Ozone effects on wheat in relation to CO_2: modelling short-term and long-term responses of leaf photosynthesis and leaf duration

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Abstract

A combined stomatal–photosynthesis model was extended to simulate the effects of ozone exposure on leaf photosynthesis and leaf duration in relation to CO_2. We assume that ozone has a short-term and a long-term effect on the Rubisco-limited rate of photosynthesis, A_c. Elevated CO_2 counteracts ozone damage via stomatal closure. Ozone is detoxified at uptake rates below a threshold value above which A_c decreases linearly with the rate of ozone uptake. Reduction in A_c is transient and depends on leaf age. Leaf duration decreases depending on accumulated ozone uptake. This approach is introduced into the mechanistic crop simulation model AFRCWHEAT2. The derived model, AFRCWHEAT2-O3, is used to test the capability of these assumptions to explain responses at the plant and crop level.

Simulations of short-term and long-term responses of leaf photosynthesis, leaf duration and plant and crop growth to ozone exposure in response to CO_2 are analysed and compared with experimental data derived from the literature. The model successfully reproduced published responses of leaf photosynthesis, leaf duration, radiation use efficiency and final biomass of wheat to elevated ozone and CO_2. However, simulations were unsatisfactory for cumulative radiation interception which had some impact on the accuracy of predictions of final biomass. There were responses of leaf-area index to CO_2 and ozone as a result of effects on tillering which were not accounted for in the present model. We suggest that some model assumptions need to be tested, or analysed further to improve the mechanistic understanding of the combined effects of changes in ozone and CO_2 concentrations on leaf photosynthesis and senescence. We conclude that research is particularly needed to improve the understanding of leaf-area dynamics in response to ozone exposure and elevated CO_2.

Keywords: CO_2, leaf duration, leaf photosynthesis; modelling, ozone, wheat

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Introduction

Increases in atmospheric ozone and CO_2 concentrations have been predicted for the future (IPCC 1992, 1994). Chronic responses (Heath 1994; Sandermann 1996) of wheat crops to ozone exposure vary with CO_2 concentration (Hertstein *et al.* 1995; McKee *et al.* 1997a; Heagle *et al.* 1998) and other climatic conditions (Heagle 1989; Bender *et al.* 1999) and are difficult to predict (Kärenlampi & Skärby 1996; Fuhrer *et al.* 1997; Bender *et al.* 1999). Mechanistic crop simulation models might be

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a useful tool to improve the understanding and prediction of ozone effects on wheat. However, little work has been done to extend these models for the combined effects of ozone and CO_2.

There is substantial experimental evidence that ozone exposure induces inhibition of leaf photosynthesis (see reviews of Reich 1987; Darrall 1989; Saxe 1991; Heath 1994; Pell *et al.* 1994) and acceleration of leaf senescence (Lehnherr *et al.* 1987; Grandjean & Fuhrer 1989; Ojanperä *et al.* 1992,1998; Fangmeier *et al.* 1993; Nie *et al.* 1993; Sandelius *et al.* 1995; Mulholland *et al.* 1997, 1998). In wheat, a reduction in carboxylation capacity appears to

be the main cause of the decline in the light-saturated rate of CO_2 uptake (Farage *et al.* 1991; Farage & Long 1995). Elevated CO_2 has been shown to protect against the ozone-induced reduction in photosynthesis in wheat primarily via a reduction in stomatal conductance (McKee *et al.* 1995). Acceleration of leaf senescence following long-term exposure to low ozone concentrations seems to be related to the increased respiratory costs associated with poorly understood detoxification and repair processes (Alscher & Amthor 1988; Amthor 1988; Mehlhorn 1990; Pell *et al.* 1994, 1997). Evidence suggests that photosynthetic response to ozone fumigation changes with development stage (Pell *et al.* 1992; Eckardt & Pell 1994; McKee *et al.* 1995; Carlsson *et al.* 1996). Some mechanistic approaches have modelled ozone effects on leaf photosynthesis (review by Kickert & Krupa 1991; Martin *et al.* 1995). However, none refers to chronic responses of wheat to ozone exposure. There is limited published information about attempts to model the combined effects of ozone and CO_2 elevation on leaf photosynthesis and little is known about the integration of responses from the leaf to the plant and crop level. It has been suggested that photosynthetic responses to ozone and CO_2 elevation should also apply in the field (McKee *et al.* 1995).

The present paper describes an attempt to assemble, into a coherent framework, experimentally derived understanding of short-and long-term effects of ozone and CO_2 on leaf photosynthesis and leaf duration considering that:

(i) ozone reduces photosynthesis and stomatal conductance via an effect on the Rubisco-limited rate of photosynthesis;

(ii) elevated CO_2 counteracts ozone damage via stomatal closure;

(iii) ozone can be detoxified and ozone damage depends on leaf age and can be repaired;

(iv) ozone enhances leaf senescence.

This approach is introduced into the mechanistic crop simulation model AFRCWHEAT2 (Porter 1993). Simulations of leaf photosynthesis, leaf duration and plant and crop growth in response to ozone exposure and elevated CO_2 are analysed and are compared with experimental data described in the literature. We also attempt to test the capability of the relationships between the two gases at the leaf level to explain observed responses at the plant and crop level.

Theory and model description

Photosynthesis and stomatal conductance model

We started with the biochemical model of leaf photosynthesis developed by Farquhar *et al.* (1980) and von Caemmerer & Farquhar (1981). Net assimilation is calculated as the minimum of the Rubisco-limited rate of photosynthesis at light-saturated conditions, A_c, and of the rate of leaf photosynthesis limited by RuP2 regeneration at subsaturating light intensities, A_q.

$$A = \min\{A_c, A_q\} - R_d \tag{1}$$

R_d represents the day respiration rate. The Rubisco-limited rate of photosynthesis is given by

$$A_c = \frac{(c_i - \Gamma^*)V_{cmax}}{c_i + K_c(1 + o_i/K_o)} \tag{2}$$

in which V_{cmax} is the maximum carboxylation velocity, Γ_* is the CO_2 compensation point in the absence of respiration, K_c and K_o are the Rubisco Michaelis–Menten constants for CO_2 and O_2 and c_i and o_i are the intercellular CO_2 and O_2 concentrations. Leaf photosynthesis at subsaturating light intensities is given by

$$A_q = \frac{(c_i - \Gamma^*)J}{4.5c_i + 10.5\,\Gamma^*} \tag{3}$$

where J is the rate of photosynthetic electron transport. We used the nonrectangular hyperbola function described in Farquhar *et al.* (1980) and Farquhar & Wong (1984) to describe J in terms of the absorbed irradiance,

$$J = \frac{J_{max} + I_c - \sqrt{(J_{max} + I_c)^2 - 4\theta J_{max}I_c}}{2\theta} \tag{4}$$

where I_c is calculated from incident irradiance, I, as follows $I_c = I(1-f_l)/2$ (Farquhar & Wong 1984) The term f_l represents the fraction of light not absorbed by the chloroplast and θ is the curvature of the hyperbolic function. The potential light-saturated rate of electron transport, J_{max}, is temperature-dependent and is calculated according to Farquhar (1988).

The photosynthesis model is coupled with a semi-empirical model of stomatal conductance for CO_2 described by Leuning (1995)

$$g_{sc} = g_0 + \frac{a_1 A}{(c_a - \Gamma)(1 + D_s/D_0)} \tag{5}$$

in which A is the net assimilation rate; D_s and c_a are the humidity deficit and the CO_2 concentration at the leaf surface, respectively; Γ is the CO_2 compensation point; g_0 is the conductance as $A \rightarrow 0$ when $I \rightarrow 0$; and D_0 and a_1 are empirical coefficients (Leuning 1995). For the present analysis we assumed that boundary layer conductance is high and constant under the different conditions. This assumption is reasonable, because there was rapid air recirculation in the chambers of the experiments used to test the model. The coupling of photosynthesis and stomatal model provides a complete solution for g_{sc}, c_i and A (Leuning 1995) and a mechanistic explanation for

Table 1 Definition and default values of parameters used in the simulations.

Parameter	Unit	Definition	Value
Stomatal conductance			
g_0	mol m^{-2} s^{-1}	Conductance as $A \to 0$ when $I \to 0$	0.01
a_1	(–)	empirical coefficient	7.0
D_0	kPa	empirical coefficient	0.75
Ozone damage			
f_{DO3}	(–)	Ratio of diffusion rates for ozone and CO_2	0.93
γ_1	(–)	Ozone short-term damage coefficient	0.060
γ_2	(nmol m^{-2} s^{-1})$^{-1}$	Ozone short-term damage coefficient	0.0045
γ_3	(μmol m^{-2})$^{-1}$	Ozone long-term damage coefficient	0.5

the decrease in stomatal conductance observed in elevated CO_2 environments (Morison 1985).

Description of leaf duration

The description of leaf duration [i.e. the time (d) between leaf emergence and complete senescence] refers to relationships used in the crop model AFRCWHEAT2 (Porter 1984, 1993; Weir *et al*. 1984). The life-span of a leaf (t_l is calculated as a thermal time-interval (°Cday). It is assumed that t_l comprises the thermal time-intervals of the emerging leaf ($t_{l,em}$) and the mature leaf ($t_{l,ma}$),

$$t_l = t_{l,em} + t_{l,ma} \qquad (6)$$

where $t_{l,ma}$ composes the thermal time during which the leaf is fully expanded ($t_{l,ep}$) and the thermal time during which the leaf is senescing ($t_{l,se}$) (Porter 1984).

$$t_{l,ma} = t_{l,ep} + t_{l,se} . \qquad (7)$$

All thermal time-intervals can be calculated from the thermal phyllochron (i.e. the thermal time between the appearance of successive leaf tips) (Porter 1984). In the present study we employed a phyllochron of 87 °C day which was derived from experiments with spring wheat cv. Minaret (Ewert & Porter 1997).

Modelling the effects of ozone

At the physiological level of plants exposed to ozone inhibition of photosynthesis and acceleration of leaf senescence are the two main alteration patterns (e.g. Lehnherr *et al*. 1987; Heath 1994; Pell *et al*. 1994). Analysing the effects of ozone exposure on photosynthesis, Darrall (1989) and Saxe (1991) distinguish between short-term (hours) and long-term (seasonal) responses. We follow this approach in our model under the assumptions that the Rubisco-limited rate of photosynthesis, A_c, decreases (i) immediately at high ozone fluxes, $f_{O3,s}(d)$ and (ii) with leaf senescence (Loreto *et al*.

1994) which is enhanced dependent on cumulative ozone uptake (f_{LS}).

$$A_c = \frac{(c_i - \Gamma^*)V_{cmax}}{c_i + K_c(1 + o_i/K_o)} f_{O3,s}(d) f_{LS} . \qquad (8)$$

In the knowledge that ozone uptake occurs primarily through the stomata (Kerstiens & Lendzian 1989; Laisk *et al*. 1989) and that the intercellular ozone concentration is virtually zero (Laisk *et al*. 1989)

$$O_{3,up} = O_{3,a} g_{sc} f_{DO3} , \qquad (9)$$

where $O_{3,a}$ is the ozone concentration at leaf surface and $O_{3,up}$ is the uptake rate corrected for differences in the diffusivities of CO_2 and ozone in air (Laisk *et al*. 1989) (f_{DO3}, Table 1).

Short-term response and recovery from ozone damage. The ability of plants to resist low-level ozone concentrations via detoxification and repair systems has been discussed frequently (Alscher & Amthor 1988; Heath 1994; Pell *et al*. 1997). However, once ozone flux exceeds a particular threshold, photosynthesis declines — an effect often linked to a loss of Rubisco activity (see Pell *et al*. 1994). Farage *et al*. (1991) and Farage & Long (1995) showed that the light-saturated rate of leaf photosynthesis and stomatal conductance in wheat responded within a few hours after ambient ozone concentration was increased from <3 nmol mol^{-1} to 200 and 400 nmol mol^{-1}. These authors consider carboxylation capacity to be the first point of damage and the major initial cause of the decline in light-saturated CO_2 uptake rate. In addition, the effects of ozone on leaf photosynthesis depend on leaf age (Saxe 1991; Pell *et al*. 1992; Nie *et al*. 1993; Eckardt & Pell 1994; McKee *et al*. 1995). A possible explanation is that the ability to protect against, and recover from, ozone damage depends on leaf age (Pell *et al*. 1992; Pell *et al*. 1994, 1997). Young leaves resist higher ozone concentrations and can recover completely from ozone damage within a period ranging from hours to a few days dependent on cultivar and conditions (Saxe 1991). In the
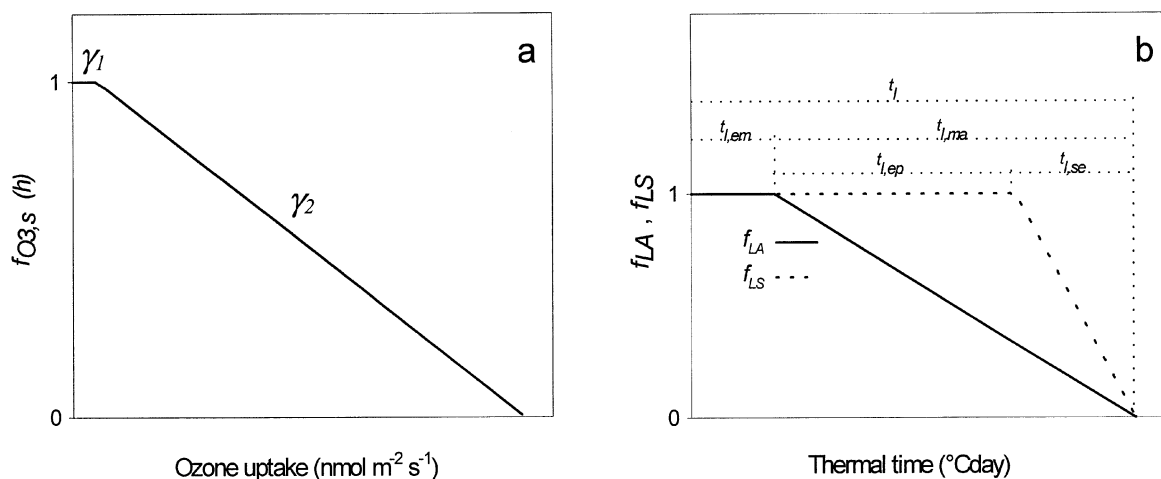
Fig. 1 Proposed relationships between (a) factor accounting for short-term ozone effect on the Rubisco-limited rate of photosynthesis ($f_{O3,s}$) and ozone uptake; and (b) factors used to simulate the recovery from ozone damage dependent on leaf age (f_{LA}), the decline in the Rubisco-limited rate of photosynthesis in senescing leaves (f_{LS}), and the thermal life-span of a leaf. Factors and coefficients are explained in the text.

present model we use a linear relationship between ozone uptake and decrease in A_c which is represented by the factor $f_{O3,s}$ calculated for every hour (see Fig. 1a).

$$
\begin{aligned}
f_{O3,s}(h) &= 1; & \text{for } O_{3,\text{up}} \leq \gamma_1/\gamma_2 \\
f_{O3,s}(h) &= 1 + \gamma_1 - \gamma_2 O_{3,\text{up}}; & \text{for } \gamma_1/\gamma_2 < O_{3,\text{up}} < (1+\gamma_1)/\gamma_2 \\
f_{O3,s}(h) &= 0; & \text{for } O_{3,\text{up}} \geq (1+\gamma_1)/\gamma_2.
\end{aligned}
\tag{10}
$$

The coefficients γ_1 and γ_2 are empirically determined (Table 1) and γ_1 takes in to account that low ozone concentrations are detoxified without direct effects on the photosynthetic system. The short-term damage factor, $f_{O3,s}(h)$, is set at a value of one if ozone uptake is low and it decreases with increasing rate of ozone uptake (Eqn 10, Fig. 1a). The coefficient γ_2 describes the decrease in A_c per unit of ozone influx. A similar approach was taken by Martin *et al.* (1995), who also used a threshold value for ozone damage above which carboxylation efficiency decreased with ozone exposure. Because we do not assume repair of ozone damage in the light we calculate the ozone-induced reduction in A_c considering the damage caused by ozone during the previous hour.

$$
f_{O3,s}(d) = f_{O3,s}(h)\, f_{O3,s}(h-1); \qquad \text{for } h = 2..24. \tag{11}
$$

However, repair of ozone damage is simulated during the night (Amthor 1988). Incomplete recovery from ozone damage of the previous day ($r_{O3,s}$) is considered in the calculation of the ozone effect on A_c of the following day.

$$
f_{O3,s}(d) = f_{O3,s}(h)\, r_{O3,s}; \qquad \text{for } h = 1. \tag{12}
$$

In the present model we assume that the capacity to recover from ozone damage depends on leaf age.

$$
r_{O3,s} = f_{O3,s}(d-1) + (1 - f_{O3,s}(d-1))f_{LA}, \tag{13}
$$

where the factor f_{LA} accounts for leaf age and is calculated over the life-span of the leaf, t_l (Fig. 1b). Young leaves can recover fully from ozone damage (Pell *et al.* 1992). Irreversible injury in emerging leaves that might occur at very high uptake rates is not considered in this study.

$$
f_{LA} = 1; \qquad \text{for } a_l \leq t_{l,\text{em}}, \tag{14}
$$

where a_l is the age of the leaf (°Cday). In mature leaves the repair capacity decreases linearly with the age of the mature leaf and falls to zero when the leaf is dead (Fig. 1b).

$$
\begin{aligned}
f_{LA} &= 1 - (a_l - t_{l,\text{em}})/t_{l,\text{ma}}; & \text{for } t_{l,\text{em}} < a_l < t_l \\
f_{LA} &= 0 & ; & \text{for } a_l \geq t_l
\end{aligned}
\tag{15}
$$

Long-term response. Wheat plants exposed to low ozone concentrations for prolonged periods have been shown to exhibit enhanced rates of senescence (e.g. Lehnherr *et al.* 1987, 1988; Nie *et al.* 1993). Reviewing results on the physiology of senescence, Huffaker (1990) suggested that degradation of Rubisco starts to increase quickly, following the triggering of a 'senescence signal'. Ozone may trigger such Rubisco degradation in mature leaves (Eckardt & Pell 1994). The mechanism underlying these effects remains unclear but stress ethylene production is considered to be involved (Heath & Taylor 1997; Pell *et al.* 1997). In the absence of further information we introduce a linear relationship between the life-span of a mature leaf, $t_{l,\text{ma}}$, and the accumulated ozone consumption.

$$
t_{l,\text{ma}} = (t_{l,\text{ep}} + t_{l,\text{se}})f_{O3,l}. \tag{16}
$$

Because $t_{l,\text{se}}$ changes with $t_{l,\text{ma}}$ ($t_{l,\text{se}} = 0.33\, t_{l,\text{ma}}$, Porter

1984) both the signal for the onset of senescence and the rate of senescence are affected due to ozone exposure represented by the factor $f_{O3,l}$ which is calculated as a function of accumulated ozone uptake and an empirically determined coefficient (γ_3, Table 1),

$$f_{O3,l} = 1 - \gamma_3 \int_0^{t=t_l} O_{3,\text{up}} \, dt. \qquad (17)$$

The coefficient γ_3 describes the reduction in the life time of a mature leaf per unit accumulated ozone uptake.

Finally, the factor that accounts for the effect of leaf senescence on A_c (Eqn 8, Fig. 1b) is calculated as,

$$
\begin{aligned}
f_{\text{LS}} &= 1; & &\text{for} \quad a_l \leq t_{l,\text{em}} + t_{l,\text{ep}} \\
f_{\text{LS}} &= 1 - \frac{a_l - t_{l,\text{em}} - t_{l,\text{ep}}}{t_{l,\text{ma}}/f_{O3,l} - t_{l,\text{ep}}}; & &\text{for} \quad t_{l,\text{em}} + t_{l,\text{ep}} < a_l < t_l \\
f_{\text{LS}} &= 0; & &\text{for} \quad a_l \geq t_l
\end{aligned}
$$

$$(18)$$

Leaf photosynthesis was simulated in hourly steps in order to account for the diurnal variation in ozone concentration and other climatic conditions and to allow comparison with results from the literature.

The crop model

The present approach is introduced into the process-orientated crop simulation model AFRCWHEAT2 (Porter 1984, 1993; Weir et al. 1984). The derived model (AFRCWHEAT2-O3) comprises five submodels: phenological and canopy development, dry matter production and partitioning and grain growth (Porter 1984; Weir et al. 1984) and includes subroutines that describe the movement of water and nitrogen within the soil profile and their uptake and effects on growth (Porter 1993). The model simulates phenology and canopy development at a high level of mechanistic detail and incorporates leaf and tiller emergence, growth and senescence (Porter 1984). Leaves are grouped into age classes. Photosynthetic rates are calculated for each leaf-age class, assuming the youngest leaves to be at the top of the canopy. The radiation interception model is based upon that described by Charles-Edwards (1978).

The time-step of the model is one day except for the assimilation rate which is simulated hourly. Water and nitrogen were assumed not to be limiting which agrees with the conditions of the controlled environment (McKee et al. 1995, 1997a,b) and open-top chamber experiments (Mulholland et al. 1997, 1998; Hertstein et al. 1999) chosen for the present analysis. AFRCWHEAT2-O3 runs with input data of temperature, radiation, VPD, CO_2 and ozone concentrations.

Model parameterization

The parameterization of the photosynthesis model was based upon Farquhar et al. (1980) with the exception of f_l and θ of the non-rectangular hyperbolic function of the relationship between the absorbed irradiance and rate of electron transport (Eqn 4), which have values of 0.23 and 0.7, respectively (see Farquhar & Wong 1984; Evans & Farquhar 1992). The stomatal model was parameterized for spring wheat cv. Minaret (Table 1). Relevant experimental data on which to quantify the two parameters representing the short-term responses to ozone were not available. Thus, we calculated both parameters based on investigations with winter wheat (cv. Avalon) performed by Farage et al. (1991) (Table 1). The parameter representing the long-term response of leaf photosynthesis to ozone exposure (Table 1) was calculated for spring wheat cv. Minaret from data of selected open-top chamber experiments of the ESPACE-wheat project (Ewert & Porter 1997). The submodels describing phenological and canopy development of the crop simulation model AFRCWHEAT2-O3 were parameterized for spring wheat cv. Minaret using independent experiments (Ewert & Porter 1997).

Experimental data

Simulations of leaf photosynthesis, leaf duration and wheat growth in response to ozone and CO_2 elevation were compared with data from two climate chamber experiments (Farage & Long 1995; McKee et al. 1995, 1997a,b) and one open-top chamber experiment (Mulholland et al. 1997, 1998) (Table 2).

We also consider results from a simulation analysis performed with the present model and a series of open-top chamber experiments carried out across Europe between 1994 and 1996 (Ewert et al. 1999). We have extended this analysis for the combined effects of ozone and CO_2 elevation on canopy processes. We selected only those experiments that obtained sufficient measurements in order to perform the present analysis. The experiments considered in this study were carried out, over periods up to three years, at Braunschweig (Germany, 52.2°N, 10.3°E), Giessen (Germany, 50.3°N, 8.41°E), Tervuren (Belgium, 50.5°N, 4.31°E), Sutton Bonington (United Kingdom, 52.8°N, 1.12°W) and Carlow (Ireland, 52.5°N, 6.55°W) and are described in more detail in Hertstein et al. (1999). A summary description of ozone and CO_2 concentrations and other climatic conditions of the selected experiments is given in Table 3. Measurements of leaf-area index and aboveground biomass were used to calculate cumulative radiation interception and radiation use efficiency (Ewert et al. 1999). All simulations

Table 2. Summary description of three experiments used for the comparison of simulated and observed data of ozone and CO_2 effects on leaf photosynthesis, leaf duration and growth of wheat.

	Farage & Long (1995)	McKee et al. (1995, 1997a,b)	Mulholland et al. (1997, 1998)
Cultivar	Avalon	Wembley	Minaret
Type of experiment	Climate chamber	Climate chamber	Open-top chamber
Ozone exposure duration	short-term, 4 and 16 h	long-term, season	long-term, season
Ozone concentration			(7 h mean)
Low ozone	< 8 nmol mol^{-1}	< 5 nmol mol^{-1}	26 nmol mol^{-1}
High ozone	200 and 400 nmol mol^{-1}	60 nmol mol^{-1}	84 nmol mol^{-1}
CO_2 concentration			(24 h mean)
Low CO_2	340 µmol mol^{-1}	350 µmol mol^{-1}	385 µmol mol^{-1}
High CO_2	–	700 µmol mol^{-1}	675 µmol mol^{-1}
Climatic conditions	(16/8 h day/night)	(16/8 h day/night)	(seasonal mean)
Radiation	300 µmol m^{-2} s^{-1} PPFD	645 µmol m^{-2} s^{-1} PAR	10.2 MJ m^{-2} day^{-1} SRAD
Air temperature	20/15 °C	24/18 °C	13.0 °C
Vapour pressure deficit	0.8 kPa	0.5 kPa	0.78 kPa

PPFD, photosynthetic photon flux density; PAR, photosyntheically active radiation; SRAD, solar radiation

Table 3. Summary statistics of seasonal climatic conditions during 12 open-top chamber (OTC) experiments with spring wheat cv. Minaret considered in the present analysis. Experiments were performed within the ESPACE-wheat project at various sites in Western Europe (see text) over three years between 1994 and 1996. All data represent the climate measured within the OTCs.

	Mean solar radiation (MJ m^{-2} d^{-1})	Mean air temperature (°C)	VPD (kPa)	CO_2, 24 h-mean (µmol mol^{-1})		Ozone, 12 h-mean (nmol mol^{-1})	
				Ambient	Elevated	Ambient	Elevated
Average	14	17.4	0.7	377.7	684.8	25.1	50.7
SD	1.5	2.7	0.1	12.4	31.3	12.5	18.6
Minimum	11	12.9	0.4	357	639	5	23
Maximum	17	22.7	0.8	401	745	41.6	85

VPD, vapour pressure deficit

were performed using the climatic data measured in each chamber treatment.

Simulations of leaf photosynthesis and wheat growth in response to elevated ozone and CO_2

Short-term responses

Responses of leaf photosynthesis, stomatal conductance and rates of ozone uptake were simulated with the combined stomatal–photosynthesis model (Fig. 2). We simulated an increase in the rate of photosynthesis and a decrease in stomatal conductance with increasing CO_2 concentrations. The simulations indicate that these responses were affected by ozone exposure (Fig. 2a,b) and that the effects of the two gases in combination were

interactive. The model simulated that the rate of ozone uptake decreases with increasing atmospheric CO_2 concentrations (Fig. 2c) as a consequence of a decrease in stomatal conductance associated with increasing CO_2 concentrations (Fig. 2b).

In the present model it was assumed that stomatal aperture declines within one hour of exposure to ozone as a result of a pollutant-induced reduction in the Rubisco-limited rate of photosynthesis. Farage et al. (1991) found both photosynthesis and stomatal conductance to be most affected in the first four hours of exposure. They also reported that reductions in the light-saturated rate of photosynthesis, stomatal conductance, and ozone uptake were less with increasing exposure duration. Because we simulate leaf photosynthesis in hourly steps, reduction in the rate of photosynthesis due
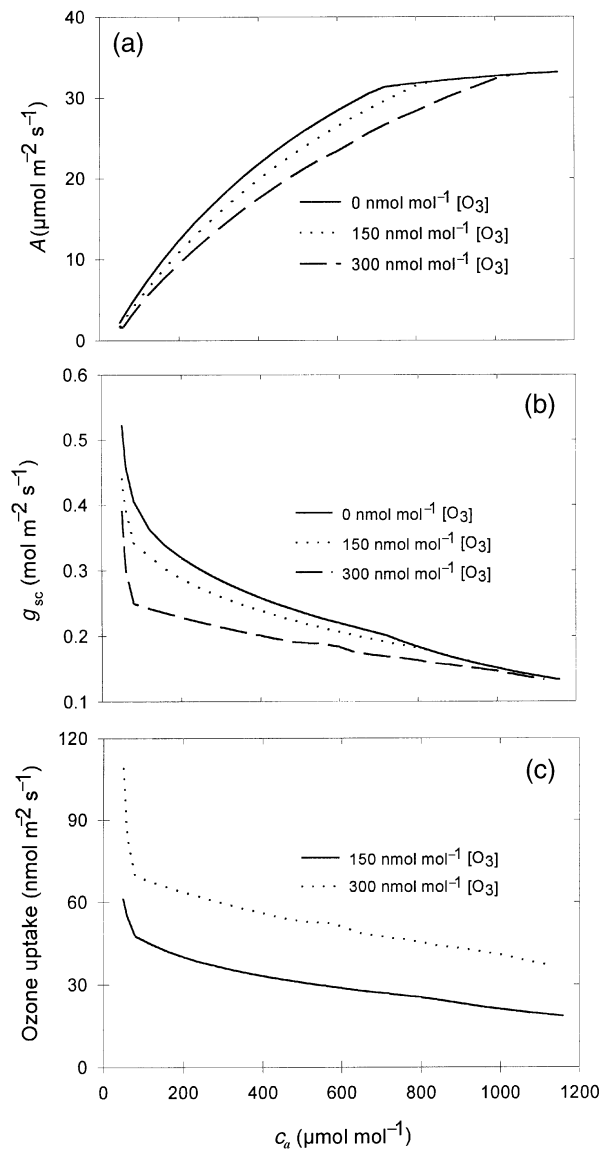
Fig. 2 Simulated effects of contrasting levels of ozone on the relationships between CO_2 concentration and (a) the rate of photosynthesis (b) stomatal conductance to CO_2 and (c) the rate of ozone uptake. Simulations were performed for a temperature of 20 °C, an irradiance of $1500\,\mu mol\,m^{-2}\,s^{-1}$ and a vapour pressure deficit of 0.5 kPa.

Fig. 3 Simulated relationships between ozone exposure duration and (a) light-saturated rate of photosynthesis, (b) stomatal conductance for CO_2, and (c) rate of ozone uptake in response to contrasting levels of ozone. Simulations were performed for a temperature of 20 °C, a vapour pressure deficit of 0.5 kPa and a CO_2 concentration of $350\,\mu mol\,mol^{-1}$.

to ozone exposure during one hour affects stomatal conductance and thus CO_2 and ozone uptake in the subsequent hour (eqn 11). Due to this feedback on stomatal conductance and ozone damage as a result of decreased photosynthesis we were able to reproduce the nonlinear relationship between the reduction in the light-saturated rate of photosynthesis, stomatal conductance and ozone uptake, and ozone exposure duration (Farage *et al.* 1991) (Fig. 3a–c). These responses were less pronounced at elevated CO_2 (not shown).

Long-term responses

Simulated responses of the seasonal time-course of leaf photosynthesis to elevated ozone and CO_2 concentrations are shown for light-saturated conditions (Fig. 4). The model simulated that the negative effect of ozone exposure on leaf photosynthesis increased with ozone exposure concentration and with leaf age (Fig. 4).
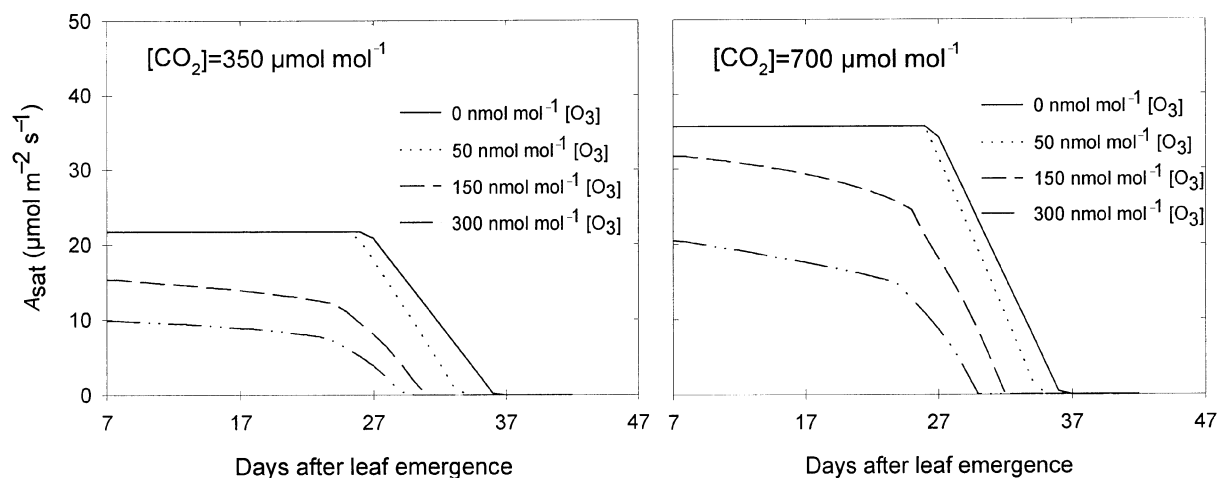
Fig. 4 Simulated effects of long-term ozone exposure on the light-saturated rate of photosynthesis for ambient and elevated CO_2. Simulations were performed for a temperature of 24 °C and a vapour pressure deficit of 0.5 kPa.
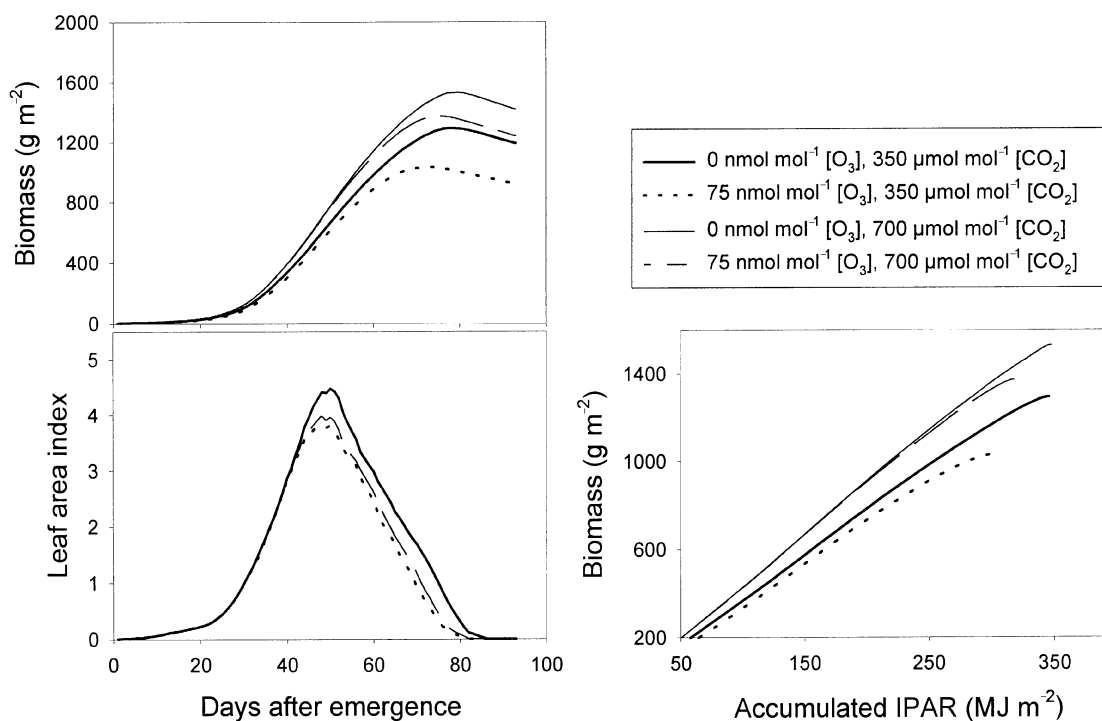

Fig. 5 Simulated effects of ozone and CO_2 elevation on biomass accumulation, leaf-area dynamics and the relationship between biomass and accumulated intercepted photosynthetically active radiation (IPAR) of spring wheat. Simulations were performed for average climatic conditions in North-West Europe (van Oijen & Ewert 1999). Crop emergence was at day of the year 117 (27 April).

Simulations indicate that elevated CO_2 reduced the ozone effect on both photosynthesis and leaf duration (Fig. 4).

Effects of ozone and CO_2 elevation on wheat growth were simulated with the crop simulation model AFRCWHEAT2-O3 for average climatic conditions in North-West Europe (van Oijen & Ewert 1999). We simulated a negative effect of ozone exposure on crop growth that was less pronounced at elevated CO_2 (Fig. 5). Because, in our model, ozone induces acceleration of leaf senescence (Fig. 4), the model simulated a decrease in the size of the canopy due to ozone exposure (Fig. 5) and, thus, a reduction in the amount of radiation intercepted
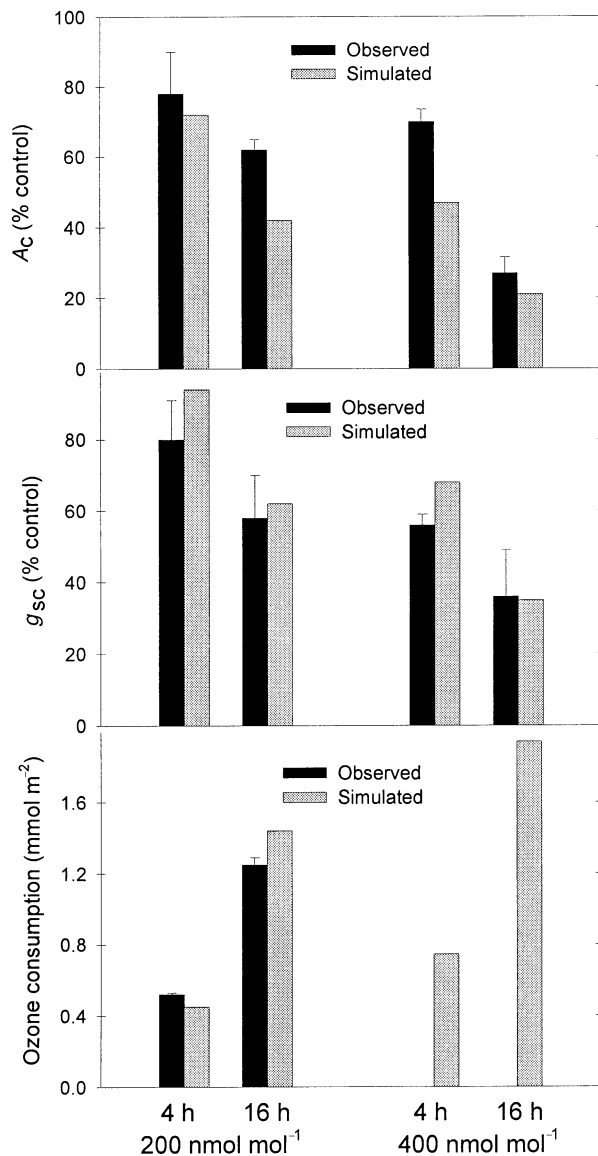
Fig. 6 Observed and simulated effects of ozone exposure on the Rubisco limited rate of photosynthesis (A_c), stomatal conductance (g_{sc}) and cumulative ozone uptake after 4 and 16 h of exposure to 200 and 400 nmol mol^{-1} ozone. Experimental data were taken from Farage & Long (1995) and refer to measurements of the carboxylation efficiency and stomatal conductance to water vapour in winter wheat (cv. Avalon). For ozone concentrations and other growing conditions see Table 2. Ozone consumption at 400 nmol mol^{-1} ozone was not observed.

by the canopy (Fig. 5). However, it is important to note that ozone exposure was simulated to reduce both the amount of radiation intercepted by the canopy and the efficiency of converting intercepted radiation into biomass (Fig. 5). Again, these responses were simulated to depend on CO_2 concentration (Fig. 5). It should be noted that there was no difference in the simulations of leaf-

area index between CO_2 treatments at low ozone (Fig. 5). This is in contrast to a number of investigations into wheat that report an increase in leaf-area index due to CO_2 elevation (e.g. Mulholland *et al.* 1998; Ewert & Pleijel 1999), which is discussed later.

Comparison of simulated and observed data

Short-term responses of leaf photosynthesis

There are limited data available for wheat which describe short-term and long-term responses of leaf photosynthesis to ozone exposure in relation to elevated CO_2. Farage & Long (1995) measured carboxylation efficiency, stomatal conductances and ozone uptake on winter wheat (cv. Avalon) after four and 16 hours of exposure to 200 and 400 nmol mol^{-1} ozone. With the present model we were able to reproduce the observed response patterns reasonably well (Fig. 6). The negative effects of ozone exposure on the Rubisco-limited rate of photosynthesis (A_c) and stomatal conductance were simulated to decrease with increasing exposure duration, which was in close agreement with the observations (Fig. 6). However, the model overestimated effects on A_c after 16 h of exposure to 200 nmol mol^{-1} ozone (Fig. 6). Farage *et al.* (1991) reported no further reduction in the light-saturated rate of photosynthesis after eight hours of fumigation with 200 nmol mol^{-1} ozone. However, earlier in this paper we simulated that photosynthetic rate was further decreased after eight hours of exposure to 150 nmol mol^{-1} ozone (Fig. 3a–c). This might suggest that the stomatal response to ozone exposure is not completely explained by the c_i-feedback from the reduction in assimilation capacity (Farage *et al.* 1991).

Long-term responses of leaf photosynthesis and leaf duration

Effects of ozone exposure in combination with CO_2 elevation on carboxylation efficiency at different stages of leaf development were investigated in spring wheat (cv. Wembley) grown in climate chambers (McKee *et al.* 1995) and spring wheat (cv. Minaret) grown in open-top chambers (Mulholland *et al.* 1997). Generally, model simulations reproduced the finding that CO_2 can protect against ozone damage in wheat (McKee *et al.* 1995; Mulholland *et al.* 1997) (Fig. 7a,b), and closely reproduced the observed ozone-induced decline in carboxylation efficiency which became more pronounced with leaf age at ambient CO_2 (Fig. 7a,b). However, the model tended to overestimate the ozone effects on carboxylation efficiency as the leaves aged at elevated CO_2 (Fig. 7a,b). Such results suggest that the protection against ozone damage
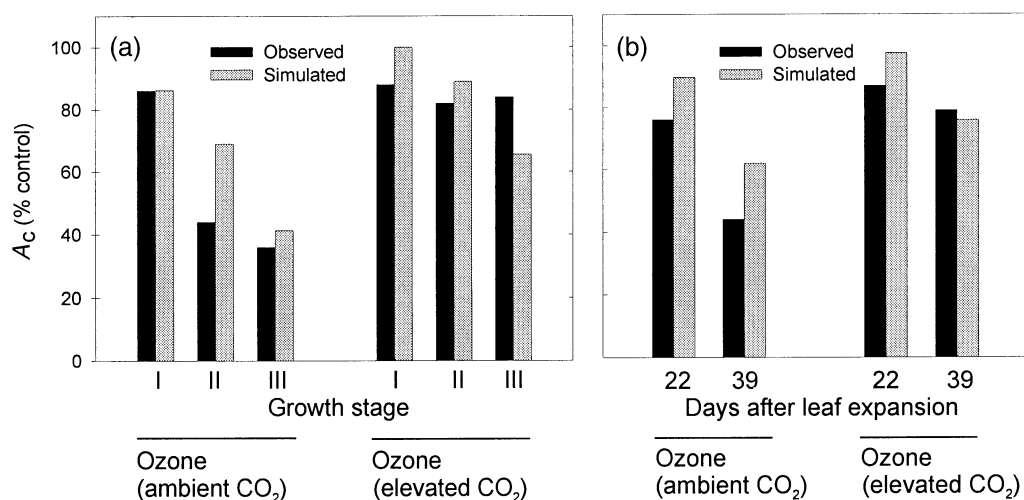
Fig. 7 Observed and simulated effects of long-term ozone exposure without and in combination with elevated CO_2 on the Rubisco-limited rate of photosynthesis (A_c) in response to flag leaf development. (a) Observed data were taken from McKee *et al.* (1995) and refer to measurements of maximum carboxylation velocity on spring wheat (cv. Wembley). For ozone and CO_2 concentrations and other growing conditions see Table 2. Measurements and simulations refer to three growth stages: panicle emergence (I), senescence of the second leaf before flag leaf (II) and senescence of first leaf before flag leaf. (b) Observed data were taken from an open-top chamber experiment conducted at Sutton Bonington, UK, in 1996 (Mulholland *et al.* 1997) and refer to measurements of the maximum carboxylation efficiency of spring wheat (cv. Minaret). For ozone and CO_2 concentrations and other growing conditions see Table 2. Measurements and simulations refer to 22 days (I) and 39 (II) days after flag leaves attained full expansion.
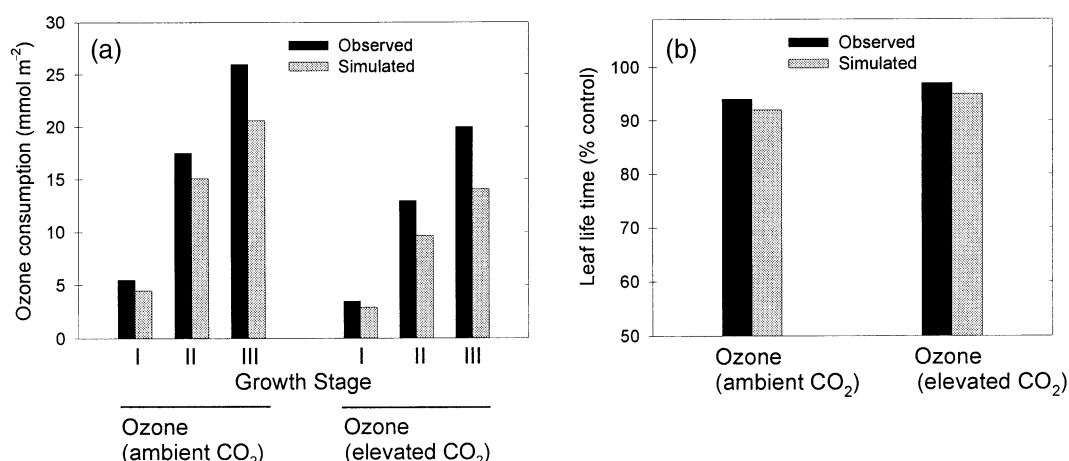

Fig. 8 Observed (McKee *et al.* 1997a,b) and simulated effects of ozone exposure ($60\,nmol\,mol^{-1}$), singly and in combination with elevated CO_2, on (a) cumulative ozone uptake over the life-span of the flag leaf and (b) flag leaf duration. For experimental and simulation conditions and growth stages see Table 2 and Fig. 7(a).

afforded by CO_2 elevation was not completely explained by the reduction in stomatal conductance, as assumed in the present model.

Results relating to the effects of elevated ozone and CO_2 concentrations on cumulative ozone uptake (McKee *et al.* 1997b) and leaf duration (McKee *et al.* 1997a) have been reported for flag leaves of spring wheat (cv. Wembley). Cumulative ozone uptake was observed and simulated to increase with increasing exposure duration and to be less pronounced under CO_2-enriched conditions (Fig. 8a). However, the observed accumulative ozone dose was predicted less accurately as the leaf aged (Fig. 8a) under both ambient and elevated CO_2. The underestimation of the absorbed dose of ozone might be a consequence of the underestimation of the extent of reduction in the Rubisco-limited rate of photosynthesis
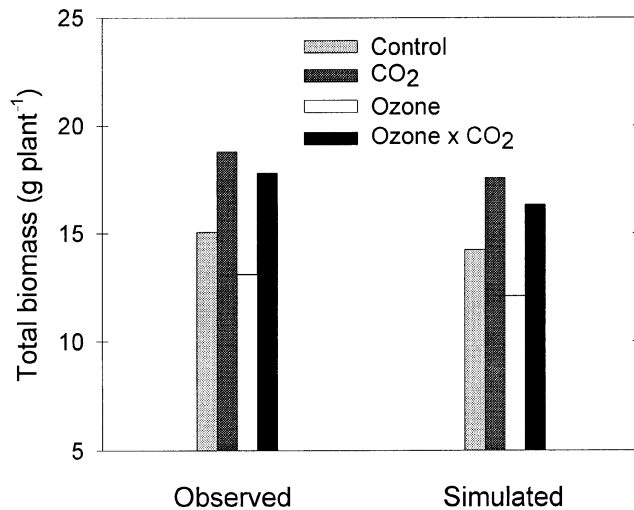
Fig. 9 Observed (McKee *et al.* 1997a) and simulated effects of elevated ozone and CO_2 on total plant biomass of spring wheat cv. Wembley grown in controlled environments. For ozone and CO_2 concentrations and other growing conditions see Table 2.

(A_c) in older leaves (Fig. 7a). However, the relative underestimation of the observed ozone dose was virtually constant at the three stages of leaf development [simulated ozone uptake was about 80% of observed uptake (Fig. 8a)]. In contrast, the relative underestimation of the observed ozone-induced reduction in A_c differed substantially among the three dates (Fig. 7a). These results suggest that the response of stomata to elevated ozone (and ozone uptake) is not explained completely by the c_i-feedback from the reduction in CO_2 assimilation assumed in the model. It might be that detoxification capacity reduces with leaf age (Pell *et al.* 1997) – a factor not considered in the present approach – or that there are direct effects of ozone on the guard cell membranes (Heath & Taylor 1997).

Finally, leaf duration has been found (McKee *et al.* 1997a) to be reduced by ozone exposure — an effect reduced under elevated CO_2. The present model closely reproduced these responses (Fig. 8b).

Plant and crop growth responses

Single effects of ozone and CO_2. The total plant biomass at maturity of spring wheat (cv. Wembley) grown in climate chambers was reduced due to ozone exposure and increased at elevated CO_2 (McKee *et al.* 1997a) (Fig. 9). The present model reproduced these results closely (Fig. 9).

Reduction and increase of total above ground biomass at maturity, as a result of ozone and CO_2 exposure, respectively, were also observed in spring wheat (cv.
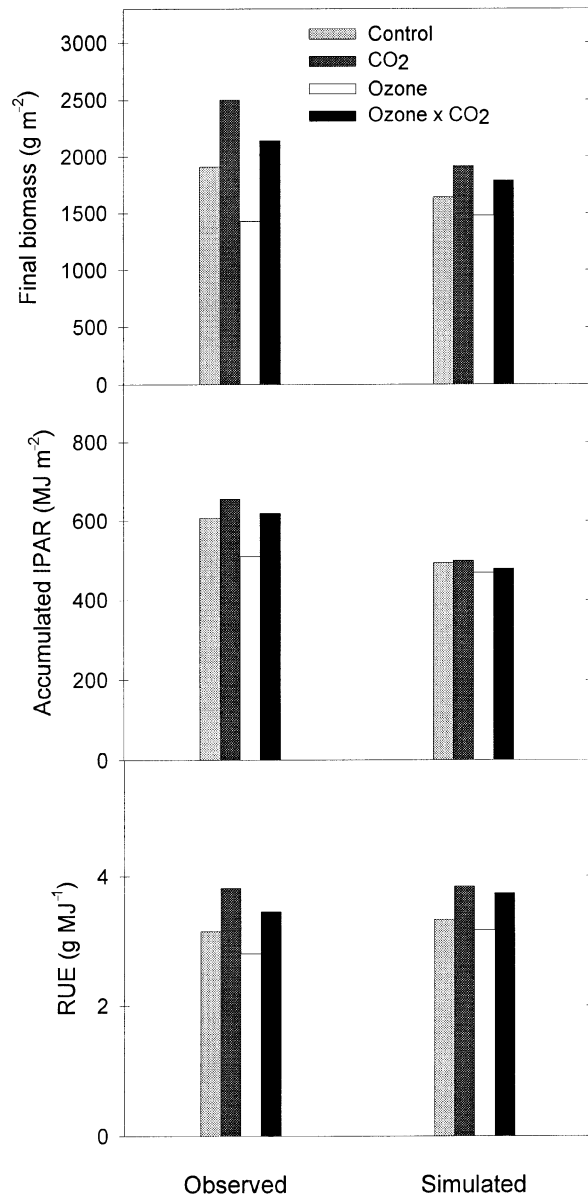

Fig. 10 Observed (Mulholland *et al.* 1998) and simulated effects of elevated ozone and CO_2 on total aboveground biomass, accumulated intercepted photosynthetically active radiation (IPAR) and radiation-use efficiency (RUE) of spring wheat cv. Minaret grown in open-top chambers. For ozone and CO_2 concentrations and other growing conditions see Table 2.

Minaret) grown in open-top chambers (Mulholland *et al.* 1998) which, again, was reproduced by the model (Fig. 10). However, the effects of the two gases were underestimated, which was due mainly to poor simulations of cumulative radiation interception (Fig. 10). Simulations of radiation-use efficiencies were in close agreement with the observations (Fig. 10), which is consistent with earlier simulations about photosynthetic
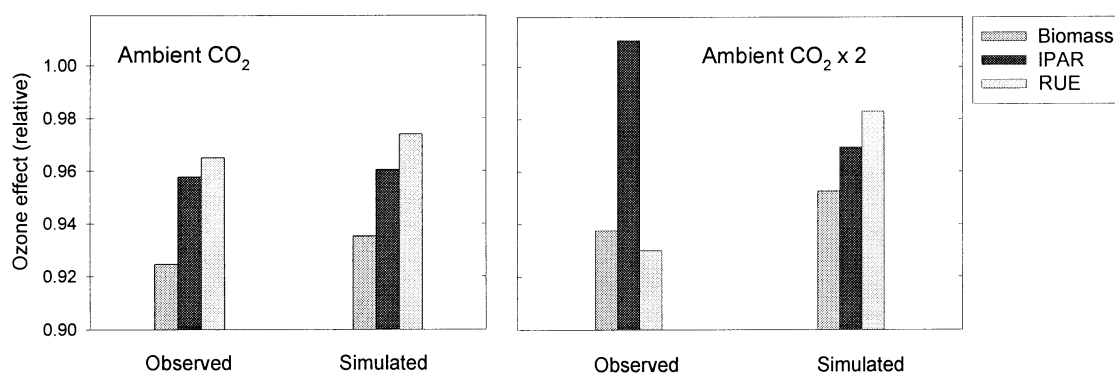
Fig. 11 Observed (ESPACE-wheat project) and simulated effects of chronic ozone exposure on total aboveground biomass, accumulated intercepted photosynthetically active radiation (IPAR) and radiation use efficiency (RUE) at ambient and elevated CO_2 of spring wheat cv. Minaret grown in open-top chambers across Europe. For ozone and CO_2 concentrations and other growing conditions see Table 3.
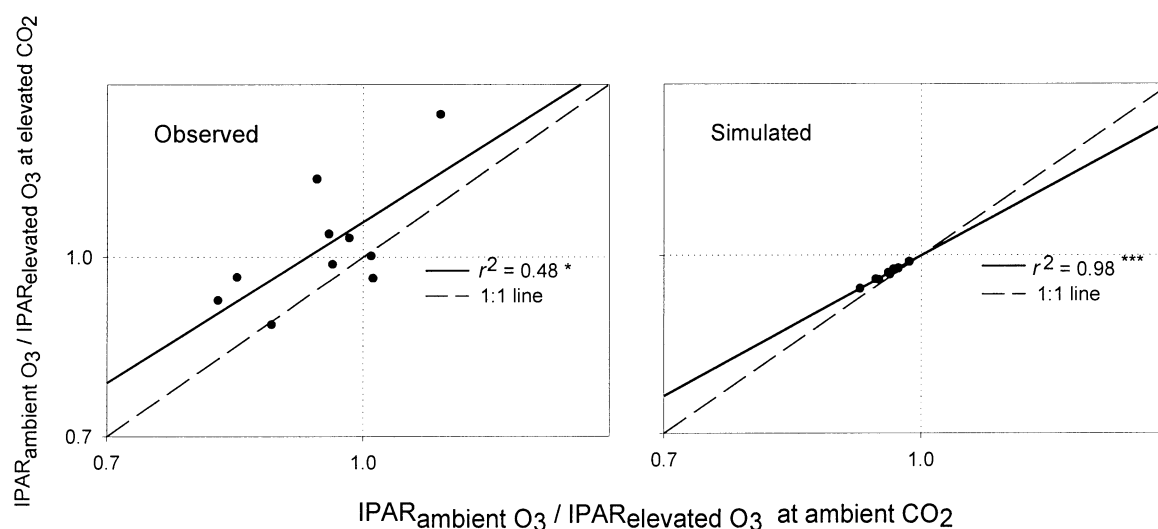

Fig. 12 Observed (ESPACE-wheat project) and simulated relationships between the relative effects of chronic ozone exposure on accumulated intercepted photosynthetically active radiation (IPAR) at ambient and elevated CO_2 of spring wheat cv. Minaret grown in open-top chambers across Europe. For ozone and CO_2 concentrations and other growing conditions see Table 3. The 1:1 line indicates where ozone effects on IPAR are the same at ambient and elevated CO_2 conditions. *P $\leq$ 0.05 and *** P $\leq$ 0.001.

responses to ozone and CO_2 elevation (Fig. 7b). Mulholland *et al.* (1998) reported that the increase in radiation interception at elevated CO_2 was due primarily to increased tillering. Because, in the present model, tillering is not affected by CO_2 we underestimate the CO_2 effect on the amount of radiation intercepted by the crop and, thus, the CO_2 effect on final biomass. These authors also reported that the ozone-induced reductions in leaf-area index and cumulative radiation interception were due to acceleration of leaf senescence and increased tiller mortality in the high ozone treatment. Our simulations about the effects of ozone exposure on leaf duration were in close agreement with the observations (data not shown). However, we do not assume a relationship

between ozone exposure and tiller mortality that might explain the underestimation of the ozone-induced reduction in cumulative radiation interception and final biomass (Fig. 10).

An analysis of a series of open-top chamber experiments with spring wheat cv. Minaret across Europe has shown that effects of CO_2 and ozone on tiller number and canopy size were observed in only some experiments (Ewert & Pleijel 1999). Also, the effects of the two gases on radiation interception and final biomass were, on average, less pronounced (Ewert *et al.* 1999) as reported by Mulholland *et al.* (1998). For these experiments the present model simulated closely the average ozone-induced reductions in final biomass, cumulative

radiation interception and radiation-use efficiency observed at ambient CO_2 (Ewert *et al.* 1999) (Fig. 11). The average CO_2 effect on biomass was underestimated, partly due to underestimation of CO_2 effects on canopy size (Ewert *et al.* 1999).

Combined effects of ozone and CO_2. For all experiments considered in this study we simulated that the negative effect of ozone on wheat biomass was less pronounced at high compared to low CO_2 (Figs 9–11). McKee *et al.* (1997a) reported that ozone exposure caused a 15% decline in total plant biomass at ambient CO_2, but that ozone had no significant effect in the presence of elevated CO_2 (Fig. 9). The data presented by Mulholland *et al.* (1998) (Fig. 10) also indicate that the ozone effect on final biomass was less at high CO_2 (14% decrease) compared to low CO_2 (25% decrease). The average ozone effects on final biomass of the ESPACE-Wheat experiments considered in this study were 8% and 6% at low and high CO_2 (Fig. 11). However, interaction between ozone and CO_2 elevation was not significant for most experiments (Bender *et al.* 1999).

From the data by Mulholland *et al.* (1998) (Fig. 10) it can also be calculated that the ozone-induced reductions in cumulative radiation interception were 16% and 5% at low and high CO_2, respectively. In contrast, the decrease in radiation-use efficiency induced by ozone exposure was somewhat independent of CO_2 concentration (11% and 10% at low and high CO_2, respectively). Similar relationships emerge from the data of the ESPACE-wheat project. Again, at ambient CO_2, both cumulative radiation interception and radiation-use efficiency were reduced due to ozone exposure (Fig. 11). However, ozone exposure did not reduce cumulative radiation interception at elevated CO_2 (Fig. 11) and the observed effect of elevated CO_2 in counteracting the ozone-induced reduction in final biomass, was completely attributable to responses of cumulative radiation interception to combined changes in ozone and CO_2 concentration (Fig. 11). The present model simulated an interaction between the two gases on both cumulative radiation interception and radiation-use efficiency that was not observed (Fig. 11). Clearly, elevated CO_2 provided a higher protective benefit against the ozone-induced reduction in cumulative radiation interception in most experiments than was simulated by the model (Fig. 12). This was due primarily to the limitations of the model in simulating CO_2 effects on leaf-area dynamics.

Discussion

In this paper we have developed and analysed an approach to modelling the effects of ozone exposure on leaf photosynthesis and leaf duration in relation to CO_2. We were able to reproduce different responses of leaf photosynthesis and leaf duration to ozone and CO_2 elevation described in the literature. However, several assumptions made in the simulations (listed below) require further investigation in order to test and to develop more mechanistic explanations.

1 *The stomatal response to elevated ozone is explained completely by the c_i-feedback from the reduction in assimilation capacity.* However, simulations have indicated that this approach does not completely account for the observed stomatal responses to short-term (Farage *et al.* 1991; Farage & Long 1995) and long-term (McKee *et al.* 1995) ozone exposure.

2 *Elevated CO_2 affords additional protection against ozone damage via an effect of CO_2 on stomatal conductance* which has been reported frequently (Allen 1990; Barnes & Pfirrmann 1992; McKee *et al.* 1995, 1997a; Heagle *et al.* 1998; Reid & Fiscus 1998; Volin *et al.* 1998). However, the effect of CO_2 in ameliorating the ozone-induced reduction in carboxylation efficiency, was underestimated as leaves aged (Fig. 7a,b). Alternatively, elevated CO_2 might ameliorate ozone-damage via the promotion of anti-oxidant production (Rao *et al.* 1995). However, there are also contributions which suggest that ozone effects on photosynthesis and plant growth are independent of CO_2 concentration (Balaguer *et al.* 1995; Barnes *et al.* 1995; Rudorff *et al.* 1996a; Olszyk & Wise 1997).

3 *There is no decrease in the photosynthetic capacity with CO_2 elevation.* This assumption contrasts with several investigations in wheat, where elevated CO_2 was observed to cause down-regulation of Rubisco in young leaves (McKee *et al.* 1995; Rao *et al.* 1995). However, other experiments found little evidence of acclimatory loss of photosynthetic capacity in young wheat leaves with growth under CO_2-enriched conditions (Nie *et al.* 1995; Mulholland *et al.* 1997; Garcia *et al.* 1998).

4 *There is no effect of CO_2 elevation on leaf senescence for conditions without ozone exposure.* This assumption contrasts with a number of studies reporting enhanced senescence of wheat leaves due to CO_2 elevation (e.g. McKee *et al.* 1997a; Nie *et al.* 1995). However, results from open-top chamber experiments have shown that CO_2 elevation can induce both increase and decrease in leaf duration (Ewert & Pleijel 1999), which is consistent with the rather inconclusive message emerging from the literature (Baker & Allen 1994; Rudorff *et al.* 1996b).

5 *A proportion of the ozone taken up into the leaf is detoxified and this has no effect on A_c and is constant throughout the leaf life-span.* However, other investigations have indicated that ozone detoxification capacity may reduce with leaf age (see Pell *et al.* 1997), which may partially explain the underestimation of the ozone effect on A_c in older leaves (Fig. 7a).

6 *The capacity to repair ozone damage decreases linearly with leaf age after full leaf expansion.* Analysing different approaches to modelling the short-term response of photosynthesis to ozone exposure, Schut (1985) showed that a model with repair positively dependent on photosynthetic rate was the most successful. We did not consider such a relationship in our model. However, Schut (1985) also reported that assuming a constant repair rate gave a reasonable approximation to experimental findings.

The present approach to modelling the effects of ozone and CO_2 elevation on leaf photosynthesis and leaf duration was considered in the mechanistic crop simulation model AFRCWHEAT2 (Porter 1993). The derived model, AFRCWHEAT2-O3, reproduced satisfactory observed response patterns of final wheat biomass to ozone and CO_2 elevation (Figs 9–11). We were also able to reproduce the observation that both cumulative radiation interception and radiation use efficiency were reduced due to ozone exposure (Mulholland *et al.* 1998; Ewert *et al.* 1999) (Figs 10,11). However, our simulations of cumulative radiation interception in response to ozone exposure and CO_2 elevation were unsatisfactory, and had some impact on the accuracy of the predictions of final biomass (Fig. 10). Because the model does not predict an effect of CO_2 on tillering, it underestimated the CO_2 effects on leaf-area index and the amount of radiation intercepted (Figs 10,11). However, such effects are not always apparent because they vary strongly dependent on environmental conditions (Ewert & Pleijel 1999; Ewert *et al.* 1999). Elevated CO_2 was observed to compensate the negative effect of ozone on leaf-area dynamics (Figs 10–12) and final biomass (Figs 10,11). Interestingly, the observed ozone-induced reduction in radiation-use efficiency was rather independent of CO_2 concentration (Figs 10,11). These relationships were not reproduced adequately with AFRCWHEAT2-O3 (Figs 10–12), primarily due to the problem of simulating CO_2 effects on leaf-area index. Our results suggest that accurate simulations of wheat growth was more limited by our modelling of canopy processes than by the present approach to model leaf photosynthesis in response to ozone and CO_2 elevation. As there is little information about adaptation mechanisms of wheat canopies to physiological stress induced by ozone, and the relative importance of such responses for crop growth and yield, we conclude that emphasis in research should be given to understanding the responses of leaf-area dynamics to ozone and CO_2 elevation.

Acknowledgements

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