Available online at www.sciencedirect.com
SCIENCE DIRECT
Environmental and Experimental Botany 56 (2006) 63–71
Environmental and Experimental Botany
www.elsevier.com/locate/envexpbot

Differential ozone sensitivity in an old and a modern Swedish wheat cultivar—grain yield and quality, leaf chlorophyll and stomatal conductance

H. Pleijel [a,*], A. Berglen Eriksen [b], H. Danielsson [c], N. Bondesson [d], G. Selldén [d]

[a] *Göteborg University, Applied Environmental Science, P.O. Box 464, SE-405 30 Göteborg, Sweden*
[b] *University of Oslo, Department of Biology, The Phytotron, P.O. Box.1066, N-0316 Oslo, Norway*
[c] *Swedish Environmental Research Institute, P.O. Box 5302, SE-400 14 Göteborg, Sweden*
[d] *Göteborg University, Botanical Institute, P.O. Box 461, SE-405 30 Göteborg, Sweden*

Received 16 June 2004; accepted 6 January 2005

Abstract

Field-grown crops of one modern ('Dragon') and one 100-year old ('Lantvete') wheat (*Triticum aestivum* L.) cultivar were exposed to charcoal-filtered air (CF) or non-filtered air plus additional ozone (NF+) in open-top chambers ($n = 6$). Ambient air plots ($n = 3$) were used to monitor the effects of the chamber enclosure. Quantitative (ear weight, harvest index and 1000-grain weight), as well as qualitative (grain concentration of N, P, K, Ca, Mg, Cd), aspects of crop yield were studied. In addition, the chlorophyll content of the top leaf was followed during the 10-week-long ozone exposure and flag leaf stomatal conductance was monitored. Ear weight was negatively affected by ozone. The effect was smaller in the lower yielding 'Lantvete' compared to the modern cultivar 'Dragon'. A similar ozone effect was found for the harvest index and 1000-grain weight. The difference between cultivars was small for 1000-grain weight, with harvest index significantly lower in 'Lantvete' compared with 'Dragon'. The crude protein concentration of the grain was higher in 'Lantvete', and was enhanced by ozone exposure. No significant treatment or cultivar effects were observed on grain Ca and Cd concentrations, but the greatest chamber effect was on Cd, which was considerably higher in grain from treatment chambers compared with ambient air (possibly due to the higher rate of transpiration in the plants enclosed in the chambers). There were strong differences with respect to grain P, K and Mg concentration between cultivars; 'Lantvete' exhibiting higher concentrations than 'Dragon' in all cases, apart from K. Weak, but significant, increases in grain P, K and Mg concentrations were evident in O_3-treated plants. The chlorophyll concentration of the flag leaf was negatively affected by ozone exposure after approximately 1 month, but chlorophyll content declined faster in 'Dragon' than 'Lantvete'. Flag leaf chlorophyll concentration was significantly lower in 'Lantvete' compared with 'Dragon' before onset of ozone effects in NF+ and during most of the experiment in CF. Stomatal conductance was also significantly lower in 'Lantvete' than in 'Dragon'. The most important conclusion drawn from the present study is that the 'older' cultivar, 'Lantvete', was less affected by ozone than the modern, bred cultivar, 'Dragon'. This observation might to a large extent be explained by the higher stomatal conductance exhibited by the modern wheat cultivar. The result is consistent with earlier investigations for Greek wheat cultivars, and suggests that the findings reported by others, based on vegetative growth, may extend to grain yield. Growth dilution effects (i.e. the lower observed concentration of an element at higher biomass yield) may explain observed differences between cultivars, and between ozone treatments, in the level of various elements, except for potassium.

Keywords: Breeding; Conductance; Cultivar; Inadvertent selection; Ozone; *Triticum aestivum*; Yield; Protein

1. Introduction

Over the past 100 years, plant breeders have succeeded in greatly improving crop yields. In wheat (*Triticum aestivum* L.) the increase in grain yield has been associated with shifts in carbon partitioning. For example, the harvest index (HI, the

* Corresponding author. Tel.: +46 31 773 2532; fax: +46 31 773 2984.
E-mail address: hakan.pleijel@miljo.gu.se (H. Pleijel).

0098-8472/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.envexpbot.2005.01.004

proportion of the above-ground biomass found in the grain at harvest) has increased, with this effect accounting for most of the recent improvement in yield (Evans, 1993).

An increase in crop yield may lead to reduced investments in other organs or functions, which can be associated with a loss of stress adaptation or by an increasing demand for the provision of additional management such as irrigation, weed and pest control (Blum, 1983; Evans, 1993). Furthermore, when selecting for higher wheat yields, breeders have produced cultivars having a high harvest index that depend more on photosynthesis for the production of carbohydrates during grain filling than on retranslocation of stored carbohydrates (Evans et al., 1975). Net photosynthesis during grain filling, as well as leaf duration, is therefore of considerable importance for the realisation of the high yield potential of these cultivars.

Based on experiments with ten genotypes of Greek wheat cultivars, bred and introduced between 1932 and 1980, Barnes et al. (1990) and Velissariou et al. (1992) concluded that ozone sensitivity had increased during the 50-year study period, and that inadvertent selection by breeders may be responsible for the observed effects (Velissariou et al., 1992). The authors concluded that the phenomenon may be related partly to selection for increased gas exchange (Velissariou et al., 1992), but there was also the suggestion that shifts in stomatal conductance were not of sufficient magnitude to fully explain the observed shifts in ozone sensitivity (Velissariou et al., 1992). A further explanation proposed by Barnes et al. (1990) was that the use of agrochemicals, many of which are known to act as antioxidants or ozone protectants (Mooi, 1982), increased dramatically during the last century and their application may thus have masked the effects of ozone during field trials.

One of the limitations of the studies conducted by Barnes et al. (1990) and Velissariou et al. (1992) was that the plants were grown in controlled environment chambers and only long enough to develop ears; studies on grain yield were not performed. Thus, it remains to be established whether the observed increase in ozone sensitivity in modern-day wheat cultivars extends to grain yield and is a common phenomenon (see Barnes et al., 1999).

In addition to effects on yield and yield components, ozone has been shown to affect elemental composition of grain (e.g. nitrogen, potassium, magnesium and calcium). This effect is of importance for the nutritional quality of wheat-based products. Typically, the reduction in yield by ozone has been associated with an increase in the concentration of these minerals (Fuhrer et al., 1990; Pleijel et al., 1998), commonly attributed to a growth dilution effect. The dilution is chiefly with carbohydrates, which in wheat grain mainly represented by starch (Kibite and Evans, 1984). Such an effect suggests that the concentration, though not the content, of minerals recorded in grain may be expected to be depressed in higher yielding genotypes and for growth promoting conditions (such as elevated CO_2), and vice versa when growth is depressed, such as by ozone (Pleijel et al., 1998). The mineral content of the grain grows at a slower rate than the biomass when biomass is increased, according to this principle.

Bread resulting from wheat flour is a major source of the highly toxic element cadmium in the human diet. This can cause kidney dysfunction and affect the mineral metabolism of the skeleton (Elinder and Järup, 1996). Shifts in Cd accumulation in grain due to environmental influences and of genetic factors is thus of importance in estimating the risks associated with this element.

The present investigation was undertaken to compare the ozone sensitivity of a 'modern' and an 'old' (early twentieth century) Swedish wheat cultivar. Ozone sensitivity was assessed in terms of grain yield, yield components, protein and other minerals, leaf chlorophyll concentration and stomatal conductance. The hypothesis was that the impact of ozone on grain yield in the 'modern' cultivar would exceed those on the 'old' cultivar and that this would, at least partly, be associated with higher stomatal conductance in the 'modern' cultivar.

2. Materials and methods

2.1. Experimental site

The experiment was situated in a wheat field at Östads säteri, 50 km North-East of Göteborg, Sweden (57°54′N, 12°24′E). The field was situated 60 m above sea level and soil comprised loamy sand, which had been used for agriculture for an extended period. During the growing season before the present experiment, the field was cultivated with potato. No major air pollutant sources are located in the vicinity of the investigation area.

2.2. Cultural practices

Spring wheat, *Triticum aestivum* L. cv. 'Dragon' and cv. 'Lantvete', was sown (240 kg seeds ha^{-1}) with 0.125 m row spacing, and the plants supplied with fertilisers (120 kg N ha^{-1}, 24 kg P ha^{-1} and 48 kg K ha^{-1}) and chemical treatments as per commercial practices in the region (Table 1). The cultivar 'Lantvete' was grown in Southern Sweden between 1900 and 1910. Since, it has been used by plant breeders for comparison with more modern cultivars, but has not been actively subject to plant breeding. Thus, it is likely to retain most of its original traits.

2.3. Experimental design

Two ozone treatments were employed in six replicate open-top chambers (OTCs) ($n = 6$) for each cultivar: charcoal-filtered air (CF) or non-filtered air with additional (+ 40 ppb) ozone (NF+). Thus a total of 24 OTCs were used for the experiment. Ambient air plots (AA, $n = 3$) were used to monitor effects of chamber enclosure. The treatments were distributed in a completely randomised design. To retain the normal diur-

Table 1
Timetable of events during the experiment

Event	Date
Sowing	5 May 1999
Fertilizer application (NPK)	5 May 1999
Emergence 'Lantvete'	18 May 1999
Emergence 'Dragon'	19 May 1999
Insecticide (Sumi-Alpha, 0.33 l ha^{-1})	25 May 1999
Herbicides (MCPA, 0.8 l ha^{-1}; Starane, 0.6 l ha^{-1})	2 June 1999
Installation of OTCs	8 June 1999
Start of irrigation	14 June 1999
Start of ozone fumigation and air filtration	16 June 1999
Insecticide (Sumi-Alpha, 0.225 l ha^{-1})	23 June 1999
Insecticide (Sumi-Alpha, 0.4 l ha^{-1}), fungicide (Amistar, 0.5 l ha^{-1})	8 July 1999
Anthesis 'Dragon' OTC	14 July 1999
Anthesis 'Lantvete' OTC	15 July 1999
Anthesis 'Dragon' AA	16 July 1999
Anthesis 'Lantvete' AA	18 July 1999
End of ozone fumigation	24 August 1999
Harvest 'Dragon' NF+	26 August 1999
Harvest 'Dragon' CF and 'Lantvete' NF+	30 August 1999
Harvest 'Lantvete' CF	31 August 1999
Harvest 'Dragon' AA and 'Lantvete' AA	9 September 1999

OTC: open-top chamber; AA: ambient air; CF: charcoal-filtered air; NF+: non-filtered air with additional ozone.

nal variation in ozone concentrations, ozone was added to the NF+ treatment each day between 08.00 and 20.00, local time. Ozone was generated from pure oxygen using electric discharge and distributed to the chambers via PTFE tubing. Charcoal filtered air was used as the reference since the ozone concentrations 100 years ago, when the genotype 'Lantvete' was grown commercially, are likely to have been significantly lower than present-day ambient air levels (Borrell et al., 1997). An addition of approximately 40 ppb ozone compared to CF was selected since daytime average ozone concentrations of approximately 50 ppb, expected to result from a CF ozone concentration of 10 ppb plus an additional 40 ppb, have resulted in significant ($P < 0.05$) repressions in the yield of modern wheat cultivars in earlier experiments performed at the same site (Pleijel et al., 1991; Gelang et al., 2000).

To facilitate measurements, the plants enclosed in each OTC were divided by a plastic strip (diameter 1.10 m, height 0.06 m). The Southern portion of each chamber remained untouched throughout the experiment and was used for the final harvest. The Northern half was used for chlorophyll measurements and sampled for determination of leaf area. At the final harvest, three samples, each comprising 25 tillers, were collected at random from the Southern part of each experimental plot. The ears were separated from the straw, grain was threshed by hand and the dry weight of straw and grain determined by drying three sub-samples at 70 °C to constant weight. The harvest index was calculated as the proportion of the above-ground biomass recorded in the grains. The remaining grain from the Southern portion of each plot was threshed and subject to chemical analyses.

2.4. Open-top chambers

The OTCs were 1.24 m in diameter and 1.60 m tall, including a frustum. To exclude rain-water, a roof was attached 0.25 m above the upper part of the frustum. The plants were irrigated every 4 days with an amount corresponding to 20 mm rain. 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}; resulting in approximately three air changes per minute in each OTC.

2.5. Pollutant monitoring

Air samples were drawn through 50 m long PTFE tubes (0.25 in. diameter), which were continuously ventilated and connected to a time-share sampling system incorporating PTFE solenoid valves. Gas concentrations were measured twice every hour in each chamber treatment and in the ambient air. Air was sampled in all NF+ chambers and in four of the six CF chambers for each cultivar. Ozone concentrations were monitored using Thermo Environmental instrumentation (355 River Oaks Parkway, San Jose, CA 95134-1991, USA) model 49 UV absorption analysers. Ozone analysers were calibrated on a monthly basis using a portable ozone generator (Monitor Labs model 8500, 12333 West Olympic Boulevard, Los Angeles, CA 90064, USA). Data were collected with the aid of a Campbell Scientific (815 West, 1800 North Logan, Utah 84321-1784, USA) CR10 data logger.

2.6. Stomatal conductance

On 15 July 1999, stomatal conductance was measured using a LiCor 6200 (LI-COR, Biosciences, 4421 Superior, St. Lincoln, NE 68504, USA). Measurements were made on flag leaves of ambient air grown 'Lantvete' plants. Stomatal conductance data for 'Dragon' have already been published by Danielsson et al. (2003); the study reporting a maximum observed stomatal conductance of 414 mmol H_2O m^{-2} s^{-1} per total flag leaf area. Measurements on 'Lantvete' distributed over the daylight hours (10.00 until 20.00) with 37 measurements made under conditions likely to yield g_{max}. The total number of stomatal conductance measurements on which the calibration for 'Dragon' was based was 163. Out of these, 36 measurements were performed within the experiment presented in this paper and 127 in an experiment with wheat cv. 'Dragon' in 1996 (Danielsson et al., 2003). The conductance measurements in 1999 on 'Lantvete' and 'Dragon' were performed on the same day.

The stomatal conductance model used was a multiplicative algorithm:

$$g_s = \max\{g_{min}; g_{max}(\min\lfloor f_{phen} f_{O_3} \rfloor f_{VPD} f_T f_{PAR} f_{time})\} \quad (1)$$

where g_{min} and g_{max} denote the relative minimum and absolute maximum stomatal conductance, respectively, allowing for individual species or genotype values to be used in the model. The factors f_{VPD}, f_T, f_{PAR} and f_{time} represent the short-term (based on hourly averages) effects of leaf-to-air vapour pressure difference, leaf temperature, photosynthetic photon fluence rate and time of day, respectively. The long term influences of phenology (mainly leaf aging) and ozone on stomatal conductance are described by f_{phen} and f_{O_3}, respectively. The model employed is based on earlier work by Jarvis (1976) and Emberson et al. (2000), and is described in detail by Danielsson et al. (2003). This paper also reports the calibration of the model, based on total leaf area and hourly average values of the different driving model variables.

2.7. Exposure indices

Four different indices were used to express ozone exposure. The first was simply the average concentration during daytime (08.00–20.00) and night-time (20.00–08.00). Secondly, the AOT40 was employed. This represents the accumulated exposure over a concentration threshold of 40 nmol mol^{-1} ozone based on hourly averages. The index has been the most widely used ozone exposure index employed in Europe as a direct consequence of co-operation on air pollution abatement within the Convention on Long-range Transboundary Air Pollution (Fuhrer et al., 1997). More recently, models to estimate stomatal ozone uptake based on multiplicative algorithms have been developed (Emberson et al., 2000) and used to derive dose-response relationships for wheat and other key crops (Pleijel et al., 2000a). In the present paper, the calibrated multiplicative algorithm presented in Danielsson et al. (2003), described in Section 2.6, was used to calculate the accumulated stomatal flux of ozone per total flag leaf area, employing (i) a flux threshold of 4 nmol m^{-2} s^{-1} ($AF_{st}4$), which provided the strongest correlation (i.e. explained the variation of yield effects in terms of ozone exposure better than any other exposure index tested) between relative yield and ozone exposure in Danielsson et al. (2003) and (ii) no flux rate threshold ($AF_{st}0$). Similar to AOT40, $AF_{st}4$ represents the calculated accumulated stomatal ozone flux over a stomatal ozone flux threshold of 4 nmol m^{-2} s^{-1} based on hourly averages, the main difference being that AOT40 is based on the ozone concentration exterior to the plants and $AF_{st}4$ is based on the calculated ozone uptake.

2.8. Climate monitoring

Air temperature and relative humidity were monitored using Rotronic (Grindelstrasse 6, CH-8303 Bassersdorf, Switzerland) YA-100 hygrometer/thermometers positioned 0.1 m above the canopy in one chamber and in one ambient plot. The water vapour pressure deficit (VPD) was calculated according to Jones (1992). The photosynthetic photon fluence rate (PPFR) was measured using a Li-Cor (LI-COR Biosciences, 4421 Superior, St. Lincoln, NE 68504, USA) LI 190SA Quantum sensor. Climate data were stored as 30-min averages using a Campbell Scientific CR10 data logger.

2.9. Chlorophyll

A non-destructive dual-wavelength chlorophyll meter (Norsk Hydro ASA, N-0240 Oslo, Norway), that measures the transmission of red and infrared light (650 and 940 nm, respectively) through the leaves, was used to estimate the chlorophyll concentration of flag leaves. Each daily value represents the mean of 10–15 measurements on leaves, each of which was measured at two or three positions. Chlorophyll measurements were started on June 14, 2 days before the onset of ozone fumigation. The chlorophyll measurements were made on the last fully developed leaf; consequently early measurements (first three measurements dates) were made on what was later to become the first leaf below the flag leaf. The remaining eight measurements were made on the flag leaf.

The chlorophyll meter values were transformed to leaf chlorophyll concentrations (mg g^{-1} f.w.) using a calibration curve based on samples taken on three occasions 7 July, 28 July and 13 August. On each of these occasions three flag leaves from each treatment were measured using the chlorophyll meter and subsequently were analysed for chlorophyll according to Arnon (1949) in the laboratory. The calibration data covered a range of chlorophyll concentrations from close to zero up to above 4 mg chlorophyll per g fresh weight. The relationship between chlorophyll meter values was very similar for the two genotypes and a common, non-linear relationship was used to transform all chlorophyll meter values to chlorophyll concentration. The chlorophyll concentration was 0.353(exp(0.00395(chlorophyll meter value))).

2.10. Chemical analyses

The concentrations of Ca, P, Mg and K were analysed using induced coupled plasma atomic emission spectroscopy (ICP-AES). Prior to analysis, samples were digested at a temperature of 550 °C, then elements were dissolved using HCl prior to dilution with HNO_3 (NMKL No. 139 1991 mod.). Cd was extracted based on HNO_3 and H_2O_2 digestion at 190 °C (NMKL No. 161 1988 mod.) with analysis made using ICP mass spectrometry. Nitrogen was determined using the Kjeldahl method and crude protein concentration was obtained by multiplying the nitrogen concentration by 6.25.

2.11. Statistical analysis

The statistical significance of the open-top chamber treatments were analysed using two-way analysis of variance (ANOVA), testing for the effects of ozone (CF versus NF+), cultivar ('Dragon' versus 'Lantvete') and interactions. The effect of open-top chambers was not tested, since the difference between the CF and AA treatments comprises both ozone (CF lower than AA) and chamber enclosure effects. The difference in ozone exposure (AOT40 and $AF_{st}4$) between the two cultivars was tested using a Student's *t*-test. Only effects that were statistically significant at $P < 0.05$ are considered significant in the following text.

3. Results

3.1. Climate

The daytime (08:00–20:00) average ambient air temperature, VPD and PPFR (photosynthetic photon fluence rate) were 18.4 °C, 0.52 kPa and 690 μmol m^{-2} s^{-1}, respectively. The corresponding values for night-time (20:00–08:00) were 11.2 °C, 0.16 kPa and 42 μmol m^{-2} s^{-1}, respectively. Chamber enclosure invariably resulted in the elevation of air temperatures (on average, by 1.3 °C during the day and 1.0 °C during the night). VPD was higher in ambient air compared with the OTCs, by 0.07 kPa during the day and 0.04 kPa during the night. Daytime reduction in photosynthetic photon fluence rate inside the open-top chambers was estimated to 15%, based on data collected by Pleijel and Wallin (1996). This may be an underestimation due to the aging of the chamber walls between 1996 and 1999 (year of present study).

3.2. Ozone exposure

Plants were exposed to ozone from anthesis to harvest and the resulting exposure is described in Table 2. Air filtration resulted in the reduction of ozone concentration by 72% and 80% during daytime and night-time, respectively, compared with ambient air. Ozone addition in the NF+ treatment resulted in greater elevation of ozone in chambers containing 'Dragon' (35 nmol mol^{-1}, 24-h average) compared with those containing 'Lantvete' (32 nmol mol^{-1}, 24-h average), i.e. 8% higher AOT40 for 'Dragon'. However, the estimated stomatal flux of ozone was considerable greater in 'Dragon' compared to 'Lantvete' in NF+; 51% greater using $AF_{st}4$ and 21% greater using $AF_{st}0$. The main reason for this difference in estimated stomatal uptake between the cultivars was the difference in maximum recorded conductance. Using Student's *t*-test it was found that there was no significant difference ($P > 0.05$) in AOT40 between the NF+ treatments with 'Lantvete' and 'Dragon'. For $AF_{st}4$, the difference between the NF+ treatments with 'Lantvete' and 'Dragon' was statistically significant ($P < 0.05$).

Table 2
Ozone exposure during daytime (08.00–20.00) and night-time expressed as the average concentration (nmol mol^{-1}) and AOT40 (the accumulated exposure over a threshold concentration of 40 nmol mol^{-1} h, μmol mol^{-1} h), $AF_{st}4$ and $AF_{st}0$ (the accumulated flag leaf stomatal flux of ozone to the total flag leaf area, above a flux threshold of 4 nmol m^{-2} s^{-1} and employing no flux threshold, respectively)

	Average 08.00–20.00	Average 20.00–08.00	AOT40	$AF_{st}4$	$AF_{st}0$
CF Dragon	9	3	0	0	2.1
CF Lantvete	9	3	0	0	1.8
NF+ Dragon	57	13	9.7	6.2	12.8
NF+ Lantvete	52	12	9.0	4.1	10.6
AA	32	15		–	–

AA: ambient air; CF: charcoal filtered air; NF+: non-filtered air with additional ozone.

3.3. Grain yield, 1000-grain weight and harvest index

Grain dry weight per ear was significantly higher ($P < 0.001$) in CF compared to NF+ (Fig. 1(a)), with substantially greater effects in 'Dragon' (40%) compared with 'Lantvete' (21%) and a significant interaction ($P < 0.05$) between ozone treatment and cultivar. The 1000-grain weight revealed a similar interaction (O_3 effect, $P < 0.01$; O_3 cultivar, $P < 0.05$). Harvest index was negatively affected by ozone ($P < 0.001$).

The difference between the two cultivars was significant for dry weight per ear ('Dragon' 52% higher, $P < 0.001$, Fig. 1(a)) and for harvest index ('Dragon' 35% higher, $P < 0.001$, Fig. 1(d)). The 1000-grain weight did not differ significantly between the cultivars (Fig. 1(c)). Consequently, the number of grains per ear was larger in 'Dragon' compared with 'Lantvete', by 36% in both CF and NF+. The average number of grain per ear (34 and 31 in CF and NF+, respectively, for Dragon; 25 and 23 in CF and NF+, respectively, for 'Lantvete') was not significantly affected by ozone. Chamber enclosure tended to enhance ear dry weight, 1000-grain weight and harvest index in both cultivars (Fig. 1).

Total above-ground biomass (Table 3) was significantly greater ($P < 0.001$) in 'Dragon' compared with 'Lantvete', and the negative effect of ozone was also highly significant ($P < 0.001$). For straw yield there was no significant ozone effect, and 'Lantvete' was higher than 'Dragon' ($P < 0.001$, Table 3); emphasizing the role of harvest index in the grain yield difference between the 'old' and the 'modern' cultivar.

3.4. Grain composition

The crude protein concentration of the grain was positively affected by ozone exposure ($P < 0.01$), by 11% and 6% in 'Dragon' and 'Lantvete', respectively (Fig. 1(b)). 'Lantvete' was considerably higher than 'Dragon' in grain crude protein concentration ($P < 0.001$), by 37% and 31% in CF and NF+, respectively. The plants grown in the OTCs had higher grain crude protein concentrations than plants grown in the ambient air.

Grain concentrations of elements other than nitrogen are presented in Table 3. No significant treatment- or cultivar-related changes were observed in Ca and Cd concentrations. The cultivar difference with respect to the P and Mg content of the grain was highly significant, 'Lantvete' having higher concentration. 'Dragon' was, however, significantly ($P < 0.001$) higher in the concentration of K, compared to 'Lantvete'. Weak, but statistically significant ($P < 0.05$)

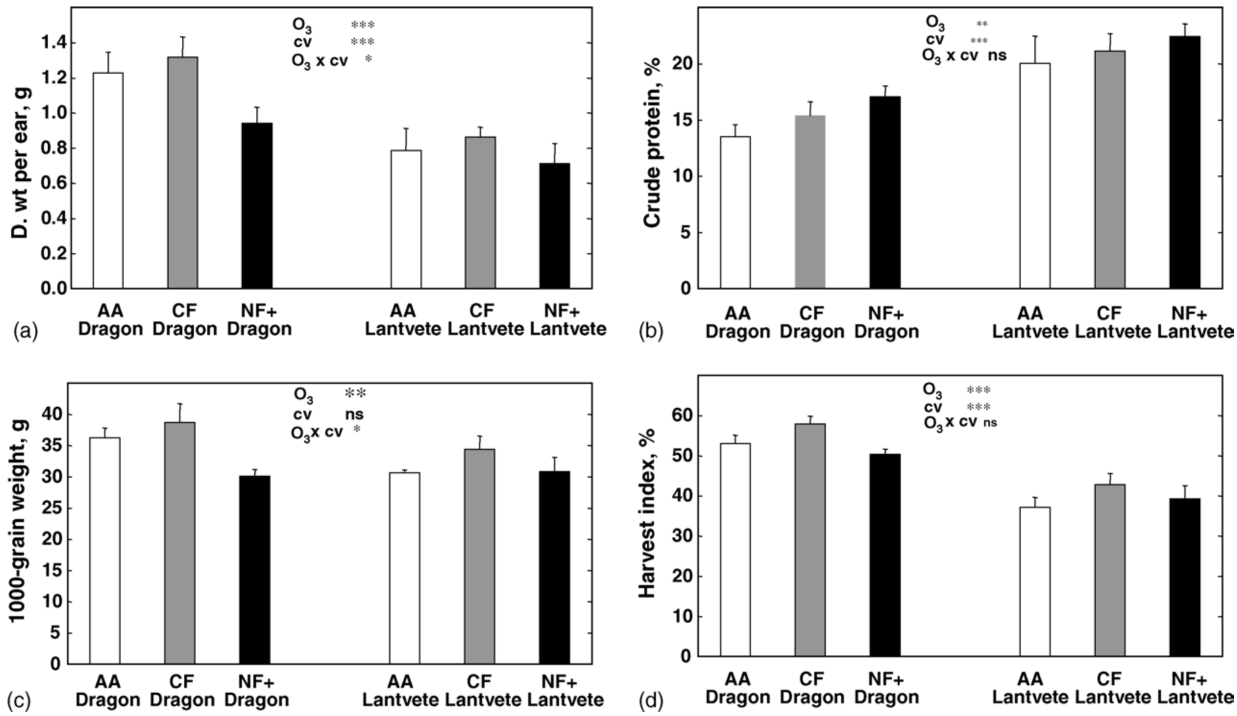

Fig. 1. The dry weight per ear (A), crude protein concentration (B), 1000-grain weight (C) and harvest index (D) of wheat cultivars 'Dragon' and 'Lantvete' grown in ambient air (AA), open-top chambers ventilated with charcoal-filtered air (CF) and non-filtered air plus ozone (NF+). The results of two-way ANOVAs, using ozone (O_3), cultivar (cv) and their interaction ($O_3 \times$ cv), are presented. AA treatment was not included in the statistical analysis.

elevation of grain P, K and Mg concentrations was observed in ozone-treated plants.

The only non-essential element studied was Cd. The behaviour of this element differed considerably from the other elements studied, in that there was a pronounced positive chamber effect both in 'Dragon' (31%) and 'Lantvete' (19%).

3.5. Flag leaf chlorophyll

Ozone exposure exerted a significant ($P < 0.001$) impact on the chlorophyll content of the leaves within 1 month of exposure; the ozone-induced decline in chlorophyll content tended to proceed at a faster rate in 'Dragon' compared with 'Lantvete'. Moreover, the chlorophyll concentration of flag leaves was significantly ($P < 0.05$ in 8 out of 11 observations) lower in 'Lantvete' compared to 'Dragon' (Fig. 2).

3.6. Stomatal conductance

The stomatal conductance for 'Dragon' was used in an earlier study, together with other data, to calibrate a multiplicative algorithm for predicting stomatal conductance (Danielsson et al., 2003). Parallel measurements performed during the course of the present study revealed a maximum conductance for 'Lantvete' of 334 mmol H_2O m^{-2} s^{-1} per total flag leaf area. The model from Danielsson et al. (2003), modified only by the use of the observed maximum conduc-

Table 3
Grain phosphorus (P, %), potassium (K, %), magnesium (Mg, %), calcium (Ca, %) and cadmium (Cd, mg kg^{-1}) content

	Lantvete CF	Lantvete NF+	Dragon CF	Dragon NF+	cv	O_3	cv × O_3	Lantvete CF/AA	Dragon CF/AA
P	0.45 ± 0.049	0.50 ± 0.027	0.37 ± 0.015	0.38 ± 0.014	***	*	ns	0.91	1.01
K	0.41 ± 0.059	0.44 ± 0.028	0.48 ± 0.023	0.52 ± 0.025	***	*	ns	0.88	1.04
Mg	0.14 ± 0.018	0.16 ± 0.006	0.13 ± 0.008	0.14 ± 0.005	**	*	ns	0.96	1.10
Ca	0.036 ± 0.009	0.034 ± 0.005	0.035 ± 0.004	0.033 ± 0.006	ns	ns	ns	1.00	1.05
Cd	0.047 ± 0.008	0.050 ± 0.005	0.046 ± 0.004	0.045 ± 0.004	ns	ns	ns	1.19	1.31
TAB	2.1 ± 0.10	1.8 ± 0.16	2.4 ± 0.18	1.9 ± 0.14	***	***	ns	0.96	0.99
SY	1.2 ± 0.10	1.1 ± 0.08	1.0 ± 0.06	0.96 ± 0.05	***	ns	ns	0.89	0.88

Total above-ground biomass per shoot (TAB, g $shoot^{-1}$), straw yield (SY, g $shoot^{-1}$) at harvest. Data for 'Lantvete' and 'Dragon' wheat grown in charcoal-filtered air (CF) and non-filtered air with additional ozone (NF+). cv: cultivar; O_3: ozone treatment; ns: not significant. Values represent mean ± S.D.

* Statistically significant at $P < 0.05$.

** Statistically significant at $P < 0.01$.

*** Statistically significant at $P < 0.001$.

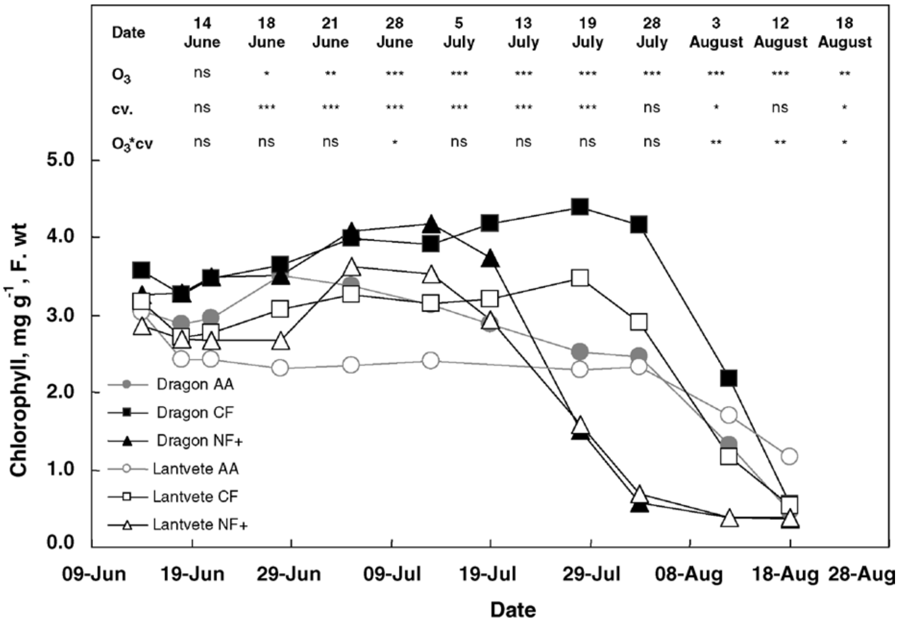

Fig. 2. Chlorophyll concentration (mg g^{-1}, f.w.), based on chlorophyll meter readings, of the last developed leaf (the flag leaf on the last eight measurements) of the wheat cultivars 'Dragon' and 'Lantvete' grown in ambient air (AA) and open-top chambers with charcoal filtered air (CF) and non-filtered air with extra ozone (NF+).

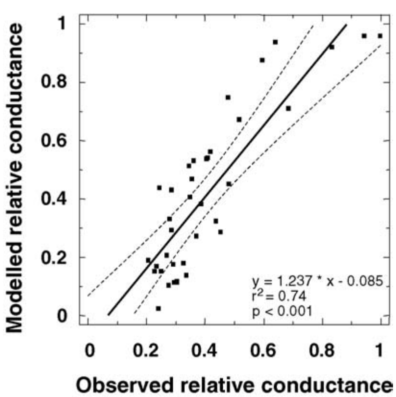

Fig. 3. Modelled vs. observed stomatal conductance, relative to the maximum observed conductance, in 'Lantvete'.

tance for 'Lantvete' revealed a strong relationship ($r^2 = 0.74$, $P < 0.001$) between predicted and observed conductance for 'Lantvete' (Fig. 3) and the intercept was not significantly different from zero.

4. Discussion

The 'old' wheat cultivar 'Lantvete' exhibited a higher straw yield, lower grain yield, lower harvest index and a higher grain protein concentration than the 'modern' cultivar 'Dragon'. Total above-ground biomass was significantly higher in 'Dragon' compared to 'Lantvete', suggesting that the higher yield of the more 'modern' cultivar cannot be fully attributed to breeding for higher harvest index in this particular cultivar.

In terms of effects on grain yield and 1000-grain weight, the 'old' cultivar appeared to be less affected by ozone than the 'modern' cultivar, and this was revealed as significant interactions between cultivar and ozone effects when data were subject to ANOVA. This is consistent with earlier results (Barnes et al., 1990; Velissariou et al., 1992).

Selection by breeders for higher crop yields may be associated with increased gas exchange via higher stomatal conductance (see Velissariou et al., 1992). Estimated $AF_{st}4$ for 'Dragon' was 51% higher than for 'Lantvete'. Since $AF_{st}4$ was zero in the CF treatments, and $AF_{st}4$ is the exposure index resulting in the strongest correlation with wheat yield over a number of Swedish experiments (Danielsson et al., 2003), it should represent a relevant measure of ozone exposure harmful to yield. The 51% higher $AF_{st}4$ can be compared with the effect of ozone on yield in the present experiment, which was 61% larger in 'Dragon' than in 'Lantvete'. The two figures match relatively well, but the larger effect on yield compared with the estimated $AF_{st}4$ may indicate that the greater ozone uptake by the modern cultivar is not sufficient to fully explain the difference in ozone sensitivity between the two cultivars. One candidate for further explanation of the difference in sensitivity between 'old' and 'modern' wheat cultivars is that the widespread use of agrochemicals, many of which act as antioxidants, may have masked the effects of ozone during field trials, resulting in an apparent increase in ozone sensitivity in more modern genotypes (Barnes et al., 1999).

Experiments have shown that the yield of 'older' wheat cultivars is subject to different constraints than 'modern' cultivars. When comparing three wheat cultivars, Stoy (1965) found that an ‘older’ cultivar, characterised by smaller flag leaves and shorter leaf duration, stored more carbohydrates in the stem and later redistributed more of the stored carbohydrates to the grain, compared with more ‘modern’ cultivars. These findings suggest that ‘older’ cultivars of wheat may depend less on current photosynthesis for grain filling than ‘modern’ cultivars. Indeed, it was suggested by Selldén and Pleijel (1995) that ozone-induced reductions in photosynthesis may not be reflected in reduced yield in ‘older’ cultivars to the same extent as in ‘modern’ cultivars (i.e. yield of ‘older’ cultivars may be expected to be less affected by ozone than that of more-modern genotypes).

Chlorophyll measurements revealed that flag leaf duration, and thus the duration of grain fill, was shortened by ozone exposure in both cultivars, consistent with earlier studies (Grandjean and Fuhrer, 1989; Ojanperä et al., 1992; Pleijel et al., 1997; Gelang et al., 2000). The ozone-induced loss of chlorophyll was faster earlier in ‘Dragon’ compared with ‘Lantvete’, consistent with the higher rate of ozone uptake exhibited by ‘Dragon’.

‘Lantvete’ exhibited higher grain protein concentration and this parameter was negatively correlated with grain yield. Pleijel et al. (1999) reported a negative relationship between grain protein concentration and grain yield across a range of experiments including ozone, carbon dioxide and irrigation treatments. According to that relationship the predicted increase in grain protein concentration due to the NF+ treatment should be around 10% in ‘Dragon’ and 6% in ‘Lantvete’. The actual values observed were 11% in ‘Dragon’ and 6% in ‘Lantvete’. The findings of the present study therefore corroborate the relationship presented by Pleijel et al. (1999) and affirm the strong link between grain yield and grain protein at conventional rates of fertiliser application. There was also a general trend toward higher concentrations of several mineral constituents in grain under elevated ozone, a finding reported previously (e.g. Fuhrer et al. (1990) for P, K, Mg and Ca) attributable to growth dilution effects.

The only non-essential element studied in the present investigation was Cd. This was included because wheat grain is a potentially important source of ingestion of this highly toxic element in humans. Pleijel et al. (2000b) showed that Cd tends to accumulate in open-top chamber grown wheat compared with plants grown in ambient air, and suggested that the effect is related to a higher rate of transpiration in the warmer, continuously stirred chamber environment compared with ambient air. Consistent with this hypothesis, Cd accumulation was found to be greater in ‘Dragon’, with higher conductance and thus higher transpiration, than in ‘Lantvete’.

The present study revealed the ‘modern’ cultivar ‘Dragon’ to be more sensitive to ozone than a cultivar introduced at the turn of the 20th century (‘Lantvete’) and this was at least partially attributable to higher stomatal conductance (and thus ozone flux) in the modern genotype. This finding is consistent with observations made earlier on Greek wheat cultivars (Barnes et al., 1990; Velissariou et al., 1992).

Acknowledgements

The experiment was funded by the Swedish Council for Forestry and Agricultural Research (SJFR) and the data evaluation by the Mistra-Research funded ASTA programme. Thanks are due to Patrik Alströmer, Östads Säteri, for making the investigation area available.

References

Arnon, D.I., 1949. Copper enzymnes in isolated chloroplasts. Polyphenoloxidase *Beta vulgaris*.

Barnes, J.D., Velissariou, D., Davison, A.W., Holevas, C.D., 1990. Comparative ozone sensitivity of old and modern Greek cultivars of wheat. New Phytol. 116, 707–714.

Barnes, J., Bender, J., Lyons, T., Borland, A., 1999. Natural and man-made selection for air pollution resistance. J. Exp. Bot. 50, 1423–1435.

Blum, A., 1983. Plant Breeding for Stress Environments. CRC Press, Boca Raton.

Borrell, P., Builtjes, P.J.H., Grennfelt, P., Hov, Ø., 1997. Photo-oxidants, Acidification and Tools; Policy Applications of EUROTRAC Results. Springer/Verlag, Berlin, p. 216.

Danielsson, H., Pihl Karlsson, G., Karlsson, P.E., Pleijel, H., 2003. Ozone uptake modelling and flux-response relationships – an assessment of ozone-induced yield loss in spring wheat. Atmos. Environ. 37, 475–485.

Elinder, C.G., Järup, L., 1996. Cadmium exposure and health risks: recent findings. Ambio 25, 370–373.

Emberson, L., Ashmore, M.A., Cambridge, H.M., Simpson, D., Tuovinen, J.-P., 2000. Modelling stomatal ozone flux across Europe. Environ. Pollut. 109, 403–413.

Evans, L.T., 1993. Crop Evolution, Adaptation and Yield. Cambridge University Press, Cambridge, UK, ISBN 0-521-22571.

Evans, L.T., Wardlaw, I.F., Fisher, R.A., 1975. Wheat. In: Evans, L.T. (Ed.), Crop Physiology: Some Case Histories. Cambridge, Cambridge University Press, pp. 101–149, ISBN 0-521-20422-4.

Fuhrer, J., Lehnherr, B., Moeri, P.B., Tschannen, W., Shariat-Madari, H., 1990. Effects of ozone on the grain composition of spring wheat grown in open-top chamber. Environ. Pollut. 65, 181–192.

Fuhrer, J., Skärby, L., Ashmore, M., 1997. Critical levels for ozone effects on vegetation in Europe. Environ. Pollut. 97, 91–106.

Gelang, J., Pleijel, H., Sild, E., Danielsson, H., Younis, S., Selldén, G., 2000. Rate and duration of grain filling in relation to flag leaf senescence and grain yield in spring wheat (*Triticum aestivum*) exposed to different concentrations of ozone. Physiol. Plant 110, 366–375.

Grandjean, A., Fuhrer, J., 1989. Growth and leaf senescence in spring wheat (*Triticum aestivum*) grown at different ozone concentrations in open-top field chambers. Physiol. Plant 77, 389–394.

Jarvis, P.G., 1976. The Interpretation of the Variations in Leaf Water Potential and Stomatal Conductance Found in Canopies in the Field, vol. B273. Philosophical Transactions of the Royal Society, London, pp. 593–610.

Jones, H.G., 1992. Plant and Microclimate: A Quantitative Approach to Environmental Plant Physiology, 2nd ed. Cambridge University Press, Cambridge/Oxford, p. 452.

Kibite, S., Evans, L.E., 1984. Causes of negative correlations between grain yield and grain protein concentration in common wheat. Euphytica 33, 801–810.

Mooi, J., 1982. The Possibilities of Using Anti-oxidants: a Literature Study. Report R.273, IPO Wageningen.

Ojanperä, K., Sutinen, S., Pleijel, H., Selldén, G., 1992. Exposure of spring wheat, *Triticum aestivum* L., cv. Drabant, to different concentrations of ozone in open-top chambers: effects on the ultrastructure of flag leaf cells. New Phytol. 120, 39–48.

Pleijel, H., Wallin, G., 1996. Effects of the open-top chamber on air turbulence and light – the possible consequences for ozone uptake by cereals. In: Kärenlampi, L., Skärby, L. (Eds.), Critical Levels for Ozone in Europe: Testing and Finalizing the Concepts. UN-ECE Workshop Report, University of Kuopio, Department of Ecology and Environmental Science, pp. 303–307, ISBN 951-780-653-1.

Pleijel, H., Skärby, L., Wallin, G., Selldén, G., 1991. Yield and grain quality of spring wheat (*Triticum aestivum* L., cv. Drabant) exposed to different concentrations of ozone in open-top chambers. Environ. Pollut. 69, 151–168.

Pleijel, H., Ojanperä, K., Danielsson, H., Sild, E., Gelang, J., Wallin, G., Skärby, L., Selldén, G., 1997. Effects of ozone on leaf senescence in spring wheat—possible consequences for grain yield. Phyton 37, 227–232.

Pleijel, H., Danielsson, H., Gelang, J., Sild, E., Selldén, G., 1998. Growth stage dependence of the grain yield response to ozone in spring wheat (*Triticum aestivum* L.). Agric. Ecosyst. Environ. 70, 61–68.

Pleijel, H., Mortensen, L., Fuhrer, J., Ojanperä, K., Danielsson, H., 1999. Grain protein accumulation in relation to grain yield of spring wheat (*Triticum aestivum* L.) grown in open-top chambers with different concentrations of ozone, carbon dioxide and water availability. Agric. Ecosyst. Environ. 72, 265–270.

Pleijel, H., Danielsson, H., Pihl Karlsson, G., Gelang, J., Karlsson, P.E., Selldén, G., 2000a. An ozone flux-response relationship for wheat. Environ. Pollut. 109, 453–462.

Pleijel, H., Danielsson, H., Gelang, J., Selldén, G., 2000b. Effects of transpiration, carbon dioxide and ozone on the content of cadmium and zinc in spring wheat grain. In: Yunus, M., Singh, N., de Kok, L.J. (Eds.), Environmental Stress: Indication, Mitigation, and Eco-conservation. Kluwer Academic Publishers, Dordrecht, pp. 207–218.

Selldén, G., Pleijel, H., 1995. Photochemical oxidant effects on vegetation – response in relation to plant strategy. Water Air Soil Pollut. 85, 111–122.

Stoy, V., 1965. Photosynthesis, respiration and carbohydrate accumulation in spring wheat in relation to yield. Physiol. Plant (Suppl. IV), 1–125.

Velissariou, D., Barnes, J.D., Davison, A.W., 1992. Has inadvertent selection by plant breeders affected the O_3 sensitivity of modern Greek cultivars of spring wheat. Agric. Ecosyst. Environ. 38, 79–89.