



Ozone effects on crops and consideration in crop models

Lisa D. Emberson^{a,*}, Håkan Pleijel^b, Elizabeth A. Ainsworth^c, Maurits van den Berg^d, Wei Ren^e, Stephanie Osborne^{a,f}, Gina Mills^f, Divya Pandey^a, Frank Dentener^d, Patrick Büker^a, Frank Ewert^g, Renate Koeble^d, Rita Van Dingenen^d

^a Stockholm Environment Institute at York, Environment Dept., University of York, YO10 5NG, United Kingdom

^b University of Gothenburg, Biological and Environmental Sciences, P.O. Box 461, 40530 Göteborg, Sweden

^c USDA ARS Global Change & Photosynthesis Research Unit, Department of Plant Biology, University of Illinois at Urbana-Champaign, 1201 W. Gregory Dr., Urbana, IL 61801, USA

^d European Commission, Joint Research Centre, via E. Fermi, 2749, Ispra, Italy

^e Department of Plant and Soil Sciences, College of Agriculture, Food and Environment, University of Kentucky, 1100 S Limestone St, Lexington, USA

^f Centre for Ecology and Hydrology, Bangor, LL57 2UW, United Kingdom

^g Institute of Crop Science and Resource Conservation INRES, University of Bonn, 53115, Germany



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ABSTRACT

We review current knowledge of the processes by which ozone will cause injury and damage to crop plants. We do this both through an understanding of the limitations to ozone uptake (i.e. ozone being transferred from some height in the atmosphere to the leaf boundary layer and subsequent uptake via the stomata) as well as through the internal plant processes that will result in the absorbed ozone dose causing damage and/or injury. We consider these processes across a range of scales by which ozone impacts plants, from cellular metabolism influencing leaf level physiology up to whole canopy and root system processes and feedbacks. We explore how these impacts affect leaf level photosynthesis and senescence (and associated carbon assimilation) as well as whole canopy resource acquisition (e.g. water and nutrients) and ultimately crop growth and yield. We consider these processes from the viewpoint of developing crop growth models capable of incorporating key ozone impact processes within modelling structures that assess crop growth under a variety of different abiotic stresses. These models would provide a dynamic assessment of the impact of ozone within the context of other key variables considered important in determining crop growth and yield. We consider the ability to achieve such modelling through an assessment of the different types of crop model currently available (e.g. empirical, radiation use efficiency, and photosynthesis based crop growth models). Finally, we show how international activities such as the AgMIP (Agricultural Modelling and Improvement Intercomparison Project) could see crop growth modellers collaborate to assess the capabilities of different crop models to simulate the effects of ozone and other stresses. The development of robust crop growth models capable of including ozone effects would substantially improve future national, regional and global risk assessments that aim to assess the role that ozone might play under future climatic conditions in limiting food supply.

1. Introduction

Ground level ozone is widely recognised as the most damaging air pollutant to vegetation due to its phytotoxicity and prevalence at high concentrations over rural/agricultural regions (Ainsworth et al., 2012). Fig. 1 shows present-day ozone concentrations in the high ozone season (i.e. the highest rolling 3 month ozone concentration). Highlighted (by the hatched areas) are those ozone concentrations that are considered damaging to crops, also shown is how these locations overlap with important agricultural regions. For some world regions, the figure

indicates how ozone concentrations are expected to increase in the future according to projected ozone pre-cursor emission scenarios. This suggests that ozone is already, and will continue to present, a serious threat to crop productivity. Ozone causes a wide variety of damage in agricultural crops including visible injury, reduction in photosynthesis, alterations to carbon allocation, and reduction in yield quantity and quality (see review papers by Fuhrer and Booker (2003), Fuhrer (2009), Fiscus et al. (2005), Ashmore, (2005), Heath (2008), Ainsworth et al. (2012) and Ainsworth (2017)). These reviews have served to build a picture of the mechanisms or processes by which ozone causes damage.

* Corresponding author.

E-mail address: l.emberson@york.ac.uk (L.D. Emberson).

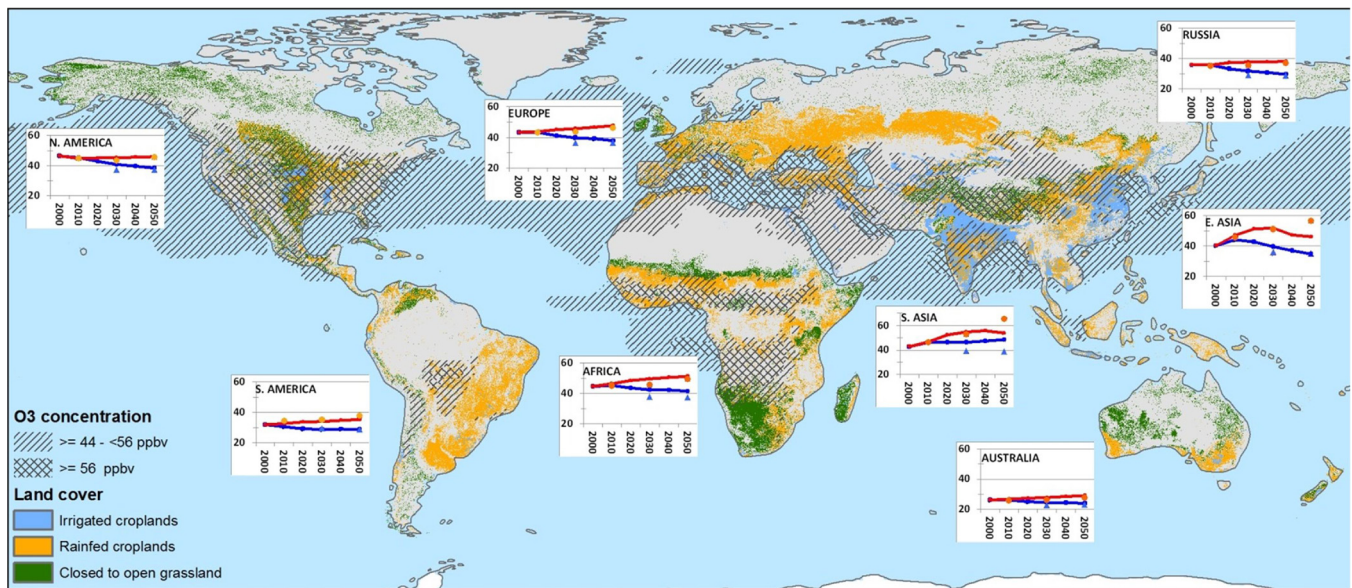


Fig. 1. Global distribution of current day ozone ‘hotspot’ regions (hatched and striped areas), where damage to sensitive crop species is likely. Ozone concentration data are computed by the global atmospheric chemistry transport model TM5 (Van Dingenen et al., 2009) for the year 2001; an ozone ‘hotspot’ region is defined as a location where the maximum of the continuous 3 month M7 (7 h monthly mean) ozone concentration is greater than 44 and 56 ppbv, corresponding to a 5 or 10% yield loss to wheat respectively, according to the concentration-response function of Lesser et al. (1990). The map shows the location of these hotspots in relation to ‘irrigated’ and ‘rainfed’ croplands and grasslands (irrigated data are from Siebert et al. (2013) and land cover from the ESA GlobCover 2009 Project (http://due.esrin.esa.int/page_globcover.php)). Also shown are estimated changes in surface ozone M7 concentrations, weighted to national and regional wheat production (FAO), according to the RCP2.6 (blue line) and RCP8.5 (red line) emission scenarios until 2050 (van Vuuren et al., 2011). Additionally, two alternative air pollution emission scenarios are taken from UNECE (2016): Current Legislation (orange circles) and Maximum Feasible Reduction (blue triangles) showing that, especially in Asia with current air pollution policies and energy projections, ozone levels may further increase. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Due to the absence of worldwide observational ozone data, risk assessments of ozone damage rely mostly on modelling methods that combine estimates of ozone concentration (derived from global or regional atmospheric chemistry transport models; CTMs) with crop distribution and production data using empirically derived statistical relationships between crop yield and ozone concentration. Several models have been developed and applied over the past few decades, these use a number of different ozone metrics and functional relationships to relate ozone concentrations to relative yield loss. Some of the common ozone metrics include: (i) daylight hour (7 h (M7) or 12 h (M12)) growing season average ozone concentrations; (ii) accumulated daylight ozone concentrations above thresholds (e.g. AOT40 and SUM06) or; (iii) continuously weighted growing season averages (W126) to emphasise the higher ozone concentrations. Weibull (M7, M12, SUM06 and W126) or linear (AOT40) dose-response relationships have commonly been used to relate the metrics to damage (for further details see Wang and Mauzerall, 2004 and Van Dingenen et al., 2009). These models have been used to quantify the extent of ozone damage (both in terms of yield and economic losses) at national (Adams et al., 1989; Wang and Mauzerall, 2004), regional (Holland et al., 2002) and global levels (Van Dingenen et al., 2009; Avnery et al., 2011, 2013). The estimated yield losses are substantial, under both current and projected future ozone concentrations. A summary of recent model findings is presented in Ainsworth (2016) with global yield losses due to current day ozone estimated at between 2 to 16% for wheat, rice, maize and soybean. These empirical concentration-based modelling methods provide an estimate of the potential ozone effects but are unable to assess the combined effects of ozone with other factors that influence ozone uptake and hence ozone damage. Such factors include soil and atmospheric water deficits under both current and climate change conditions (Emberson et al., 2000a; Tai et al., 2014).

Semi-empirical ozone-effect modelling methods have been developed more recently; these use the same statistical relationships as described above but are ‘built in’ to process-based ecosystem modelling

frameworks. This allows these models to assess the direct and indirect impacts of ozone on plant growth and ecosystem processes such as photosynthesis, carbon allocation and transpiration (Reich, 1987; Felzer et al., 2004, 2005, 2009; Ollinger et al., 1997; Ren et al., 2007a,b, 2011, 2012; Tai et al., 2014). Most land-ecosystem models are process-based to simulate the dynamics in ecosystem function and structure as influenced by global environmental changes, from cell, leaf, community, ecosystem, to regional and global level, and from daily, monthly, seasonal, to yearly and longer scales. Some of these models have incorporated ozone effects (as one of multiple environmental drivers) on ecosystem carbon and water dynamics using the statistical relationships described above, the most popular index being AOT40 (used by Felzer et al., 2004; Ollinger et al., 1997; Ren et al., 2007a). These models are constructed so that the general influences of ozone work directly on photosynthesis $f(O_3)$ and then indirectly on stomatal conductance (g_{sto}). For example, in the study of Ren et al. (2007a), they assume a linear relationship between canopy conductance (g_{sto}) and gross primary productivity (GPP): $g_{sto} = f(GPPO_3)$; $GPPO_3 = GPP * f(O_3)$; $f(O_3) = 1 - (\alpha \times g_{sto} \times AOT40)$, here $GPPO_3$ is the result of GPP limited by ozone and α is an empirical ozone response coefficient. In addition to similar requirements as empirical models, extensive model calibration is needed to specify α (e.g. for different crop types) and other parameters; and spatially explicit, time series environmental data sets including AOT40 are needed to drive these semi-empirical models. These models present ozone effects on carbon and water gain and loss in an ecosystem at multiple spatial and temporal scales and also address the interactive effects of ozone with other environmental factors (climate, land use and management practices, CO₂, nitrogen deposition etc...). Some models (e.g. the Terrestrial Ecosystem Model – TEM (Felzer et al., 2004)) run at a monthly time step to represent long-term exposure of plants to ambient ozone. Other models run on a daily time step and try to capture incident ozone effects over a short-period of time (Dynamic Land Ecosystem Model – DLEM (Ren et al., 2007a)), for these models uncertainty in daily response

patterns is unavoidable due to the original season-long based dose-response relationship. In summary, there are three key limitations in the use of these approaches: firstly, those that use threshold indices such as AOT40 omit ozone concentrations below 40 ppb which are known to contribute to ozone damage. Unequivocal evidence for this comes from the fact that AOT30 was found to be as equally statistically significant as AOT40 in terms of estimating plant damage (Karenlampi and Skarby, 1996). Secondly, the time-step of these approaches prevents the capture of the hourly co-variation in ozone concentration and environmental variables which are known to influence stomatal ozone uptake (Emberson et al., 2000b). Finally, the use of season-long statistical relationships of ozone damage in combination with monthly or daily plant process based modelling suggests a level of sophistication by which ozone effects are simulated that is not actually achieved due to this mismatch in temporal resolution.

Over the last decade there has been a move to a flux-based approach to assess the statistical relationship between ozone and crop yield (Emberson et al., 2000a; Pleijel et al., 2007). This flux-based approach accounts for the stomatal control of ozone uptake and the fact that ozone causes damage only once taken up via the stomata. Hence, especially under conditions of varying water supply to crops (when stomatal conductance changes in accordance with water availability), these flux-based models perform statistically better than concentration-based models (Pleijel et al., 2007) and have also been shown to be better predictors of the distribution of damage when combined with CTMs to estimate regional ozone damage (Mills et al., 2011). However, current empirical flux-based models still have their shortcomings. The ones that most seriously limit their application in practical assessments of impacts and mitigation/adaptation options are: (i) they are unable to incorporate the interactive effects of environmental stress that might influence the plants ability to detoxify ozone once taken up via the stomata (see Section 3.2); (ii) they focus on cohorts of fully expanded upper canopy leaves (or the flag leaf in the case of wheat) assuming that these are primarily responsible for determining ozone effects on yield; (iii) they only assess the relative impacts of ozone on yield, while neglecting the effects of variables other than ozone and (iv) hence, they are unable to provide estimates of absolute yield which would be important to understand the role of ozone in yield gaps under particular environmental conditions.

The final group of modelling approaches are dynamic process-based models that consider effective ozone flux (the stomatal ozone flux which exceeds the detoxification capacity of the plant) and effects on photosynthesis (Martin et al., 2000; Massman, 2004). These approaches have been scaled to estimate consequent effects on carbon (C) assimilation in crops (Ewert and Porter, 2000; Tao et al., 2017); forest canopies (Martin et al., 2001; Deckmyn et al., 2007) and for ecosystem plant functional types in the MOSES-TRIFFID land surface model (Sitch et al., 2007). However, few of these process-based ozone models have been applied at a large scale due to limited ozone flux data and complicated model parameterisation and little attention has currently been devoted to developing such models for crops. An obvious way to achieve a robust, broad geo-spatial application of these models this is to develop a more comprehensive mechanistic approach to modelling ozone effects on crop physiology, growth and yield.

In this paper, we make the case to do this by developing and incorporating modules and parameterisations capable of simulating ozone damage into existing crop growth models. First, we review our current understanding of how ozone causes damage at the cellular level and describe the current ‘process-based’ modelling methods that have been developed to simulate these mechanisms. We then identify additional features of ozone damage that scale to the whole canopy and indicate the factors that need to be taken into consideration to model these processes and feedbacks. This includes damage caused directly by ozone as well as environmental conditions that might influence the sensitivity of crops to ozone, or result in feedbacks that could enhance or dampen ozone damage. With this understanding, we identify a

number of plant physiological traits that might enhance (or reduce) ozone sensitivity and consider how future global climate change might influence ozone sensitivity. Finally, we consider existing crop models, assess their suitability to incorporate these ozone damage mechanisms and review past and current efforts underway to incorporate the influence of ozone. A summary of priorities for future modelling is provided to guide research in this area.

2. Pathways leading to ozone damage

2.1. Determinants of ozone dose to the crop canopy

To assess the impacts of ozone on crops, it is first necessary to define the ozone concentration; $[O_3]$, to which the crop canopy is exposed. This may sound simple enough but in practice, with the need for reliance on national, regional and sometimes global modelling to provide the required geographical coverage of ozone concentrations, this can be a complex task; and one that is not easily evaluated given the sparsity of surface ozone monitoring sites/networks. CTMs incorporating ozone dry deposition schemes (that estimate ozone loss to vegetated/non-vegetated surfaces) are used to estimate the changing ozone profile with height above the canopy. Dry deposition schemes are an essential part of such models, and removal of ozone by dry deposition is one of the dominant terms in the overall ozone budget of the troposphere (e.g. Stevenson et al., 2006). They also determine the transfer of ozone from the lowest atmospheric layer modelled in the CTMs (often at the surface of the planetary boundary layer, i.e. ~ 50 m above the ground surface (Emberson et al., 2001)) to the plant canopy. The importance of incorporating this term becomes obvious when considering that the $[O_3]$ at crop canopy height is commonly $\sim 10\%$ lower than the concentration at 20 m above the surface (LRTAP Convention, 2010).

Many ozone deposition modelling schemes have been developed. All models have in common the use of a series of resistances (an example is given in Fig. 2) to describe the transfer of ozone through: (i) the atmosphere (determined by mechanical (wind) and thermal driven turbulence both of which depend on land surface properties and meteorology and; (ii) across the crop canopy boundary layer (a quasi-laminar surface boundary where transfer is determined both by mechanical (wind speed), but also molecular diffusion gradients). These processes deliver ozone to the canopy top, but ozone deposition (determined by uptake via the stomata, deposition to the external plant parts, in-canopy transfer and deposition to the underlying soil surface) will determine the sink strength to ozone and hence the concentration gradient that exists between the CTM $[O_3]$ reference height and the canopy. All of these processes need to be estimated to determine the $[O_3]$ at the top of the crop canopy. The quantitative consideration of these deposition processes provide an estimation of the ‘external $[O_3]$ ’. It is from this ‘external $[O_3]$ ’ to which plants are exposed that the different pathways by which ozone causes crop damage are then detailed in Fig. 3.

All ozone deposition models have at their core an estimate of stomatal conductance; g_{sto} , which is important because it is a large determinant of the vegetation sink strength. Different schemes use different methods to estimate g_{sto} . The most commonly used in many of the global CTMs is based on Wesely (1989), which calculates g_{sto} using a multiplicative model accounting for photosynthetically active radiation (PAR) and air temperature. Dry deposition schemes used in CTMs with more regional applications (e.g. the Community Multiscale Air Quality (CMAQ) suite of models) follow a scheme developed by Pleim et al. (2001) and Xiu and Pleim (2001); this also uses a multiplicative g_{sto} scheme, but also includes root zone soil moisture and air humidity terms in addition to air temperature and PAR. The DO_3SE dry deposition model (Emberson et al., 2001) was the first developed with the specific remit of estimating both ozone deposition and ozone impacts. This model can use either multiplicative or coupled photosynthesis-stomatal conductance (A_n-g_{str}) methods to estimate g_{sto} ; both models

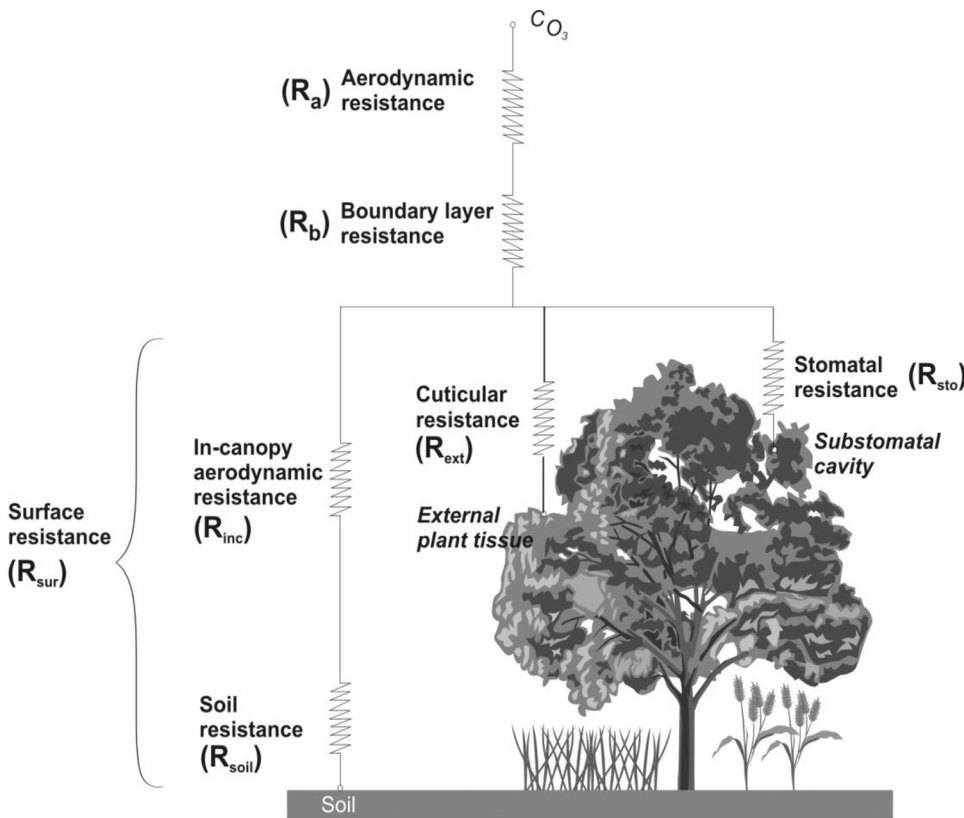


Fig. 2. A standard dry deposition scheme that is used to transform ozone concentrations $[O_3]$ from some height above the canopy (denoted by C_{O_3}) to the within-canopy sinks. The scheme shown here is for the DO_3SE model (Emberson et al., 2001; Bükér et al., 2012), which can be used for a range of different land cover types including crops. This scheme assumes the key resistances to ozone deposition are the aerodynamic resistance (R_a), the quasi-laminar sub-layer resistance (R_b) above the canopy, and the surface resistance (R_{sur}). R_{sur} comprises two resistance paths in series, the stomatal and non-stomatal resistance. The latter represents a) within canopy aerodynamic resistance (R_{inc}) and subsequent b) soil resistance to decomposition at the soil surface (R_{gs}) which encompasses features such as leaf litter and ground vegetation under forest canopies, as well as c) resistance to adsorption to the external plant parts (R_{ext}) including cuticle, bark etc.

perform similarly well when compared against observations (Bükér et al., 2007). These models represent the canopy as a ‘big-leaf’ split into sun and shade fractions according to the reduction in irradiance that occurs with increasing penetration into the canopy according to Beer’s law. However, we know that $[O_3]$ and wind speed will also vary with

canopy depth; we also know that cohorts of leaves of a similar age (and hence exposure of ozone) will also tend to have a particular canopy location. Modelling would ideally take account of these different factors both in terms of estimations of ozone uptake (and hence canopy deposition) but also ozone damage; the latter would require a better

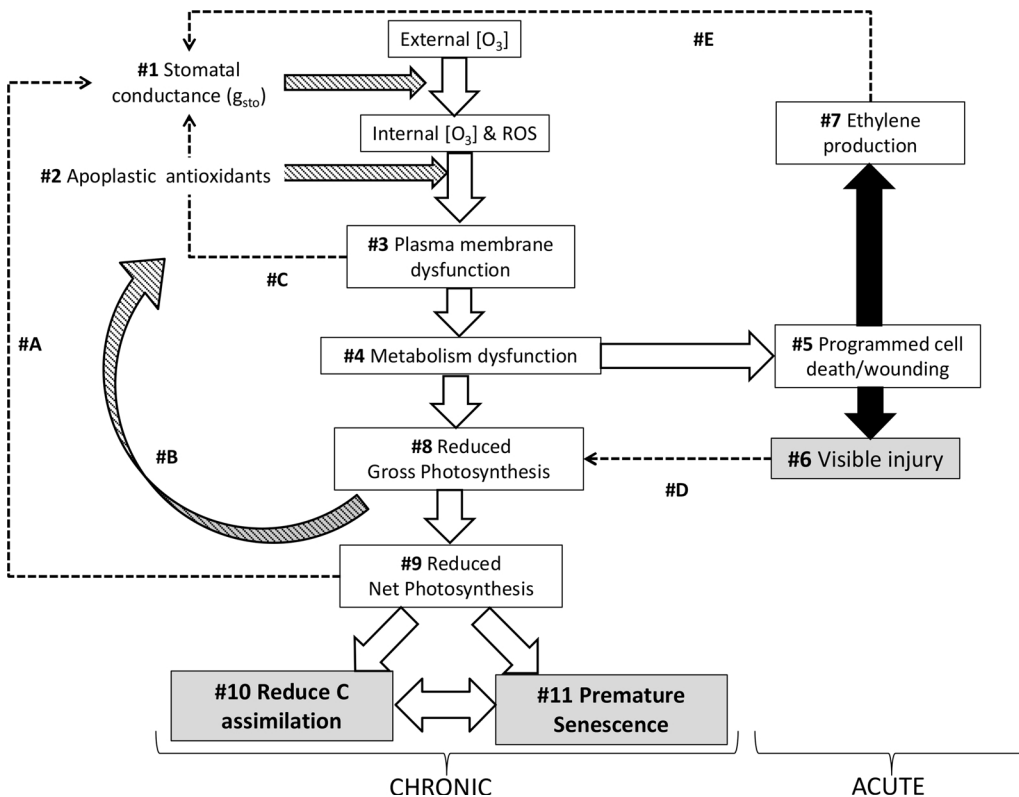


Fig. 3. The cellular and metabolic pathways by which ozone (and its Reactive Oxidant Species (ROS) derivatives) lead to damage and injury (indicated by shaded boxes) in crops. The movement of ozone or its products is shown as open arrows; also shown as hatched arrows are the possible limitations to, or prevention of, ozone penetration into the cell; particular responses to ozone are denoted by #1–11. Feedbacks to stomatal conductance (g_{sto}), which will influence stomatal ozone flux, are shown by dotted line arrows and denoted by #A–E. Visible ozone injury is defined as acute injury (generally associated with infrequent very high episodes of ozone exposure) whilst reductions in C assimilation and premature leaf senescence are characterised as chronic injury (associated with more frequent mid-level ozone concentrations). Diagram based on Heath (1994) and Heath (2008).

understanding of the influence of ozone dose on C assimilation of leaves of different ages and their role within the canopy in terms of contribution to yield.

2.2. Determinants of the fate and impact of absorbed ozone at the cellular level

In order to develop process-based modelling methods that can assess ozone damage, it is crucial to understand what happens to ozone once taken up via the stomata. Although there is a wealth of experimental data describing responses to ozone, there are a number of factors which make a clear definition of processes and pathways extremely difficult. These include: (i) the fast rate with which ozone reacts with organic substrates to form a wide variety of reactive products; (ii) the large number of cellular sites within the leaf that respond to ozone and its reactive products; (iii) the cascade of metabolic and physiological responses that ensue at different scales in space and time; and (v) genetic variation in response of crops to ozone, both within and among species. Fig. 3 summarises our current understanding of possible pathways of ozone damage at the cellular level. Here we distinguish three key ozone responses that can be incorporated into crop models but recognise that the pathways to these different types of damage and injury will most likely be strongly interlinked: **a) visible injury**, **b) reduced C assimilation**, and **c) premature leaf senescence**. The first term is often defined as an acute response while the latter two are referred to as chronic responses to ozone. All these responses scale up from the cell, to the leaf, to the crop canopy and will influence the final productivity of the crop. This scaling is further discussed in Section 3.

Fig. 3 shows pathways of ozone (and the reactive oxygen species (ROS) formed on its oxidation) as open arrows as it moves into the plant tissue. Also shown (as hatched arrows) are the possible limitations to, or prevention of, ozone (and its derivatives) penetration into the cell, representing the ‘damage limitation’ mechanisms that may be operating. Particular plant responses to ozone are noted as #1–11 in Fig. 3. Finally, feedbacks within the system are shown as dotted line arrows and noted as #A–E in Fig. 3. The sequence is as follows: (1) ozone enters the tissue (determined by g_{sto} ; for the purposes of calculating ozone flux it is generally assumed that internal ozone concentration is negligible, i.e. the gradient is entirely determined by the external $[O_3]$ (Laisk et al., 1989)) #1, Fig. 3; (2) ozone has a very short life-time inside the leaf but forms other ROS (e.g. hydrogen peroxide (H_2O_2), superoxide (O_2^-), and hydroxyl radical (HO) (Heath, 2008)) on dissolution in the aqueous layers surrounding, and within, the apoplast (the cell wall continuum); (3) ozone and other ROS will encounter **apoplastic antioxidants** (#2, Fig. 3) that can counteract the harmful effect of these ‘toxicants’. Together ozone uptake and the ability to detoxify ozone and ROS determine the ‘effective ozone flux’ into the leaf (i.e. the ozone dose that exceeds the detoxification capacity; Musselman et al., 2006). A number of potential mechanisms influence the levels of apoplastic antioxidants: (a) activity of certain enzymes (e.g. ascorbate peroxidase, superoxide dismutase, and glutathione reductase (Chernikova et al., 2000)); (b) production of antioxidant compounds (e.g. ascorbic acid, β -carotene, polyamines and glutathione) (Chernikova et al., 2000; Baier et al., 2005; Dizengremel et al., 2008) and; (c) production of unsaturated monoterpenes (volatile organic compounds (VOCs) emitted by crop species that remove ozone in the atmosphere) (Fiscus et al., 2005). The potential benefits of such mechanisms must be counterbalanced against the costs, both in terms of additional metabolic demand for C to support these processes (#B, Fig. 3; see also Section 2.2) and in the manufacture of substrates (Sharkey and Yeh, 2001). For example, studies have found increased rates of mitochondrial respiration in many crop species including soybean, wheat, rice, and common bean to enhance ozone detoxification and repair of cellular damage (Ainsworth et al., 2012). (4) If apoplastic antioxidant capacity is overcome, either by depletion of antioxidants or a rapid entry of ozone, ozone (and derived ROS) can react with the next level of organization – the plasma membrane.

Reactions within the plasma membrane may generate compounds that have a longer half-life and slightly lower reactivity than ozone (and derived ROS). These compounds can penetrate deeper into the cell causing metabolic changes; together these reactions will produce a ‘cascade of responses’ that lead to ozone damage to crop physiological processes, growth and yield.

The plasma membrane is believed to play a critical role as the initial site of ‘ozone-attack’. Initial targets for ozone and its ROS derivatives include plasma membrane lipids, susceptible amino acids in plasma membrane proteins or apoplastic enzymes, and a variety of organic metabolites localized in the apoplast (Fiscus et al., 2005). These apoplast and plasma membrane reactions will result in **plasma membrane dysfunction** (#3, Fig. 3), which can lead to membrane leakage affecting cells and membrane bound organelles (Heath, 2008) or shifts in signal transduction proteins within the membrane (Rossard et al., 2006). Subsequent cellular changes include (i) shifts in ion and solute concentrations that result in a de-polarizing and hence dysfunction of the membrane; (ii) changes in cell wall constituents (e.g. its ascorbate acid concentration; Castillo and Heath, 1990) which can detoxify ozone and ROS reducing the amount of ozone present at the membrane but at a cost of ascorbate oxidation or loss; (iii) a triggering of protein cascades leading to newly formed proteins, via transcription factor activations (Evans et al., 2005); (iv) ozonolysis of the double bonds in unsaturated fatty acids of cell membranes (Iriti and Faoro, 2008) and; (v) peroxidation of membrane lipids leading to formation of organic radicals and organic peroxides that can initiate a cascade of free radical generation (Sharma et al., 2012). At high concentrations, the production of ROS derived from ozone can lead to cell death (#5, Fig. 3) similar to that experienced by plant cells upon pathogen attack (Overmyer et al., 2003). Even in the absence of cell death, the cellular changes described above lead to altered signaling and gene expression within cells, causing metabolism dysfunction (#4, Fig. 3). This ultimately results in physiological changes including decreased photosynthesis (#8, Fig. 3) and g_{sto} (#1, Fig. 3), increased respiration rates (see section 2.3 and Fig. 6), and premature leaf senescence (#11, Fig. 3) (Ainsworth et al., 2012). These physiological changes can subsequently impact plant development and growth.

In terms of modelling, there are two main approaches for estimating the detoxification capacity of plants. The first is empirically based and relies on the development of flux-response relationships that integrate both stomatal ozone flux and the response it induces (e.g. crop yield) over the course of a full growing season (see Section 1). Here, different instantaneous detoxification or ‘y’ thresholds are tested (usually in incremental steps; see Pleijel et al. (2007)) and the threshold is defined as that which gives the best linear regression model between stomatal ozone flux and damage. Objective criteria can be used to establish this ‘y’ threshold, i.e. the regression which gives the least squares difference and an intercept closest to a relative yield of 1 at zero ozone concentration (Büker et al., 2015). For some crops (wheat, potato, tomato), ‘y’ thresholds of $6 \text{ nmol } O_3 \text{ m}^{-2} \text{ PLA s}^{-1}$ have been defined (CLRTAP, 2011). Importantly, Musselman et al. (2006) also hypothesised that the level of detoxification capacity will vary diurnally with photosynthesis since this will determine plant defenses. They suggest a lower sensitivity during the morning-to-noon period and a higher sensitivity in the early afternoon (when ozone concentrations also tend to be highest). The second approach involves cellular level process based modelling; Plöchl et al. (2000) developed a method that estimated detoxification capacity based on the rate with which ozone reacts with ascorbate in the apoplast. This model accounts for stomatal ozone flux and the replenishment of apoplastic ascorbate on consideration of various species-specific physico-chemical characteristics (including g_{sto} , mesophyll cell wall thickness and tortuosity, chloroplast volume, apoplast pH and ascorbate to ozone reaction stoichiometry). The model determines the flux of ozone which impinges on the plasma membrane as a measure of detoxification capacity. Neither of these modelling methods consider the ‘costs’ of maintaining such defense mechanisms which might, for

example, be expected to require increased respiration rates (see Section 2.3 for further discussion). Modelling methods have however, been developed to incorporate the influence of leaf senescence in reducing stomatal ozone flux and thereby the yield loss estimates made using empirically derived flux-response relationships that trigger senescence once a threshold accumulated flux is exceeded (Pleijel et al., 2007; Ewert and Porter, 2000).

The physical changes in the plasma membrane can cause a series of **cellular and metabolic** responses (#4 ‘metabolism dysfunction’ in Fig. 3). An important response is that of ozone and associated ROS attacking the guard cells that form the stomatal complex, since this then influences subsequent ozone uptake. Short duration ozone exposures resulting in increases in H_2O_2 have been linked to Abscisic Acid (ABA)-induced closure of the stomata through an activation of the calcium influx in guard cells (Pei et al., 2000; Schroeder et al., 2001). In contrast, prolonged exposures can cause this stomatal response to become sluggish (McAinsh et al., 2002) with implications for plant response to water stress (see Section 3.3). Theories have also been put forward that high ozone fluxes and production of ROS derivatives may cause damage to the permeability of the guard cell membranes so that the cell loses osmotically-active materials. This would lead to increases in water potential resulting in the guard cells losing water, and hence turgor (rigidity), to adjacent cells (Alscher and Wellburn, 1994); either mechanism will result in loss of control over gas exchange and will affect g_{sto} and hence feedback on ozone uptake (process #C, Fig. 3). It’s important to note that such changes in g_{sto} , which are not influenced by changes in photosynthesis, are consistent with many field studies where the reduction in g_{sto} with increasing ozone concentration is much less consistent than the reduction in photosynthesis (e.g. Betzelberger et al., 2010). Other metabolic responses include the **production of plant hormones** (e.g. the wounding hormone – ethylene as well as salicylic acid and jasmonic acid (Tamaoki, 2008)). These metabolic responses represent signaling pathways to induce the expression of defense genes. Ozone and ROS can also lead to changes in **plant proteins and enzymes** resulting from altered amount, activity or signals for the production of each protein (i.e. the amount of mRNA) (Heath, 2008). It is these changes that are thought to be the ‘primary drivers’ of alterations to gross photosynthesis (also defined as carboxylation; Wohlfahrt and Gu (2015)) (see #8, Fig. 3) and net photosynthesis (the latter taking into account gross photosynthesis less photorespiration and dark respiration).

It is still uncertain exactly how these ‘primary drivers’ of cellular and metabolic responses lead to more specific alterations to gross **photosynthesis**. There is some evidence that the light reactions, that control photosynthetic electric transport as well as the occurrence of photo-inhibition, are affected by ozone (Fiscus et al., 2005), though these studies tend to rely on laboratory rather than field experiments, using rather high, short duration ozone exposures (e.g. Guidi et al., 2000). However, it is the carbon reduction reactions of photosynthesis (collectively represented by the Calvin cycle enzymes) that are widely considered to have the greatest sensitivity to ozone and ROS, leading to reductions in carboxylation efficiency and carbon assimilation capacity (Fiscus et al., 2005; Heath, 2008; Ainsworth et al., 2012). One of the most critical enzymes for the Calvin cycle is Ribulose 1,5-bisphosphate carboxylase/oxygenase also known as ‘rubisco’. A recent review by Galmés et al. (2013) of ~20 experimental studies finds that ozone causes a decline in the concentration, rather than the activation state of rubisco. This has been attributed to a decline in synthesis of the messenger RNA of the rubisco enzyme (Heath, 2008; Ainsworth et al., 2012). However, Eckardt and Pell (1994) showed a substantial decline in rubisco content under conditions where additional synthesis should be minimal, suggesting that the primary cause of the decline in rubisco content was enhanced degradation rather than reduced production. Furthermore, some of the messenger RNA signals (Pell et al., 1997; Miller et al., 1999) were shown to be equivalent to those due to senescence (Gielen et al., 2007), thereby linking this reduction in rubisco

content to an early decline in the leaf function.

The ozone effect on photosynthesis is further complicated by feedbacks, since ozone damage to photosynthesis will in turn reduce the plant’s detoxification ability, which may lead to an enhancement of maintenance respiration to replace lost antioxidants (Amthor and Cumming, 1988) (#B, Fig. 3; Massman, 2004; see also Section 2.3 and Fig. 5). Conversely, the decrease in photosynthesis can also lead to increased internal CO_2 concentration that can result in a decrease in g_{sto} (#A, Fig. 3 and #4, Fig. 5), leading to a reduction in ozone uptake.

It is useful to consider these potential feedbacks in relation to commonly used approaches to model photosynthesis as well as coupled net photosynthesis-stomatal conductance ($A_{net-g_{sto}}$) models that could in theory account for the feedbacks mentioned in Fig. 3. These coupled models aim to provide a consistent estimate of the exchange of CO_2 across the stomata driven by the supply and demand of CO_2 for photosynthesis. These $A_{net-g_{sto}}$ models generally consist of a combination of two separate modules: (i) one that estimates net C assimilation or net photosynthesis (A_{net}), normally based on the mechanistic and biochemical Farquhar model (Farquhar et al., 1980) and (ii) an $A_{net-g_{sto}}$ model that estimates g_{sto} based on an empirical linear relationship between A_{net} and g_{sto} (e.g. Leuning, 1990). Fig. 5 shows the three sites of action where ozone (or ROS and the metabolic responses caused by ozone) can affect this coupled $A_{net-g_{sto}}$ model. According to the evidence reviewed above, ozone may damage the guard cells (#1, Fig. 5) thereby altering gas exchange and therefore the supply of CO_2 (internal CO_2 concentration (C_i)) for photosynthesis; (#2, Fig. 5) reduce the rate of electron transport in the light reactions; and reduce the concentration of rubisco (#3, Fig. 5). Both (#2 and #3, Fig. 5) would see changes in the carboxylation efficiency (V_{cmax}), and hence C assimilation. Finally, reductions caused by processes (#2 and #3, Fig. 5) will see an increase in C_i which will cause a feedback and reduction in g_{sto} through the coupled $A_{net-g_{sto}}$ model (#4, Fig. 5).

As well as affecting net photosynthesis, thereby reducing C assimilation (#10, Fig. 3), the action of ozone and its ROS derivatives in the apoplast can also trigger unregulated cell death and hyper-sensitive responses leading to programmed cell death (Pell et al. (1997) and Sandermann et al. (1998); #5, Fig. 3) that can manifest as the appearance of visible injury (#6, Fig. 3; Günthardt-Goerg and Vollenweider (2007)). Visible injury begins with water-logging (i.e. the loss of intracellular water), leads to chlorosis (bleaching of chlorophyll due to the extreme damage or death of cells) and necrosis (due to the loss of all cellular components) (Heath, 2008). These cell death responses can also lead to the production of the plant hormone ethylene via a wounding response (#7, Fig. 3), which in turn can induce a feedback on g_{sto} and lead to a reduction in stomatal ozone flux (see #E, Fig. 3). These types of injury generally occur under high levels of ozone uptake and are a mark of **acute injury**, and decrease the per unit leaf area available for photosynthesis, as indicated by the feedback response in #D, Fig. 3. Visible injury gives a direct visual indication of ozone damage and is a characteristic symptom in certain crops. Visible injury becomes particularly important in leafy crops such as spinach and lettuce (since it may affect their retail value) and clover grown as fodder plant (e.g. Heagle and Johnson, 1979; Heggstad, 1991; De Temmerman et al., 2002; Karlsson et al., 1995).

There is a growing consensus that accelerated leaf ageing and senescence are among the most important responses to ozone exhibited by field-grown crops (#11, Fig. 3). Senescence is the age-dependent program of degradation and degeneration that allows for nutrients to be re-distributed to other organs (Lim et al., 2007). However, under ozone stress, this process can occur earlier and more rapidly in leaves as well as at the whole plant or crop canopy scale. The causes of this early and accelerated senescence are not completely understood, but at the molecular level, exposure to ozone can induce the expression of many genes involved in natural senescence (Miller et al., 1999). An early event in senescence is the transcriptional up-regulation of ROS-responsive genes (Breeze et al., 2011), which can be promoted in the

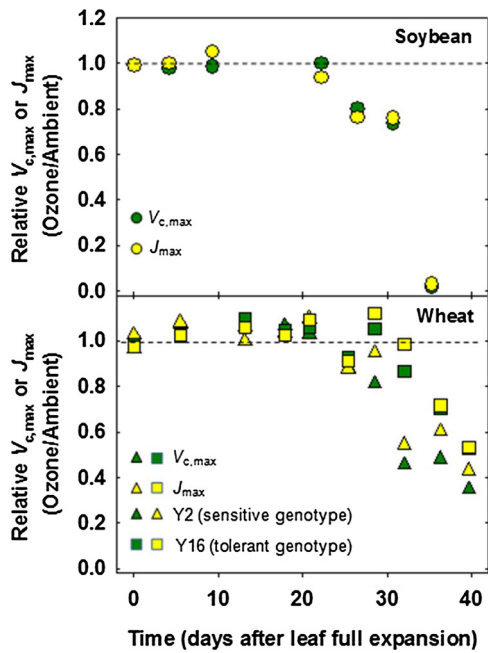


Fig. 4. $V_{c,max}$ and J_{max} of a soybean leaf (upper panel) and a flag leaf in two genotypes of wheat (lower panel) decline faster in leaves exposed to elevated ozone. A value of 1 indicates that $V_{c,max}$ or J_{max} was the same in plants exposed to elevated ozone ($1.2 \times$ ambient for soybean and $1.5 \times$ ambient for wheat) as those grown in ambient ozone. A value of less than 1 indicates that $V_{c,max}$ or J_{max} are lower in plants exposed to elevated ozone. Data were adapted from Morgan et al. (2004) for soybean and Feng et al. (2011) for wheat.

presence of ozone. Elevated ozone may also inhibit sugar export from leaves (Grantz and Farrar, 2000), which could trigger early onset of leaf senescence. This early and accelerated senescence will reduce

photosynthesis (through a loss of chlorophyll content and photosynthetic capacity) and hence C assimilation. An interesting question is whether it is the direct action of ozone on the photosynthetic mechanism or indirect action of ozone on leaf senescence that leads to the reductions in C assimilation. Fig. 4 provides some evidence that the latter mechanism is more important. This shows the response of soybean and wheat to elevated ozone in FACE experiments. Soybean measurements were made on a cohort of leaves through time and showed a more rapid loss of $V_{c,max}$ and J_{max} in plants exposed to $\sim 1.2x$ ambient ozone (with ambient ranging between 30 and 90 ppb) in the Mid-West USA (Morgan et al., 2004; Fig. 4). Similar results were found for wheat plants exposed to $\sim 1.5x$ ambient (with ambient typically between 20 to 80 ppb) in a FACE experiment conducted in Eastern China. Differences in the yield response of the two wheat cultivars were attributed in part to differences in the onset of leaf senescence (Fig. 4; Feng et al., 2011). Critically, these experiments show that the initial maximum photosynthetic capacity in the leaf was not altered by elevated ozone, but that the rate of loss of photosynthetic capacity was accelerated (Morgan et al., 2004; Feng et al., 2011). This observation, that plants are more sensitive to elevated ozone concentrations during reproductive periods towards the end of the growing season (Fiscus et al., 2005), is consistent with the observation at the FACE experiments that maximum capacity of photosynthesis is often not decreased by elevated ozone, but that the rate of leaf senescence is accelerated. Further studies that explore how different leaf cohorts respond to ozone uptake over time would be helpful to understand whole canopy response in terms of C assimilation and influence on yield.

2.3. Determinants of the fate and impact of absorbed ozone at the canopy level

Fig. 6 summarises the cellular and metabolic effects of ozone described in the previous section and shows how these lead to canopy level effects through influence on C allocation to different plant-parts

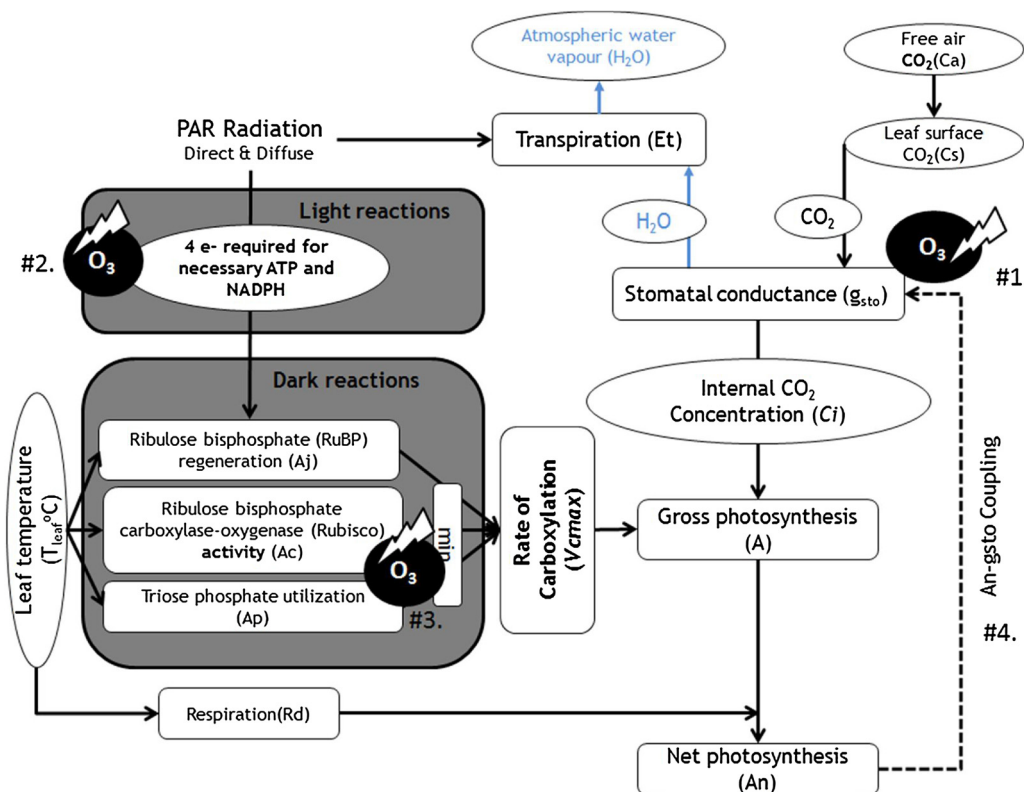


Fig. 5. Schematic representation of how the ozone influences on photosynthetic injury can be incorporated into existing coupled models of photosynthesis and stomatal conductance (A_n-g_{sto}). These A_n-g_{sto} models allow estimates of A_n (through the biochemical representation of light (driven by PAR (Photosynthetically Active Radiation)) and dark reactions of photosynthesis based on von Caemmerer and Farquhar (1981) and associated estimates of g_{sto} according to empirical regression models (Leuning et al., 1995). This scheme calculates g_{sto} so that the supply of internal CO_2 concentration (C_i) meets the demand determined by the rate limiting step (A_j (RuBP regeneration), A_c (Rubisco activity) or A_p (Triose phosphate utilization)) for gross photosynthesis (A), which translates into net photosynthesis after allowance for respiration (R_d). This scheme will also determine the loss of H_2O vapour via the stomata (which will influence transpiration (E_t)). The components of this scheme which are considered to be directly affected by ozone damage are shown as lightning strikes; all effects (both direct and indirect) are numbered #1-4, with descriptions of the processes included

in Section 2.2.

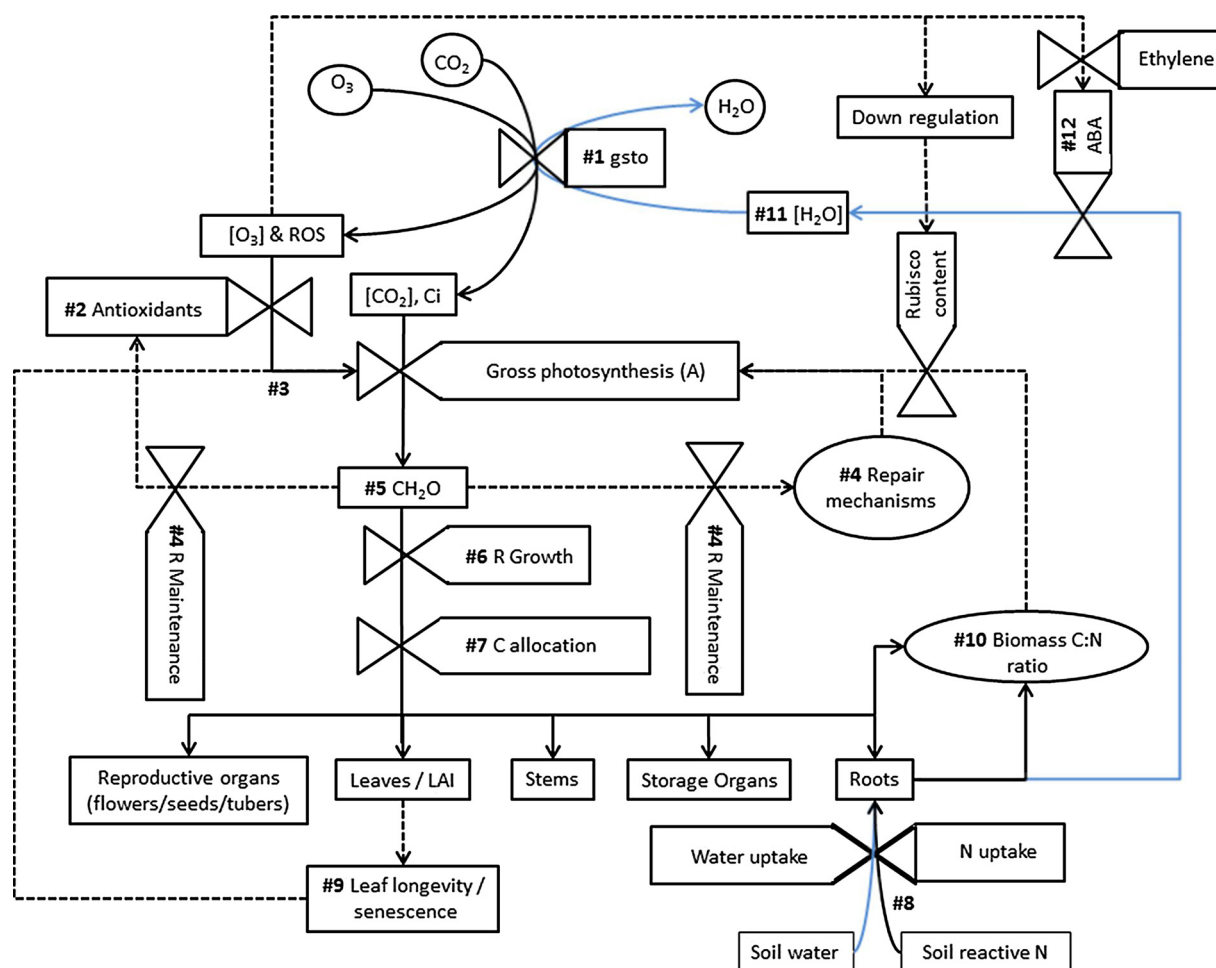


Fig. 6. Schematic representation of how the cellular and metabolic effects of ozone described in Section 2.2 will lead to canopy level effects through influence on C allocation to different plant-parts such as grains, leaves (and Leaf Area Index (LAI)), roots and storage organs (described in Section 2.3). Changes in allocation to these different plant organs may also cause feedbacks that will in turn alter the uptake of ozone (e.g. through changes in g_{sto}) or modify the crop's ability to take up nutrients and soil water with consequent effects on crop physiology, associated biochemistry and ultimately C assimilation. The #1-12 are key processes or feedbacks that are described in more detail in Section 2.3. Flows of matter (C, H_2O , ozone and N) are indicated by solid arrows; relationships between processes are indicated by broken lines).

such as grains, leaves (thus affecting Leaf Area Index (LAI)), stems, storage organs and roots. Changes in allocation to these different plant organs may also cause feedbacks that will in turn alter the uptake of ozone (e.g. through changes in g_{sto}) or modify the crop's ability to access nutrients and soil water with consequent effects on crop physiology, associated biochemistry and ultimately C assimilation, growth and yield.

In brief, g_{sto} (#1, Fig. 6) controls the exchange of gases (CO_2 , ozone and H_2O). Upon entering the leaf, ozone (and its ROS derivatives) may be partially detoxified by antioxidants (#2, Fig. 6) leaving an effective ozone flux to damage gross photosynthesis (#3, Fig. 6). The demands on C to support maintenance and repair processes increase as more carbohydrates are used in dark respiration (termed 'R Maintenance' in Fig. 6) to drive the production of secondary metabolites and antioxidants (Biswas et al., 2008; Andersen, 2003; #4, Fig. 6). This decrease in leaf-level carbon assimilation (#5, Fig. 6) and the associated increase in carbon demand leaves less photosynthate available for growth respiration (#6, Fig. 6) and to transfer to developing leaves and other plant parts (#7, Fig. 6). In response to this C constraint, the normal source-sink balance of the plant changes due to preferential reallocation of photosynthate to some organs; this response varies by crop species, cultivar and developmental stage (Grantz et al., 2006). Studies on several annual crops (e.g. wheat, barley, rice, beans and soybean) have identified the reproductive phase as being the most

ozone sensitive phase (Soja et al., 2000; Pleijel et al., 1998; Younglove et al., 1994). Studies have shown that ozone exposures can enhance C allocation to reproductive organs (i.e. flowers and seeds) during the reproductive phase, compromising the growth of other organs, especially roots (Pell et al., 1997; Sandermann et al., 1998; Morgan et al., 2003; Grantz and Yang, 2000; Grantz et al., 2006; Betzelberger et al., 2010) whilst other studies have shown the proportion of above-ground biomass allocated to seeds to be negatively affected (Betzelberger et al., 2010; Pleijel et al., 2014). Therefore, there is uncertainty in the direction of the response in terms of ozone influence on harvest index as well as the likelihood that the yield loss will to some extent depend on whether the harvestable part of the crop is tubers, roots, leaves or seeds.

The preferential retention and favoured partitioning of C towards above ground plant parts means that ozone effects on root growth become apparent earlier, and to a greater degree, than effects on shoot growth (Grantz et al., 2006; Andersen, 2003). These changes in root development interfere with plant-microbe interactions and rhizospheric processes as well as nutrient uptake. These alterations in root growth may also make plants more vulnerable to water-stress conditions (see #8, Fig. 6).

The triggering of early leaf senescence by ozone (see Section 2 and #9, Fig. 6) will shorten the period between flowering to maturity thus providing less time for an already depleted C resource to allow complete grain filling (Gelang et al., 2000). The resulting loss in

photosynthetic capacity in annual crops is unlikely to be regained were ozone exposure to be reduced later in the growth period, although the further decline in leaf vitality may proceed at a slower rate. Enhanced senescence will also reduce the length of time over which nutrients will be taken up from the soil (Broberg et al., 2015). Accelerated leaf senescence leads to nitrogen remobilization from vegetative tissue (Wang and Frei, 2011). The amino acids resulting from this remobilization compensate for the decrease in grain filling time and the nitrogen shortage from reduced root uptake; this means that protein synthesis can remain relatively unchanged. In contrast, synthesis of carbohydrates in seeds, which depend primarily on concurrent C fixation, may lead to a decrease in grain starch concentration. As such, protein deposition may be favoured over starch accumulation in the grain. This is because the production and translocation of carbohydrates to the grain is more sensitive to ozone than protein accumulation (Pleijel et al., 1999) and results in seeds grown under ozone stress tending to have a protein content that is less diluted by carbohydrate accumulation, resulting in the often observed increase of grain protein concentration (Pleijel and Uddling, 2012). However, the absolute amount of protein produced (also known as the “protein yield”) is reduced by ozone, but to a lesser extent than biomass. The net effect is increased protein concentration (i.e. the amount of protein produced per unit area is reduced by ozone, but the protein concentration of the grain is increased), as observed in the majority of the 25 experimental studies analysed by Wang and Frei (2011).

These changes in nitrogen (N) uptake and remobilization under ozone stress, which may both be driven by reduced N uptake capacity of the roots and by a smaller demand of N from the shoot impaired by ozone stress, may also be expected to alter C:N ratios in plant biomass (#10, Fig. 6). No studies were found that investigated this for crops, but studies on forest trees found reductions in C:N ratios in roots, stems and leaves caused by an increase in N concentrations whilst the C concentration remained stable (Cao et al., 2016). This could have important implications for decomposition rates and hence nutrient cycling. The reduction in carbon allocation tends to concentrate other nutrients. In a meta-analysis of 42 experiments conducted on wheat, Broberg et al. (2015) found that ozone exposure increased protein as well as N, P, K, Mg, Ca and Zn concentrations in wheat grains. Similarly, an increase in both N and Mg concentrations in potato tubers was reported (Fangmeier et al., 1994).

Ozone has also been found to interfere with stomatal functioning causing increases in conductance, sluggish stomatal response to environmental factors or stomatal closure depending on species and ozone exposure (Mills et al., 2016). For crops, the majority of papers published up until 2013 indicated that the dominant effect on stomata is an ozone-induced closure (17 out of 22 experiments, 12 out of 16 species; Mills et al., 2016). This would lead to a reduction in evapotranspiration and water use efficiency (WUE), including reduced ability to uptake soil water and an increase in sensible heat flux (as seen, for example for soybean, VanLoocke et al., 2012; Bou Jaoudé et al., 2008; see (#11, Fig. 6)). Although stomatal closure seems to be the dominant response in crops, recent research has suggested that under chronic ozone exposure, ozone-induced elevated production of stress ethylene can lead to a dampening of the Abscisic Acid (ABA) signal (#12, Fig. 6; Wilkinson and Davies, 2009, 2010) that would normally lead to decreases in g_{sto} to conserve water in drying soils (Mills et al., 2009). This could result in the crop losing control of stomatal closure, exacerbating water loss and enhancing ozone uptake that would otherwise be limited by soil water stress, thus creating a feedback loop that enhances ozone damage.

2.4. Ozone impacts on yield

Seeds are one of the major plant compartments for C storage and their yields depend on the degree to which the C demand can be met under ozone stress. Wheat has been extensively studied for ozone

effects. A highly consistent response of wheat to ozone exposure is a decline in the harvest index (Pleijel et al., 2014). This sign of efficiency loss in crop yield production under ozone exposure is consistent with the pattern outlined above, in that the reproductive phase is the more sensitive and the duration of seed growth is shortened. It is also important to note that the ozone effect on wheat grain yield is dominated by a reduction in grain mass, while the effect on grain number is small (Broberg et al., 2015). In an experiment conducted on wheat with five different levels of ozone exposure Gelang et al. (2000) observed that grain mass at harvest correlated strongly with grain filling duration, which in-turn was strongly correlated with flag leaf duration as expressed by chlorophyll content. Araus and Labrana (1991) suggested that at approximately 50% senescence, wheat flag leaves are no longer net producers of photosynthates.

Other crops vary in their yield response to ozone. A meta-analysis by Feng and Kobayashi, (2009) looked at yield components for six different crops and for example found that soybean and barley showed greater reductions in the mass rather than the number of grains or pods. By contrast, for rice they found that the contribution of reduced grain number is greater than that of grain mass in ozone-induced yield loss. In potato, (where tubers rather than seeds are the plant storage organs used for species' reproduction as well as food production) the shortening of green leaf area duration was found to be a key factor to the ozone effect on tuber yield in potato, with the size rather than the number of the tubers being affected (Piikki et al., 2004; Vandermeiren et al., 2005). Therefore, the response of yield components to ozone is dependent on the species under investigation but is often related to the shortening of the maturation period.

As described in Section 2.3, it is not only the quantity of grain that is affected but also the quality of grains. Crop quality traits can be divided into different groups and what is considered an important quality trait varies considerably among different crops. However, some generalisations are possible with respect to the effects of ozone on crop quality:

- One category of quality traits is the concentration of N and different minerals of the yield. It is a very general observation that the concentration of N and minerals in crop yield under ozone exposure is positively affected, while the unit-area yield (important from a human and animal feed nutrition perspective) of N and minerals decreases. This is the net result of a smaller uptake of N/minerals (due to a shorter uptake period and reduced root vs. shoot growth) and an even larger negative effect of biomass accumulation in the yield. This net-negative balance applies not only to cereals like wheat (Broberg et al., 2015), but has also been found in potato, a root crop, where tuber production was affected (Piikki et al., 2008). These effects are all well explained by the sequence of ozone effects outlined in Section 2.3. The consequences of reductions in protein per unit area on human nutrition may become particularly important in those world regions where ozone concentrations are high and access to nutritious food-stuffs is limited (e.g. by purchasing power of individual households or by choice of diet (i.e. vegetarian diets)).
- Secondly, certain physical properties are of importance as quality traits. These include seed or tuber size and density. They are predominantly negatively affected by ozone and include e.g. grain mass and density (“volume weight”) in cereals (Broberg et al., 2015) and tuber size in potato (Vandermeiren et al., 2005). In many cases, these properties influence market price and processing quality of the harvested crop. These effects can be understood as a result of the shortened period of seed or tuber filling resulting from the enhanced canopy senescence from ozone stress (Section 2.3).
- Finally, there is a rather diverse set of quality traits that vary strongly among crops and are mostly related to different organic compounds (vitamins, organic acids, enzyme activity etc...) and their influence on the processing of the crop products (baking, starch production, chip production, beer production etc...) (Broberg

et al., 2015). For this category, both negative and positive effects of ozone have been observed and responses have been very variable, partly because they interact strongly with abiotic and biotic factors (e.g. the α -amylase activity in wheat grain). These responses cannot be easily predicted or modelled based on the processes treated in Section 2.3 and further empirical studies would be required to implement them in the modelling of ozone effects on crops.

The complexity described above in the pattern of crops' responses to ozone exposure at the whole canopy and plant organ level highlights the importance of using process based models that can couple together a cascade of responses that result from instantaneous ozone damage and have cumulative effects on leaf to whole canopy physiology, growth and end of season quantity and quality of yield. There are four core effects that need to be captured by modelling: – (1) A higher ozone sensitivity in the reproductive, compared to the vegetative phase; (2) Acceleration of leaf senescence by ozone and the associated effect on photosynthetic capacity; (3) A reduction in harvest index and change in components of yield (e.g. grain mass, grain number); and (4) An increase in seed protein (and mineral) concentration and a reduction in protein (and mineral) yield. It may be that all four aspects can be explained to a large extent as different consequences of the promotion of leaf senescence and the consequent alterations in biomass allocation patterns by ozone. In addition, to date, there has been a focus on the uppermost leaf-level (e.g. the flag leaf in wheat), since this leaf-level intercepts a large fraction of the photosynthetically active radiation and is less senescent than lower leaves, thus being more productive during seed filling. As mentioned previously, the contribution that effective ozone flux may have on damage at the whole canopy level is an issue that requires further consideration and could be incorporated through the use of multi-layer models that represent the influence on flux of vertical profiles of ozone and micrometeorology (Oue et al., 2008).

3. Which plant traits, management techniques and climatic conditions modify the plants' responses to ozone?

Intra- and inter-specific variation in the response of plants to elevated $[O_3]$ has been documented across a number of species and varieties (reviewed in Ainsworth (2017) and Mills et al. (2007)). Modelling methods aiming to accurately simulate ozone damage to crops must be able to capture this naturally occurring variation in sensitivity. In this section, we consider the particular plant physiological and genetic traits that have been linked to high ozone sensitivity, as well as factors relating to crop management and climate that can increase or mitigate the vulnerability of crops to ozone. An important aspect of modelling will be whether models are able to account for these trait differences in estimates of ozone sensitivity and how strongly these differences are expressed in different plant varieties.

3.1. Factors that modify ozone uptake

Variation in g_{sto} is a key determinant of ozone dose to the inter-cellular airspace of the leaf, and is associated with yield sensitivity to ozone. The association between g_{sto} and ozone damage to crops is illustrated by a comparison of 20 wheat cultivars released over the past 60 years, which found that newer lines with greater g_{sto} were more sensitive to ozone compared to older lines (Biswas et al., 2008). Similarly, Osborne et al. (2016) found that the ozone sensitivity of 49 soybean cultivars increased by an average of ~30% between 1960 and 2000, partly due to an increased g_{sto} . g_{sto} determines the dose of ozone that enters plants, but it also controls the entry of CO_2 , which is required for photosynthesis and ultimately for growth and yield. Breeding for low stomatal conductance in order to exclude ozone is therefore likely to result in a penalty in terms of C assimilation, and *visa-versa*, breeding for optimising CO_2 uptake and yields may result in higher sensitivity to ozone. For this reason, it is not necessarily beneficial to

select for low g_{sto} as a means of protecting the net effect on grain yield (Ainsworth, 2008); varieties with a low g_{sto} may confer a yield advantage in very polluted regions and years, but could result in a substantial yield penalty in cleaner locations and years.

Similarly, plants with adaptations that enable stomata to remain open under environmental stress may have greater sensitivity to ozone. For example, varieties with drought-adaptive traits such as extensive root systems, better hydraulic adaption, maintenance of leaf elongation and high levels of membrane stability (Liu et al., 2004) that enable the maintenance of high g_{sto} under drought stress would result in a higher ozone dose to the plant (Blum, 1996). However, it is these traits that enable plants to be productive under drought stress, and so selecting against them may be unlikely to lead to greater productivity.

A further consideration when estimating the vulnerability of a particular crop to ozone is the likelihood of co-occurrence of high ambient ozone concentrations with the crop-growing season. Ozone concentrations show strong seasonal profiles; in temperate regions, ozone levels peak in the spring and summer months, driven by high rates of photochemical ozone production. In tropical regions under the influence of monsoons, the highest ozone concentrations tend to occur in the months preceding the onset of the monsoon. This is due to the enhanced vertical mixing that occurs during the monsoon throughout the atmosphere, distributing ozone more evenly across the atmospheric column rather than concentrating ozone at the ground level (as will tend to occur during dry seasons). Heavy rains will wash soluble pollutants out of the atmosphere and (thick) cloud cover will limit ozone forming photochemical reactions (Reddy et al., 2008; David and Nair, 2011). The timing of sensitive periods in the crop calendar is therefore crucial in determining ozone exposure, and the consequent impact on yield. South Asian wheat-rice rotations in the Indo-gangetic plain region provide a good example of this; the sensitive reproductive phase of the winter (*Rabi*) wheat crop occurs in February to March when ozone concentrations are high. By contrast, the rice crop occurs in the summer monsoon (*Kharif*) season during the months of June to November when ozone concentrations are far lower (Ramanathan et al., 2008).

Similarly, management can influence ozone vulnerability. Globally, approximately 25% of major crops are now irrigated (Portmann et al., 2010), allowing growers to shift crop calendars to periods when radiation and temperature are optimal for crop growth, conditions which can also favour ozone formation. A global modelling study by Teixeira et al. (2011) found that yield losses for irrigated crops are usually equal or greater than for rain-fed crops, especially in India, due to co-occurrence of crop growth calendars with seasonal peaks in ozone formation. They also found that shifting crop calendars could reduce regional ozone damage for specific crop-location combinations (e.g. up to 25% for rain-fed soybean in India), but this had little implication at the global level. The relevance of this with regard to the effectiveness of climate change adaptation strategies has not been well studied (see Section 3.3)

Finally, the development of crop varieties with different maturity traits designed to avoid stressors such as heat and drought (Crasta et al., 1999) may also influence ozone vulnerability through changes in stomatal ozone flux. Jamir (2011) explored cultivars with different heat stress traits including 'early maturing' heat avoidance varieties and 'late' or 'timely' sown heat tolerant varieties. The late sown crops were pushed towards those times of year when temperatures were high which limited ozone flux, but also resulted in a shortened grain-filling period.

3.2. Factors that modify response to internal ozone

Some traits which have been associated with ozone tolerance do not influence ozone uptake, but instead relate to the internal response to ozone (see Section 2). While molecular and biochemical characterisation of the antioxidant capacity of numerous genotypes within a species has not yet been performed, there is evidence from both between-

species comparisons and within-species comparisons that variation in ROS quenching mechanisms play an important role in ozone tolerance (Inada et al., 2012; Yendrek et al., 2015). There is also theoretical evidence to suggest the leaf mass per unit area may be important in determining effective ozone flux, by influencing the length of the pathway over which ozone (and ROS) is transported to the cellular sites of damage (Tingey and Taylor, 1982). The theory that leaf structure plays a role in ozone sensitivity is supported by studies that found ozone-sensitive genotypes of green ash and black cherry have a thinner palisade mesophyll layer and ratio of palisade to spongy mesophyll as compared to ozone-tolerant varieties (Bennett et al., 1992; Ferdinand et al., 2000).

Whether targeting particular plant physiological traits associated with limiting ozone uptake or increasing ozone resistance will be beneficial for yield in practice will depend on the concurrent environmental stress factors, as well as the species and location-specific crop growth calendar. Crop modelling can theoretically be used as a tool to identify which plant traits might be beneficial in protecting yield against ozone under future climates and that therefore should be targeted by plant breeders.

3.3. How might global climate change impact the response of crops to ozone?

Accurate simulation of ozone impacts on yield under future climate scenarios requires an understanding of how future changes in atmospheric CO₂, and other environmental variables, can influence g_{sto} and plant function. Increasing levels of CO₂ are the main driver of climate change. The 21st century will see an increase in global atmospheric CO₂ concentrations from present levels of approximately 400 ppm, to concentrations ranging from 421 to 936 ppm by 2100 (IPCC, 2014). Numerous controlled, semi-controlled and field experiments have investigated the effect of elevated CO₂ on crop yield (reviewed in Ainsworth et al., 2002; Long et al., 2006; Taub et al., 2008 and Mills et al., 2016). A widely observed response to elevated CO₂ is reduced g_{sto} , which is driven in the short term by in-leaf chemical signalling induced by high intracellular CO₂ (Ainsworth et al., 2008), and in the long-term by reduced stomatal density (Gray et al., 2000). Reduced g_{sto} limits ozone flux to the apoplast, and consequently elevated CO₂ has been seen to significantly ameliorate damaging ozone effects in a number of crop species (Feng et al., 2008; Morgan et al., 2003; Feng and Kobayashi, 2009). How ozone and elevated CO₂ will interact in real crop systems is less clear, as very few CO₂-ozone interaction experiments have been conducted in open field conditions. Evidence from FACE experiments carried out to date suggests that ozone impacts on yield, conducted in laboratory and mesocosm studies (Ainsworth, 2008; Long et al., 2005), may have been underestimated, and CO₂ fertilisation effects overestimated. However, drought stress and increasing temperatures also modulate the response of crops to rising CO₂ concentrations (e.g., Manderscheid et al., 2014; Gray et al., 2016), and modelling efforts are underway to understand uncertainties in predicting crop responses to combinations of climate change factors (Bassu et al., 2014; Li et al., 2015).

Furthermore, the global rise in atmospheric CO₂ will take place against a backdrop of other environmental changes, with global mean temperatures projected to increase by between 1 and 5 °C, and drought and heat stress events likely to become more frequent (Dai, 2011; IPCC, 2014). A meta-analysis of responses in rice to combined elevated CO₂, ozone and temperature showed that high temperature damage negated any yield benefits from elevated CO₂ (Ainsworth, 2008). High temperatures reduce yield by inducing faster maturation and hence a shorter grain fill period (Erda et al., 2005), and can induce floret sterility in cereals such as rice (Matsui et al., 2014) and wheat (Wheeler et al., 2009, 1996) but physiological responses differ among crops (Rezaei et al., 2015). Damaging effects of high growth temperatures may also be exacerbated at high CO₂ due to reduced evaporative

cooling of the leaf canopy (Bernacchi et al., 2006). Stomata have been assumed to close at high temperatures and under drought stress (Jarvis, 1976), leading to the notion that ozone damage in crops may be ameliorated under future climate extremes. However, this effect is not consistent across existing ozone-drought interaction experiments carried out in crops (Feng and Kobayashi, 2009), with the damaging effect of drought stress on yield often outweighing the positive impact of ozone exclusion (Fangmeier et al., 1994). There is also some evidence that chronic ozone exposure can impair ABA-mediated stomatal regulation, leading to excessive water loss and greater-than-additive negative impact of co-occurring drought and ozone (Mills et al., 2009), although this effect is yet to be observed in crop species. How the different components of global change - including ozone, CO₂, temperature, and weather extremes - might combine and interact to influence crop yield has been little studied, and remains a key uncertainty in modelling ozone impacts under future climates.

4. How suitable are existing crop models for assessing ozone damage and injury?

A large variety of models and model approaches exist that aim to assess the effects of changes in environmental conditions and management practices on the phenological development, growth in biomass and yields of agricultural crops. Crop models can vary substantially in the modelling approach taken including the mechanistic detail used to simulate physiological processes and relationships determining crop growth and yield (Ewert et al., 2015). Simulation results can therefore differ substantially among models as for example recently shown in comparison studies with 27–30 wheat crop models tested against field experiments (Asseng et al., 2013, 2014). Similar results were also obtained for models simulating maize and rice (Rosenzweig et al., 2013; Li et al., 2015). Differences in the modelling approach and mechanistic detail among crop models reflect the differences in the original aims for which the model was developed (e.g. operational impact assessments vs synthesizing scientific understanding, focus on specific crops vs. development of more generic models for different crops, consideration or not of abiotic stresses due to drought or heat, etc...) and the availability of basic data to run the model for the envisaged applications. Obviously, these differences in modelling approach and detail will determine the way ozone effects can be incorporated. For example, a crop model using the concept of radiation use efficiency (RUE) will only be able to model ozone effects through the development of empirical relationships describing the influence of ozone on RUE (Kobayashi and Okada, 1995) and not on photosynthesis or stomatal conductance unless RUE is modelled in greater physiological detail.

Overviews of the differences in crop models are presented elsewhere (Asseng et al., 2013; Ewert et al., 2015; Rosenzweig et al., 2013) and shall not be repeated here. Briefly, models can differ in whether or not and how they model processes such as leaf area dynamics and light interception, light utilization, assimilate partitioning, yield formation, phenology, root distribution and depth, consideration of environmental constraints, types of heat, water and other abiotic stresses, water dynamics and evapotranspiration, nutrient dynamics and related soil models used, consideration of effects of elevated CO₂, the number of cultivar parameters and climate variables required to run the model. Importantly, these processes are simulated with a temporal resolution of typically one day or higher depending on the model and are interlinked allowing the simulation of feedback loops that account for the complexity of crop response dynamics to environmental conditions and management.

A simplified scheme of key growth and developmental processes and interlinkages often considered in crop models is presented in Fig. 7; this scheme also describes the primary and secondary processes and relationships by which ozone exposure is assumed to influence crop growth as modelled in AFRCWHEAT2-O3 (Ewert and Porter, 2000). In principle, the effects of ozone at canopy level, as extensively described

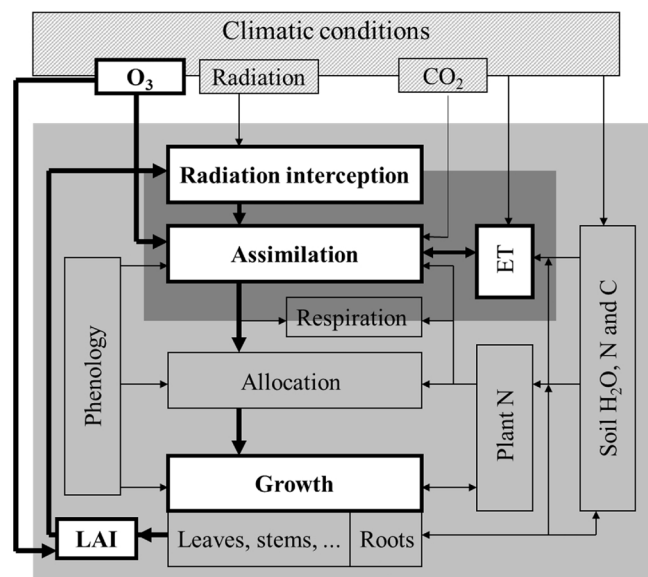


Fig. 7. Growth and developmental processes that are commonly described in process-based crop simulation models and effects of ozone exposure as modelled in AFRCWHEAT2-O3 (Ewert and Porter, 2000). Processes and relationships that are shown in white boxes and bold arrows represent the primary modelled ozone effects. Other processes that will be indirectly affected are represented by the smaller arrows. Grey areas indicate the temporal resolution of different processes ranging from seconds-to-hours (dark grey) and to days (grey). The figure is based on Fig. 1 in Ewert, (2004).

above, account for the underlying cellular and metabolic responses (Section 2 and Fig. 6) that could be considered in a crop model. However, because of the differences in model structure and detail, each model will likely implement these effects differently. Based on the empirical evidence about ozone effects on crops some general considerations about the modelling of ozone effects on individual processes within crop models are given in Table 1.

So far, only relatively few attempts have been made to consider ozone effects in mechanistic crop growth models (e.g. Kobayashi et al., 1990; Ewert and Porter, 2000; van Oijen et al., 2004; Tao et al., 2017) and only one study is known of in which different crop models have been compared for simulating the effects of ozone using different implementations (Ewert et al., 1999; van Oijen and Ewert, 1999). This comparison has shown, that ozone effects can be simulated fairly well but that interactions with other climate variables and elevated CO₂ can diverge depending on the model (Ewert et al., 1999; van Oijen and Ewert, 1999). The extensive capabilities of such implementations to assess ozone impacts across a range of environments and production systems differing in management intensity is presently constrained by lack of experimental data available for model calibration and testing. Model inter-comparison studies can also provide insights into the uncertainty due to model structure.

Finally, it is important to give consideration to the soil-plant-energy balance type of models that are included in land surface exchange schemes (e.g. the MOSES-TRIFFID land surface model (Sitch et al., 2007)). Currently these may lack some of the finer detailed aspects of crop modelling (developmental stage, carbon allocation algorithms) and also lack the capacity to be parameterised for a wide range of specific-species/varieties; together this makes it difficult to use these models to explore the effect of ozone on assimilation and growth processes. However, these models do offer the opportunity to simulate the

Table 1
Considerations for modelling ozone effects on crops using crop models.

Consideration	Comment
Time step	The vast majority of crop models used for practical assessments use a time step of one day. This implies that co-variation of ozone concentrations with physiological processes that determine ozone uptake and detoxification cannot be captured. This problem could be overcome by using smaller time steps for ozone related process, or it could be circumvented by considering that daily peak ozone levels tend to coincide – more or less – with peak temperature, radiation and water demand. This would require the use of Gaussian integration or simply using the model with one-day time step, with a simplifying parameterisation to account for non-linear effects. Obviously, models using such simplification will lack the ability to describe effects of deviating patterns in any of these variables.
Carbon assimilation	Some crop models use the biochemical Farquhar model to estimate C assimilation, thus allowing for the accommodation of ozone flux and damage mechanisms to photosynthesis described in Section 2.2 and Fig. 5. More common is the calculation of new biomass from intercepted PAR and radiation use efficiency (RUE), which requires the development of empirical relationships to describe the influence of ozone on RUE. Other models use an intermediate approach, e.g. calculating gross photosynthesis from PAR interception at various depths in the canopy, with explicit consideration of maintenance respiration. Here the effects of ozone on gross photosynthesis and maintenance respiration could potentially be distinguished, although this would require a higher level of empiricism than in the Farquhar based approach; these methods would also lack the feedback from internal CO ₂ concentration to g_{sto} (#4 in Fig.5).
Canopy development	All crop models distinguish at least: emergence, a phase of vegetative growth and a phase of reproductive growth ending at maturity, which can be user-defined as an additional phase or assumed to be reached when leaf senescence is complete and photosynthesis has stopped. Most models keep track of the daily development of newly formed leaves (or leaf units) in age classes or leaf cohorts, albeit (in most cases) without considering leaf geometry or the position of such classes within the canopy. Hence, impacts of ozone such as visible leaf injury and accelerated leaf ageing can easily be modelled to affect only those leaf cohorts that are present at the time of exposure.
Assimilate partitioning of biomass	Daily allocation of newly formed biomass to different plant parts vary strongly between crop models. Some models use a rather rigid approach; others are more flexible, whereas still others just apply a harvest index to total biomass at the end of the season to calculate yield. We note that in the latter category the harvest index is not a constant but calculated on the basis of the duration of the grain filling period and several stress factors. Most models that do explicitly calculate biomass allocation on a daily basis, allow part of the biomass formed during the vegetative stage to be reallocated to grains during the reproductive phase. These model attributes could be useful for incorporating the senescence enhancing effects of ozone.
Water uptake/transpiration and stomatal ozone uptake	In most crop models, crop water uptake is calculated as the minimum of potential transpiration (by the leaf canopy) and potential uptake (by the roots). Potential transpiration depends on atmospheric demand, LAI and one or more crop parameters that integrate the radiative interception properties of the canopy and the canopy resistance to water vapor transfer under well-watered conditions. Water uptake depends on root density and soil water content but is often estimated as potential transpiration multiplied by a soil water content dependent stress factor (0-1). Ozone uptake can be accommodated in such models by assuming proportionality with transpiration; with the effects of ozone on g_{sto} represented by an empirical relationship between ozone uptake and one of the crop parameters. Only a few crop models explicitly model g_{sto} to estimate transpiration.

exchange of gases (CO₂, H₂O and potentially ozone) at various scales from cellular, to leaf, canopy and ecosystem levels incorporating the influence of multiple global changes including ozone. Future development of these models that improve the characterisation of specific and variety specific traits would improve the power of these tools.

5. Recommendations to ensure development of suitable crop models to simulate ozone damage

In order to develop crop growth models to include the effects of ozone as described in Section 4, it will be imperative to identify experimental datasets that can be used to test and calibrate models. These datasets will ideally provide hourly, or at the very least daily data describing ozone and meteorological conditions over the course of the crop growing season and will also provide details of yield, ideally compared against a low ozone control. The most common datasets providing this information will be from ozone filtration/fumigation experiments conducted in Open Top Chambers or FACE studies (see Ainsworth et al. (2012) for further details). Although the results of many of such experimental investigations have been reported in the literature (Mills et al., 2007; Emberson et al., 2009), the challenge will be in finding enough datasets whose digital archives are still available; with these datasets providing a good representation of crop species (and varieties) sensitive to ozone under a representative set of global conditions. The availability of datasets will most likely be limited to more recently conducted experiments (i.e. those performed in the last decade) and to the global regions of North America, Europe and Asia. Ideally, new exposure experiments should be conducted which are targeted at the needs of the crop modelling community and focus on responses of current crop varieties to expected future ozone profiles.

It may be extremely beneficial to conduct this crop model development using model inter-comparison approaches similar to those described by Asseng et al. (2013). One means of achieving this, currently being explored, is to connect the ozone risk assessment experimental and modelling community with the AgMIP (Agricultural Model Inter-comparison and Improvement Project; <http://www.agmip.org/>). This would allow a comparison of models of varying levels of complexity, to enable a better understanding of which model processes should be incorporated to capture ozone effects, as well as an understanding of the limitations associated with different types of modelling approaches. This research can also help to identify uncertainties in ozone crop modelling and additional experimental investigation that would need to be conducted in the future to overcome these limitations.

One approach that could be helpful in the model development might be to establish a ‘module library’ that could be shared by modellers allowing the development of particular ozone model components that could be exchanged among modellers. It will also be important to identify templates that allow the delivery of empirical data in the required format to be readily used within crop modelling frameworks. This will require collaboration between the ozone and crop modelling communities to define data demands and determine the standards of data files to be exchanged.

Ultimately, this work would provide a better understanding of ozone impacts on crops and how these might be expected to interact with other environmental and global change factors. This will increase the accuracy with which crop productivity losses due to ozone can be assessed, which in turn will allow improved evaluations of the benefits of national, regional and global air quality policies that target reductions of ozone precursor emissions. Improved understanding will also allow the identification of agricultural management options to cope with ozone stress, as well as optimized management to cope with multiple stressors. Options focussing on ozone could include the development of crop breeding of ozone resistant varieties or the adoption of farm management practices that would see crops ‘avoid’ the worst effects of ozone episodes.

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