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OXIDATIVE STRESS

Effects of Ozone on Leaf Senescence, Photochemical Efficiency and Grain Yield in Two Winter Wheat Cultivars

S. Burkart[1], J. Bender[2], B. Tarkotta[2], S. Faust[2], A. Castagna[3], A. Ranieri[3] & H.-J. Weigel[2]

1 Thünen-Institute of Climate-Smart Agriculture Braunschweig, Germany
2 Thünen-Institute of Biodiversity Braunschweig, Germany
3 Department of Crop Biology, University of Pisa Pisa, Italy

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Correspondence
S. Burkart
Thünen-Institute of Climate-Smart Agriculture
Bundesallee 50
D-38116 Braunschweig
Germany
Tel.: +49 531 596 2546
Fax: +49 531 596 2599
Email: stefan.burkart@ti.bund.de

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Abstract

The adverse effects of tropospheric ozone (O_3) on crop photosynthesis, growth and yield have been documented in numerous studies over the last decades, but little information from field experimentation exists on how modern European winter wheat cultivars respond to O_3. Two winter wheat cultivars (Astron and Pegassos) differing in development characteristics were exposed to non-filtered ambient air or non-filtered air plus 30 ppb and non-filtered air plus 60 ppb O_3 (8 h day^{-1}) in open-top field chambers. At several dates during growth, green leaf area was determined by destructive harvests. Leaf gas exchange, pigment content and xanthophyll cycle activity, and photochemical efficiency by chlorophyll a fluorescence were measured. O_3 exposure induced accelerated senescence with no difference between cultivars. Photosynthesis declined especially in Pegassos; however, stomatal conductance was hardly affected by O_3. Pigment contents were reduced by O_3 exposure, and de-epoxidation index increased. Photochemical efficiency (F_v/F_m) declined, whereas actual quantum yield (Φ_{PSII}) did not respond to O_3. O_3 exposure reduced grain yield in both cultivars. However, yield of Pegassos was more affected by O_3 exposure than yield of Astron, suggesting a higher O_3 sensitivity of Pegassos. The data presented in this manuscript indicate a need to test whether high-yield varieties such as Pegassos are particularly sensitive to O_3 exposure.

Introduction

Tropospheric ozone (O_3) is a widespread secondary air pollutant found in all industrialized countries worldwide, and it is considered the most significant phytotoxic pollutant in the atmosphere affecting vegetation. Predictive models indicate that O_3 concentrations will continue to increase at a rate of 0.5–2 % per year in the Northern hemisphere during the next several decades (IPCC 2007). To date, the background O_3 concentration in the Northern hemisphere is within the range of 23–34 ppb (Vingarzan 2004). Current levels of O_3 pollution are known to be high enough to decrease carbon assimilation and to suppress growth and biomass production in many crop and wild plant species (Bender and Weigel 2003, 2011, Ashmore 2005, Mills et al. 2010, Pleijel 2011). Moreover, O_3 has been shown to mitigate possible benefits due to expected climate change in Europe (Clausen et al. 2011). The observed decline in the yield of major crops may imply a growing threat from ozone pollution to the security of food supplies (Weigel and Bender 2012, Wilkinson et al. 2012).

Ozone enters leaves through stomata, and it is thought to rapidly react with the aqueous phase of the mesophyllic cell wall matrix to form highly reactive oxygen species (ROS). As a consequence of this oxidative stress, accelerated senescence and leaf damages were often observed as one of the harmful effects on plants caused by O_3 (Wohlgemuth et al. 2002, Fuhrer and Booker 2003). Photosynthetic membranes are seen as main targets of ROS (Fiscus et al. 2005). Impairments of light reactions of photosynthesis can be detected by studying chlorophyll fluorescence, which is an acknowledged non-invasive tool, even before visible symptoms emerge (Sayed 2003). O_3 stress is known to reduce both the maximum (F_v/F_m) and effective quantum yield (Φ_{PSII}) of photosystem II photochemistry (Carrasco-Rodriguez and del Valle Tascon 2001, Calatayud 2007, Feng

et al. 2008). O_3-related damages of photosynthetic processes will increase the need to dissipate excessive energy due to overreduction of the photosystems. The de-epoxidation of violaxanthin (*V*) via antheraxanthin (*A*) to zeaxanthin (*Z*) is an important process in the dissipation of excess energy and protection of the photosynthetic membranes from injury (Niyogi 1999). The de-epoxidation state of the system (DEPS), that is, the proportion of de-epoxidized epoxide groups in total epoxide groups, indicates the degree of activation of light protection processes. A higher de-epoxidation state is related to lower light harvesting capacity and more energy dissipation in the pigment (Demmig-Adams and Adams 1992). Moreover, carotenoids such as β-carotene and *Z* are known to act as protectors of photosynthetic membranes against ROS (Young et al. 1997, Jahns et al. 2009). A stimulation of the xanthophyll cycle activity was observed especially in O_3-sensitive species (Castagna and Ranieri 2009), indicating enhanced non-photochemical dissipation processes.

Winter wheat is the predominant cereal crop across Europe. Wheat is known to be one of the most ozone-sensitive crops (Mills et al. 2007), but the vast majority of the research on wheat has been performed on spring wheat cultivars (Feng et al. 2008, Grünhage et al. 2012). Exposure or dose–response functions gained from these studies are used for quantifying effects of ozone on wheat yield and for the derivation of critical levels for O_3 on crops in Europe (Mills et al. 2007, 2011, Grünhage et al. 2012). However, there is a substantial variability in O_3 responses between cultivars (Bender et al. 1999, Feng et al. 2008, Gonzalez-Fernandez et al. 2010), and knowledge about O_3 effects on modern winter wheat cultivars is particularly scarce. For example, it is unclear whether winter wheat cultivars differing in ontogenesis and performance also differ in their sensitivity to O_3.

The objective of this study was to test how photosynthesis and senescence of winter wheat cultivars respond to chronic O_3 exposure and whether the responses can be related to yield responses and to differences in growth characteristics between the cultivars.

Material & Methods

Plant material, growth conditions and experimental design

An open-top field chamber (OTC) experiment was conducted in 2006 in an agro-ecosystem at the Johann Heinrich von Thünen-Institute (vTI) in Braunschweig, Lower Saxony, Germany (10°26′E, 52°18′N, 79 m a.s.l.), to examine the effects of O_3 on plant growth, yield and physiology in two field-grown winter wheat cultivars (CV): *Triticum aestivum* cv. Astron (*Ast*) and Pegassos (*Peg*). Astron is a medium late ripening blending wheat cultivar, with medium self-shading capacity (Saaten-Union 2012a). Pegassos is a medium early ripening quality wheat cultivar, with high self-shading capacity and drought resistance (Saaten-Union 2012b).

For the last 30 years, the local climate at the field site is characterized by a mean annual air temperature of 8.8 °C and a total precipitation rate of 618 mm year^{-1} (Germany's National Meteorological Service). The soil at the site is a luvisol of a loamy sand texture, with a pH of 6.3–6.5. For at least 30 years, the field has been used for agriculture. After ploughing and preparation of the field with a cultivator, plants were sown with 0.12-m row spacing in October 2005 (230 kg seeds ha^{-1}). Agricultural management measures were carried out according to local farming practices (Table 1). Total nitrogen added to the respective experimental area was 188 kg N ha^{-1}. Pesticide treatments were performed on demand to control insects and fungal infection. None of the pesticides used (Table 1) have been reported to affect crop responses to O_3. From begin of May until mid-June, experimental plots were regularly irrigated as needed to prevent drought stress. Total irrigation during this period was 68 mm. Plants were exposed to O_3 in OTCs, 3 m in diameter by 2.3 m height (for details refer to Weigel and Jäger 1988) beginning on 9 May 2006 (stem elongation). The centres of the OTCs were separated from each other by at least 12 m. Plants were fumigated with O_3 for 54 days (Table 1). Three O_3 treatments were employed in four replicate open-top chambers (n = 4): non-filtered (NF) ambient air, NF with additional 30 ppb (NF+) or NF with additional 60 ppb O_3 (NF++) for 8 h day^{-1} (10:00–18:00). Starting from May 22nd, O_3-dosage in NF ++ was reduced to 40 ppb due to the occurrence of visible symptoms in the highest ozone treatment.

Table 1 Timetable of events during the 2006 open-top chamber experiment GS = plant growth stages according to Tottman and Broad (1987)

Event/growth stage	Date
Sowing	07 Oct 2005
Emergence	16 Oct 2005
GS 31 (first node)	04 May 2006
GS 65 (mid-anthesis)	14 June
GS 92 (grain maturity)	22 July
Installation of OTCs	April 2006
Start of ozone fumigation	09 May
End of ozone fumigation	02 July
Days of exposure	54
Fertilizer application (kg N ha^{-1})	10 Mar (48)
	21 April (90)
	22 May (50)
Insecticides application (*Karate Zeon*™)	31 May
Fungicides application (*Opus Top*™)	08 May
	31 May
	21 June

Assessment of senescence and yield

Five to seven plants per cultivar and OTC were harvested at four harvest dates (04.05.2006 tillering, growth stage (GS) 31; 17.05.2006 booting, GS 39; 07.06.2006, pre-anthesis; 20.06.2006 grain filling, GS 71) and separated in leaves, stems and ears. The final harvest was done at grain maturity. Leaves were separated in green and yellow/dead leaves, and the projected area of green leaves (GLA) was measured with a LAI 3100 instrument (Li-COR, Lincoln, NE, USA). At maturity, grain yield was determined after drying to constant weight and threshing.

Chlorophyll fluorescence

Chlorophyll a fluorescence measurements were taken with a pulse-modulated chlorophyll fluorometer (FMS 2; Hansatech Instruments Ltd, Norfolk UK) *in situ* on flag leaves (Schreiber et al. 1986, Krause and Weis 1991). There is a discrepancy between the need to select the most representative part of the leaf and to avoid using yellow/dead leaves for fluorescence measurements. Anyway, for the measurements, only leaf parts that were still green were chosen, putting up with a possible bias in the results.

Maximum photochemical efficiency of photosystem II (F_v/F_m, Bolhàr-Nordenkampf et al. 1989) was measured daily from May 9th to July 2nd. Leaf clamps were fixed on the mid of flag leaf of two of the marked plants to pre-darken the leaves for 30 min. After opening the gate of the leaf clamp, a weak pulsed measuring light was given to obtain ground fluorescence F_0. A short intense light pulse was then given to close all reaction centres, and maximum fluorescence F_m was recorded. F_v/F_m was calculated as

$$F_v/F_m = (F_m - F_0)/F_m. \quad (1)$$

The actual quantum yield of photosystem II reaction centres (Φ_{PSII}, Genty et al. 1989) was measured twice per week on mid of unshaded flag leaves of all marked plants. The leaf was fixed in the leaf holder and kept under natural light conditions. A weak pulsed measuring light was given to obtain steady-state fluorescence F_s. A short intense light pulse was then given to close all reaction centres, and then the maximum fluorescence F_m' was recorded. The actual quantum yield of photosystem II was calculated as

$$\Phi_{PSII} = (F'_m - F_s)/F'_m. \quad (2)$$

Pigment analysis

Total chlorophyll content was estimated with a SPAD Chlorophyll meter (Minolta SPAD-502 (Uddling et al. 2007)) two times a week on several spots of flag leaves (except leaf base and apex) of the marked plants.

Flag leaves of at least 10 plants were harvested at anthesis (growth stage 61). Pigments were analysed by means of HPLC, according to the method of Castagna et al. (2001). Leaves were homogenized in the dark in 100 % HPLC-grade acetone with sodium ascorbate and filtered through 0.2-μm filters. The separation was performed using a Zorbax ODS column (Chrompack SA, 5 mm particle size, 250 × 4.6 mm diameter). Pigments were eluted using 100 % solvent A (acetonitrile/methanol, 75/25, v/v) for the first 15 min, followed by a 2.5-min linear gradient to 100 % solvent B (methanol/ethylacetate, 68/32, v/v) which continued isocratically until the end of the 32-min separation. The flow rate was 1 ml min^{-1}. The pigments were detected by their absorbance at 445 nm. To quantify pigment content, known amounts of a pure standard were injected into the HPLC system and an equation, correlating peak area to pigment concentration was formulated. Pigment concentration was expressed as nmol g^{-1} fresh weight. The de-epoxidation index (DEPS, %) was calculated as

$$DEPS = (A/2 + Z)/(V + A + Z)^*100, \quad (3)$$

where A, Z and V represent the content in antheraxanthin, zeaxanthin and violaxanthin, respectively.

Leaf gas exchange

Leaf gas exchange was measured on flag leaves using an open gas-exchange system (LI-6400; LI-COR). Gas-exchange measurements were taken from May 5th to June 25th, from 08:30–19:00 h. The sequence of OTCs measured was randomized each day to exclude any bias caused by diurnal variation. Measurements were taken under natural light conditions using the transparent cuvette. Photosynthetic photon flux density (PPFD, μmol m^{-2} s^{-1}) and air temperature (T, °C) were recorded at the leaf level. The water vapour concentration of air entering the chamber was not controlled and therefore tracked ambient conditions. The chamber [CO_2] was set to 380 μmol mol^{-1}. Readings were taken once the coefficient of variation (stability variables: sample cell (CO_2), sample cell (H_2O) and flow) of the infra red gas analyzer output was below 0.7 %. Each reading took at least eight minutes to ensure that g_s had enough time to equilibrate to the vapour pressure deficit (VPD, kPa) in the leaf chamber. Net photosynthesis (P_N, μmol m^{-2} s^{-1}), stomatal conductance (g_s, mol m^{-2} s^{-1}) and the ratio of leaf internal/ambient CO_2 concentration (C_i/C_a) were calculated using the equations of von Von Caemmerer and Farquhar (1981).

Data processing

The effects of O_3 on leaf growth and senescence, gas exchange and fluorescence as dependent variables were

tested by three-factorial analysis of variance (date × O_3 × CV) for n = 4 replicated chambers. Average values of the variables calculated for each chamber half were used as statistical unit. Data were analysed with the R Software Package V. 2.12.0 (http://www.r–project.org/) as a completely randomized design with NF, NF+ and NF++ treatments (n = 4 each) each split for cultivar (total n = 24). As pigments and yield were only analysed for one date (anthesis and final harvest, resp.), two-way ANOVA was applied in this case. P-values lower than 0.05 were considered as significant.

Results

Ozone concentration

O_3 concentration in the NF-chambers superseded 40 ppb in the first half of May and at the end of the measuring period, and 60 ppb in mid-June (Fig. 1). In the OTCs with O_3 enrichment, maximum values of O_3-concentration reached 60 ppb in NF+ and 80 ppb in NF++ in these periods. From the end of May, O_3-dosage was reduced to +25 ppb (NF+) and +40 ppb (NF++) due to the occurrence of visible injuries. The 8-h seasonal means for the whole exposure period were 36 ppb in NF, 51 ppb in NF+ and 58 ppb in NF++. The resulting average O_3 exposure (accumulated exposure over threshold 40 ppb; AOT40) was 1.70 ppm.h in NF, 6.46 ppm.h in NF+ and 10.04 ppm.h in NF++.

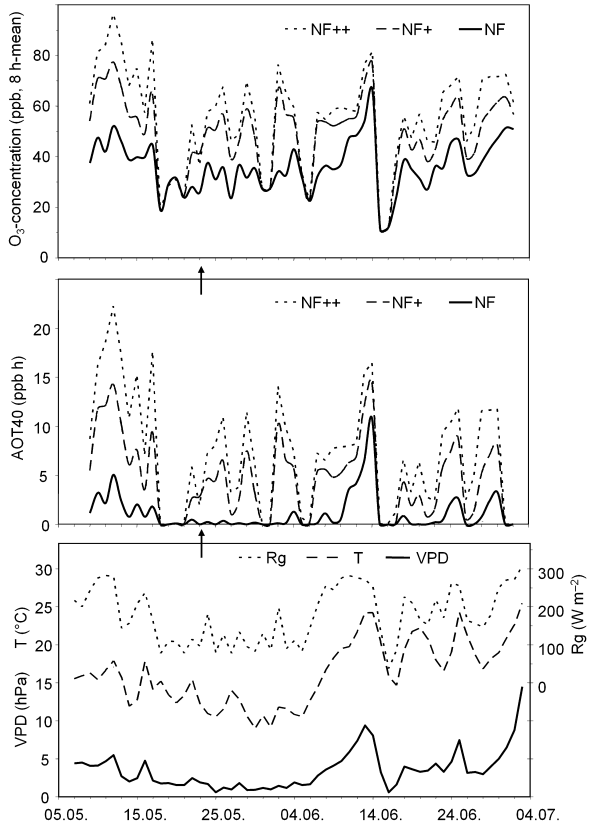

Fig. 1 Seasonal courses of O_3-concentrations (8 h-mean, 10–18 h), AOT40 and weather parameters. NF: Non-filtered ambient air, NF+: NF + 30 ppb O_3, NF++: NF + 60 ppb O_3. VPD vapour pressure deficit (hPa), *T* temperature (°C), Rg global radiation (W m^{-2}). Arrows indicate the start of the reduction in the amount of O_3 added in NF++ to 40 ppb.

Visual symptoms of senescence

Ozone exposure caused leaf damages in *Ast* and *Peg*, visible as spots, necrosis and yellowing. In the course of the season, different patterns of injury between the cultivars were observed: a rather homogenous yellowing occurred in *Peg*, whereas in *Ast*, increasing proportions of the leaf blade completely died off with a corresponding loss of green leaf area. The green parts, however, remained nearly completely green, with no substantial change in colour.

Green leaf area

Green leaf area (GLA) increased to ca. 60 cm^2 per plant at GS 39 and remained constant until anthesis in NF plants (Fig. 2). GLA of *Ast* tended to be lower than of *Peg*, but this was not significant (P = 0.23 at GS 31). In both cultivars, O_3 exposure induced a significant decline of GLA starting from the 2nd harvest in both O_3-treatments (P < 0.001). The reduction in GLA due to O_3-fumigation reached 75 % at GS 56, with no differences between NF+ and NF++ or between cultivars.

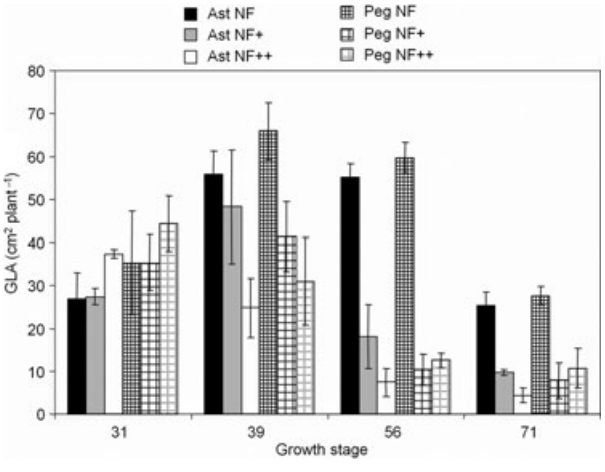

Fig. 2 Green leaf area as determined from destructive harvest at different growth stages. NF: Non-filtered ambient air, NF+: NF + 30 ppb O_3, NF++: NF + 60 ppb O_3. Error bars represent standard error of mean (n = 4).

Gas exchange

Due to high variation of the data, three-way ANOVA indicated that there was neither any difference between cultivars in g_s, nor any O_3 effect on g_s (Fig. 3a), although g_s of *Peg* seemed to be slightly reduced by O_3 after anthesis. In both cultivars, O_3 exposure reduced P_N after booting stage ($P = 0.049$, Fig. 3b). At grain filling stage, O_3 exposure reduced P_N by 30 % in *Ast* and 43 % in *Peg*. P_N of *Peg* tended to be lower than that of *Ast* especially at the end of the season ($P = 0.095$). The ratio internal/ambient CO_2 concentration (C_i/C_a) did not differ between cultivars, but was increased by O_3 at the 2nd and 3rd measuring date ($P = 0.026$, Fig. 3c). There was no O_3 x CV interaction on leaf gas-exchange parameters at any growth stage.

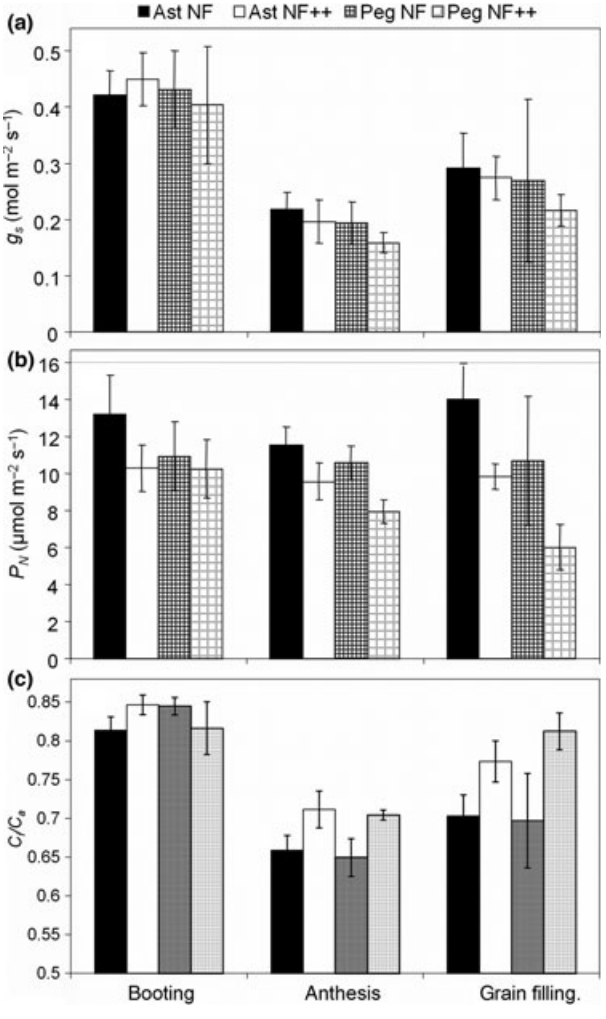

Fig. 3 A Stomatal conductance (g_s), B net photosynthesis (P_N) and C the ratio internal/ambient CO_2 concentration (C_i/C_a) averaged across different measuring periods (booting 17.05.–24.05., anthesis 07.06.–14.06., ripening 25.06.). NF: Non-filtered ambient air, NF++: NF + 60 ppb O_3. Error bars represent standard error of mean (n = 4).

Pigments

In both cultivars, SPAD values (estimate of total chlorophyll) of flag leaves declined in the course of the growth period ($P < 0.001$, Fig. 4). At all growth stages, the SPAD value of *Ast* was higher than that of *Peg* ($P < 0.001$). The O_3-exposure reduced SPAD at all growth stages ($P < 0.001$). There were no interactions between cultivar and O_3 at any growth stage, although the SPAD values tended to reflect the differences in visual symptoms described above.

As found for SPAD values, the concentrations of all pigments measured were significantly lower in *Peg* than in *Ast* ($P = 0.031$ for total chlorophylls, $P = 0.030$ for total carotenoids, $P = 0.060$ for β-carotene, $P = 0.032$ for the $V + A + Z$ sum, $P = 0.017$ for total xanthophylls) when averaged across all O_3-treatments, while the de-epoxidation state of the xanthophyll cycle was similar between the two cultivars (Fig. 5). O_3 exposure resulted in a clear decline in pigment concentrations ($P < 0.001$), with no $O_3 \times$ CV interaction for any pigment. The small differences in pigment concentrations between NF+ and NF++ were not significant. The percentage of decrease was higher for chlorophylls (−41 % and −54 % in *Ast*, and −55 % and −64 % in *Peg*, in NF+ and NF++ samples, respectively) than for carotenoids (−34 % and −44 % in *Ast*, and −41 % and −50 % in *Peg*, in NF+ and NF++ samples, respectively). Statistical analysis showed a significant increase in DEPS due to O_3 exposure ($P = 0.013$) with no

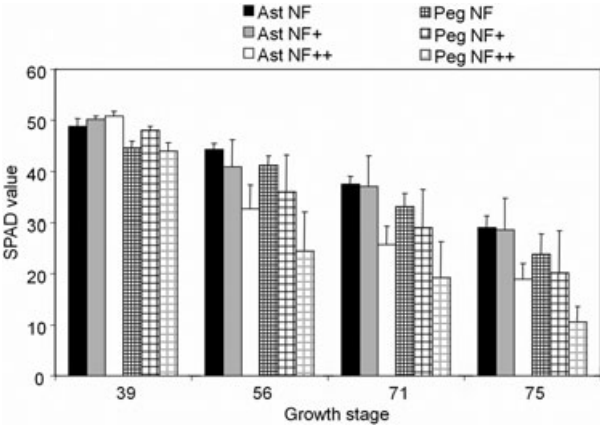

Fig. 4 Relative chlorophyll content measured with SPAD chlorophyll meter at different growth stages. NF: non-filtered ambient air, NF+: NF + 30 ppb O_3, NF++: NF + 60 ppb O_3. Error bars represent standard error of mean (n = 4).

difference between NF+ and NF++, whereas there were no differences in DEPS between cultivars.

Chlorophyll a fluorescence

Throughout the measuring period, F_v/F_m (potential quantum yield, Fig. 6a) was higher in *Ast* than in *Peg* ($P = 0.016$). At GS 39, O_3-fumigation did not affect F_v/F_m. However, starting with GS 56, F_v/F_m responded to O_3-fumigation in both cultivars ($P < 0.001$). Moreover, especially in NF++, F_v/F_m declined much more in *Peg* than in *Ast* at GS 56 ($P = 0.068$). These changes in F_v/F_m at GS56 were mainly caused by a decrease in F_v ($P = 0.024$), whereas F_0 remained unaffected by O_3 (Fig. 6c,d). Towards the end of the measuring period, F_v/F_m recovered in both cultivars. After recovery, there were no significant responses of F_v/F_m to O_3 nor any interactions between O_3 and cultivars. However, the recovery of F_v/F_m at GS 71 was mainly due to a decrease in F_0 ($P = 0.086$) which appeared to be more pronounced in *Peg*, but this difference between cultivars was not significant.

With respect to climatic conditions and growth stages, the measuring period was divided into three sub-periods: 1: May 23rd to June 6th, 2: June 7th to June 19th, 3: June 28th. Φ_{PSII} (actual quantum yield) varied with PPFD (Fig. 6b), but was not affected by O_3-fumigation at any period ($P = 0.27$). As was found for F_v/F_m, Φ_{PSII} of *Peg* was lower than of *Ast* at all growth stages ($P = 0.004$). There was no $O_3 \times$ CV interaction on Φ_{PSII} at any growth stage.

Grain yield and biomass

Two-factorial ANOVA showed that grain yield of *Peg* was significantly higher than yield of *Ast* (+29.1 % in NF, +19.6 % in NF+ and +10.5 % in NF++, $P = 0.004$, Table 2). Similar results were found for straw mass (1031.5 ± 31.8 gm^{-2} in *Peg*, 832.0 ± 34.2 g m^{-2} in *Ast*, data not shown). O_3-fumigation reduced grain yield of both cultivars ($P < 0.001$). In *Ast*, the reduction of grain yield amounted −13.1 % in NF+ and −19.4 % in NF++, whereas in *Peg* O_3-fumigation reduced grain yield by −19.5 % in NF+ and −31.1 % in NF++. However, a

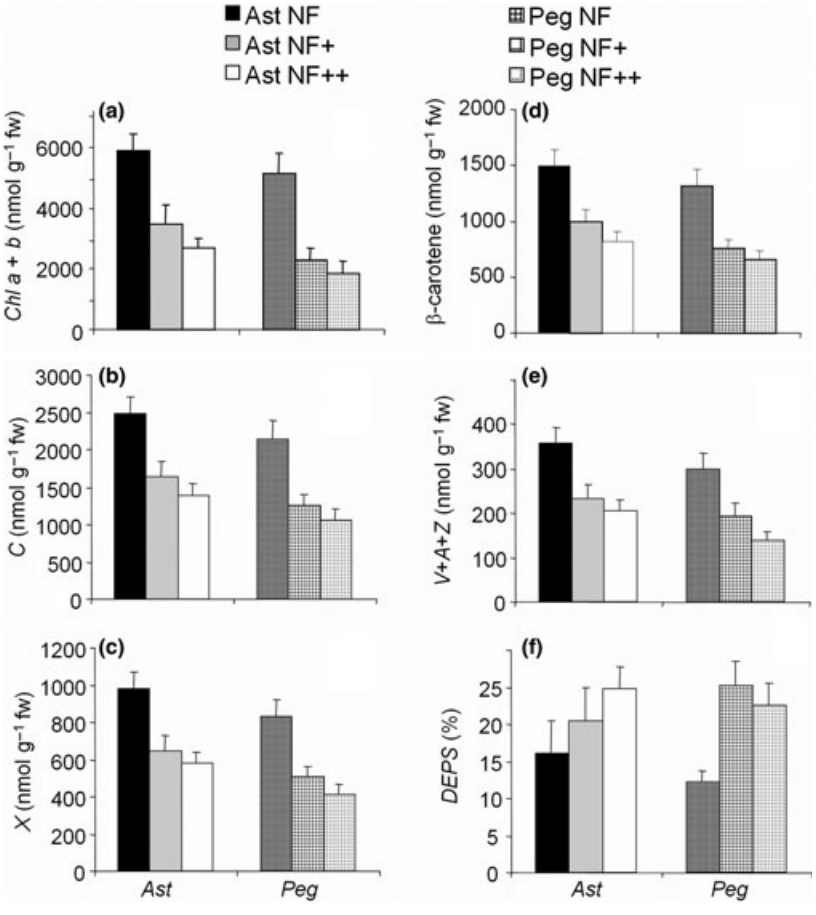

Fig. 5 Content of A chlorophyll a + b (*Chl*), B total carotenoids (*C*), C total xanthophylls (*X*), D *β*-carotene, E the sum of violaxanthin (*V*), antheraxanthin (*A*) and zeaxanthin (*Z*), and (F) de-epoxidation index (DEPS) at anthesis. NF: Non-filtered ambient air, NF+: NF + 30 ppb O_3, NF++: NF + 60 ppb O_3. Error bars represent standard error of mean (n = 4).

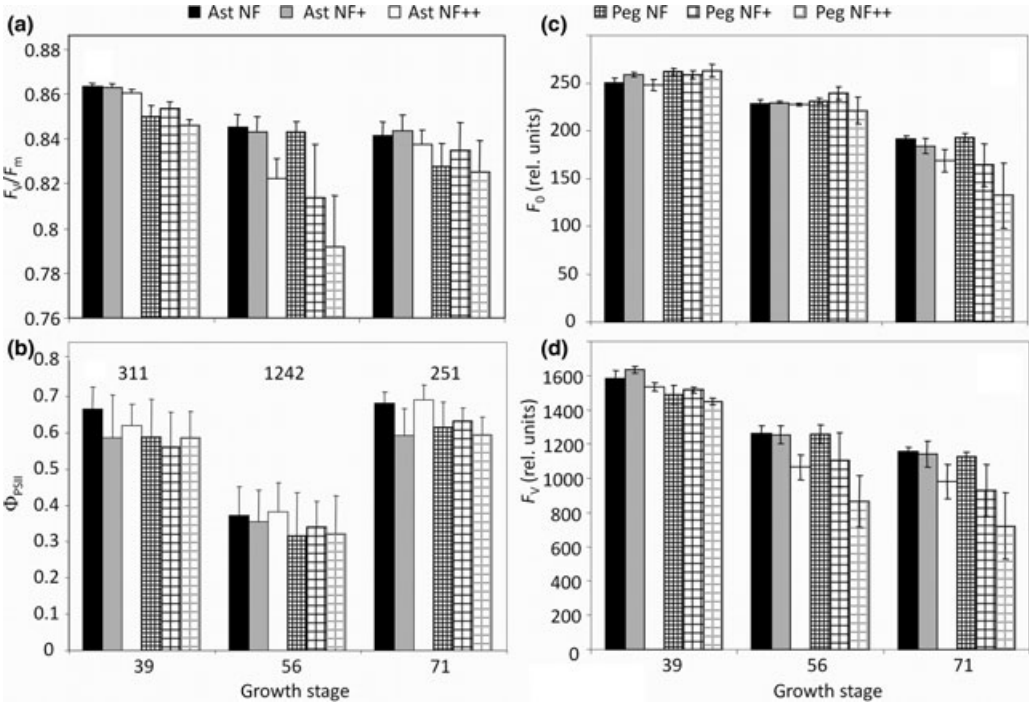

Fig. 6 (a) Maximum quantum yield (F_v/F_m) of photosystem II, (b) effective quantum yield (Φ_{PSII}) of photosystem II, (c) ground fluorescence (F_0) and (d) variable fluorescence (F_v) at different growth stages. NF: Non-filtered ambient air, NF+: NF + 30 ppb O_3, NF++: NF + 60 ppb O_3. Error bars represent standard error of mean (n = 4). Please note for panel B: Values of Φ_{PSII} were averaged across three different measuring periods. Hence, growth stage numbers in panel B cover 23.05.–06.06. (GS 39), 07.06.–19.06. (GS 56), 28.06. (GS 71). Numbers above columns indicate mean photon flux densities (μmol m^{-2} s^{-1}) during measurements.

Table 2 Grain yield (g m^{-2}) as determined at maturity (GS 92). NF: non-filtered ambient air, NF+: NF + 30 ppb O_3, NF++: NF + 60 ppb O_3. Values represent means ± standard error of means (S.E., n = 4)

	Astron	Pegassos
NF		
Mean	688.1	888.4
±S.E.	30.1	30.4
NF+		
Mean	598.1	715.3
±S.E.	50.6	49.8
NF++		
Mean	554.3	612.5
±S.E.	54.1	52.8

significant O_3 × CV interaction on grain yield could not be proven (P = 0.32).

Discussion

In this study, chronic O_3 exposure decreased photosynthesis and caused accelerated senescence in two winter wheat cultivars. Both cultivars responded to the chronic O_3 exposure by reducing green leaf area, via an accelerated senescence and resulting in a loss in grain yield. This was also found in many but not all studies with wheat (Reichenauer et al. 1998, Ewert and Pleijel 1999, Sild et al. 2002).

Our data showed only a slight decrease in stomatal conductance in *Peg* caused by O_3 fumigation, especially after anthesis. However, the overall reduction in g_s of wheat due to O_3 as reported by Feng et al. (2008) could not be confirmed in the present study, which may be due to small effects and the high variability of the data. Moreover, both the differences in the means of g_s between the cultivars and the effects of O_3 on g_s were smaller than the O_3-effects on P_N. Ozone exposure led to a decline in net CO_2 fixation in both cultivars, which is in line with many other experiments with wheat (Feng et al. 2008). However, in several studies, photosynthetic and growth responses to O_3 could not be related to variations in g_s (Reichenauer et al. 1998, Herbinger et al. 2002, Akhtar et al. 2010, Feng et al. 2011). Although g_s likewise tended to decrease in this study in response to O_3, the reduction in photosynthesis was mainly due to decreases in CO_2-fixation processes (Singh et al. 2009) because *Ci/Ca* was increased under O_3 fumigation, whereas Φ_{PSII} did not respond to O_3.

The ozone-induced decrease in both chlorophyll and carotenoid concentration suggests a generalized negative impact of the pollutant on photosynthetic pigments, although chlorophylls tended to be more affected than carotenoids. Carotenoids, apart from their role as secondary light-absorbing pigments, are able to reduce the chlorophyll triplet state and to prevent the formation of harmful singlet oxygen or to scavenge it after its production by the interaction of triplet chlorophyll with O_2 (Young et al. 1997). It is well known that when absorbed light exceeds plant photochemical requirement as may occur also under normal light irradiation, but in the presence of environmental constraints, this excess energy may be transferred to the ever-present oxygen, leading to the formation of ROS and, ultimately, to damage at the photosystem level (Castagna et al. 2001, Ort 2001, Demmig-Adams and Adams 2006). In this respect, O_3 stress resembles plant responses to many other abiotic stresses such as drought (Singh et al. 2012), UV-B radiation (Lidon et al. 2012) or heat stress (Dias et al. 2011). CO_2 assimilation is the major sink for the reducing equivalents and energy produced by the primary photochemical reactions. Therefore, a diminished CO_2 fixation will induce a decreased demand for ATP and NADPH and, consequently, may lead to an over-reduction in PSII. Ozone-treated samples, which exhibited a decline in net CO_2 fixation in comparison with their respective controls, might be expected to activate the xanthophyll cycle to dissipate the large amount of excitation energy absorbed by the antenna. The increase in DEPS observed in both wheat cultivars underly the thylakoid capacity to react and acclimate to O_3, allowing a decrease in the PSII photochemical rate and subsequent alleviation of excitation pressure on the reaction centres.

In this study, chronic O_3 exposure reduced the performance of photosynthetic electron transport chain. The exposure to O_3 induced a decrease in F_v/F_m more in *Peg* than in *Ast*. Similar to our results, F_v/F_m was found to decline under O_3 exposure in soybean (Chen et al. 2009), in *Quercus mongolica* (Wang et al. 2009b) and in wheat at grain filling (Wang et al. 2009a). However, after reducing the amount of O_3 added after 22nd May, F_v/F_m recovered to nearly initial values especially in *Peg*. These changes in F_v/F_m may indicate a plasticity of the photosynthetic system (Bussotti et al. 2011). Reichenauer et al. (1998) showed that a recovery of F_v/F_m may occur even overnight but not in all wheat cultivars of their study. The decrease in F_0 as observed in this study, however, indicates an increased thermal dissipation and not an inactivation of photosystem II (Guidi et al. 2000, 2002, Leipner et al. 2001, Bussotti et al. 2011), which is in line with the observed increase in DEPS. The decrease in F_v under O_3 fumigation may be attributed to the pigment losses.

In contrast to other experiments with wheat (Feng et al. 2008), O_3 fumigation impaired the performance of photosynthetic electron transport (Φ_{PSII}) only marginally. Adams and Demmig-Adams (2004) showed that Φ_{PSII} cannot be seen as a measure of Pn. Together with the sensitivity of F_v/F_m to O_3, this is a further evidence that it is not the electron transport chain *per se* that is impaired by O_3 but the light harvesting capacity due to pigment loss and the use of reductive equivalents. This may reflect a strategy described by Bussotti et al. (2011) for poplar showing that leaves try to maintain the efficiency of the remaining green leaf area around necrotic regions at an optimal level. Literature, however, is unequivocal although most studies reported a decrease in Φ_{PSII} under O_3 fumigation depending on species, cultivars and exposure duration (Ciompi et al. 1997, Endo et al. 2005, Calatayud et al. 2006, Degl'Innocenti et al. 2007, Wang et al. 2009a). Moreover, in this study, senescence behaviour differed between cultivars, thus preferring leaf parts that are still alive for fluorescence measurements may cause a bias in the data. Only a few studies addressed the spatial heterogeneity of visible damages by likewise selecting green leaves or areas for measurement (Guidi et al. 2000, Carrasco-Rodriguez and del Valle Tascon 2001). Moreover, visible damages do not necessarily entail changes in fluorescence parameters and *vice versa* (Carrasco-Rodriguez and del Valle Tascon 2001, Konishi et al. 2005).

The observed O_3-induced yield reductions in both cultivars (yield reduction in NF++ of 19 % and 31 % in *Ast* and *Peg*, respectively) suggest that modern winter wheat cultivars exhibited a similar high O_3 sensitivity as spring-sown cultivars do. The meta-analysis performed by Feng et al. (2008) revealed that O_3 exposure to an average of 72 ppb (7-h seasonal mean) reduces wheat grain yield by 29 % (95 % confidence interval: 24–34 %). The majority of studies considered in this meta-analysis were performed with spring wheat. Nevertheless, a direct comparison between spring wheat and winter wheat in the responses to elevated O_3 is difficult to draw due to the lack of comparative experiments with the two wheat types in which they are simultaneously exposed to O_3. However, in the present study, grain yield of *Peg* responded more sensitively to O_3 exposure as compared to *Ast* which is correlated with the overall higher growth and yield performance of *Peg*. Photosynthesis was also more affected by O_3 in *Peg* than in *Ast*, as indicated by a larger reduction in *Pn* and the decrease in F_v/F_m. The physiological or genetic basis for the variation in O_3 sensitivity between genotypes is poorly understood (Fiscus et al. 2005); however, the differences in O_3 sensitivity between the two cultivars cannot be attributed to differences in g_s and thus to differences in O_3 uptake or flux. Moreover, there are examples where the O_3 response of grain yield did not necessarily correlate to the extent of leaf

damage (Picchi et al. 2010). The data presented in this manuscript indicate a need to test whether high-yield varieties such as *Peg* are particularly sensitive to O_3 exposure.

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