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1 **How is ozone pollution reducing our food supply?**

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1

2 **Abstract**

3

4 Ground level ozone pollution is already decreasing global crop yields (from

5 approximately 2.2-5.5 % for maize to 3.9-15 and 8.5-14 % for wheat and soybean), to

6 differing extents depending on genotype and environmental conditions, and this

7 problem is predicted to escalate given climate change and increasing ozone precursor

8 emissions in many areas. Here we summarise how ozone pollution affects yield in a

9 variety of crops, thus impacting global food security. Ozone causes visible injury

10 symptoms to foliage, comprising patches of necrotic tissue; it induces early

11 senescence and abscission of foliage; it can reduce stomatal closure and thereby

12 carbon uptake, and/or directly reduce photosynthetic carbon fixation; it can moderate

13 biomass growth via carbon availability or more directly; it can decrease translocation

14 of fixed carbon to edible plant parts (grains, fruits, pods, roots) either due to reduced

15 availability at source, redirection to synthesis of chemical protectants, or due to

16 reduced transport capabilities via phloem; decreased carbon transport to roots reduces

17 nutrient and water uptake and affects anchorage; ozone can moderate or bring forward

18 flowering and induce pollen sterility; it induces ovule and/or grain abortion; and

19 finally it reduces the ability of some genotypes to withstand other stresses such as

20 drought, high vapour pressure deficit (VPD) and high photon flux density (PFD) via

21 effects on stomatal control. This latter point is emphasized here, given predictions that

22 atmospheric conditions conducive to drought formation that also give rise to intense

23 precursor emission events will become more severe over the coming decades.

24

3   **Introduction**

4

5   Although essential in the upper atmosphere, gaseous ozone is also present in the lower

6   atmosphere where it is a pollutant formed from the effects of sunlight on vehicular

7   and industrial emissions. In the northern hemisphere, mean background ozone

8   concentrations have risen from approximately 10-15 ppb in pre-industrial times to the

9   current level of 35 ppb (Vingarzin, 2004; see Fuhrer, 2009). Effects of phytotoxic

10   ozone on crop yields have been studied extensively in the last 30 years, and several

11   syntheses of these findings exist (e.g. Mills et al., 2007; Emberson et al., 2009; Feng

12   et al., 2009; Pleijel, 2011). On the whole, wheat and soybean are particularly

13   sensitive; potato, rice and maize are moderately sensitive, whilst barley has been

14   found to be ozone resistant, with South Asian varieties of food staples being at least as

15   sensitive as those grown in the USA and Europe (Emberson et al., 2009). Global

16   models indicate that ozone reduced crop yields by a larger margin than the reduction

17   attributable to climate change in the year 2000 (Van Dingenen et al., 2009; The Royal

18   Society, 2008). Estimated reductions of global yields ranged from 2.2-5.5 % for

19   maize, to 3.9-15 % and 8.5-14 % for wheat and soybean (Avnery, 2011a). Economic

20   losses for Europe in 2000, based on ozone effects on 23 crops, were estimated to be

21   6.7 billion Euros (Holland et al., 2006); and global crop production losses in the same

22   year were estimated to have been 79-121 million metric tons worth \$11-18 billion

23   (USD2000; Avnery, 2011a). In a recent meta-analysis of 30 experiments representing

24   nine countries in North America, Europe and Asia, and spanning 18 wheat genotypes,

1 average wheat yield improvement by removing ozone from the air entering open

2 topped chambers in the field was 9% (Pleijel, 2011).

3   Crop plants are often irrigated, or planted to coincide with adequate rainfall,

4 which, as well as maximizing growth, induces the opening of stomatal pores in the

5 leaf surface for gas exchange for photosynthesis. It is through these open pores that

6 ozone gains entry to the plant. Yield is reduced by cumulative ozone effects on

7 several aspects of growth and productivity over the course of the growing season, and

8 more immediately visible effects are induced by ozone "episodes" or concentration

9 peaks frequently superimposed on the background level in spring and summer

10 months. Such episodes, lasting for several days and covering scores to thousands of

11 square kilometres, occur when local climatic conditions conducive to ozone formation

12 and persistence coincide with significant precursor emissions. Ozone concentrations

13 are elevated to peaks of above 60 ppb each day, with concentrations as high as 150-

14 180 ppb being recorded in years such as 2006 in many areas of the Mediterranean

15 Basin and central Europe (EAA Technical report, 2006).

16   The visible injury symptoms manifest as chlorotic or necrotic patches on the

17 leaf surface in the form of stippling, which can coalesce to form larger injured areas,

18 and these leaves often senesce and abscise early. Other visible yield impediments

19 stem from reductions in general plant biomass growth, and reduced flowering. Less

20 visible ozone effects include reduced grain/pod filling, and/or ovule and grain

21 abortion in later developmental stages (see below). Thus, in field crops such as some

22 varieties of wheat, rice, maize, bean, soybean, oil seed rape and sorghum, ozone

23 reduces grain size, grain weight, grain nutritional quality and/or grain number (e.g.

24 Mulholland et al., 1998; Biswas and Jiang, 2011). In pod crops such as bean, pods are

25 smaller, or of a reduced number. In tuber crops such as potato, tuber size rather than

1 tuber number is reduced. Crop quality, for example protein or oil content of pods or

2 grains, can also be affected (see Fuhrer, 2009).

3   Furthermore, ground-level background ozone concentrations are still

4 increasing (by an average of 0.3 ppb per year), as a result of increased human activity,

5 and this is predicted to reduce global yields still further, particularly given that climate

6 change will exacerbate ozone formation in some regions (The Royal Society, 2008;

7 see Fuhrer, 2009). Avnery et al. (2011b) predict that global yield losses in the year

8 2030 will be worth up to $35 billion (USD), with losses of 4-26 % expected for

9 wheat, 9.5-19 % for soybean and 2.5-8 % for maize dependent on scenario used. The

10 frequency of episodes is also predicted to increase, and this may be especially severe

11 in Southern Asia and in Africa (The Royal Society, 2008). Not only does this threaten

12 agricultural sustainability in general, but our research shows that current global

13 models may have underestimated the potential impact of future ozone concentrations

14 on crop damage, because ambient ozone concentrations can prevent stomata of some

15 sensitive genotypes from closing as fully as usual in response to stresses such as

16 drought and high VPD (see below). This is relevant to the vulnerability of "local"

17 food supply in an unpredictable climate.

18   Here we describe how ozone is reducing our food supply, with emphasis on its

19 effects in a variable and changing environment.

20

21 <u>Mechanisms for ozone-induced yield reduction</u>

22

23 Figure 1 depicts ozone impacts on carbon gain and use affecting yield. It should be

24 noted that whilst some genotypes may be more vulnerable to one in particular of these

25 mechanisms, it is most likely that several of these interact to curtail productivity, and

1   that these interactions will be altered by the environment (e.g. Guidi et al., 2010;

2   Biswas and Jiang, 2011). It should also be noted that the effects of ozone described in

3   this section refer in the main to those known to occur in relatively unstressed plants,

4   however we describe, within a separate section below, the fact that ozone effects on

5   plants experiencing abiotic stress such as a lack of soil moisture can respond very

6   differently. Thus we include separate sections below describing ozone effects in

7   plants experiencing additional abiotic stress (ozone by environment interactions),

8   particularly in terms of plant signalling and stomatal behaviour, that may then impact

9   on our food supply.

10

11   *Visible injury*

12   Inside leaves, ozone interacts with the contents of the sub-stomatal apoplast and with

13   adjoining cell membranes and walls, to form reactive oxygen species (ROS) such as

14   hydrogen peroxide, superoxide, and hydroxyl radicals (reviewed in Fiscus et al.,

15   2005). This induces a chain reaction whereby further oxidative bursts occur within

16   adjoining cells. ROS that remain un-scavenged can cause a variety of foliar injury

17   symptoms such as inter-veinal necrosis and early senescence and abscission as

18   described above. Such symptoms have been confirmed on 27 crop species growing in

19   farmer's fields across Europe (Mills et al., 2011), and are caused by free-radical

20   induction of unregulated and/or programmed cell death. Evidence is growing that

21   these processes are in part mediated via the plant hormones ethylene, jasmonic acid

22   and salicylic acid (see Fiscus et al., 2005 and Kangasjärvi et al., 2005, for reviews).

23   Visible injuries are one way in which ozone can eventually reduce yield, even

24   if they only occur at early vegetative stages of crop growth. Leaf injury, senescence

25   and/or abscission directly reduce yields in leafy biomass crops such as alfalfa, lettuce,

1   spinach, chicory and cabbage. However in non-foliage crops visible injuries also

2   affect yield (e.g. of grain and pod crops) by reducing the amount of leaf area *per se*, or

3   the amount of healthy green leaf area available for carbon fixation for further biomass

4   growth and/or grain filling (e.g. Mulholland et al., 1998; McKee and Long, 2001; see

5   below*)*.

6

7   *Carbon acquisition, transport and use*

8   Many yield-depressive ozone effects on biomass growth and grain filling are only

9   measurable post harvest, as effects are often cumulative across the full growing

10   season (Wittig et al., 2007; 2009; Singh and Agrawal, 2010). Farmers may be

11   unaware that such crop losses are caused by ozone. Such reductions in biomass

12   growth have traditionally been linked to ozone-induced reductions in biochemical

13   photosynthetic carbon fixation processes (reviewed in Fiscus et al., 2005). Ozone-

14   induced stomatal closure has also been implicated (Torsethaugen et al., 1999;

15   McAinsh et al., 2002; Overmeyer et al., 2008) in reducing carbon availability.

16   Furthermore, logically, direct reductions of shoot and root cellular expansion via

17   ozone-induced ethylene generation can be implied (see Wilkinson & Davies, 2010;

18   and below).

19   Reductions in whole plant biomass induce yield reductions in grain and pod

20   crops by reducing the availability of leaf surface area to fix and provide carbon (C) for

21   reproductive parts (Figure 1). The Figure depicts the various different contributions to

22   the reduced availability of photosynthate for grains or pods, stemming from a)

23   reduced photosynthetic efficiencies, b) reduced stomatal conductance (gs), c) reduced

24   green foliage biomass (e.g. McKee & Long, 2001) as a result of reduced new growth,

25   shortening of the life-cycle, injury, senescence and abscission, d) reduced root

1 biomass, e) reduced partitioning of available C to grains/pods in favour of synthesis of

2 protective chemicals (Betzelberger et al., 2010; see below), or as a result of reduced

3 phloem translocation efficiency (e.g. Grantz, 2006; McKee & Long, 2001).

4 The ozone-induced reduction in root biomass (which indirectly reduces yield)

5 is understood to arise either from a reduction in C translocation from the shoot to the

6 root via the phloem (either due to reduced availability at source or as a result of the

7 blockage of phloem sieve plates with callose tissue – e.g. Wittig et al., 2009; Grantz,

8 2006; Asensi-Fabado et al., 2010); and/or from an effect of ozone on the

9 concentrations of the plant hormones such as ethylene that control root growth

10 (suggested in Wilkinson & Davies, 2010). Ozone-induced reductions in root biomass

11 impact indirectly on the shoot and therefore on grain/pod production via a reduction in

12 the ability of the plant to take up the nutrients and water required to sustain growth

13 and yield. In addition root biomass crops such as potato, onion and carrot are directly

14 vulnerable to reductions in root biomass (e.g. Asensi-Fabado et al., 2010).

15

16 *Effects on reproductive parts*

17 There are direct effects of ozone to reduce bud formation and flowering, to cause

18 pollen sterility, and to induce flower, ovule or grain injury and abortion which are

19 discussed in detail by Black et al. (2000; 2007) and Mulholland et al. (1998). Some of

20 these effects may potentially occur through plant hormones such as ethylene (see

21 below).

22

23 *Ozone and growth stage*

24 Some studies suggest that ozone exposure in the vegetative phase is most yield

25 depressive, whilst others describe that the reproductive stage is most vulnerable (e.g.

1 Heagle, 1989; Mulholland et al., 1998), with, for example, the sensitivity of seed crop

2 yield to ozone having been shown to be greatest during the period between flowering

3 and seed maturity in some studies (e.g. Lee et al., 1988; Pleijel et al., 1998). However,

4 Singh and Agrawal (2010) showed that the presence of ethylene diurea (EDU – a

5 compound that can be used to protect plants against ozone damage via an unknown

6 mechanism, that is used as an experimental tool) was necessary at all stages of wheat

7 crop production, in order to prevent ozone-induced yield penalties, implying that

8 ozone effects on yield were more cumulative. Vulnerability at a given key stage of

9 crop development, or vulnerability that arises more cumulatively, is likely to be

10 genotypically determined, and/or to be dependent on prevailing environmental

11 conditions.

12

13 The basis of genotypic sensitivity to ozone

14

15 Crop species differ widely in their susceptibility to ozone, and lists of

16 species/genotypes that fall into sensitive and resistant categories can be accessed

17 elsewhere (e.g. Heagle et al., 1989; Mills et al., 2007). There is a wide intra- as well

18 as inter-specific variation in plant sensitivity to ozone (e.g. Brosche et al., 2010;

19 Biswas *et al.,* 2008; Maggs and Ashmore, 1998) and recent studies have identified

20 some genetic loci associated with ozone resistance and/or susceptibility in wheat and

21 rice (e.g. Ainsworth et al., 2008; Frei et al., 2010), amongst other crops.

22   However it must be noted that the definition of crop "sensitivity" to ozone can

23 be rather imprecise. For example crops can be sensitive to ozone regarding the visible

24 injury symptoms seen on foliage at early growth stages, but, apart from in foliage

25 crops (such as leafy salad crops and alfalfa) this does not necessarily give rise to an

1 equivalent negative impact on grain/pod/fruit yield in fully developed crops.

2 Indeterminate species often possess sufficient compensatory flexibility to avoid

3 reductions in seed production in spite of ozone effects at the vegetative stage (e.g.

4 Black et al., 2007). Sawada and Kohno (2010) have shown in rice, and Picchi et al.

5 (2010) in wheat, that cultivars in which grain yields are most impacted by ozone are

6 those which showed the least visible injury symptoms to foliage. Picchi et al. (2010)

7 proposed that this may be related to genotypic variation in the extent of the stomatal

8 closure response to ozone. Cultivars where ozone closes stomata could be said to be

9 relatively ozone insensitive in reference to visible injury, as the ozone "dose" will be

10 reduced subsequent to this closure, preventing further foliar injury. However

11 prolonged stomatal closure reduces C fixation, and thereby the amount of assimilate

12 available for grains/pods/leaves, thus these cultivars may be more "sensitive" to ozone

13 in terms of yield.

14   Nevertheless there is scope for the impacts of increased ozone concentrations

15 on food security to be mitigated by choosing/developing genotypes with greater ozone

16 tolerance with respect to yield. Genotypic variability in sensitivity to ozone can arise

17 through several mechanisms:

18

19 *The extent of detoxification*

20 Some of the genotypic variability in ozone tolerance has been attributed to levels of

21 detoxifying antioxidants (ascorbic acid - AsA, glutathione, tocopherol, carotenoids,

22 flavonoids, and phenolics) and/or ROS scavengers (superoxide dismutase, catalase, or

23 peroxidases) present in and/or generated by certain genotypes in response to ozone

24 stress (Blokhina et al., 2003; Conklin and Barth, 2004; Eltayeb et al., 2007). Naturally

25 occurring genetic variation in ozone tolerance of visible leaf injury in rice was

1 dissected into two distinct quantitative trait loci (QTLs; Frei et al., 2008). These were

2 developed into two chromosome segment substitution lines (Frei et al., 2010).

3 Tolerance in one of the lines was related to lower expression of genes encoding

4 ascorbic oxidase, and AsA catabolism was proposed to be reduced such that this line

5 had higher concentrations of apoplastic AsA when exposed to ozone. Genes related to

6 ethylene and jasmonic acid metabolism were also differentially regulated between the

7 tolerant and sensitive lines.

8

9 *Changes in stomatal conductance*

10 Inherent rates of stomatal conductance (gs) have also been linked to variability of

11 susceptibility of wheat and rice varieties to ozone. In general, modern wheat cultivars

12 released in the last two decades are the most vulnerable, and these also display highest

13 gs (Biswas et al., 2008; but see Biswas and Jiang, 2011). It is thought that breeding

14 for high yield has a functional link to increased stomatal CO_2 influx. Unfortunately,

15 large stomatal apertures also allow larger ozone flux to the internal leaf parts (Brosche

16 et al., 2010). Evidence for this hypothesis is provided by some of our findings in

17 clover clones: Figure 2 shows that an ozone-sensitive clover accession (NC-S) has a

18 higher level of gs in drying soil than its ozone-resistant counterpart (NC-R; originally

19 selected by Heagle et al., 1994). Importantly, this difference is particularly dramatic in

20 the presence of ozone, and we propose that ozone may prevent full stomatal closure

21 under drought. Poor stomatal closure under drought implies both excessive water loss

22 and inwardly directed ozone flux, giving rise to poor plant performance (Wilkinson &

23 Davies, 2009; 2010; see below). Whilst clover is an important pasture crop, we expect

24 that this mechanism will also dictate some of the varietal sensitivity to ozone in

25 human staples. For example it was recently demonstrated that genotypic variation in

1   the sensitivity of stomatal aperture to external factors may be linked to yield tolerance

2   to ozone in wheat (Biswas and Jiang, 2011; see below).

3

4   *Changes in photosynthetic capacity*

5   Flowers et al. (2007 – snap bean), Biswas and Jiang (2011 – wheat), Betzelburger et

6   al. (2010 - soybean) and Pang et al. (2009 – rice) amongst others, have determined

7   that genetic variability in ozone susceptibility in terms of yield is related to direct

8   ozone effects on biochemical photosynthetic processes. However it is important to

9   note that genetic variability in photosynthetic capacity, will, to some extent, depend

10 on genetic variability in both gs-related parameters, and in the efficiency of ozone

11 detoxification, which both contribute to the final amount of ROS present at the

12 photosynthetic apparatus.

13 *The extent of ethylene production*

14 The production of the gaseous plant hormone ethylene from shoot tissues is frequently

15 observed in plants exposed to ozone pollution (see Kangasjärvi *et al.,* 2005;

16 Wilkinson & Davies, 2009; 2010), including many crop species (see Wilkinson &

17 Davies, 2009), and the extent of ozone-induced ethylene production has been shown

18 to be commensurate with visible leaf injury and abscission (e.g. Mehlhorn et al., 1991;

19 Tamaoki et al., 2003; Overmyer 2008). Recently ethylene accumulation has been

20 associated with stomatal closure *per se*, as well as being correlated with genetic

21 variability in the extent of ozone-induced stomatal closure (Kangasjärvi et al., 2005;

22 see Wilkinson & Davies, 2009; 2010). It has also been shown to be involved in ozone-

23 induced disruption of the plant's defence mechanisms against abiotic stresses such as

24 drought, by altering the sensitivity of stomata to a second plant hormone, abscisic acid

1   (ABA; Wilkinson & Davies, 2009; 2010; see below), and ozone-sensitivity in terms

2   of leaf injury is greater in ethylene over-producers (see Kangasjärvi *et al.,* 2005), than

3   in ethylene-insensitive *Arabidopsis* mutants (Overmyer et al., 2008). We predict that

4   genotypic variability in ethylene production of human staples will also determine

5   yield responses to ozone, because a) there is much data demonstrating variable

6   ethylene generation by crop plants in response to ozone (see above), and b) because

7   outside of ozone biology, ethylene is also known to play a role in leaf and root

8   growth, in carbon partitioning to grains, and in grain abortion (see below).

9

10   Ozone by environment interactions

11

12   There is a great deal of variability in injury, yield, growth and stomatal responses to

13   ozone (e.g. Wittig et al., 2007; 2009), some as a result of inherent genetic variability

14   as described above, and/or some that arises as a result of variable growth conditions.

15   Interactions between ozone stress and atmospheric CO_2 concentrations have been

16   reviewed elsewhere (e.g. Long et al., 2005). Here we highlight the fact that ozone

17   episodes frequently co-occur with climatic conditions that also induce soil drying.

18   It is widely understood that drought-induced stomatal closure will limit ozone

19   uptake, thereby mitigating ozone-induced crop yield losses (e.g. Fuhrer, 2009;

20   Fagnano et al., 2009). However several studies have established that drought is not

21   always protective in the presence of ozone concentrations in the ambient range (see

22   Wilkinson & Davies, 2009; 2010; Biswas and Jiang, 2011), and we show here that

23   some genetic variability in ozone sensitivity may be related to the extent to which

24   ozone reduces the sensitivity of stomatal closure to soil drying (Fig 2). Paoletti and

25   Grulke (2010) also describe that stomata open and close less sensitively in response to

1 changes in light intensity, and Biswas and Jiang (2011) very recently demonstrated

2 that faster stomatal control in response to changing light intensity occurred in ozone-

3 stressed plants of a more ozone-tolerant primitive tetraploid wheat species than in

4 those of a less tolerant recently released genotype of hexaploid wheat. However,

5 stomatal control became slower in the tetraploid species when ozone stress was

6 combined with drought, and the primitive species lost ozone tolerance.

7 We have shown that in drying soil, or when leaves are detached from the

8 plant and allowed to dry out, stomata of some species close much less sensitively in

9 ozone-polluted air (Mills *et al*., 2009; Wilkinson & Davies, 2009; 2010; Figs 2-3).

10 This effect will reduce the protective effect of drought-induced stomatal closure on

11 the ozone dose that these plants receive, and on the regulation of plant water loss (e.g.

12 McLaughlin et al., 2007), and therefore increase vulnerability to the drought episode

13 (particularly when combined with a reduced root biomass – Wittig *et al*., 2009), with

14 secondary impacts on leaf water potential and xylem cavitation likely. We predict that

15 this will eventually cause secondary reductions in growth and yield, and/or increased

16 injury, abscission, senescence and death (Wilkinson & Davies, 2009; 2010),

17 particularly if the vulnerable plants begin to experience additional/subsequent stresses

18 such as wind, biotic attack, high light/VPD or flood/storm conditions. Our recent data

19 (in preparation) describe a growing number of species, including some crops (notably

20 *Phaseolus vulgaris*), that exhibit ozone-induced reductions in the stomatal closing

21 response either in the presence or absence of soil drying. We describe below the

22 potential mechanism behind these effects, and behind the linked ozone effect whereby

23 stomata also close less sensitively in response to other stresses such as high salinity,

24 light and VPD (e.g. Robinson et al., 1998; Paoletti and Grulke, 2010; Biswas and

1 Jiang, 2011). We speculate on how genetically widespread these effects might be, thus

2 exploring their consequences for food security.

3

4 <u>Ozone-induced disruption of plant signalling.</u>

5

6 The concentration of ABA in plant tissues usually increases linearly with soil drying.

7 Generally stomata close and leaves grow more slowly in response to ABA sent

8 upwards from the roots, reducing the loss of valuable water to the air (for reviews see

9 Davies and Zhang, 1991; Wilkinson and Davies, 2002; Tardieu et al., 2010). ABA

10 also moderates stomatal aperture and leaf growth in response to other stresses such as

11 high VPD, high salinity and temperature extremes. Signals from the aerial

12 environment (e.g. VPD, light, CO_2, temperature and air-borne pollutants) can directly

13 affect concentrations of signalling molecules such as ABA. Ozone has been shown to

14 increase ABA concentrations in some genotypes, but to decrease it or have no effect

15 on it in others (see Wilkinson & Davies, 2009). The sensitivity of guard cells, leaf

16 cells and/or cells of reproductive organs to a given concentration of ABA can also be

17 altered by the aerial environment. In some genotypes ozone increases plant calcium

18 concentrations and this is associated with ozone-induced stomatal closure and reduced

19 C fixation, possibly by increasing stomatal sensitivity to ABA (McAinsh et al., 2002).

20 We proposed that the implications of a recently defined effect of ozone to reduce the

21 responsiveness of stomata to ABA signalling (Wilkinson & Davies, 2009; 2010)

22 could have as yet unexplored and serious consequences for food security, via the

23 effect of ozone to increase gaseous ethylene production.

24   It has long been known that ozone increases the generation of ethylene from

25 the leaves of sensitive plants (see above). Outside of ozone science, ethylene has

1   traditionally been regarded as a shoot growth inhibiter and a promoter of ripening,

2   senescence and abscission (Abeles et al., 1992; Morgan and Drew, 1998). However,

3   evidence has also recently been emerging for a role for ethylene in stomatal

4   movements in the unpolluted plant (Desikan et al., 2006; Tanaka et al., 2007). This

5   directed our investigations to show (Wilkinson & Davies, 2009) that an ozone-

6   induced up-regulation of ethylene was responsible for the observed ozone-induced

7   reduction in stomatal sensitivity to ABA, drought and other closing stimuli described

8   above (Figs 2-3). A "cartoon" of the effect of ozone to disrupt the stomatal response

9   to ABA via ethylene is shown in Figure 4. We propose that this may occur in response

10   to any stress that produces ABA (or increases its access to xylem transport pathways)

11   such as high VPD/temperature, high salinity, high light stress or nutrient deficiency

12   (Rossel et al., 2006; Wilkinson & Davies, 2002; Wilkinson & Hartung, 2009).

13   We have predicted (Wilkinson & Davies, 2009; 2010; Wilkinson & Hartung,

14   2009) that many crop plants (in addition to *Phaseolus vulgaris*) will be susceptible to

15   ozone via disruption of the stomatal signalling mechanism in this way, by searching

16   the literature for those in which ozone is known to up-regulate ethylene. These

17   include varieties of pea and pinto bean (Mehlhorn et al., 1991), potato (Sinn et al.,

18   2004), tomato (Bae et al., 1996), snap bean (Elagoz & Manning, 2005) and some

19   wheat and rice cultivars (e.g. Tiwari et al., 2005); and for those in which stomatal

20   responses to the environment are known to be "sluggish", e.g. snap bean (Paoletti and

21   Grulke, 2010). Recently Biswas and Jiang (2011) showed differential stomatal control

22   in wheat species under combined ozone and drought stress, although it was difficult to

23   directly implicate this effect in yield differences.

24   In addition to its effects on senescence, abscission and stomatal closure, it has

25   long been known that ethylene can directly reduce shoot growth and root growth in

16

1   non-polluted plants (Abeles et al., 1992; Morgan & Drew, 1997; Wilkinson & Davies,

2   2010; Pierik et al., 2007). We have proposed (Wilkinson & Davies, 2010) that ozone

3   also reduces leaf and root biomass via the up-regulation of ethylene production in

4   ozone sensitive species, in addition to or instead of the general reduction in carbon

5   fixation which has traditionally been assumed to be the basis for ozone-induced

6   reductions in plant biomass growth. It is likely that ethylene also has a more direct

7   impact on grain yield in response to ozone stress. A clear link between ethylene and

8   yield susceptibility to heat stress has been demonstrated in certain wheat genotype

9   classes (Hays et al., 2007). Yang et al. (2006) also demonstrated that higher

10   ABA/ethylene ratios were associated with superior grain quality and higher grain

11   filling rates (Figure 5). The importance of the biology of stress ethylene in the

12   regulation of grain filling and yield in rice is already recognized (e.g. Naik and

13   Mohapatra, 2000). It seems reasonable to assume that ozone-induced ethylene will

14   have similarly direct detrimental effects on grain yields and quality. This remains to

15   be tested and will generate valuable knowledge related to mitigating ozone effects on

16   yield.

17

18   *Implications for food security*

19

20   There is clear evidence that the effects of ozone pollution described here are already

21   occurring in many areas of the world (e.g. Europe - Mills et al., 2011 ; SE Asia -

22   Emberson et al., 2009). Furthering our understanding of the mechanisms by which

23   ozone impacts on crop species, including how ozone interferes with hormonal

24   signalling in response to a variable environment, may help us to develop new ways of

25   protecting food crops from the current and increasing threat from ozone. Since the

highest ozone concentrations are predicted to occur in those areas of the world where the population is increasing most rapidly (The Royal Society, 2008), and where water is likely to be most scarce (Bates et al., 2008), there are urgent needs for global cooperation in reducing ozone precursor emissions, development of management methods for protecting crops from ozone in the field under variable environments, and selecting and breeding for new ozone-tolerant varieties of our most important staple food crops.

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4

5   **Figure Legends**

6

7   **Figure 1**. Effects of ozone on carbon gain and carbon use that impact on crop yield.

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9   **Figure 2**. Stomatal conductance (gs) in sensitive (NC-S) and resistant (NC-R) clover

10   accessions in drying soil (50 % full capacity, assessed via daily weighing of evapo-

11   transpirational water loss) in the presence (70 ppb) and absence (15 ppb) of elevated

12   ozone concentrations; temperature maintained at 21-23°C; photon flux density 6-700

13   μmol m^{-2} s^{-1} during a 14h photoperiod. Ozone exposure was carried out in 1.0 m^{3}

14   boxes inside a greenhouse. Measurements were initiated 10 days after transfer to

15   ozone treatments, plants purchased as root plugs and allowed to re-grow in a

16   greenhouse for 2 weeks prior to ozone treatment initiation. Gs was measured weekly

17   at 10.00 h using a porometer (AP4, Delta-T Devices, Cambridge, UK); n=12-16, two

18   gs measurements for each of 6-8 plants; SE and significant differences (*$P < 0.05$,

19   **$P < 0.01$, Student's *t*-test).) between NC-R and NC-S are shown.
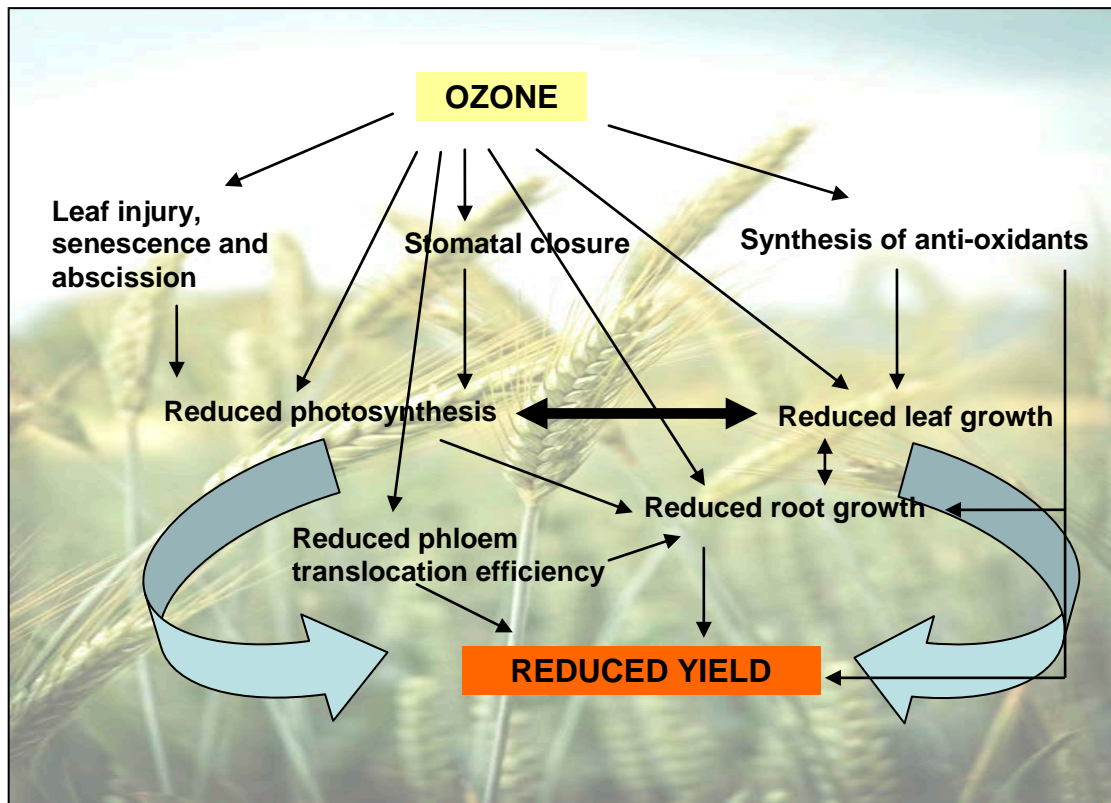
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21   **Figure 3**. Effect of ozone concentration (ctrl = 15 ppb; + ozone = 70 ppb) on the

22   response of the pasture forb *Leontodon hispidus* stomatal conductance (gs) to soil

23   drying. Re-calculated from Wilkinson & Davies (2009; *Plant, Cell & Environment*

24   32, 949-959). Significant differences (*$P < 0.05$, **$P < 0.01$, Student's *t*-test) between

25   well-watered (WW) and droughted (DD) treatments are shown.

1

Figure 4. Model describing effects of ozone-induced ethylene production on ABA-induced stomatal closure. Under stresses such as soil drying (and high PFD, high VPD) ABA is up-regulated, and it closes stomata so that leaves retain water. However in some sensitive genotypes ozone-induced ethylene causes stomatal guard cells to lose their sensitivity to ABA such that stomatal pores remain more open. Plants will take up more ozone and lose more water than they would have done in the absence of ozone.

Figure 5. Relationships between grain-filling rate and concentrations of ABA (a), ethylene (b), and the ratio of ABA to ACC (c) in superior (closed symbols) and inferior (open symbols) grains of wheat (cvs Yangmai 6 and Yangmai 11) during linear grain growth. Treatments were well-watered (WW), moderately soil-dried (MD) and severely soil-dried (SD). Reprinted with kind permission from *New Phytologist*. From Yang et al., 2006, *New Phytologist* **171**, 293-303.
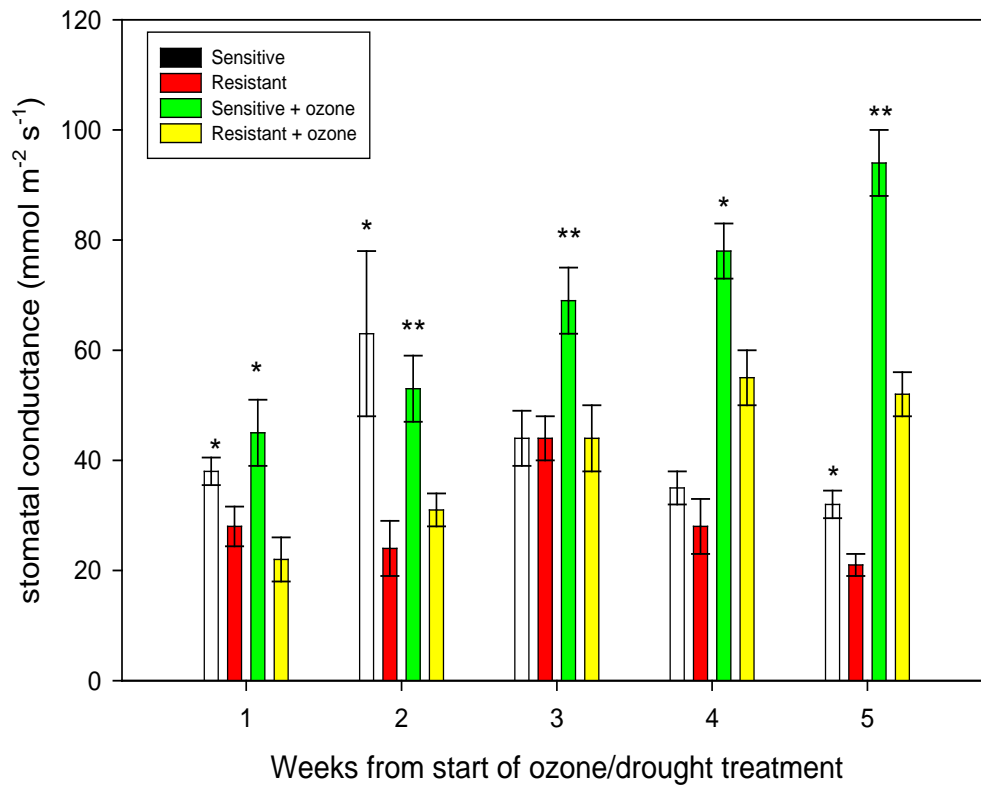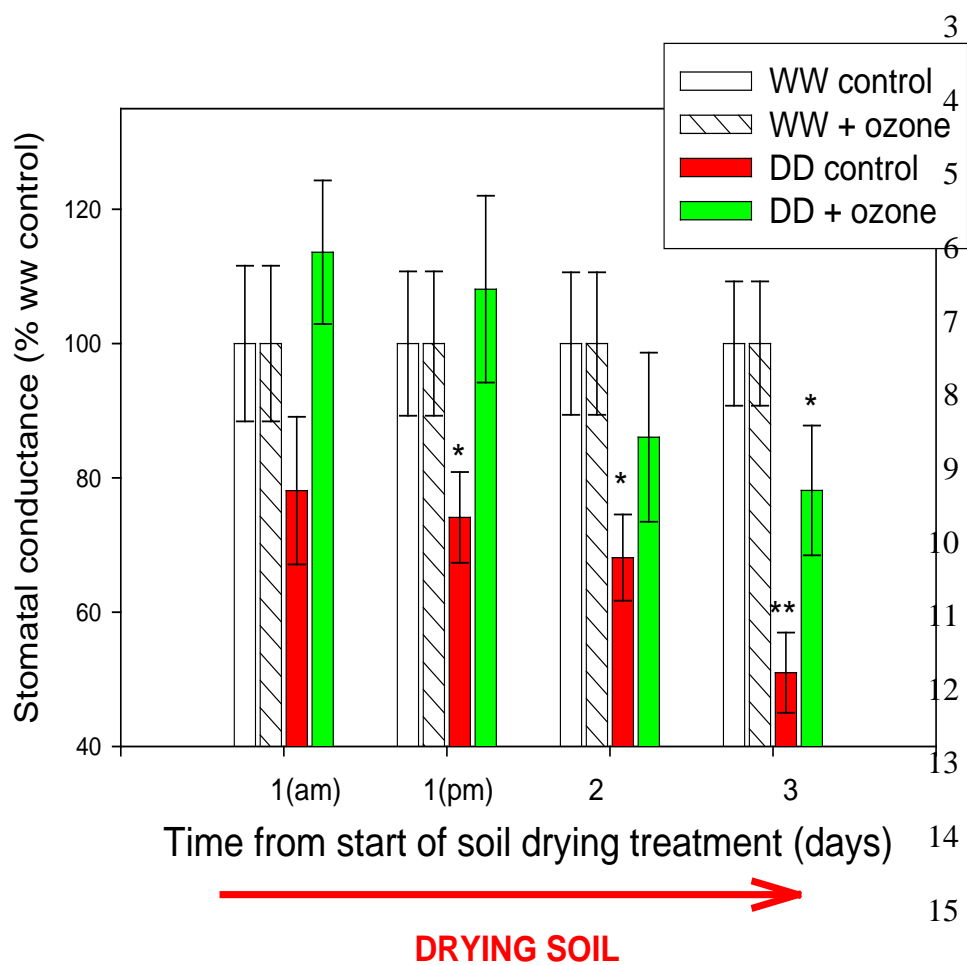
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4   Figure 1

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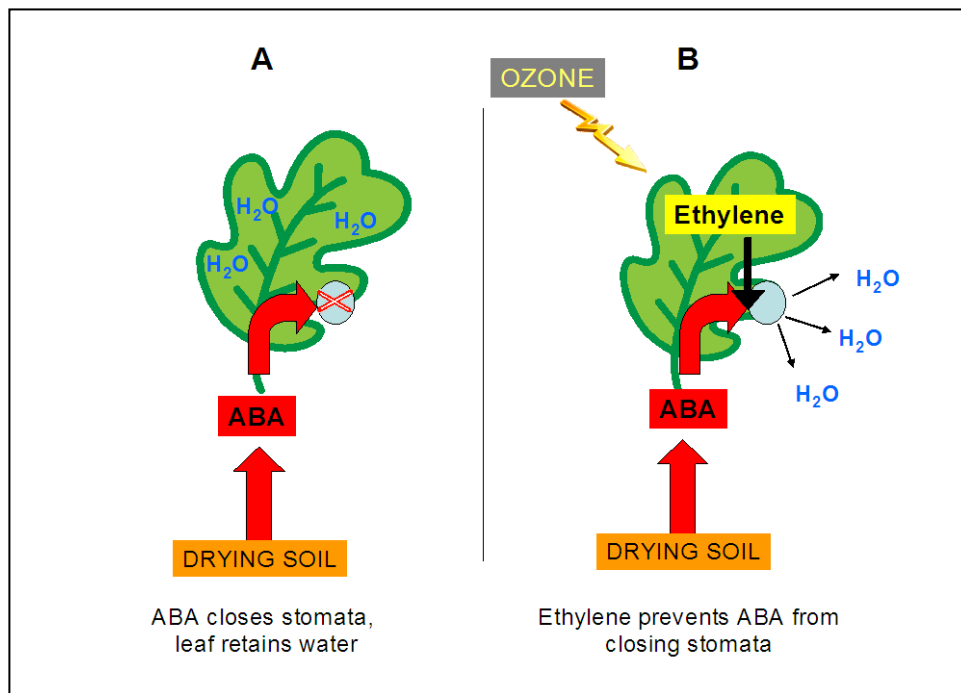
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Stomatal conductance (% ww control)

1(am)   1(pm)   2   3

Time from start of soil drying treatment (days)

DRYING SOIL

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23   Figure 5