiments, the treatment effect on grain composition and quality was minor compared with the effect on grain yield.

One aspect of this study is the effect of altitude on the response of grain yield to O_3. Results from the site at 900 m can be compared with those of a study at 485 m (Fuhrer *et al.*, 1989). Exposure-response curves offer the possibility of determining potential effects at equivalent exposures. The major limitation of this comparison between sites is the difference in time between the two studies. But at the lower site it was observed that the year-to-year variation in the response is small, even when years differ significantly in weather conditions. During the 3-year experiment, the radiation-weighted mean O_3 concentration corresponding to a 25% reduction in grain yield calculated with the Weibull function averaged 92 $\mu g\ m^{-3}$ at the lower site. This value is less than the concentrations calculated in the present study (113 and 137 $\mu g\ m^{-3}$ in 1989 and 1990, respectively), indicating that wheat grown at 900 m is less sensitive to O_3 than at 485 m. This is in contrast to the findings by Winner *et al.* (1989) who observed increasing O_3 injury with increasing altitude for 3 out of 5 surveyed plant species. The difference between the higher and lower sites revealed here could be caused by the differences in the physical and pollution climate, or by different soil conditions. Furthermore, it must be taken into account that under changing temperature and pressure, the mass concentration at equivalent partial pressure or volume fraction changes, and, as pointed out by Larsen & Vong (1991), changes in the deposition of O_3 at equivalent mass concentrations must be considered. The latter could account for about 2 to 5% of the difference in the concentration calculated for a yield reduction of 25%. Clearly, the amount of experimental data from higher and lower elevations is too limited at present to draw far-reaching conclusions. But, the apparent effect of altitude on the sensitivity of agricultural crops to O_3 revealed here can be regarded as preliminary indication of a problem that should be studied in more detail.

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