Gartenbauwissenschaft, **66** (1). S. 27–33, 2001, ISSN 0016–478X. © Verlag Eugen Ulmer GmbH & Co., Stuttgart

Responses of Strawberry to Long-Term Elevated Atmospheric Ozone Concentrations – I. Leaf Gas Exchange, Chlorophyll Fluorescence, and Macronutrient Contents

Einfluß eines erhöhten Ozongehaltes auf Erdbeere. I. Blattgaswechsel, Chlorofluoreszenz und Mikronährstoffegehalt

N. Keutgen and F. Lenz
Institut für Obstbau und Gemüsebau, University Bonn

Summary

Strawberry plants of cvs 'Elsanta' and 'Korona' were grown during three months for eight hours five days a week in an atmosphere that contained 65 ppb ozone. This ozone stress was comparable to an AOT40 of 13200 ppb-h, an elevated burden that had already been recorded in Germany. Net photosynthetic rates of both strawberry cultivars were not affected by this ozone burden. Exposure during leaf growth had no major and lasting effect on chloroplast development and attainment of photosynthetic capacity, but photochemical quenching was reduced in old leaves. An earlier start of chlorophyll degradation and an in tendency reduced leaf number in the less ozone tolerant cv. 'Elsanta' indicated an acceleration of leaf ageing processes. The fact that Ca concentrations in leaves of the more tolerant cv. 'Korona' were about twice as high as in cv. 'Elsanta' favoured the hypothesis that Ca played an important role in strawberry plant tolerance to ozone stress.

Zusammenfassung

Erdbeerpflanzen der Sorten 'Elsanta' und 'Korona' wurden drei Monate lang fünf Tage pro Woche in einer Atmosphäre von 65 ppb Ozon angezogen. Dieser Ozonstress entsprach einem AOT40 von 13200 ppb-h, einer erhöhten Belastung, die schon in Deutschland gemessen wurde. Die Nettophotosyntheseraten der beiden Erdbeersorten wurden durch diese Ozonbelastung nicht reduziert. Während des Blattwachstums trat kein größerer und bleibender Schaden weder bei der Entwicklung der Chloroplasten noch beim Aufbau der Photosynthesekapazität auf. Bei alten Blättern nahm jedoch die photochemische Fluoreszenzlöschung ab. Im Falle der weniger ozontoleranten Sorte 'Elsanta' wiesen ein früher einsetzender Chlorophyllabbau und eine tendenziell reduzierte Blattzahl auf eine Beschleunigung von Blattalterungsprozessen hin. Die Tatsache, dass die Ca-Konzentrationen in Blättern der ozontoleranteren Sorte 'Korona' ungefähr doppelt so hoch waren wie bei 'Elsanta' befürwortete die Hypothese, dass Ca bei der Toleranz von Erdbeerpflanzen auf Ozonstress eine wichtige Rolle spielt.

Abbreviations: *A*, net photosynthesis rate; *Chl*, chlorophyll; C_i, intercellular CO_2 concentration; $\Delta F/Fm'$, effective quantum yield of PS II; *E*, transpiration rate; *F*, actual fluorescence of illuminated leaves; *Fm*, maximal fluorescence of dark-adapted leaves; *Fm'*, maximal fluorescence of light-adapted leaves; *Fo*, basal fluorescence of dark-adapted leaves; *Fo'*, quenched *Fo*; *Fv/Fm*, optimal quantum yield of PS II; *Fv'/Fm'*, excitation capture efficiency of PS II reaction centres; g_s, stomatal conductance of H_2O; PAR, photosynthetically active radiation; PS I or II, photosystem I or II; *qN*, non-photochemical quenching; *qP*, photochemical quenching; *R*, respiration.

Introduction

Ozone is a major atmospheric pollutant, which is still a threat for fruit and crop yield despite various national control measures. Ambient concentrations of ozone in the air over rural areas of Europe are normally below 80 ppb (AARNES et al. 1993), but well above the background level of 20 ppb (VOLTZ and KLEY 1988). In the efforts to correlate the atmospheric ozone concentrations with specific plant stress responses, scientists in Europe have applied the concept of critical levels. The AOT40 (= Accumulated exposure Over a Threshold of 40 ppb), which was used in the UN-ECE studies (FUHRER 1994), is one of the most popular among ozone exposure statistics (LEGGE et al. 1995). It was shown that an AOT40 of 5300 ppb-h accumulated over three consecutive months significantly reduced relative grain yield by about 10 % in spring wheat (FUHRER 1994) and this ozone burden was subsequently considered as critical. Comparatively high AOT-40 values ranging from 8600 to 17200 ppb-h, were recorded between 1992 and 1994 in southern Germany (THIEL 1994). They may represent the maximal ozone threat that can be expected in Germany. In the present experiments with the strawberry cvs 'Elsanta' and 'Korona', 13200 ppb-h ozone were supplied over three consecutive months, which was well within the range of rather elevated but realistic ozone concentrations. Earlier studies have shown strawberry to be comparatively tolerant to ozone (MCCOOL et al. 1986, TAKEMOTO et al. 1989), but more recent investigations (KEUTGEN et al. 1997, 1998) indicated slight differences in the ozone sensitivity between European cultivars, which seemed to be related to the stomatal aperture and density. The present research intended to study the effects of ozone on the development and performance of the photosynthetic apparatus. Leaves of the first, second and fifth

whorl were studied to evaluate gas exchange and chlorophyll fluorescence of leaves of different stages grown for different time periods under the ozone enriched atmosphere. Fruit yield of strawberry plants was recorded to assess the long-term ozone effects important for horticultural practice. In addition, macronutrient contents of leaves were studied to correlate their concentrations with the ozone sensitivity of the cultivars in order to identify relationships that may help to explain the different ozone sensitivities.

Materials and Methods

Plant material

One hundred cold-stored strawberry (*Fragaria* x *ananassa* Duch.) plantlets of cvs 'Elsanta' and 'Korona' were planted in 5 l plastic pots containing quartz sand in April 1996 and March 1997. Plants were grown under greenhouse conditions at the University of Bonn, Germany (about 51° N, 7° E). They were watered every evening and received 500 ml of a modified Hoagland nutrient solution once a week (SRUAMSIRI and LENZ 1985). For ozone application 21 plants per cultivar were selected for uniformity and transferred into one of three plant chambers for acclimatisation at the end of April 1996 and March 1997. Pots were moved to different positions in the chambers once a week to avoid position effects. Spider mites and further insect populations within the plant chambers were controlled with Decis® (deltametrin) and Kiron® (fenpyroximat), but two-spotted spider mite (*Tetranychus urticae* Koch) populations developed visible symptoms (small yellow spots on the upper leaf surface) in mid May 1997. Euparen® (dichlofluanid) and Metasystox® (oxydemeton-methyl) were applied to protect strawberries against fungal infections.

Ozone fumigations lasted from May to July 1996 and from April to June 1997, respectively. One set of plants received 65 ± 15 ppb ozone for eight hours during the light period, five days a week for three months. The second set was grown in charcoal-filtered (no ozone) and the third in unfiltered air (5–10 ppb ozone). The plant chambers used for this ozone fumigation experiment were placed in a glasshouse. Each plant chamber consisted of an aluminium frame with a ground area of 1.21 m^2 and 0.8 m height, the inner side of the frame being covered with Teflon film (0.13 mm thickness; Norton Pampus GmbH, Willich, Germany). Light intensity within the plant chambers varied according to the natural sunlight (about 500 µmol $m^{-2}s^{-1}$ PAR at midday). Shading by the glass of the glasshouse and the Teflon cover was nearly compensated by a high pressure sodium lamp (Philips SON-T AGRO 400), supplying an additional PAR of 100 µmol $m^{-2}s^{-1}$. To mimic the open-top chamber design, the plant chambers received air from outside the greenhouse taken at about 2 m height by a fan, purified for all but the third chamber by passing through an air-purification filter, a charcoal filter and a particle filter to remove most of the hydrocarbons, ozone, and oxides of sulphur and nitrogen. An air flow through each of the plant chambers of about 60 m^3h^{-1} was maintained. Before the air entered into the first plant chamber, ozone was generated by two Hg lamps (series 78-2046-2; Seefelder Meßtechnik, Seefeld, Germany). To minimise nitrogen oxides generated by the Hg lamps, the ozone-enriched air as well as the air of the second chamber passed through a quartz-wool filter before the gas entry into the plant chambers and, finally, through a distributor at the top of each plant chamber to produce a turbulent environment that reduced boundary layer resistance, temperature and pollutant stratification. The homogenous distribution of ozone concentrations within each chamber was examined and continuously measured with the ozone analyser MLU400 (MLU, Essen, Germany). Plant chamber temperature was up to 3 °C higher than outside the glasshouse, ranging from 10 to 30 °C. Air humidity within the chambers varied between 40 % and 80 % and the CO_2 concentration was about 360 ppm.

Gas exchange measurements were performed in mid July 1996 and in mid June 1997. Stomatal density was determined in June 1997, only. In 1996, chlorophyll fluorescence measurements were performed and leaf samples for chlorophyll and macronutrient contents were taken. In case of gas exchange and chlorophyll fluorescence measurements as well as for analyses of leaf chlorophyll contents, plants from all the three chambers (ozone-enriched, charcoal-filtered and unfiltered air) were investigated. However, for analyses of macronutrients, only leaf samples from plants grown in ozone-enriched or charcoal-filtered air were compared.

Photosynthetic activities

Measurements of net photosynthesis (A), respiration (R), transpiration (E), stomatal conductance (g_s), and internal CO_2 concentration (C_i) of strawberry leaves of the first, second and fifth whorl were performed in mid July 1996 and mid June 1997. Leaves of the fifth whorl showed visible signs of ageing such as a distinct reduction of chlorophyll content. Gas exchange data were collected between 9.00 and 12.00 in the morning, using the portable porometer type CIRAS-1 (PP Systems, Hitchin, Herts., England) with integrated automated gas supply. The leaves were clamped in a broad Parkinson leaf chamber connected to the CIRAS-1. The measurements were conducted *in situ* without interrupting the ozone application in chamber 1, but ozone concentrations dropped to about 20 ppb. Environmental conditions within the leaf chamber during the measurements were as follows (mean values): temperature about 28 °C, light intensity ca. 860 µmol $m^{-2}s^{-1}$ PAR, CO_2 concentration ca. 370 ppm and relative humidity about 32 %. Stomatal density of strawberry leaves from the second whorl was counted end of June 1997 using a microscope with a 400 times magnification (Zeiss Axioskop, Zeiss, Germany).

Chlorophyll fluorescence was measured on leaves of the first, second and fifth whorl in mid July 1996 at about 25 °C and an ambient CO_2 level of 500–600 ppm with a portable pulse amplitude modulation fluorometer (PAM 2000, Heinz Walz GmbH, Effeltrich, Germany). Strawberry plants were transferred to the fluorescence laboratory in the afternoon and dark-adapted for 30 min. The fluorescence measurement on the adaxial leaf surface was as described by SCHREIBER et al. (1995). The leaf was initially exposed to a weak modulated measuring beam (< 0.1 µmol m^{-2} s^{-1}) to estimate *Fo*. Then, the leaf was exposed to a 800 ms saturation pulse of white light to assess *Fm*. The ratio (*Fm-Fo*)/*Fm*

Table 1: Fruit number per plant, fruit yield in g fresh mass and total sugar content of class 'I' and 'Extra' fruits in cv. 'Elsanta'.
Zahl der Früchte pro Pflanze, Fruchtertrag in g Frischmasse und Gesamtzuckergehalt von 'Elsanta'-Früchten der Klasse 'I' und 'Extra'.

	1. year filtered no O_3	1. year filtered 65 ppb O_3	1. year unfiltered 5–10 ppb O_3	2. year filtered no O_3	2. year filtered 65 ppb O_3	2. year unfiltered 5–10 ppb O_3
fruit number	10.8 ± 2.7 ab	8.7 ± 1.8 ab	11.3 ± 3.5 a	9.3 ± 2.4 ab	8.0 ± 2.0 b	8.5 ± 2.8 ab
fruit yield	102.3 ± 19.7 ab	82.5 ± 20.7 b	110.7 ± 37.5 a	84.1 ± 8.4 ab	78.7 ± 17.3 b	95.0 ± 20.3 ab
total sugar	53.5 ± 4.1 a	48.6 ± 5.8 b	n.d.	52.1 ± 4.6 a	50.7 ± 3.6 ab	n.d.

The values represent means ± S.D. The number of replicates is 7. In a row, different letters indicate significant differences at the 5 % level by Duncan or Wilcoxon tests [n.d. = not determined].

Table 2: Fruit number per plant, fruit yield in g fresh mass and total sugar content of class 'I' and 'Extra' fruits in cv. 'Korona'.
Zahl der Früchte pro Pflanze, Fruchtertrag in g Frischmasse und Gesamtzuckergehalt von 'Korona'-Früchten der Klasse 'I' und 'Extra'.

	1. year filtered no O_3	1. year filtered 65 ppb O_3	1. year unfiltered 5–10 ppb O_3	2. year filtered no O_3	2. year filtered 65 ppb O_3	2. year unfiltered 5–10 ppb O_3
fruit number	9.7 ± 3.0 a	9.6 ± 1.1 a	9.3 ± 3.5 ab	7.3 ± 2.0 ab	6.4 ± 2.6 b	7.4 ± 2.4 ab
fruit yield	78.4 ± 24.4 a	81.6 ± 10.1 a	82.8 ± 26.0 a	58.3 ± 16.3 ab	50.9 ± 22.3 b	66.4 ± 21.1 ab
total sugar	52.6 ± 4.5 a	51.1 ± 3.2 ab	n.d.	49.8 ± 3.0 b	46.0 ± 5.4 c	n.d.

The values represent means ± S.D. The number of replicates is 7. In a row, different letters indicate significant differences at the 5 % level by Duncan or Wilcoxon tests [n.d. = not determined].

= *Fv/Fm* is a convenient measure of the potential maximal PS II quantum yield of a given sample. Thirty seconds after the saturation pulse, continuous actinic light (about 100 µmol m^{-2} s^{-1}) was applied to determine the steady-state fluorescence yield (*F*). After the fluorescence response had peaked, saturation pulses were triggered every 20 s over a period of 300 s until a quasi steady-state was reached and *Fm*' could be assessed. *Fo*' was determined upon darkening of the leaf sample and application of weak far-red background light. Then, *ΔF/Fm*', *qP*, *Fv*'/*Fm*', and *qN* were calculated:

$$\Delta F/Fm' = (Fm'-F)/Fm',$$
$$qP = (Fm'-F)/(Fm'-Fo'),$$
$$Fv'/Fm' = \Delta F/Fm'/qP$$
$$qN = 1-(Fm'-Fo')/(Fm-Fo).$$

Photochemical quenching, *qP*, is considered to represent the fraction of open PS II reaction centres, whereas nonphotochemical quenching, *qN*, mainly represents an increase in non-radiative dissipation of the light energy.

Analyses of leaf chlorophyll and macronutrient contents

Leaf samples were collected end July 1996, freeze-dried to constant weight and ground. The contents of total *Chl*, *Chl* a and *Chl* b were determined in methanol extracts as described by ŠESTÁK (1971). Total nitrogen content was determined by a modified Kjeldahl digestion procedure and phosphorus, potassium, calcium, and magnesium contents after digestion with HNO_3 and H_2O_2 according to CHEN et al. (1997).

Strawberry fruit analyses

Fruit harvest extended from June to middle of July in 1996 and from May to middle of June in 1997. Fruit number per plant and fresh mass of class 'I' and 'Extra' fruits were determined as well as their contents of glucose, fructose, and sucrose (BOEHRINGER 1995). Total sugar content was regarded as the sum of these sugars. In case of fruit number and fresh mass, plants from all the three chambers (ozone-enriched, charcoal-filtered and unfiltered air) were investigated. However, for analyses of sugar contents, only fruits from plants grown in ozone-enriched or charcoal-filtered air were compared.

Data analyses

The experimental data were analysed with the SPSS statistical package (SPSS INC., Chicago, USA). A 5 % probability level was accepted to indicate significant differences. The data were tested for normal distribution and variance homogeneity and were compared by Duncan-tests. In some instances a logarithmic transformation of the data was necessary to stabilise the variances. If the variances were not homogeneously distributed, the non-parametric Wilcoxen-test was used and the probability level corrected according to Bonferroni. Additionally, a multifactorial ANOVA was performed on the total sugar content of strawberry fruits.

Results

Strawberry cvs 'Elsanta' and 'Korona' were only slightly affected by 13200 ppb-h ozone applied over three months. In cv. 'Elsanta' the ozone treatment tended to reduced leaf number one month after the ozone application was finished and chlorophyll content tended to decline somewhat earlier in old leaves during the last six weeks of the experiment. In cv. 'Korona', however, this ozone-effect could not be observed (cf. KEUTGEN and LENZ 1997). Yield of class 'I' and 'Extra' fruits and number of fruits was in tendency reduced in both strawberry cultivars, and this tendency was more obvious in 'Elsanta' than in 'Korona' (Tables 1–2). Total

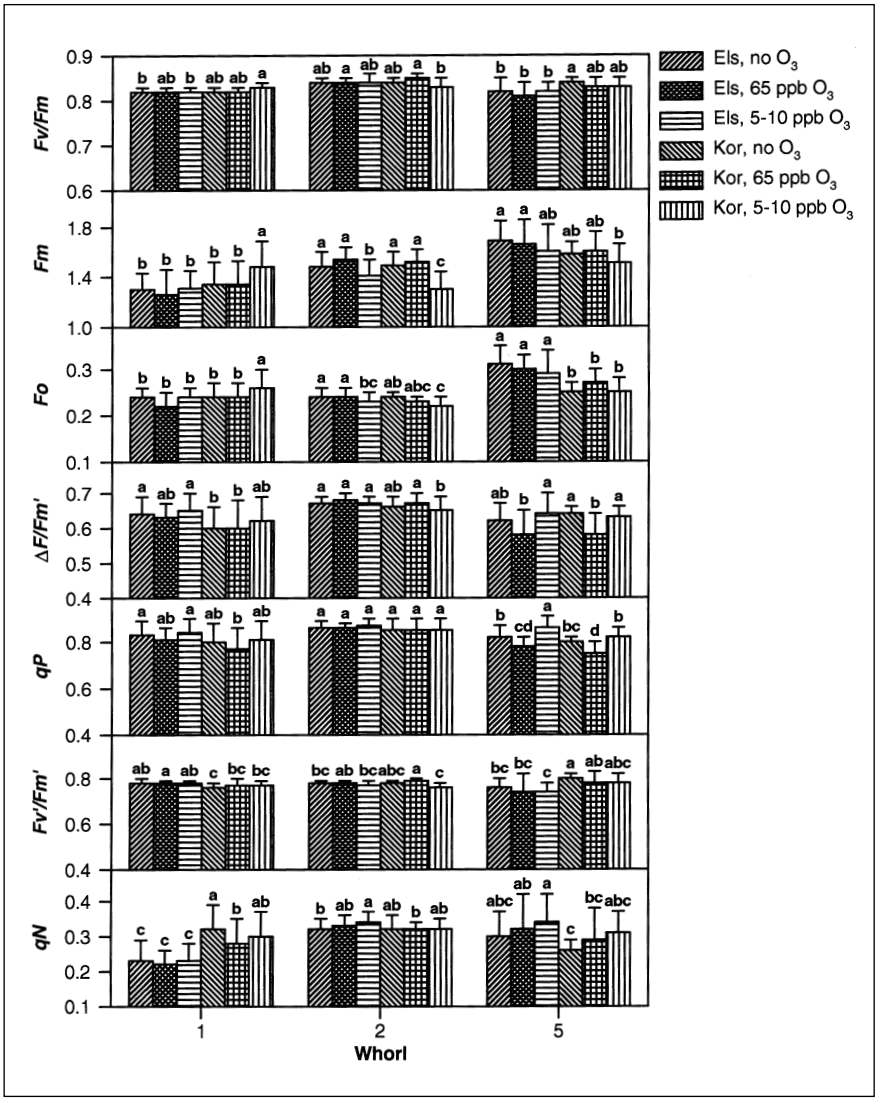


Fig. 1: Responses of strawberry cvs 'Elsanta' (Els) and 'Korona' (Kor) leaf chlorophyll fluorescence parameters to a long term exposure of 13200 ppb-h (65 ppb, eight hours per day) ozone. For comparable whorls, different letters indicate significant differences at the 5 % level by Duncan or Wilcoxon tests. Bars represent the standard deviation (n = 21).
Veränderungen der Chlorophyllfluoreszenzparameter der Erdbeersorten 'Elsanta' (Els) und 'Korona' (Kor) durch eine langfristige Ozonbegasung von 13200 ppb-h (65 ppb, acht Stunden pro Tag). Für vergleichbare Wirtel zeigen unterschiedliche Buchstaben signifikante Unterschiede auf den 5 %-Niveau nach Duncan oder Wilcoxon an. Fehlerbalken repräsentieren die Standardabweichung (n = 21).

sugar content of strawberry fruits was significantly reduced in one of two years in both strawberry cultivars. The multifactorial ANOVA revealed a significant ozone effect on the total sugar content in cvs 'Elsanta' and 'Korona', but in cv. 'Korona' also an influence of the year, which may point to an effect of the comparatively strong spider mite infection in April 1997 reducing fruit sugar content as well.

Leaf gas exchange of strawberry cv. 'Korona' was not affected, that of cv. 'Elsanta' only slightly affected by 65 ppb ozone in leaves of the second and fifth whorl (Table 3). For cv. 'Elsanta', A and E were not affected at any leaf stage, but g_s increased significantly and C_i in tendency in leaves of the second and older whorls. The ozone-induced increase of g_s was accompanied by an increase in stomatal density by about 14 % in leaves of the second whorl. Stomatal density of cv. 'Elsanta' varied between 150 and 300 per mm^2, that of cv. 'Korona' between 200 and 400 per mm^2 in control leaves. Differences in respiration among the treatments were not observed (Table 3).

Chlorophyll fluorescence parameters *Fv/Fm*, *Fm*, *Fo*, Δ*F/Fm'*, *qP*, *Fv'/Fm'*, and *qN* indicated hardly any ozone effect on PS II in leaves of the first and second whorls. However, in leaves of the fifth whorl, Δ*F/Fm'* and *qP* were distinctly reduced in cvs 'Elsanta' and 'Korona' (Fig. 1). Total *Chl*, *Chl* a, *Chl* b contents and a/b ratio of strawberry leaves from the second whorl were unaffected by ozone. Nevertheless, *Chl* content was larger in cv. 'Korona', whereas a/b ratios were similar for both cultivars (Table 4).

Leaf contents of N, P, K, Ca, and Mg were not affected by the ozone treatment (Table 5). It is noteworthy, however, that the Ca content was about twice as high in cv. 'Korona' when compared to cv. 'Elsanta'. Mg content was also somewhat elevated in cv. 'Korona', whereas K content was in tendency higher in leaves of cv. 'Elsanta'.

Discussion

The growth of the strawberry cvs 'Elsanta' and 'Korona' was only slightly affected by 13200 ppb-h ozone applied during three months. Visible differences between the control variants with 5–10 ppb or without ozone and the plants treated with 65 ppb ozone were hardly

Table 3: Responses of the strawberry cvs 'Elsanta' (Els) and 'Korona' (Kor) leaf gas exchange to a long-term exposure of 13200 ppb-h (65 ppb eight hours per day) ozone.
Veränderungen des Gaswechsels der Erdbeersorten 'Elsanta' (Els) und 'Korona' (Kor) durch eine langfristige Ozonbegasung von 13200 ppb-h (65 ppb, acht Stunden pro Tag).

	Els, filtered no O_3	Els, filtered 65 ppb O_3	Els, unfiltered 5–10 ppb O_3	Kor, filtered no O_3	Kor, filtered 65 ppb O_3	Kor, unfiltered 5–10 ppb O_3
			first whorl			
A	6.1 ± 2.9 ab	6.7 ± 3.7 ab	5.1 ± 3.2 b	8.6 ± 3.8 a	8.1 ± 2.1 a	7.2 ± 2.4 ab
E	2.7 ± 0.8 a	2.8 ± 0.8 a	2.6 ± 0.9 a	2.9 ± 0.8 a	2.9 ± 0.8 a	2.8 ± 1.1 a
Ci	293 ± 42 ab	302 ± 28 a	281 ± 45 abc	258 ± 41 c	268 ± 31 bc	257 ± 43 c
g_s	162 ± 64 ab	179 ± 49 ab	130 ± 49 b	192 ± 75 a	185 ± 46 a	164 ± 88 ab
			second whorl			
A	13.7 ± 5.1 a	13.9 ± 5.5 a	13.5 ± 4.4 a	13.7 ± 4.8 a	14.5 ± 5.6 a	14.0 ± 4.7 a
E	3.6 ± 1.4 a	3.9 ± 1.4 a	3.6 ± 1.6 a	4.1 ± 1.8 a	4.2 ± 1.5 a	3.9 ± 1.5 a
Ci	226 ± 38 a	235 ± 35 a	229 ± 36 a	243 ± 34 a	232 ± 27 a	236 ± 32 a
g_s	202 ± 58 b	270 ± 55 a	206 ± 60 b	297 ± 100 a	303 ± 80 a	284 ± 66 a
R	1.6 ± 0.4 a	1.6 ± 0.7 a	1.6 ± 0.9 a	1.4 ± 0.6 a	1.6 ± 0.4 a	1.2 ± 0.4 a
stom.d.	192 ± 47 b	222 ± 44 a	189 ± 46 b	272 ± 65 a	260 ± 74 a	264 ± 60 a
			fifth whorl			
A	5.2 ± 3.1 ab	7.8 ± 4.1 a	5.4 ± 3.1 ab	6.8 ± 3.7 ab	5.5 ± 2.2 ab	4.4 ± 2.3 b
E	2.5 ± 1.4 ab	3.4 ± 1.1 a	2.6 ± 1.0 ab	3.3 ± 1.2 a	2.8 ± 1.0 ab	2.2 ± 1.1 b
Ci	274 ± 33 ab	294 ± 50 a	275 ± 48 ab	272 ± 32 ab	279 ± 35 ab	252 ± 53 b
g_s	112 ± 60 bc	193 ± 59 a	111 ± 43 bc	188 ± 73 a	150 ± 43 ab	94 ± 58 c

The values represent means ± S.D. The number of replicates is 14 for the first and fifth whorl and 35 for the second whorl with the exception of respiration (14 measurements) and stomatal density (stom.d. = 48 measurements). In a row, different letters indicate significant differences at the 5 % level by Duncan tests [*A*, *R* in µmol CO_2 $m^{-2}s^{-1}$, *E* in mmol H_2O $m^{-2}s^{-1}$, C_i in ppm, g_s in mmol $m^{-2}s^{-1}$].

Table 4: Responses of chlorophyll contents in mg g^{-1} dry mass of leaves from the second whorl of strawberry cvs 'Elsanta' (Els) and 'Korona' (Kor) to a long-term exposure of 13200 ppb-h ozone.
Veränderungen der Chlorophyllgehalte in mg g^{-1} Trockenmasse von Blättern aus dem zweiten Wirtel der Erdbeersorten 'Elsanta' (Els) und 'Korona' (Kor) durch eine langfristige Ozonbegasung von 13200 ppb-h (65 ppb, acht Stunden pro Tag).

	Els, filtered no O_3	Els, filtered 65 ppb O_3	Els, unfiltered 5–10 ppb O_3	Kor, filtered no O_3	Kor, filtered 65 ppb O_3	Kor, unfiltered 5–10 ppb O_3
Chl a	6.0 ± 0.4 c	6.2 ± 0.4 bc	6.2 ± 0.3 bc	7.0 ± 0.3 a	6.6 ± 0.4 ab	6.9 ± 0.3 a
Chl b	2.1 ± 0.2 b	2.3 ± 0.3 ab	2.3 ± 0.4 ab	2.6 ± 0.3 a	2.5 ± 0.2 ab	2.5 ± 0.2 ab
tot. *Chl*	8.1 ± 0.6 c	8.5 ± 0.7 bc	8.5 ± 0.6 bc	9.6 ± 0.6 a	9.1 ± 0.6 ab	9.4 ± 0.5 a
a/b	2.8 ± 0.2 a	2.7 ± 0.2 a	2.8 ± 0.3 a	2.7 ± 0.2 a	2.7 ± 0.1 a	2.8 ± 0.1 a

The values represent means ± S.D. The number of replicates is 7. In a row, different letters indicate significant differences at the 5 % level by Duncan tests.

Table 5: Macronutrient contents in mg g^{-1} dry mass of leaves from the second whorl of strawberry cvs 'Elsanta' and 'Korona' partly exposed to 13 200 ppb-h ozone for three months.
Mineralstoffgehalte in mg g^{-1} Trockenmasse von Blättern aus dem zweiten Wirtel der Erdbeersorten 'Elsanta' (Els) und 'Korona' (Kor), die teilweise einer langfristigen Ozonbegasung von 13200 ppb-h (65 ppb, acht Stunden pro Tag) ausgesetzt waren.

	Elsanta, filtered air, no O_3	Elsanta, filtered air, 65 ppb O_3	Korona, filtered air, no O_3	Korona, filtered air, 65 ppb O_3
N	25.4 ± 3.3 a	28.3 ± 3.5 a	24.5 ± 3.2 a	26.8 ± 2.9 a
P	4.4 ± 1.2 a	4.3 ± 0.8 a	3.9 ± 1.6 a	3.2 ± 0.9 a
K	17.3 ± 2.4 ab	18.5 ± 3.0 a	15.5 ± 3.0 ab	14.2 ± 2.6 b
Ca	11.0 ± 3.5 b	14.4 ± 2.7 b	22.1 ± 5.3 a	21.7 ± 3.8 a
Mg	4.8 ± 1.0 b	4.8 ± 0.4 b	6.5 ± 1.4 a	6.1 ± 0.6 a

The values represent means ± S.D. The number of replicates is 7. In a row, different letters indicate significant differences at the 5 % level by Duncan and Wilcoxon tests.

detectable, but during the last six weeks of and after ozone application the fumigated plants of cv. 'Elsanta' had less leaves (cf. KEUTGEN and LENZ 1997), because older leaves died somewhat earlier. Differences in leaf net photosynthesis rate could not be detected between ozone fumigated and control leaves of the first, second and fifth whorl (Table 3). Chlorophyll contents of leaves of the second whorl were not affected by ozone (Table 4), indicating that exposure to 65 ppb ozone during leaf growth had no major and lasting effect on chloroplast development and attainment of photosynthetic competence. A similar result was obtained for

wheat exposed to 150 ppb ozone (NIE et al. 1993). However, ozone fumigation did have a detrimental effect on the ability of fully developed leaves to maintain chlorophyll contents in cv. 'Elsanta'. The accelerated decline of chlorophyll content of old leaves during the last six weeks of the experiment, which was indicated by a visible chlorophyll reduction, was consistent with ozone inducing accelerated senescence of the leaf tissue and also indicated by the in tendency reduced leaf number in the oldest whorl(s) of cv. 'Elsanta' due to an earlier leaf fall at the end of the experiment (cf. KEUTGEN and LENZ 1997). Accelerated leaf senescence is a widespread response to ozone (LEHNHERR et al. 1987, NIE et al. 1993, KANGASJÄRVI et al. 1994) and breakdown of chlorophyll may be one of the earliest symptoms of senescence. Another symptom is closure of stomata leading to a decline in transpiration (PEÑARRUBIA and MORENO 1995). This decline was not detected in cv. 'Elsanta'. Instead, stomatal density increased due to ozone fumigation, increasing stomatal conductance of leaves of the second and fifth whorl. The increased stomatal density might have hidden the closure of stomata due to accelerated leaf senescence in cv. 'Elsanta'. For a detailed discussion on stomatal responses of strawberry cvs 'Elsanta' and 'Korona' to ozone, the interested reader is referred to KEUTGEN et al. (1998) and further literature mentioned therein.

Exposure to ozone during leaf growth had no significant effect on *Fv/Fm* in leaves of the first, second and fifth whorl, indicating that the efficiency with which individual PS II complexes utilise absorbed photons for photochemistry did not change. Because *Fv/Fm* has been reported to remain constant during senescence (JENKINS et al. 1981) this result could be expected. The absence of any decrease in *Fv/Fm* also indicated that there wasn't any photoinhibitory damage to PS II reaction centres due to ozone.

In leaves of the fifth whorl, however, PS II efficiency was affected by ozone stress, as $\Delta F/Fm'$ and *qP* were distinctly reduced in cvs 'Elsanta' and 'Korona' (Fig. 1). According to SCHREIBER et al. (1995), effective quantum yield $\Delta F/Fm'$ is considered to be determined by the product of reaction centre 'openness', *qP*, and excitation capture efficiency of open centres, Fv'/Fm'. Because Fv'/Fm' was not affected by ozone in leaves of the fifth whorl, the decrease of $\Delta F/Fm'$ can be attributed to a reduction of open PS II reaction centres in both strawberry cultivars.

To summarise, leaf photosynthesis was only slightly affected by ozone in the strawberry cvs 'Elsanta' and 'Korona' and CO_2-uptake rates did not differ significantly. Hence, the in tendency reduced fruit yield and sugar contents of fruits cannot be explained by an impairment of CO_2 assimilation. An increased respiration of the strawberry cultivars in response to ozone stress is also improbable, because dark respiration measurements on single leaves of the second whorl did not point to any significant difference (Table 3). However, an increase of whole plant respiration due to ozone stress cannot be excluded yet. The slightly reduced fruit yield of up to ca. 10% to 20% may reflect the consumption of carbohydrates in the plant defence metabolism against the oxidative stress induced by ozone.

Strawberry cv. 'Elsanta' was somewhat more sensitive to 65 ppb ozone than cv. 'Korona', which was indicated by the slightly more pronounced decline of chlorophyll content and reduced leaf number one month after the ozone application (KEUTGEN and LENZ 1997). This ozone response is typical of accelerated senescence, which is associated with ozone-induced peroxidation of plant membrane lipids and as a consequence modification of membrane permeability (KANGASJÄRVI et al. 1994). The elevated ozone-sensitivity of cv. 'Elsanta' may thus be related to certain membrane properties. Noteworthy, the Ca content of 'Elsanta' leaves was about half when compared with cv. 'Korona' (Table 5). Calcium measurement studies indicate that the concentration of calcium in the cytoplasm in plant cells is maintained in the micromolar range, whereas concentrations in the cell wall and in organelles are in the millimolar range (REDDY 1995). There is ample evidence that Ca plays an important role in plant responses to environmental and biotic stresses. This evidence is based on the well-recognised importance of Ca^{2+} in membrane structure and function as well as in cell wall structure. For example, it is essential to have Ca^{2+} in the extracellular solution to ensure the maintenance of selective permeability, i.e., membrane integrity (PALTA 1996). In addition, Ca^{2+} stabilises cell membranes by bridging phosphate and carboxylate groups of phospholipids at the membrane surface (LEGGE et al. 1982). Studies on leaf senescence show that tissue Ca content often influences various senescence characteristics, e.g., protein and chlorophyll content or rate of respiration. Most Ca entering the tissues accumulates in cell walls and membranes that are thought to be sites of its antisenescence action (CHÉOUR et al. 1990). In consequence, Ca may represent an important component in ozone tolerance, if exposure of plants to ozone results in accelerated senescence as proposed by NIE et al. (1993) and MEYER et al. (1997) for wheat and herein for strawberry. The lower Ca content in leaves of cv. 'Elsanta' corresponds well with the higher sensitivity of this cultivar to ozone. The Mg content was also significantly higher in cv. 'Korona' than in cv. 'Elsanta', but this difference seems to reflect differences in chlorophyll contents of the two cultivars, as Mg represents the central metal atom in chlorophyll.

Conclusion

Net CO_2 assimilation rates of single leaves were not significantly affected by 65 ppb ozone applied over three months in both strawberry cvs 'Elsanta' and 'Korona', indicating that the performance of the photosynthetic system on the whole was not impaired. Yield and sugar content of fruits were in tendency reduced by elevated ozone concentrations, but for horticultural practice ozone does not represent a threat for strawberry production, at least in Germany, because yield reduction was small in comparison with the supplied ozone burden.

'Elsanta' and 'Korona' reacted slightly different to the long-term 13200 ppb-h ozone stress and cv. 'Elsanta' turned out to be less tolerant. The reasons for the different sensitivities are still unknown. Whole plant photosynthesis and respiration reflecting plant growth were not investigated, and hence it is still uncertain whether one or both of these parameters contributed to the reduction of strawberry fruit yield and differences

between the cultivars. Research on the importance of certain metabolites involved in ozone defence such as phenols, ascorbate and glutathione as well as the activity of special enzymes and plasma membrane properties are either in progress or planned and will be reported in subsequent articles.

We are grateful to the Deutsche Forschungsgemeinschaft for funding (DFG Le281/39-1).

Literature

AARNES, H., C. BAUMANN, A. B. ERIKSEN, A. SOLÅS, A. SUNDBYE and K. A. SØLVERNES 1993: Photosynthesis in ozone-exposed duckweed (*Lemna gibba*). Physiol. Plant. **87**, 256–262.

BOEHRINGER GmbH 1995: Methoden der enzymatischen BioAnalytik und Lebensmittelanalytik mit Test-Combinationen. Mannheim, Boehringer Mannheim Biochemica, Germany.

CHEN, K., G. HU and F. LENZ 1997: Effects of CO_2 concentrations on strawberry. V. Macronutrient uptake and utilization. Angew. Bot. **71**, 189–194.

CHÉOUR, F., C. WILLEMOT, J. ARUL, Y. DESJARDINS, J. MAKHLOUF, P. M. CHARES and A. GOSSELIN 1990: Foliar application of calcium chloride delays postharvest ripening of strawberry. Journal of the American Society for Horticultural Science **115**, 789–792.

FUHRER, J. 1994: The critical level for ozone to protect agricultural crops – An assessment of data from European open-top chamber experiments. In: Fuhrer, J. and B. Achermann (eds), Critical levels for ozone: an UN-ECE Workshop report. Swiss Federal Research Station for Agricultural Chemistry and Environmental Hygiene, Liebefeld-Bern, Switzerland, 42–57.

JENKINS, G. I., N. R. BAKER, M. BRADBURY and H. W. WOOLHOUSE 1981: Photosynthetic electron transport during senescence of primary leaves of *Phaseolus vulgaris* L. III. Kinetics of chlorophyll fluorescence emission from intact leaves. Journal of Experimental Botany **32**, 999–1008.

KANGASJÄRVI, J., J. TALVINEN, M. UTRIAINEN and R. KARJALAINEN 1994: Plant defence systems induced by ozone. Plant, Cell and Environment **17**, 783–794.

KEUTGEN, N., K. CHEN and F. LENZ 1998: Stomatal responses of strawberry leaves to moderate ozone. Gartenbauwissenschaft **64**, 70–74.

KEUTGEN, N. and F. LENZ F. 1997: Auswirkungen von Ozon auf Erdbeeren. Erwerbsobstbau **39**, 74–77.

KEUTGEN, N., H. OBAID and F. LENZ 1997: Different sensitivities of the strawberry cultivars 'Elsanta', 'Korona', 'Polka', and 'Tenira' to ozone. Gartenbauwissenschaft **62**, 157–162.

LEGGE, A. H., L. GRÜNHAGE, M. NOSAL, H.-J. JÄGER and S. V. KRUPA 1995: Ambient ozone and adverse crop response: An evaluation of North American and European data as they relate to exposure indices and critical levels. Angew. Bot. **69**, 192–205.

LEGGE, R. L., E. THOMPSON, J. E. BAKER and M. Lieberman 1982: The effect of calcium on the fluidity and phase properties of microsomal membranes isolated from postclimacteric Golden Delicious apples. Plant Cell Physiology **23**, 161–169.

LEHNHERR, B., A. GRANDJEAN, F. MÄCHLER and J. FUHRER 1987: The effect of ozone in ambient air on RubisCo activity decreases photosynthesis and grain yield in wheat. Journal of Plant Physiology **130**, 189–200.

MCCOOL, P. M., R. C. MUSSELMANN, R. R. TESO and R. J. OSHIMA 1986: Determining crop yield losses from air pollution. Calif. Agr. **40**, 9–10.

MEYER, U., B. KÖLLNER, J. WILLENBRINK and G. H. M. KRAUSE 1997: Physiological changes on agricultural crops induced by different ambient ozone exposure regimes. I. Effects on photosynthesis and assimilate allocation in spring wheat. New Phytologist **136**, 645–652.

NIE, G.-Y., M. TOMASEVIC and N. R. BAKER 1993: Effects of ozone on the photosynthetic apparatus and leaf proteins during leaf development in wheat. Plant, Cell and Environment **16**, 643–651.

PALTA, J. P. 1996: Role of calcium in plant responses to stress: Linking basic research to the solution of practical problems. HortScience **31**, 51–56.

PEÑARRUBIA, L. and J. MORENO 1995: Senescence in plants. In: M. Pessarakli (ed.), Handbook of plant and crop physiology, Marcel Dekker Inc., New York, USA, 461–482.

REDDY, A. S. N. 1995: Calcium as a messenger in stress signal transduction. In: Pessarakli, M. (ed.), Handbook of plant and crop physiology, Marcel Dekker Inc., New York, USA, 719–732.

SCHREIBER, U., W. BILGER and C. NEUBAUER 1995: Chlorophyll fluorescence as a nonintrusive indicator for rapid assessment of in vivo photosynthesis. In: Schulze, E.-D. and M. M. Caldwell (eds), Ecophysiology of Photosynthesis, Springer Verlag, Berlin Heidelberg, Germany, 49–70.

ŠESTÁK, Z. 1971: Determination of chlorophyll a and b. In: Šesták, Z., K. J. Catský and P.G. Jarvis (eds), Plant photosynthetic production. Manual of methods. Dr W. Junk N.V. Publishers, The Hague, The Netherlands, 672–701.

SRUAMSIRI, P. and F. LENZ 1985: Photosynthese und stomatäres Verhalten bei Erdbeeren. I. Einfluß von Licht. Gartenbauwissenschaft **50**, 78–83.

TAKEMOTO, B. K., A. BYTNEROWICZ and D. OLSZYK 1989: Physiological responses of field-grown strawberry (*Fragaria* x *ananassa* Duch.) exposed to acid fog and ambient ozone. Environ. Exp. Bot. **29**, 379–389.

THIEL, A. 1994: Ausmaß ozonbedingter Vegetationsschäden bei landwirtschaftlichen Kulturpflanzen (Insbesondere bei Sommerweizen). Critical levels for ozone of UN-ECE 1994. Umweltinstitut München e.V. Deutschland.

VOLTZ, A. and D. KLEY 1988: Evaluation of the Montsouris series of ozone measurements made in the 19th century. Nature **332**, 240–242.

Eingegangen: 20. 1. 2000 / 9. 5. 2000

Anschrift der Verfasser: N. Keutgen and Prof. em. F. Lenz, Institut für Obstbau und Gemüsebau der Universität Bonn, Auf dem Hügel 6, D-53121 Bonn, Germany, e-mail. Keutgen_N@yahoo.com.