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# Growth stage dependence of the grain yield response to ozone in spring wheat (*Triticum aestivum* L.)

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**Abstract**

Field grown spring wheat (*Triticum aestivum* L.) was exposed to three different ozone treatments in open-top chambers: non-filtered air (NF), non-filtered air with the same ozone dose (2500 nl $l^{-1}$ h above 40 nl $l^{-1}$) during 38 days before anthesis (NF+pre), and during 26 days after onset of anthesis (NF+post). In addition, ambient air plots (AA) were used. During the respective treatment periods in NF+pre and NF+post, the 12 h daytime (between 08:00 and 20:00 hours) long-term average of the ozone concentration was between 40 and 45 nl $l^{-1}$. The ozone concentration was around 20–25 nl $l^{-1}$ in the NF treatment, as well as in the NF+pre and NF+post treatments, during the NF+post and the NF+pre periods, respectively. The grain yield in NF+post was 11% lower than in NF and this difference was statistically significant. The difference in grain yield between the NF+pre treatment and the NF treatment was small (2%) and not statistically significant. Similar effects as for grain yield were obtained for straw yield and 1000-grain weight, with the harvest index and the water content of the grain at harvest being uninfluenced by the treatments. The protein content of the grain was 15% in the NF+post treatment, which was significantly higher than the contents in the NF treatment (13.7%) and in the NF+pre treatment (13.9%). The chlorophyll content of the flag leaves declined earlier in the NF+post treatment compared with the NF and NF+pre treatments. The difference in this parameter between NF+pre and NF was small. It is concluded that ozone exposure is much more effective in decreasing the grain yield of wheat between anthesis and end of grain filling than ozone exposure before anthesis. © 1998 Elsevier Science B.V. All rights reserved.

*Keywords:* Anthesis; AOT40; Ozone; Protein; Wheat; Yield

## 1. Introduction

Today there is abundant evidence that present ambient ozone concentrations are high enough to cause significant yield loss in wheat (*Triticum aestivum* L.) in the USA (Heck et al., 1988) as well as in Europe (Fuhrer et al., 1989, 1992; Pleijel et al., 1991; De Temmerman et al., 1992; Skärby et al., 1993). Although pooled data from a number of open-top chamber studies with field-grown spring wheat show a fairly consistent response pattern and can be related to the ozone exposure index, accumulated exposure over threshold 40 nl $l^{-1}$ (AOT40), with high correla-

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tion (Fuhrer and Achermann, 1994), a variation among different experiments in the relative yield loss, caused by a certain number of AOT40 hours during the growing season, exists.

It has been suggested (e.g. Lee et al., 1988), that sensitivity to ozone by seed crops is maximal during the period between flowering and seed maturity, with sensitivity lower before and after that period. Thus, it could be hypothesised that a certain amount of AOT40 would produce a larger effect if given mainly during the suggested period of maximum sensitivity than if it is given over the whole growing period of the crop. Thus, one possible reason for the reported differences in sensitivity could be that ozone exposure was performed only after anthesis in some experiments and both before and after anthesis in others. This is supported by closed-chamber experiments with wheat (Soja, 1996) and with beans (Younglove et al., 1994; Vandermeiren and De Temmerman, 1996), where sensitivity to ozone varied with the developmental stage and the highest sensitivity was found for the period after flowering.

The present study tested the hypothesis that ozone exposure, defined as a certain number of nl $l^{-1}$ h above 40 nl $l^{-1}$ (AOT40), has a larger effect on the grain yield of spring wheat when administered during and after anthesis than before anthesis.

## 2. Materials and methods

### 2.1. Experimental site

The experiment was conducted in a spring wheat field at Östad, 50 km northeast of Göteborg, Sweden (57°54′N, 12°24′E). The field was situated 60 m above the sea level and the soil was a loamy sand. No major air pollution sources are located in the vicinity of the investigation area.

### 2.2. Open-top chambers

The open-top chambers (OTCs) were 1.24 m in diameter and 1.6 m tall including the frustum. To exclude rain water, a roof was attached 0.25 m above the upper part of the frustum in order to control the water availability to the plants by irrigation. Air was blown day and night through a circular perforated annulus situated 0.1 m above the canopy at a rate corresponding to approximately 10 $m^3$ $min^{-1}$.

Table 1
Timetable of events during the experiment

| Event | Date | Julian day |
|---|---|---|
| Sowing | 03 May 1995 | 123 |
| NPK fertilisation | 03 May 1995 | 123 |
| Installation of chambers | 24 May 1995 | 144 |
| Start of irrigation | 24 May 1995 | 144 |
| Frequency and amount of irrigation | Every other day, 10 mm | |
| Herbicide, MCPA | 16 June 1995 | 167 |
| Insecticide, Pirimor | 28 July 1995 | 209 |
| Start of ozone exposure, NF+pre | 31 May 1995 | 151 |
| End of ozone exposure, NF+pre | 09 July 1995 | 190 |
| Start of ozone exposure, NF+post | 09 July 1995 | 190 |
| End of ozone exposure, NF+post | 03 August 1995 | 215 |
| Removal of chambers and harvest | 03 August 1995 | 242 |

### 2.3. Cultural practices

Spring wheat *Triticum aestivum* L. cv. 'Dragon' was sown (240 kg seeds $ha^{-1}$) with 0.125 m row spacing. The fertiliser application rate was 120 kg N $ha^{-1}$, 24 kg P $ha^{-1}$ and 48 kg K $ha^{-1}$. Table 1 gives further details of the cultural practices. Irrigation was made with 10 mm water every second day. A herbicide and an insecticide were used to control weeds and aphids, respectively.

### 2.4. Experimental design

Three different chamber treatments were used each with five replicate chambers ($n$=5). The plants in the NF treatment received non-filtered air through the entire experiment. In the NF+pre treatment, ozone exposure was started on 31 May at growth stage 13, and ended 38 days later, at growth stage 59. The growth stages are according to the decimal code of Tottman and Makepeace (1979). The plants had then received an ozone dose of approximately 2500 nl $l^{-}$ h AOT40 (Fig. 1). In the NF+post treatment the ozone exposure was started at growth stage 59 and was stopped when an ozone dose of approximately 2500 nl $l^{-1}$ h AOT40 was achieved, which occurred after 26 days (Fig. 1). In addition to the chamber treatments, five ambient air (AA) plots were included

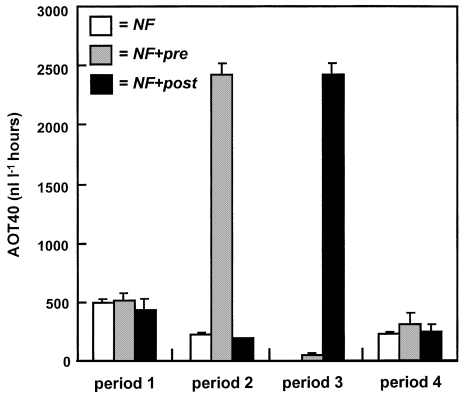


Fig. 1. Ozone exposure as AOT40 for the four periods: Period 1, Establishment; Period 2, Pre-anthesis exposure period; Period 3, Post-anthesis exposure period; Period 4, The remaining period until harvest. NF+pre, exposure before anthesis; NF+post, exposure during and after anthesis. Error bars show standard error.

in the experimental design in order to study the effect of the open-top chambers. The treatments were distributed in a completely randomised design.

### 2.5. Pollutant monitoring

Air samples were drawn through 50 m long Teflon (PTFE) tubes (1/4″ diameter), which were continuously ventilated and connected to a time-share sampling system with PTFE solenoid valves. Air was sampled in all chambers with the exception of one NF chamber. However, the variation between different chamber replicates of the different treatments was small (Table 2). Ozone concentrations were monitored using Thermo Environmental 49 UV absorption analysers. The ozone analysers were calibrated on a monthly basis using a portable ozone generator (Monitor Labs 8500). Data were collected with a Campbell Scientific CR10 data logger.

### 2.6. Climate monitoring

Air temperature and relative humidity were monitored using Rotronic YA-100 hygrometer/thermometers at 0.1 m above the crop in one chamber and at one ambient plot. Photosynthetically active radiation (PAR) was measured using a Li-Cor LI 190SA Quantum sensor in the ambient air. Vapour pressure deficit (VPD) was calculated according to Jones (1983). Climate data were stored using a Campbell Scientific CR10 data logger.

### 2.7. Harvests

To facilitate the harvest, each circular plot was marked by a plastic strip with a diameter of 1.10 m and a height of 0.06 m. The circular plot was divided into two equal parts. The southern part was used for the final harvest, when all above-ground plant material was harvested. The ears were separated from the straw and the number of ears was counted. The grain from each plot was threshed by hand and weighed. The water contents of the grain and the straw were determined by drying three subsamples at 70°C to constant weight. The rest of the grain was sent to an authorised agricultural laboratory (AnalyCen, Lidköping, Sweden) for determination of crude protein content (Kjeldahl nitrogen multiplied by 6.25) and 1000-grain weight.

The northern half of each plot was used for destructive harvests during the experiment, for the determi-

Table 2
Average ozone concentrations (over 12 h, during 08:00–20:00 hours local time) in the different treatments during the different treatment periods (Period 1, 13–30 May 1995; Period 2, 31 May to 09 July 1995; Period 3, 09 July to 03 August; Period 4, 04–30 August 1995)

| Treatment | *n* | Period 1 | | Period 2 | | Period 3 | | Period 4 | |
|---|---|---|---|---|---|---|---|---|---|
| | | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| AA, 1 m | 1 | 36 | | 33 | | 32 | | 32 | |
| NF | 4 | 34 | 0.3 | 25 | 0.3 | 20 | 0.3 | 26 | 0.2 |
| NF+pre | 4 | 35 | 0.3 | 40 | 0.8 | 23 | 1.9 | 27 | 1.8 |
| NF+post | 4 | 38 | 1.5 | 27 | 0.3 | 45 | 0.9 | 27 | 0.8 |

*n*: Number of chambers per treatment with ozone measurements.
SE: Standard error.

nation of the chlorophyll content of the flag leaves. The chlorophyll *a* and chlorophyll *b* content was determined according to Arnon (1949). On each of the sampling occasions, the flag leaves of ten plants per plot were harvested, cut into small pieces and then mixed. One subsample per plot was analysed for chlorophyll.

### 2.8. Statistical analysis

For all harvest parameters a one-way analysis of variance (ANOVA) was performed. Least significant differences (LSD) were calculated for the determination of significant differences between individual treatments when the *F*-test of the ANOVA was significant (Snedechor and Cochran, 1967). One AA plot, one NF+pre plot and one NF+post plot were excluded from the analyses, because of the occurrence of a large amount of couch grass (*Agropyron repens*) in one part of the experimental field. Thus, there were four replicates of these three treatments left for statistical analysis. This also explains why the number of chambers with ozone measurements in NF+pre and NF+post is four in Table 2.

## 3. Results

### 3.1. Ozone exposure

The growing period of the crop was divided into four parts: Period 1 (13–30 May) was the establishment phase of the plants when no ozone was added in any treatment, Period 2 (31 May-9 July) during which ozone was added to the NF+pre treatment, Period 3 (9 July-3 August) during which ozone was added to the NF+post treatment, and Period 4 (4–30 August), approximately from the end of grain filling until harvest, during which time no ozone was added to any of the treatments. During Periods 2 and 3 the 12-hour daytime ( between 08:00 and 20:00 hours) period average of the ozone concentration was 40 and 45 nl $l^{-1}$ in NF+pre and NF+post treatments, respectively. The ozone concentration was below 30 nl $l^{-1}$ in the NF treatment, as well as in the NF+pre treatment during Period 3 and in the NF+post treatment during Period 2 (Table 2). Fig. 1 presents the AOT40s of the different periods of the experiment. During Period 2, the AOT40 in the NF+post treatment was 195 nl $l^{-1}$ h and during Period 3 the AOT40 in NF+pre was 43 nl $l^{-1}$ h. During Period 1, the plants received an AOT40 of 437–519 nl $l^{-1}$ h and during Period 4 the AOT40 was 245–305 nl $l^{-1}$ h. Both the NF+pre and the NF+post plants received approximately 2500 nl $l^{-1}$ h AOT40 before or during and after anthesis, respectively.

The ozone concentration at plant level in the ambient air (AA) was higher than in the NF chambers during all periods except for Period 1, when the difference was small (Table 2). The reason for this is that the open-top chambers were installed at the end of Period 1. Due to deposition to plants, chamber walls and soil, the ozone concentration inside an open-top chamber is almost always lower than in the ambient air surrounding the chamber.

### 3.2. Climate

Table 3 shows the daytime and night-time temperatures inside and outside the chambers during Periods 2 and 3. The maximum chamber effect on temperature, +1.3°C, was obtained during daytime for Period 2. The average temperature was higher during Period 3 than during Period 2. This difference was more pronounced for daytime (4.6°C in the ambient air and 3.9°C in the chambers) than for night-time conditions (2.4°C in the ambient air and 2.5°C in the chambers).

Fig. 2 shows the average diurnal variation in the photosynthetically active radiation (PAR) in the ambient air during Periods 2 and 3. Fig. 3 presents the corresponding average diurnal variation of VPD in the NF chamber. It can be inferred that Period 3 was characterised by slightly more sunshine and lower VPD compared with Period 2.

Table 3
Daytime (08:00–20:00 hours) and night-time (20:00–08:00 hours temperatures (°C) in the ambient air (AA) and in the open-top chambers (OTC) during Period 2 (NF+pre) and Period 3 (NF+post)

| | AA daytime | AA night-time | OTC daytime | OTC night-time |
|---|---|---|---|---|
| Period 2 | 16.0 | 11.1 | 17.3 | 11.7 |
| Period 3 | 20.6 | 13.5 | 21.2 | 14.2 |

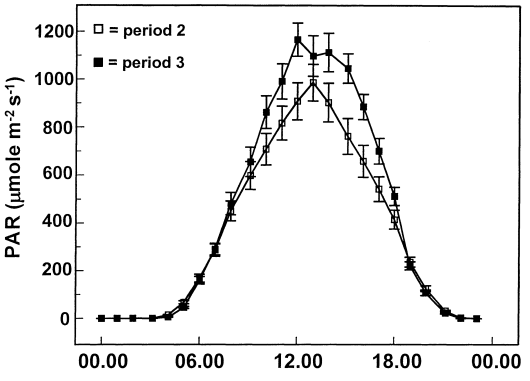


Fig. 2. Average diurnal variation in the photosynthetically active radiation (PAR) in the ambient air during Period 2 (NF+pre) and Period 3 (NF+post). Error bars show standard error.

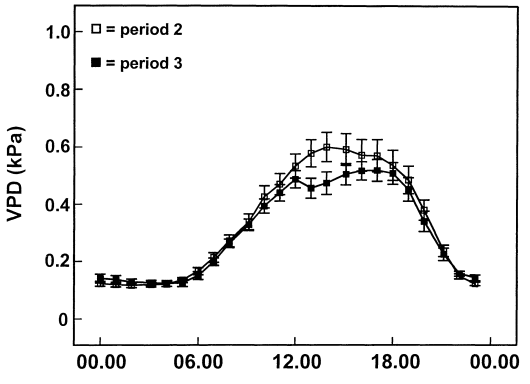


Fig. 3. average diurnal variation in vapour pressure deficit (VPD) in kPa in the chamber during Period 2 (NF+pre) and Period 3 (NF+post). Error bars show standard error.

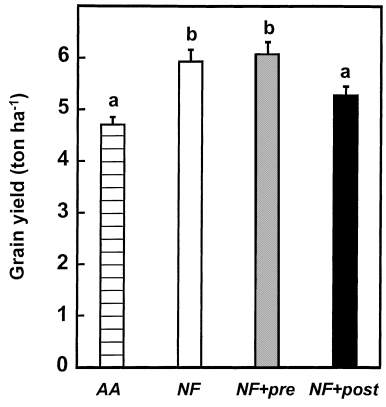


Fig. 4. Grain yield of spring wheat exposed to ambient air (AA), to non-filtered air (NF) or non-filtered air plus extra ozone either before (NF+pre) or during and after (NF+post) anthesis. Error bars show standard error. Significanct differences (ANOVA and LSD-test at the 95% level) are indicated by different letters above the bars.

### 3.3. Grain yield and protein content

The grain yield (Fig. 4) in the NF+post treatment was 11% lower than in the NF treatment and this difference was statistically significant. The difference in grain yield between the NF+pre treatment and the NF treatment was small (2%) and not statistically significant. There was a significant negative effect of the NF+post treatment compared with the other two treatments on the 1000-grain weight, but the harvest index and the water content of the grain at harvest was not significantly affected by any of the ozone treatments (Table 4). Fig. 5 shows that the protein content of the grain was significantly higher

Table 4
Number of ears per $m^2$, harvest index, straw yield, 1000-grain weight and water content of the grain in the different treatments at the final harvest (AA, NF, NF+pre, NF+post).

| | AA | | NF | | NF+pre | | NF+post | |
|---|---|---|---|---|---|---|---|---|
| | Mean | SE | Mean | SE | Mean | SE | Mean | SE |
| Ears ($m^{-2}$) | 565 | 20 ns | 614 | 14 ns | 610 | 36 ns | 552 | 13 ns |
| Harvest index | 0.47 | 0.011 a | 0.53 | 0.013 b | 0.53 | 0.011 b | 0.52 | 0.008 b |
| Straw yield (ton $ha^{-1}$) | 5.29 | 0.21 ns | 5.32 | 0.17 ns | 5.37 | 0.27 ns | 4.84 | 0.062 ns |
| 1000-grain yield (g) | 36.1 | 0.23 a | 41.7 | 0.70 b | 41.5 | 0.52 b | 38.1 | 1.14 a |
| Water content (%) | 10.9 | 0.20 ns | 9.8 | 0.18 ns | 9.8 | 0.16 ns | 9.6 | 0.14 ns |

SE: Standard error.
Different letters behind the standard error value of a certain parameter denotes a statistically significant difference ($P$=0.05).
ns: No significant treatment effects.

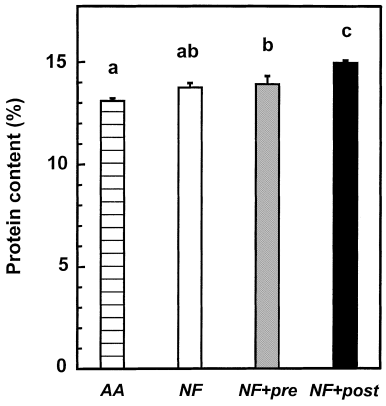


Fig. 5. Crude protein content of the grain of spring wheat exposed to ambient air (AA), to non-filtered air (NF) or non-filtered air plus extra ozone either before (NF+pre) or during and after (NF+post) anthesis. Error bars show standard error. Significant differences (ANOVA and LSD-test at the 95% level) are indicated by different letters above the bars.

in the NF+post treatment, 15.0% compared with 13.7% in the NF treatment and 13.9% in the NF+pre treatment. In AA the protein content, at 13.3%, was even lower.

### 3.4. Chlorophyll content of the flag leaves

Measurements of the chlorophyll content of the flag leaves started at anthesis and continued until two weeks after the end of grain filling (Fig. 6). The

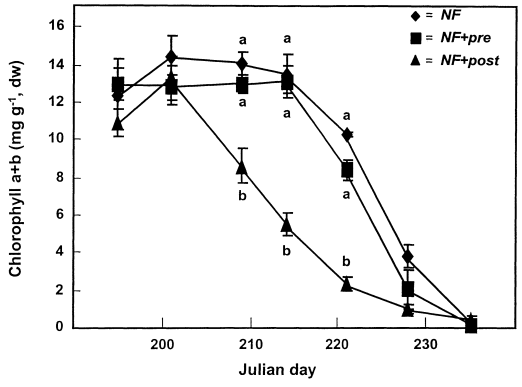


Fig. 6. Changes in chlorophyll *a* and *b* content of wheat flag leaves, expressed on a dry weight basis, with time in the different chamber treatments. The NF+post treatment was started on Julian day 190. Error bars show standard error. Significant differences on a certain sampling occassion (ANOVA and LSD-test at the 95% level) are indicated by different letters.

chlorophyll contents of the flag leaves from the NF and NF+pre treatments did not differ significantly from each other at any time. The chlorophyll content of the NF+post treatment started to decrease one week after anthesis.

## 4. Discussion

In this experiment, a certain AOT40 exposure (2500 nl $l^{-1}$ h) resulted in a negative effect on grain yield, as well as on straw yield and 1000-grain weight, when administered during and after anthesis, but not when administered before anthesis. It is therefore concluded that ozone exposure is much more effective in decreasing the grain yield of wheat between anthesis and the end of grain filling than before anthesis. This accords with what has been observed in beans, where ozone exposure after flowering had a much larger effect on pod yield than exposure before flowering (Younglove et al., 1994; Vandermeiren and De Temmerman, 1996) and in winter wheat (Soja, 1996). The increase in the protein content of grain accompanying ozone effects on the grain yield of wheat found in a number of studies, was detected in the present study only in the NF+post treatment. This further supports the conclusion that the post-anthesis period is more sensitive to ozone exposure in wheat than the pre-anthesis period.

The effect of ozone treatments on the chlorophyll content of the flag leaves was clear. Only the NF+post treatment had any effect on the chlorophyll (*a* and *b*) content and thus on the senescence of the flag leaves. Several studies have shown that the duration of flag leaves of wheat is shortened by ozone (Grandjean and Fuhrer, 1989; Ojanperä et al., 1992), and a shortening of leaf duration is likely to influence grain yield negatively (Evans, 1993; Pleijel et al., 1995).

The higher sensitivity to ozone after anthesis is consistent with observations at the biochemical level. Nie et al. (1993), for instance, showed that the ozone sensitivity in wheat leaves increase with leaf age. This can be explained by a higher concentration of anti-oxidants in young leaf tissue compared to old. Luwe et al. (1993) found that the higher content of ascorbate in young leaves protected them against visible leaf injury.

The weather differed between Period 2 (the NF+pre treatment period) and Period 3 (the NF+post treatment periods). The VPD was on average lower and the amount of PAR was, on average, higher during Period 3. Considering the effect on stomata of these factors (McLaughlin and Taylor, 1981), the ozone uptake by the plants may have been greater during Period 3 (Pleijel et al., 1994). On most days, however, the stomatal response to light is likely to have been saturated because the influence of light on stomatal conductance is small once light saturation of net photosynthesis is reached at about 600–700 μ mole $m^{-2}$ $s^{-1}$ (Larcher, 1995). The difference in PAR between Period 2 and Period 3 was small in the range below 700 μ mole $m^{-2}$ $s^{-1}$ (Fig. 2). The relatively modest difference in VPD, which was, on average, rather low on absolute terms, even for the warmest hours of the day, is not likely to have accounted for the clear difference between the NF+pre and NF+post treatments. It can, however, not be entirely excluded that the difference in VPD and light conditions between the two treatment periods modified to some extent the difference in ozone effects during the two exposure periods.

When defining air quality standards or critical ozone levels based on effects on crop yield loss in cereals and using integrative exposure indices of the AOT type, it is important to consider the delimitation of the integration period for the index. Presently in Europe, the suggested period for integrating ozone exposure as AOT40 is over a 3-month period at the so called Level I, which does not differentiate between different crop species or consider modifying factors (Fuhrer and Achermann, 1994; Kärenlampi and Skärby, 1996). For a certain plant, the three-month integration period may be too long a period to agree with the period of maximum sensitivity to ozone. For a so-called Level II approach, aiming at a yield loss assessment for spring wheat, a shortening of the period of integration should be considered in order to improve the predictions of yield loss by a given ozone exposure.

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