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Tropospheric ozone pollution reduces the yield of African crops

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Running Title: Ozone reduces the yield of African crops

Abstract

Northern, Southern and Equatorial Africa have been identified as among the regions most at risk from very high ozone concentrations. Whereas we know that many crop cultivars from Europe, north America and Asia are sensitive to ozone, almost nothing is known about the sensitivity of staple food crops in Africa to the pollutant. In this study cultivars of the African staple food crops *Triticum aestivum* (wheat), *Eleusine coracana* (finger millet), *Pennisetum glaucum* (pearl millet) and *Phaseolus vulgaris* (bean) were exposed to an episodic ozone regime in solardomes in order to assess whether African crops are sensitive to ozone pollution. Extensive visible leaf-injury due to ozone was shown for many cultivars, indicating high sensitivity to ozone. Reductions in total yield and 1000-grain weight were found for *T. aestivum* and *P. vulgaris*, whereas there was no effect on yield for *E. coracana* and *P. glaucum.* There were differences in sensitivity to ozone for different cultivars of an individual crop, indicating that there could be possibilities for either cultivar selection or selective crop breeding to reduce sensitivity of these crops to ozone.

Keywords

Millet, wheat, bean, 1000-grain weight, visible leaf-injury, photosynthesis

1 Introduction

1.1 Tropospheric ozone as a global pollutant

Ozone is a major air pollutant at ground-level and is formed when precursor molecules including NO_x and VOC's (volatile organic compounds) react in the presence of sunlight. Tropospheric ozone concentrations vary spatially and tend to be highest in parts of the USA, southern Europe, southern Asia and equatorial Africa (Cooper et al., 2014; Dentener et al., 2006; Stevenson et al., 2006). Trends in tropospheric ozone concentrations vary geographically. Although there is very little measured data on ozone concentration trends in Africa, ozone concentrations in many regions of Asia have been increasing over recent years. For example, most of the regions of India have been increasing over the period 2005-2010 and this is consistent with reported trends in coal and petroleum consumption (Lal et al., 2012). Ozone concentrations have also been increasing over the last 10 years in South Korea (Shin et al., 2017) and in many regions of China, including the Yangtze River Delta (Wang et al., 2018) and are projected to increase further in many regions of Asia (Ma et al., 2016, Sun et al., 2016, Xu et al., 2016).

In Kenya and other countries of Africa, coal and petroleum consumption are also increasing rapidly, due in part to increased vehicle use, and ozone precursor emissions are anticipated to increase further due to NO release from soils as additional N is added to increase crop yield (Hickman et al., 2017). Northern and Southern Africa have been identified as among the regions most at risk from very high ozone concentrations under a 'policy fail' SRES A2-type emissions scenario (Royal Society, 2008; Laban et al.,

2018) unless measures are taken to reduce precursor emissions (Wild et al., 2012). One of the sources of precursors is biomass burning, and regions of tropical biomass burning have elevated ozone compared to the rest of the tropics (Anderson et al., 2016). Evidence from India has shown that air masses originating from areas of crop residue burning on the Indo-Gangentic Plain can enhance ozone concentrations downwind by up to 32 ppb (Kumari et al., 2018). Similarly, the ozone concentrations in Rwanda during the dry season are increased by transport of precursors during biomass burning in northern and southern Africa (DeWitt et al., 2019). Ozone concentrations are projected to rise further in many developing regions, including Africa (Huang et al., 2018). In addition to anticipated changes in precursor emissions, African regions are also predicted to have an increase in surface mean ozone concentrations due to projected changes in climate (Racherla and Adams, 2006).

Although other air pollutants are also present in tropical regions, including fine particles $PM_{2.5}$ (Brauer et al., 2012), ozone concentrations are increasing rapidly and there is less potential to control concentrations locally than for some other pollutants such as $PM_{2.5}$ due to the hemispherical transport of ozone precursors. Although there is some sporadic monitoring of air pollutants including ozone in some parts of Africa, particularly in south Africa (Laban et al., 2018), there is currently very little monitoring of ambient ozone concentrations across most of Africa (Schultz et al., 2017) and where data is available time series are often short. Reported values often tend to be as 24 hour mean values, which may not be representative of daytime mean concentrations when plants are active, and these daytime concentrations would be expected to be much higher than those during the night due to the diurnal profile of ozone concentrations observed in urban areas e.g. Hanoi (Sakamoto et al., 2018).

Values presented may also be from urban monitoring stations (e.g. from Nairobi city, Kimayu et al., 2017), where concentrations would be anticipated to be lower than for rural regions due to scavenging in the urban environment by titration with NO. Similarly, in China it has been shown that a rural area 100km downwind of Beijing had ozone concentrations much higher than in the city, with mean values of 58 ppb in the rural area compared to 36 ppb in Beijing city (Xu et al., 2011), and a maximum value of 198 ppb. Thus, air pollution is a relevant problem for rural and agricultural areas in developing countries in addition to the more well-known air pollution problems within cities.

1.2 Differential sensitivity of plants to ozone pollution

Ozone enters plants through stomatal pores on the leaf surfaces and once inside the leaf can react with plant surfaces to form reactive oxygen species (Wilkinson et al., 2012). These can damage cell membranes and structures within the apoplast and cause a cascade of biochemical reactions that can damage photosynthetic apparatus (Caregnato et al., 2013) and ultimately lead to cell death and promote premature leaf senescence (Fiscus et al., 2005; Schraudner et al., 1997). Some crops and cultivars are much more sensitive to ozone than others (Mills et al., 2007). Reasons for differential sensitivity include the ability to exclude ozone by stomatal regulation (Hoshika et al., 2013; Salvatori et al., 2013), the rate of induction of detoxification of reactive oxygen species (ROS) to protect photosynthetic appparatus (Di Baccio et al., 2008) and the plasticity of resource partitioning to replace damaged leaves (Grantz et al., 2006). However, we do not yet know about the sensitivity of African crops.

1.3 Impact of ozone on crop yields

Food supply for a rising global population is one of the priorities for the 21st century, with agricultural productivity being one of the contributing factors (e.g United Nations Sustainable Development Goal 2, UN General Assembly, 2015). Many crops have been shown to be sensitive to ozone pollution, including staple foods such as rice (Akhtar et al., 2010), wheat (Wahid, 2006) and soybean (Betzelberger et al., 2012). Analysis of compiled datasets from many experiments has shown a wide range in ozone-sensitivity between different crops, based on ozone concentration (Mills et al., 2007, 2018a; Mills and Harmens, 2011). Model-based studies using dose-response functions from such datasets have indicated potential crop yield reductions due to ozone across wide regions of the world (Mills et al, 2018a,b,c; Van Dingenen et al., 2009, Avnery et al., 2011). Experimental investigations have shown impacts of ambient ozone concentrations on a wide range of crop species in Europe and the USA by comparing responses of plants in filtered air to those in non-filtered air (Pleijel et al, 2018; Marzuoli et al., 2017; De Temmerman et al, 2007). However, the majority of current information on crop sensitivity to ozone is based on studies from Europe and the USA, with some additional more recent studies in India and China (Tomer et al., 2015; Chen et al, 2008; Singh and Agrawal, 2017; Feng et al., 2017; Feng et al., 2015). There is very little information on responses to ozone of tropical crop species, particularly those relied on by subsistence farmers, such as pearl and finger millet. Sub-Saharan Africa continues to have the highest proportion of individuals that are undernourished in terms of food quantity and nutrition and no information currently exists on the sensitivity to ozone for some of the Sub-Saharan African major crop species.

1.4 Inter-continental variations in ozone sensitivity

It has been shown that ozone sensitivity of soybean cultivars has increased progressively over time, and it was suggested that this was due to selective breeding strategies for increased stomatal conductance and yield, which may have inadvertently selected for greater ozone sensitivity (Osborne et al., 2016). Similarly for wheat, it has been shown that the stomatal conductance of cultivars has increased over time, and this also correlates to an increase in sensitivity to ozone (Biswas et al., 2008), although in the case of wheat this conclusion has largely been based on comparisons between old and modern cultivars (e.g. Pleijel et al., 2006). However, there is also some evidence of different sensitivity of cultivars of a single crop type used in different continents, which might be due to differing selection criteria in different locations, perhaps due to requirements for suitability in a particular climate. Soybean cultivars from India and China were more sensitive to ozone than those from Europe and the USA (Osborne et al, 2016). A greater sensitivity of Asian cultivars of wheat and rice compared to USA cultivars has also been shown (Emberson et al., 2009). This may be due to differences in breeding strategies between different regions, although it was recognised that other factors such as experimental methodology and co-occurring pollutants may also be part of the explanation, in addition to differential cultivar sensitivity.

1.5 Aims and rationale for selection of crops and cultivars

In Africa, the staple foods vary by region and include wheat and millet, which had a total African production of 23 065 000 Tonnes and 13 642 000 Tonnes respectively in 2016 (FAOstat). Dry beans (*Phaseolus vulgaris*) are mainly grown for subsistence and provide a major component of dietary protein (FAO.org; Broughton et al., 2003), with an African production of 6 789 000 Tonnes in 2016 (FAOstat). There are over 40 000 varieties of bean, including indigenous, unimproved landraces and improved modern cultivars (Graham and Ranalli, 1997).

The aim of this study was to evaluate ozone sensitivity of a range of food crops grown in tropical regions in Africa. This information will be used to indicate whether ozone is likely to be a problem for crops in sub-Saharan Africa and similar regions. Several varietes of each crop were tested to determine whether there is scope for selecting cultivars with reduced ozone sensitivity, thereby preventing or limiting yield losses due to the pollutant.

2 Methods

2.1 Seeds and planting

Triticum aestivum (wheat) seeds of the Kenyan cultivars 'Kenya Korongo', 'Kenya Wren', 'Kenya Hawk 12', 'Eagle 10', 'Njoro BWII' were obtained from the Kenya Agriculture and Livestock Research Organistion (KALRO; Njoro-Kenya). *Triticum aestivum* seeds of the UK cultivar 'Skyfall' were obtained from RAGT Seeds (UK) to compare the sensitivity of the Kenyan cultivars with an ozone-sensitive cultivar of the UK (Harmens et al., 2018). *Eleusine coracana* (finger millet) of cultivars GuluE, P224, KNE624, KNE814, U15, Okhale, and *Pennisetum glaucum* (pearl millet) of cultivars Okashana, Shibe, ICMV221, KATPM1 were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT-Nairobi, Kenya). *Phaseolus vulgaris* (bean) of the widely grown cultivars 'Black Turtle', 'Pinto' 'Orca' (syn 'Ying Yang') and 'Cannellini' were obtained from Jungle Seeds Ltd, UK.

Cereal crops were planted in 6.5 l pots (diameter 21 cm, height 25 cm), and beans were planted in 7.5 l pots (diameter 26 cm, height 21 cm). All pots were filled with John Innes No 3 soil based compost (J. Arthur Bowers, UK), which contains loam soil and added nutrients, to prevent possible confounding impacts of growing in sub-optimal soil conditions. Seeds were sown on 28th April 2017 and seedlings were thinned to 9 per pot (*T. aestivum*), 4 per pot (*E. coracana*), 2 per pot (*P. glaucum*) and 1 per pot (*P. vulgaris*). Four replicate pots were used per cultivar per ozone treatment, and all plants were in the vegetative stage when ozone exposure started. Due to the differences in required growing conditions, *T. aestivum* was grown and exposed to ozone in unheated solardomes, whereas for the other crops, heated solardomes were used with an increase in temperature of approximately 7 ˚C above ambient (discussed in section 2.2). A summary of key dates and details of treatments is shown in Table 1. Note that the ozone exposure time was much shorter for *P. glaucum* than for the other crops, as this grew too tall to fit in the solardomes. All plants were kept well-watered for the duration of the experiment.

2.2 Ozone system

Plants were exposed to ozone in solardomes – hemispherical glasshouses (3m diameter, 2.1m high) at Abergwyngregyn, near Bangor, North Wales, UK. The solardomes were ventilated at a rate of two air changes per minute. Ozone was generated using an ozone generator (G11, Ozone Industries Ltd), supplied by an oxygen concentrator (Sequal 10, Pure O2). Charcoal filtered air was injected with ozone to give required concentrations using solenoid valves operating using pulse-width modulation. Concentrations were computer controlled using LabView (Version 2012, National Instruments, Texas, USA) and followed an episodic ozone regime (with five consecutive ‘high’ days in every 7-day week), to represent a profile that might be experienced in agricultural areas of Sub-Saharan Africa, based on profiles that were experienced in Mediterranean Europe in the 1990’s and 2000’s (Figure 1). The ozone concentration inside each solardome was measured for 5 minutes every 30 minutes using two ozone analysers (Envirotech, UK; 49i Thermo Scientific, UK) of matched calibration.

Heating was achieved using air conditioning units (Toshiba Super Digital Inverter, Toshiba-Aircon, UK) that were electronically controlled (Easy I/O, The Netherlands). The temperature regime used was to represent that of African countries such as Kenya, which has daily mean temperature between 20-28˚C. Crops such as millets and beans tend to be grown in the warmer regions and wheat tends to be grown in higher altitude, cooler regions. In one ambient temperature solardome and three heated solardomes, temperature and relative humidity were continuously measured (Skye Instruments, UK) and in one ambient temperature solardome Photosynthetically Active Radiation (PAR) was also continuously monitored (Skye Instruments, UK). Climatic conditions and airflow rates were matched between solardomes, however, to minimise any chamber effects all plants and ozone treatments were moved between solardomes every four weeks. Ozone concentrations and climatic conditions of the treatments are shown in Table 2, and further details of ozone exposure to individual cultivars is given in the Supplementary Information.

2.3 Leaf injury assessments

Visible leaf injury assessments of all plants was carried out after 2 weeks by counting all leaves per plant and catergorising each leaf as ‘healthy’, ‘mild’ ozone injury (<5%

of the leaf affected, based on visual assessment), ‘moderate’ ozone injury (5%-25% of the leaf affected) or ‘severe’ ozone injury (>25% of the leaf affected).

2.4 Plant physiological measurements

Light-saturated rate of net photosynthesis (A_{sat}) and stomatal conductance for water vapour (g_s) were measured simultaneously using a portable infrared gas analyser (LI-6400XT, LI-COR, Nebraska, USA). Measurements for *T. aestivum* were made on the flag leaf. Measurements for *P. glaucum* and *E. coracana* were made on typical leaves from the upper canopy. For *P. vulgaris* in the medium and high ozone treatments, many leaves initially showed symptoms of ozone damage, then quickly senesced. Therefore, for *P. vulgaris* measurements were made on healthy leaves of the upper canopy. All measurements were between the times of 10:00 and 16:30 and used a LED light attached to the leaf cuvette, set at photosynthetic photon flux density of 1500 µmol m^{-2} s^{-1}. The CO_2 concentration of the air entering the leaf cuvette was set at 400 ppm, and the leaf temperature was regulated at 20˚C for *T. aestivum* and 35 ˚C for *P. glaucum, E. coracana* and *P. vulgaris,* to reflect the temperature within the solardomes on the measurement days for these plants. Dates of these measurements are indicated in Table 1, with typically 32 measurements in total per day.

Additional stomatal conductance measurements were made to establish maximal stomatal conductance, g_{max}, for each species using a porometer (AP4, Delta-T Devices, Cambridge, UK). A minimum of 100 measurements were made per variety, and g_{max} was determined as the 95th centile of the measured values. Stomatal conductance for ozone was calculated using the conversion factor of 0.663 to account for the difference in the molecular diffusivity in air of water vapour to that of ozone

(Massman, 1998). This was then expressed in terms of projected leaf area (PLA) using measured proportion of stomatal conductance from the abaxial surface as 0.8, 0.5, 0.7 and 0.5 for *T. aestivum, E. coracana, P. glaucum* and *P. vulgaris* respectively. For all measured leaves, chlorophyll content was determined using a hand-held chlorophyll meter (CCM200plus; Opti-Sciences, Hudson, USA) recording Chlorophyll Content Index (CCI).

2.5 Plant harvest

Plants were harvested when seeds were ready. Grains were extracted from *T. aestivum* ears using a hand-thresher (Minibatt+, Reichhardt Electronic Innovations, Germany). Grains were extracted by hand for the other crops, although this was only partially successful for *E. coracana* and *P. glaucum*.

2.6 Statistical methodology

2.6.1 Leaf Injury

To investigate if the number of leaves per injury category varied with ozone treatment, a multinomial logistic regression model was run using the statistical program R (R Core Team 2016), with the 'multinom' function from the nnet package (Venables & Ripley 2002; for further detail see Supplementary Information). A categorical response variable was created from the injury counts, which were performed after two weeks of ozone exposure. The four categories were 1) Number of leaves with no injury; 2) Number of leaves with mild injury; 3) Number of leaves with moderate injury; 4) Number of leaves with severe injury. Predictor variables in the model were ozone treatment (low, medium, high), and crop cultivar. The optimal model was chosen using top down selection with examination of Akaike Information Criterion (AIC) values, following Zuur et al. (2009). This process was repeated using the data for each crop type. Predicted probabilities of the different possible outcomes of the injury counts were plotted for each crop type. Post-hoc testing was carried out by comparing the simulated predicted probabilities per cultivar for a chosen level of damage using paired t-tests.

2.6.2 Relationship between A_{sat}, g_s and chlorophyll content index (CCI)

Linear mixed-effects models (normal error) were used to investigate the relationships between A_{sat}, g_s and CCI, including a random effect of Pot ID and the categorical predictor 'Week' to control for any effects of taking repeat measurements in three different weeks for each crop species. Firstly, the effect of CCI and ozone on A_{sat} was investigated for each crop, with models including CCI and ozone (plus their interaction) as predictor variables. For the *T. aestivum* model, CCI was found to decrease with higher ozone ($p < 0.001$), therefore both predictors were not included together in the A_{sat} model. For the *P. vulgaris* and *P. glaucum* models, CCI was log transformed as A_{sat} values increased then began to level off with higher values of CCI. Marginal R^2 (R^2M; proportion of variance explained by the fixed effects) was used to report model fit. The effect of ozone on g_s was then investigated for each crop, including an interaction with 'Week' in the model set. Lastly, the effect of g_s on A_{sat} was investigated for each crop. For crops showing no effect of ozone on g_s, models included both ozone and g_s (plus their interaction) as predictor variables.

2.6.3 Yield and 1000-grain/seed weight

To investigate the effect of ozone on the total yield and 1000-grain weight, linear models (normal error) were used, including ozone, crop cultivar and their interaction as categorical predictors in the model. For *P. vulgaris*, an additional covariate of pot number was included to control for any initial variations in plant size (pots per treatment were labelled 1-4 based on their starting size). Linear models (with normal error, after reviewing distribution of model residuals) were also used to determine if ozone had an effect on the number of ears per pot and the number of grains per ear for *T. aestivum*. Post-hoc testing was carried out using Tukey's range test

All models were run using R and residuals were examined for normality and even spread. Data transformations were carried out if necessary. For each model set, top down model selection was carried out, following Zuur et al., (2009). For the mixed-effects models (package lme4; Bates et al., 2015), p-values were obtained using Likelihood Ratio Tests (LRT) with the drop command, and model fitted values were obtained using the 'predict' function. The R package 'MuMIn' was used to calculate Marginal R^2 for the optimal mixed models (Barton, 2018).

3 Results

3.1 Visible injury

All crops and cultivars tested showed visible leaf injury after two weeks of ozone exposure (Figure 2 and S1). For *T. aestivum*, all cultivars showed similar responses within each level of ozone, with the probability level of visible injury being present increasing with increasing ozone (no interaction between ozone and cultivar). At low ozone, very little visible injury was recorded on *T. aestivum* leaves. *T. aestivum* cultivars showed a difference in sensitivity to ozone. At high ozone, sensitivity was of the order 'Skyfall' > 'Korongo' > 'Wren' > 'Njoro' > 'Hawk' > 'Eagle' for moderate injury. Post-hoc testing for high ozone showed a significant difference between the predicted probabilities of moderate injury for each cultivar. The two most sensitive cultivars ('Skyfall' and 'Korongo') also showed the highest probability of severe injury on leaves at high ozone.

The *E. coracana* cultivars behaved differently depending on the ozone treatment (the optimal model contained an interaction between ozone and cultivar). There was no visible injury present in the low ozone treatment. At medium ozone, there were clear differences between cultivars.'GuluE' showed very low ozone sensitivity, while 'P224' and 'U15' were the most sensitive cultivars. At high ozone, all cultivars behaved similarly and all showed severe injury (although 'GuluE' was still showing a higher predicted proportion of mild injury than the other cultivars). At high ozone, sensitivity was of the order 'P224' > 'U15' > 'KNE814' > 'Okhale' > 'KNE624' > 'GuluE' for severe injury. Post-hoc testing for high ozone showed a significant difference between the predicted probabilities of severe injury for each cultivar.

In terms of visible injury, *P. glaucum* was less sensitive than *T. aestivum* and *E. coracana*, but some injury was still apparent on all cultivars in both the 'high' and 'medium' ozone treatments. All cultivars showed similar behaviour at each level of ozone, with the probability level of visible injury being present increasing with increasing ozone (there was no interaction between ozone and cultivar). None of the cultivars showed any severe injury, even at high ozone. For *P. glaucum* at high ozone, sensitivity was of the order 'Okashana' > 'Shibe' > 'ICMV221' > 'KATPM1' for moderate injury. Post-hoc testing for high ozone showed a significant difference between the predicted probabilities of moderate injury for each cultivar.

There was no visible injury present in the low ozone treatment for *P. vulgaris*. The 'Turtle' bean cultivar responded slightly differently to the other bean cultivars, and data for this cultivar was analysed separately (Figure S2). The extent of visible leaf injury was variable between the different cultivars of *P. vulgaris* in the medium ozone treatment, and this remained consistent at high ozone (no interaction between ozone and cultivar) (Figure 2). The most extensive and severe leaf damage was shown for the 'Pinto' cultivar ,. Sensitivity to high ozone for *P. vulgaris* was in the order 'Pinto' > 'Cannellini' > 'Orca' for severe injury, with post-hoc tests showing a significant difference between the predicted probabilities for each cultivar. Turtle beans showed a higher predicted probability of severe injury in the medium ozone treatment, compared to the high ozone treatment (Figure S2).

3.2 Relationship between Asat, gs and chlorophyll index

For *T. aestivum* there was a tight positive relationship between A_{sat} and CCI, which was linear within the range tested ($p < 0.001$, model $R^2M = 0.89$; Figure 3a). Relationships between A_{sat} and CCI showed more scatter in the data for *E. coracana, P. glaucum* and *P. vulgaris* (Figure 3b-d). For *E. coracana* the relationship was also linear within the range tested ($p < 0.001$, model $R^2M = 0.67$), however, for *P. glaucum* and *P. vulgaris* the relationship was logarithmic, showing some evidence of a plateau being reached at higher values of CCI and a higher R^2 for the relationship for these crops when a logarithmic function was used ($p < 0.001$ for both crops, model R^2M = 0.44 and 0.70 respectively). For *T. aestivum*, CCI decreased with increasing ozone (Table S6), over time. After five weeks of ozone exposure, there was no difference in CCI between treatments, however after six and seven weeks, the CCI was lower in the high ozone compared to the other ozone treatments (Time * ozone $p < 0.001$). For the other crops, there was no significant effect of ozone on the CCI of the measured leaves ($p > 0.05$) (Table S6).

The ozone treatment did not affect the slope of the A_{sat} vs. chlorophyll relationship for *E. coracana* or *P. glaucum* ($p > 0.05$ for ozone * CCI). This was not tested for *T. aestivum* as the effect of ozone on A_{sat} could not be separated from an indirect effect via reduced chlorophyll. For *P. vulgaris*, model results indicated an interaction between chlorophyll index and ozone ($p < 0.001$), with a steeper slope and higher A_{sat} values for the high ozone treatment. This is thought to be because the high ozone treatment caused many leaves to sensesce prematurely, therefore measurements were made on healthy, young leaves.

Stomatal conductance (g_s) was significantly reduced with increasing ozone for *T. aestivum* ($p < 0.001$; Table S7). The effect of ozone on g_s for this species was gradual, with no difference between low and high treatments on 28th June (Week 5), but an ozone effect recorded on g_s for subsequent weeks (Time * ozone, $p < 0.001$, $R^2M=0.51$). There was also an effect of ozone on g_s for *P. vulgaris* ($p < 0.001$, $R^2M=0.47$), however the highest values of g_s were recorded in the highest ozone treatment (Table S7). Again, this is thought to be because healthy leaves were measured at high ozone for *P. vulgaris*, due to premature leaf drop. There was no significant effect of ozone on g_s for *E. coracana* or *P. glaucum*, with g_s values remaining similar in each ozone treatment (Table S7). Positive, tightly coupled relationships between A_{sat} and g_s were found for *E. coracana*, *P. glaucum* and *P. vulgaris* ($R^2M = 0.86$, 0.63 and 0.66 respectively, $p < 0.001$ for all, Fig 4b-d) and less so for *T. aestivum* ($R^2M = 0.52$, $p < 0.001$; Figure 4a). The relationships were all linear within the range tested, although it should be noted that for *T. aestivum* this relationship was largely driven by datapoints with very low A_{sat}, when chlorophyll content had also been severely reduced and there was a large effect of senescence and/or ozone damage. For the crops tested (*E. corcana* and *P. glaucum*), ozone did not negatively affect the slope of the A_{sat} vs. g_s relationships, in fact, for *E. coracana*, at high levels of g_s, A_{sat} was slightly higher in the high ozone treatment (g_s * ozone, $p < 0.01$). All model results are summarised in the Supplementary Information, Table S8.

3.3 Yield and grain weight

3.3.1 *T. aestivum*

For *T. aestivum*, there was a decrease in the total yield at high ozone ($p < 0.001$), with the mean yield at high ozone (22 g ± 1.2 SE) significantly lower than both low (37 g ± 1.2 SE) and medium (40 g ± 0.8 SE) ozone. The average yield across cultivars in the low and medium ozone treatments was not significantly different. There was a statistically significant difference in the response of different wheat cultivars to ozone (ozone and cultivar interaction, $p < 0.001$). For the African cultivars, ozone sensitivity was ranked 'Korongo' > 'Wren'> 'Njoro' > 'Hawk' > 'Eagle' (Figure 5a; Table S1). 'Korongo' was the most sensitive of the African cultivars overall, with the average yield at high ozone 53% lower than at low ozone. In comparison, the least sensitive African cultivar, 'Eagle', only showed a 10% decrease in average yield in the high compared to the low ozone treatment.

For all Kenyan cultivars, there was a decrease in 1000-grain weight with increasing ozone ($p < 0.001$, Figure 6a). The average 1000-grain weight at high ozone was between 18 and 36% lower than at the low ozone treatment for the different cultivars. Although there was some variation in sensitivity, differences between cultivars were less marked than for total yield (Table S2) and there was no overall difference in the response of different *T. aestivum* cultivars to ozone (ozone and cultivar interaction, p

> 0.05) for 1000-grain weight. The number of *T. aestivum* ears per pot was higher in the high ozone treatment ($\bar{x}$ = 27 ± 5 sd) than the low ozone treatment ($\bar{x}$ = 24 ± 4 sd) ($p < 0.05$). However, the number of grains per ear were found to decrease with increasing ozone ($p < 0.001$). This varied with cultivar (ozone * cultivar interaction, $p < 0.001$). The African cultivar 'Korongo' and the UK cultivar 'Skyfall' showed a significant reduction in the calculated number of grains per ear with increasing ozone treatment, whereas the other African cultivars showed no significant differences (data not presented).

3.3.2 *E. coracana*

Overall, there was no clear effect of ozone on total seed head weight for *E. coracana* (Figure 5b). There was some evidence of a delay in seed development with increasing ozone treatment, of up to 10 days, but this was not quantifiable statistically. Seed heads were harvested when they were ready so that harvest occurred at the same developmental stage. Results showed that the average total seed weight in the high ozone treatment was 32% higher than in the low ozone treatment, ($p = 0.051$), and there was no overall difference in the response of each cultivar to the ozone treatments (interaction, $p > 0.05$). For the average weight per seed head, there was no significant difference in the effect of ozone between cultivars, and the overall effect of ozone was to increase the weight per seed head (average seed head weight was 4.04 g ± 0.4 SE for the low ozone treatment, 4.95 g ± 0.37 SE for medium ozone and 4.80 g ± 0.34 SE for high ozone, $p < 0.05$) Figure 6b).

3.3.3 *P. glaucum*

P. glaucum showed no overall significant ozone effect on total yield, and no difference in response between cultivars (Figure 5c). Similarly there was no overall effect of ozone on the weight per seed head (Figure 6c) or significant difference on the effect of ozone between cultivars. *P. glaucum* showed a high variation between replicates per treatment.

3.3.4 *P. vulgaris*

For *P. vulgaris*, there was a decrease in total yield with increasing ozone ($p < 0.01$), with variation in the response of the different cultivars to ozone (ozone and cultivar interaction, $p < 0.05$; Figure 5d, Table S3). The cultivar 'Pinto' was the most sensitive to ozone, with the average yield at high ozone 75% lower than that in low ozone ($p < 0.05$). Turtle and Orca beans showed a 50% decrease in average yield at high compared to low ozone, although this was not statistically significant due to the high variation in yield within ozone treatments. The total yield of Cannellini also did not vary with ozone treatment ($p > 0.05$). The weight per bean seed of *P. vulgaris* was significantly reduced ($p < 0.01$) at high compared to low ozone, with the magnitude of effect dependent on cultivar (ozone and cultivar interaction, $p < 0.05$) (Figure 6d; Table S4). For Pinto, the weight per bean seed was 53% lower in the high ozone compared to the low ozone treatment ($p < 0.001$). Turtle and Cannellini beans showed an average decrease in individual bean weight in high compared to low ozone of 33% and 16% respectively, although this was not statistically significant, and there was no effect of ozone on the individual bean weight of the cultivar 'Orca'. Model results for all crop species are summarised in the Supplementary Information, Table S9.

3.4 G_{max}

G_{max} was variable both between crop species and between cultivars of the same species (Table 3). G_{max} was approximately four times higher for *T. aestivum*, which had the highest g_{max} of the crops tested, than for *E. coracana*, which had the lowest. *P. glaucum* and *P. vulgaris* g_{max} were similar to each other, and approximately 25% less than that of *T. aestivum*. For the *T. aestivum*, *E. coracana* and *P. vulgaris* cultivars tested, g_{max} varied by approximately ± 20% compared to the mean for the species. For the crops showing a clear effect of ozone on yield/1000-grain weight (*T. aestivum* and *P. vulgaris*), the cultivars with the higher g_{max} were not those ranked as the most sensitive to ozone in terms of yield. This was also the case for the visible ozone injury ranking (data not presented).

4 Discussion

This study has shown that ozone pollution in sub-Saharan Africa has the potential to reduce crop yield, particularly for wheat and beans, which are among the staple food crops for the region. Large decreases in yield of Kenyan *T. aestivum* cultivars were found. *T. aestivum* is known to be one of the most sensitive crops to ozone pollution of those crops that have been tested (Mills et al., 2007; Mills and Harmens, 2011), and for total yield the ozone-sensitivity of the Kenyan cultivar 'Korongo' was similar to the ozone-sensitivity of the European cultivar 'Skyfall', which has previously been identified as a very sensitive European cultivar (Harmens et al., 2018). Dry beans, commonly grown as a source of protein in many parts of the world, particularly in subsistence agriculture in tropical countries, were also very sensitive to ozone pollution and showed large reductions in yield, especially for the cultivar 'Pinto'. There is less documented evidence of sensitivity to ozone for beans compared to that for wheat, however, the results from this study match those of previous studies, largely on European and American peas and beans, which have also demonstrated that these are sensitive to ozone compared to other crops (Mills et al., 2007; Mills and Harmens, 2011).

In addition to total yield, there were large reductions in 1000-grain/seed weight for *T. aestivum* and *P. vulgaris* (for the Pinto cultivar). 1000-grain/seed weight is related to commodity value, particularly when crops are graded for sale. There is not a straightforward link between grain size and nutritional quality, as growth dilution of some nutritional aspects can occur, e.g. the protein content of wheat tends to increase as grain size decreases, however, this may not compensate for the reduction in yield so that total protein yield of wheat is still reduced (Pleijel et al., 1999). The seed coat is relatively thick for *P. vulgaris*, and is a much larger proportion of the total weight for small beans compared to large beans. A previous study using adzuki beans showed that protein content was higher in large beans (26.1%) compared to small beans (22.3%) for the variety 'WSU 262' (Baik and Czuchajowska, 1999), therefore, it would be anticipated that there would be a considerable reduction in total protein content in small beans compared to large beans, in addition to the reduction in total yield for this crop.

For *T. aestivum*, the ranking of cultivars for ozone sensitivity in terms of visible leaf injury was the same as that for ozone sensitivity in terms of yield reduction. However, for *P. vulgaris* the ranking of sensitivity to ozone of the cultivars was different for these two measures. This could be explained by the different strategies of growth by different crops, as *P. vulgaris* can continue to grow leaves to replace those damaged by ozone, whereas for cereal crops such as *T. aestivum* no new leaves are grown following the emergence of the flag leaf. Therefore, for *P. vulgaris,* a range of ozone tolerance mechanisms can be relevant in addition to those that maintain photosynthetic function of existing leaves.

For the crops *T. aestivum* and *P. vulgaris* it is likely that the reduction in yield is due to an ozone-induced reduction in chlorophyll, which therefore reduces photosynthetic capacity during the grainfill stage, as A_{sat} was closely coupled to chlorophyll content for these species. This is futher supported by the reduction in grain size for these crops, indicating a lack of photosynthate at this time. Ozone-induced reductions in chlorophyll content were found in the current study, and reductions have previously been shown for a variety of crops including wheat and maize (Bagard et al., 2015) and soybean (Betzelberger et al., 2010), with these studies relating the reduction in chlorophyll content to reduced photosynthesis.

It has previously been shown for several crops that the time of highest sensitivity to stress is during anthesis and grain filling, e.g. temperature for *T. aestivum* (Ferris et al., 1998) and sorghum (Prasad et al., 2015), drought for *P. glaucum* (Mahalakshmi et al., 1987) and ozone for *P. vulgaris* (Salvatori et al., 2013). Anthesis has been identified as the most ozone-sensitive growth stage in wheat due to g_{max} being highest at this time (Pleijel et al., 2007). There is also evidence from other studies that abiotic stress can cause the grainfill stage to be shortened (Prasad et al., 2008) or the rate of grainfill to be reduced (Dias and Lidon, 2009), highlighting the importance of maintaining photosynthetic activity during this time.

The effect of ozone on the staple tropical food crops *E. coracana* and *P. glaucum* has not previously been determined. The current study suggests that the total yield of *E.*

coracana was not significantly affected by ozone, however, there was a lot of variation in the collected data for each cultivar. This study has shown that individual seed head weight of *E. coracana* increased slightly when ozone was increased. The current study has also indicated no significant effects of ozone on the yield of *P. glaucum*, however, the exposure period for *P. glaucum* was during the vegetative stages only (because it grew too large for the ozone exposure facility) and therefore an impact on yield may have become apparent if *P. glaucum* was exposed to ozone during the anthesis and/or grain-filling stages. The data for *E. coracana* and *P. glaucum* was based on seedhead weight rather than grain weight, however, we believe that this is representative of yield as when seeds were successfully extracted these comprised >90% of the total seedhead weight.

Both *E. coracana* and *P. glaucum* are C_4 plants and the nature of the C_4 pathway means that photosynthetic machinery is further isolated from the external air, as a consequence of adaptation to reduce CO_2 leakage. These adaptations in C_4 plants include a high concentration of chloroplasts in tightly fitted bundle-sheath cells, with the loosely arranged mesophyll cells (also containing chloroplasts) between these and the air spaces within the leaf (Esau, 1977). Although there was no evidence of reduced chlorophyll content and a subsequent limitation to photosynthesis, visible leaf-injury was extensive (particularly for *E. coracana*) very early in the ozone exposure period, indicating a detrimental impact causing leaf damage early in the exposure period. However, the extent of visible leaf-injury to ozone during this time may not be indicative of the sensitivity to ozone during anthesis and grainfill for this species. It is possible that up-regulation of antioxidant defence pathways occurred within these species so that they were more resilient to subsequent stress (Di Baccio et al, 2008;

Chen and Gallie, 2005). Xanthophyll pigments are used to effectively dissipate excess energy from photosynthesis pigments to reduce energy loss from photorespiration in C_4 plants as well as in C_3 plants (Shay and Kubien, 2012), therefore, increased xanthophyll production in leaves in response to ozone stress may simultaneously increase photosynthetic efficiency.

Interestingly, for some crops A_{sat} was tightly coupled to g_s, whereas for others, A_{sat} was tightly coupled to chlorophyll content. This may be related to the photosynthetic pathway of the species used as A_{sat} and g_s were tightly coupled for the C_4 plants *E. coracana* and *P. glaucum*. The C_4 photosynthetic pathway uses water more efficiently than the C_3 type, as carbon dioxide can be concentrated around Rubisco in the bundle sheath cells of C_4 plants. This may be particularly important when carbon dioxide supply is limited due to water stress, as water-use efficiency is increased and photosynthesis can occur with very low stomatal conductions, and is therefore more commonly found in plants of arid conditions. In our study we kept the plants well-watered, however, it has previously been shown that photosynthesis in water stressed *Setaroa sphacelata* (C_4) was mainly limited by stomatal rather than non-stomatal (biochemical) limitations (Da Silva and Arrabaça, 2004), implying that in arid field conditions C_4 plants would still have reduced stomatal conductance and, therefore, ozone uptake. In contrast, for *T. aestivum* and *P. vulgaris* there was a close coupling to chlorophyll index, showing non-stomatal limitations to photosynthesis for these C_3 species and indicating that light-saturated photosynthesis is more determined by the capacity for light harvesting and CO_2 fixation rather than by stomatal control of CO_2 uptake.

The tight relationships between chlorophyll content and A_{sat} for *T. aestivum* and *P. vulgaris* also suggests that crop management strategies to maintain leaf chlorophyll content and delay leaf senescence during ozone exposure may help to reduce yield losses. However, whereas standard farming practices may be to apply additional nitrogen-based fertiliser to increase leaf greenness, this may not be effective if the cause of the leaf senescence is ozone exposure (Harmens et al., 2017; Broberg et al., 2017). Strategies to increase detoxification capacity within the leaves, or to reduce stomatal flux by either reducing stomatal opening or reducing ambient ozone concentrations could be more effective in this case. It has previously been shown that biochemical and molecular responses were good indicators of ozone-sensitivity of wheat cultivars in open-top chamber experiments (Fatima et al., 2018; Feng et al., 2016), including antioxidants such as ascorbate, total thiol and glutathione.

The large variation in sensitivity to ozone of the different cultivars tested indicates that there is potential for selection of ozone tolerant cultivars in regions where ozone concentrations are already high, or are set to increase further in the future, to maintain crop production in the longer term. There are already strategies to improve resistance to drought and heat stress for several tropical crops including *P. glaucum* (Serraj et al., 2005). In the near future this could include selection of existing cultivars that may be more resistant to the local pollution climate, but in the longer term selective breeding could be used to increase the prevalence of traits or genes associated with ozone resistance. An ideotype for an ozone-tolerant crop has been suggested, which includes target traits of increased water-use efficiency, low stomatal conductance, high antioxidant capacity and balanced redox homeostasis and programmed cell-death pathways, as ozone-sensitivity can be a consequence of some traits identified when developing high-yielding varieties (Mills et al., 2018c). Studies have identified some genetic loci associated with ozone resistance and/or susceptibility in wheat and rice (e.g. Ainsworth et al., 2008; Frei, 2015, Frei et al., 2010). These could be related to biochemical processes and the efficiency of detoxification (Biswas and Jiang, 2011) or ozone induced stomatal closure to reduce ozone uptake (Kangasjärvi et al., 2005).

5 Conclusions

Tropospheric ozone pollution can cause visible leaf-damage to tropical crop plants, which can result in reduced chlorophyll content in some cases. Crop yield and 1000-grain weight were reduced by ozone for *T. aestivum* and *P. vulgaris*, whereas the response for the C4 species *P. glaucum* and *E. coracana* was less clear. The Kenyan cultivars of *T. aestivum* tested in this study were in a similar range of ozone sensitivity as European cultivars, which are known to be ozone-sensitive. Therefore, there is a risk that tropospheric ozone can reduce food production in tropical regions, including Africa, where there is a high dependence on subsistence agriculture. Thus, there is an urgent need to measure the ozone concentrations in subsistence agriculture regions and to test a wider variety of tropical and temperate crops from these areas using the representative growing conditions in order to quantify the reduction in food production that may occur due to ozone pollution in these regions in current and future climate and environmental conditions.

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7 References

Ainsworth, E.A., Rogers, A., Leakey, A.D.B. (2008) Targets for crop biotechnology in a future high-CO_2 and high-O_3 world. Plant Physiology 147:13-19.

Akhtar, N., Yamaguchi, M., Inada, H., Hoshino, D., Kondo, T., Fukami, M., Funada, R., Izuta, T. (2010) Effects of ozone on growth, yield and leaf gas exchange rates of four Bangladeshi cultivars of rice (*Oryza sativa* L.). Environmental Pollution 158:2970-2976.

Anderson. D.C., Nicely, J.M., Salawitch, R.J., Canty, T.P et al. (2016) A pervasive role for biomass burning in tropical high ozone/low water structures. Nature Communications 7, Article number 10267.

Avnery, S., Mauzerall, D.L., Liu, J.F., Horowitz, L.W. (2011) Global crop yield reductions due to surface ozone exposure:1. Year 2000 crop production losses and economic damage. Atmospheric Environment 45:2284-2296.

Bagard, M., Jolivert, Y., Hasenfratz-Sauder, M.-P., Gerard, J., Dizengremel, P., Le Thiec, D. (2015) Ozone exposure and flux-based response functions for photosynthetic traits in wheat, maize and poplar. Environmental Pollution 206:411-420.

Baik, B.K., Czuchajowska, Z. (1999) Paste particle and bean size as related to sweetened Azuki paste quality. Cereal Chemistry 76:122-128.

Barton, K. (2018) R-package 'MuMIn', Model selection and model averaging based on information criteria (AICc and alike).

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models using lme4. Journal of Statistical Software, 67:1-48.

Betzelberger, A.M., Gillespie, K.M., McGrath, J.M., Koester, R.P., Nelson, R.L., Ainsworth, E.A. (2010) Effects of chronic elevated ozone concentration on antioxidant capacity, photosynthesis and seed yield of 10 soybean cultivars. Plant, Cell and Environment 33:1569-1581.

Betzelberger, A.M., Yendrek, C.R., Sun, J.D., Leisner, C.P., Nelson, R.L., Ort, D.R., Ainsworth, E.A. (2012) Ozone exposure response for U.S. soybean cultivars: linear reductions in photosynthetic potential, biomass, and yield. Plant Physiology 160:1827-1839.

Biswas, D.K., Xu, H., Li, Y.G., Sun, J.Z., Wang, X.Z., Han, X.G., Jiang, G.M. (2008) Genotypic differences in leaf biochemical, physiological and growth responses to ozone in 20 winter wheat cultivars released over the past 60 years. Global Change Biology 14:46-59.

Biswas, D.K., Jiang, G.M. (2011) Differential drought-induced modulation of ozone tolerance in winter wheat species. Journal of Experimental Botany 62:4153-4162.

Brauer, M., Amann, M., Burnett, R.T., Cohen, A., Dentener F., Ezzati, M. et al. (2012) Exposure assessment for estimation of the global burden of disease attributable to outdoor air pollution. Environ. Sci. Technol 46:652-660.

Broberg, M.C., Uddling, J., Mills, G., Pleijel, H. (2017) Fertilizer efficiency in wheat is reduced by ozone pollution. Science of the Total Environment 607:876-880.

Broughton, W.J., Hernandez, G., Blair, M., Beebe, S., Gepts, P., Vanderleyden, J. (2003) Beans (Phaseolus spp.) – model food legumes. Plant and Soil 252:55-128.

Caregnato, F.F., Bortolin, R.C., Divan, A.M., Moreira, J.C.F. (2013) Exposure to elevated ozone levels differentially affects the antioxidant capacity and the redox homeostasis of two subtropical *Phaseolus vulgaris* L. varieties. Chemosphere 93:320-330.

Chen, Z. Gallie, D.R. (2005) Increasing tolerance to ozone by elevating foliar ascorbic acid confers greater protection against ozone than increasing avoidance. Plant Physiology 138:1673-1689.

Chen, Z., Wang, X.K., Feng, Z.Z., Zheng, F.X., Duan, X.N., Yang, W.R. (2008) Effects of elevated ozone on growth and yield of field-grown rice in Yangtze River Delta, China. Journal of Environmental Sciences 20:320-325.

Cooper, O.R., Parrish, D.D., Ziemke, J., Balashov, N.V., Cupeiro, M., Galbally, I.E., Gilge, S., Horowitz, L., Jensen, N.R., Lamarque, J.F. & Naik, V. (2014) Global distribution and trends of tropospheric ozone: An observation-based review. Elementa: Science of the Anthropocene 2, 000029. DOI: 10.12952/journal.elementa.000029.

Da Silva, J.M., Arrabaça, M.C. (2004) Photosynthesis in the water-stressed C-4 grass *Setaria sphacelata* is mainly limited by stomata with both rapidly and slowly imposed water deficits. Physiologia Plantarum 121:409-420.

De Temmerman, L., Legrand, G., Vandermeiren, K. (2007) Effects of ozone on sugar beet grown in open-top chambers. European Journal of Agronomy 26:1-9.

Dentener, F., Stevenson, D., Ellingsen, K., van Noije, T., Schultz, M., Amann, M., Atherton, C., Bell, N., Bergmann, D., Bey, I., et al. (2006) The global atmospheric environment for the next generation. Environmental Science & Technology 40:3586-3594.

DeWitt, H.L., Gasore, J., Rupakheti, M., Potter, K.E., Prinn, R.G., Ndikubwimana, J.D., Nkusi, J., Safari, B. (2019) Seasonal and diurnal variability in O_3, black carbon, and CO measured at the Rwanda Climate Observatory. Atmospheric Chemistry and Physics 19:2063-2078.

Dias, A.S. and Lidon, F.C. (2009) Evaluation of grain filling rate and duration in bread and durum wheat, under heat stress after anthesis. Journal of Agronomy and Crop Science 195:137-147.

Di Baccio, D., Castagna,A., Paoletti, E., Sabastiani, L., Ranier, A. (2008) Could the differences in O_3 sensitivity between two poplar clones be related to a difference in antioxidant defense and secondary metabolic response to O_3 influx? Tree Physiology 28:1761-1772.

Emberson, L.D., Büker, P., Ashmore, M.R., Mills, G., Jackson, L.S., Agrawal, M., Atikuzzaman, M.D., Cinderby, S., Engardt, M., Jamir, C., Kobayashi, K., Oanh, N.T.K., Quadir, Q.F. & Wahid, A. (2009) A comparison of North American and Asian exposure-response data for ozone effects on crop yields. Atmospheric Environment 43:1945-1953.

Esau, K. (1977) Anatomy of seed plants. Wiley, New York. 576 pp.

Fatima, A., Singh, A.A., Mukherjee, A., Agrawal, M., Agrawal, S.B. (2018) Variability in defence mechanism operating in three wheat cultivars having different levels of sensitivity against elevated ozone. Environmental and Experimental Botany 155:66-78.

Feng, Z., Tang, H. & Kobayashi, K. (2017) Effects of ozone on crops in China. In: Takeshi Izuta (Ed.). *Air pollution impacts on plants in East Asia*. Springer, Tokyo, Japan, pp.175-194.

Feng, Z., Wang, L., Pleijel, H., Zhu, J., Kobayashi, K. (2016) Differential effects of ozone on photosynthesis of winter wheat among cultivars depend on antioxidant enzymes rather than stomatal conductance. Science of the Total Environment 572:404-411.

Feng, Z., Hu, E., Wang, X., Jiang, L., Liu, X. (2015) Ground-level O_3 pollution an its impacts on food crops in China: A review. Environmental Pollution 199:42-48.

Ferris, R., Ellis, R.H., Wheeler, T.R., Hadley, P. (1998) Effect of high temperature stress at anthesis on grain yield and biomass of field-grown crops of wheat. Annals of Botany 82:631-639.

Fiscus, E.L., Booker, F.L., Burkey, K.O. (2005) Crop responses to ozone: uptake, modes of action, carbon assimilation and partitioning. Plant Cell and Environment 28:997-1011.

Frei, M., Tanaka, J.P., Chen, C.P., Wissuwa, M. (2010) Mechanisms of ozone tolerance in rice: Characterization of two QTLs affecting leaf bronzing by gene expression profiling and biochemical analysis. Journal of Experimental Botany 61:1405-1417.

Frei, M. (2015) Breeding of ozone resistant rice: Relevance, approaches and challenges. Environmental Pollution 197:144-155.

Graham, P.H. and Ranalli, P. (1997). Common bean (*Phaseolus vulgaris* L.). Field Crops Research 53:131-146.

Grantz, D.A., Gunn, S., Vu, H.B. (2006). O_3 impacts on plant development: a meta-analysis of root/shoot allocation and growth. Plant Cell and Environment 29:1193-1209.

Harmens, H., Hayes, F., Sharps, K., Mills, G., Calatayud, V. (2017). Leaf traits and photosynthetic responses of *Betula pendula* saplings to a range of ground-level ozone concentrations at a range of nitrogen loads. Journal of Plant Physiology 211:42-52.

Harmens, H., Hayes, F., Mills, G., Sharps, K., Osborne, S., Pleijel, H. (2018) Wheat yield responses to stomatal uptake of ozone: Peak vs rising background ozone conditions. Atmospheric Environment 173:1-5.

Hickman, J.E., Huang, Y., Wu, S.L., Diru,W., Groffman, P.M., Tully, K.L., Palm, C.A. (2017) Non-linear response of nitric oxide fluxes to fertiliser inputs and the impacts of agricultural intensification on tropospheric ozone pollution in Kenya. Global Change Biology 23:3193-3204.

Hoshika, Y., Watanabe, M., Inada, N., Koike, T. (2013) Model-based analysis of avoidance of ozone stress by stomatal closure in Siebolds beech (*Fagus crenata*). Annals of Botany 112:1149-1158.

Huang, Y., Hickman, J.E., Wu, S. (2018) Impacts of enhanced fertilizer applications on tropospheric ozone and crop damage over sub-Saharan Africa. Atmospheric Environment 180:175-125.

Kangasjärvi, J., Jaspers, P., Kollist, H. (2005) Signalling and cell-death in ozone-exposed plants. Plant Cell and Environment 28:1021-1036.

Kimayu, J.M., Gikuma-Njuru, P., Musembi, D.K. (2017) Temporal and spatial variability of tropospheric ozone in Nairobi City, Kenya. Physical Science International Journal 13:Article PSIJ.31452.

Kumari, S., Verma, N., Lakhani, A., Tiwari, S., Kandikonda, M.K. (2018) Tropspheric ozone enhancement during post-harvest crop-residue fires at two downwind sites of the Indo-Gangetic Plain. Environmental Science and Pollution Research 25:18879-18893.

Laban, T.L, Van Zul, P.G., Beukes, J.P., Vakkari, V., Jaars, K., Boduas-Dedekind, N., Josipovic, M. Thompson, A.M., Kulmala, M., Laakso, L. (2018) Seasonal influences on surface ozone variability in continental South Africa and implications for air quality. Atmospheric Chemistry and Physics Discussion. DOI: doi.org/10.5194/acp-2017-1115.

Lal, D.M., Ghude, S.D., Patil, S.D., Kulkarni, S.H., Jena, C., Tiwari, S., Srivastava, M.K. (2012) Tropospheric ozone and aerosol long-term trends over the Indo-Gangetic Plain (IGP), India. Atmospheric Research 116:82-92.

Ma, Z., Xu, J., Quan, W., Zhang, Z., Lin, W. et al. (2016) Significant increase of surface ozone at a rural site, north of eastern China. Atmospheric Chemistry and Physics 16:3969–3977.

Mahalakshmi, V., Bidinger, F.R., Raju, D.S. (1987) Effect of timing of water deficit on pearl millet (*Pennisetum americanum*). Field Crops Research 15:327-339.

Marzuoli, R., Finco, A., Chiesa, M., Gerosa, G. (2017) A dose-response relationship for marketable yield reduction of two lettuce (*Lactuca sativa* L.) cultivars exposed to tropospheric ozone in Southern Europe. Environmental Science and Pollution Research 24:26249-26258.

Massman, W.J. (1998) A review of the molecular diffusivities of H_2O, CO_2, CH_4, CO, O_3, SO_2, NH_3, N_2O, NO, and NO_2 in air, O_2 and N_2 near STP. Atmospheric Environment 32:1111- 1127.

Mills, G., Buse, A., Gimeno, B., Bermejo, V., Holland, M., Emberson, L., Pleijel, H. (2007) A synthesis of AOT40-based response functions and critical levels of ozone for agricultural and horticultural crops. Atmospheric Environment 41:2630-2643.

Mills, G. and Harmens, H. (Eds.) (2011). Ozone pollution: A hidden threat to food security. Centre for Ecology & Hydrology, Bangor, UK. ISBN: 978-1-906698-27-0.

Mills, G., Sharps, K., Simpson, D., Pleijel, H., Broberg, M., Uddling, J. et al. (2018a) Ozone pollution will compromise efforts to increase global wheat production. Global Change Biology 24:3560-3574.

Mills, G., Pleijel., H., Malley, C.S., Sinha, B., Cooper, O.R. et al. (2018b) Tropospheric Ozone Assessment Report: Present-day tropospheric ozone distribution and trends relevant to vegetation. Elementa-Science of the Anthropocene 6:Article 47.

Mills G., Sharps K., Simpson D., Pleijel H., Frei M. et al. (2018c) Closing the global ozone yield gap: Quantification and cobenefits for multistress tolerance. Global Change Biology 24:4869-4893.

Osborne, S.A., Mills, G., Hayes, F., Ainsworth, E.A., Büker, P., Emberson, L. (2016) Has the sensitivity of soybean cultivars to ozone pollution increased with time? An analysis of published dose–response data. Global Change Biology 22:3097-3111.

Pleijel, H., Mortensesn, L., Fuhrer, J., Ojanpera, K., Danielsson, H. (1999) Grain protein accumulation in relation to grain yield of spring wheat (*Triticum aestivum* L.)

grown in open-top chambers with different concentrations of ozone, carbon dioxide and water availability. Agriculture, Ecosystems & Environment 72:265-270.

Pleijel, H., Eriksen, A.B., Danielsson, H., Bondesson, N. & Sellden, G. (2006) Differential ozone sensitivity in an old and a modern Swedish wheat cultivar – grain yield and quality, leaf chlorophyll and stomatal conductance. Environmental and Experimental Botany 56:63–71.

Pleijel, H., Danielsson, H., Emberson, L., Ashmore, M.R., Mills, G. (2007) Ozone risk assessment for agricultural crops in Europe: Further development of stomatal flux and flux-response relationships for European wheat and potato. Atmospheric Environment 41:3022-3040.

Pleijel, H., Broberg, M.C., Uddling, J., Mills, G. (2018) Current surface ozone concentrations significantly decrease wheat growth, yield and quality. Science of the Total Environment 613:687-692.

Prasad, P.V.V., Djamaguiraman, M., Perumal, R., Ciampitti, I.A. (2015) Impact of high temperature stress on floret fertility and individual grain weight of grain sorghum: sensitive stages and thresholds for temperature and duration. Frontiers in Plant Science 6:Article 820.

Prasad, P.V.V., Pisipati, S.R., Ristic, Z., Bukovnik, U., Fritz, A.K. (2008) Impact of nighttime temperature on physiology and growth of spring wheat. Crop Science 48:2372-2380.

R Core Team (2016) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Racherla, P.N. and Adams, P.J. (2006) Sensitivity of global tropospheric ozone and fine particulate matter concentrations to climate change. Journal of Geophysical Research-Atmospheres 111:Article D24103.

Royal Society (2008) Ground-level ozone in the 21st century: future trends, impacts and policy implications. Science Policy Report 15/08, Royal Society, London.

Sakamoto, Y., Shojo, K., Bui, M.T., Pham, T.H., Vu, T.A., Ly, B.T., Kajii, Y. (2018) Air quality study in Hanoi, Vietnam in 2015-2016 based on a one-year observation of NOx, O3, CO and a one-week observation of VOCs. Atmospheric Pollution Research 9:544-551.

Salvatori, E., Fusaro, L., Mereu, S., Bernardini, A., Puppi, G., Manes, F. (2013) Different O_3 response of sensitive and resistant snap bean genotypes (*Phaseolus vulgaris* L.): The key role of growth stage, stomatal conductance, and PSI activity. Environmental and Experimental Botany 87: 79-91.

Schultz, M.G., Schroder, S., Lyapina, O., Cooper, O.R. et al. (2017) Tropospheric Ozone Assessment Report: Database and metrics data of global surface ozone observations. Elementa-Science of the Anthropocene 5:Article 58.

Schraudner, M., Langebartels, C., Sandermann, H. (1997) Changes in the biochemical status of plant cells induced by the environmental pollutant ozone. Physiologia Plantarum 100:274-280.

Serraj, R., Hash, C.T., Fizvi, S.M.H., Sharma, A., Yadav, R.S., Bidinger, F.R. (2005) Recent advances in marker-assisted selection for drought tolerance in pearl millet. Plant Production Science 8:334-337.

Shay, P.E., Kubien, D.S. (2012) Field analysis of photoprotection in co-occurring cool climate C3 and C4 grasses. Physiologia Plantarum 147:316-328.

Shin, H.J., Park, J.H., Park, J.S., Song, I.H., Park, S.M., Roh, S.A., Son, J.S., Hong, Y.D. (2017) The long term trends of tropospheric ozone in major regions of Korea. Asian Journal of Atmospheric Environment 11:235-253.

Singh, A.A., Agrawal, S.B. (2017) Tropospheric ozone pollution in India: effects on crop yield and product quality. Environmental Science and Pollution Research 24:4367-4382.

Stevenson, D.S., Dentener, F.J., Schultz, M.G. et al., (2006) Multimodel ensemble simulations of present-day and near-future tropospheric ozone. Journal of Geophysical Research-Atmospheres 111:D08301.

Sun, L, Xue, L, Wang, T, Gao, J, Ding, A, et al. (2016) Significant increase of summertime ozone at Mount Tai in Central Eastern China. Atmospheric Chemistry and Physics 16:10637–10650.

Tomer, R., Bhatia, A., Kumar, V., Kumar, A., Singhm R., Singh, B. & Singh, S.D. (2015) Impact of Elevated Ozone on Growth, Yield and Nutritional Quality of Two Wheat Species in Northern India. Aerosol and Air Quality Research 15:329-340.

UN General Assembly (2015) Transforming our world : the 2030 Agenda for SustainableDevelopment, 21 October 2015, A/RES/70/1, available at: http://www.refworld.org/docid/57b6e3e44.html [accessed 6 November 2018]

Van Dingenen, R., Dentener, F. J., Raes, F., Krol, M. C., Emberson, L. & Cofala, J. (2009) The global impact of ozone on agricultural crop yields under current and future air quality legislation. Atmospheric Environment 43:604-618.

Venables, W. N., Ripley, B. D. (2002) Modern Applied Statistics with S. Fourth Edition. Springer, New York. ISBN 0-387-95457-0.

Wahid, A. (2006) Influence of atmospheric pollutants on agriculture in developing countries: A case study with three new wheat varieties in Pakistan. Science of the Total Environment 371:304-313.

Wang, Y.Y., Du, H.Y., Xu, Y.Q., Lu, D.B., Wang, X.Y., Guo, Z.Y. (2018) Temporal and spatial variation relationship and influence factors on surface urban heat island

and ozone pollution in the Yangtze River Delta, China. Science of the Total Environment 631-632:921-933.

Wild, O., Fiore, A.M., Shindell, D.T., Doherty, R.M., Collins, W.J., Dentener, F.J., Schultz, M.G., Gong, S., MacKenzie, I.A., Zeng, G., Hess, P. (2012) Modelling future changes in surface ozone: a parameterized approach. Atmospheric Chemistry and Physics 12:2037-2054.

Wilkinson, S., Mills, G., Illidge, R., Davies, W.J. (2012) How is ozone pollution reducing our food supply? Journal of Experimental Botany 63:527-536.

Xu, W., Lin, W., Xu, X., Tang, J., Huang, J., et al. (2016) Long-term trends of surface ozone and its influencing factors at the Mt Waliguan GAW station, China–Part 1: Overall trends and characteristics. Atmospheric Chemistry and Physics 16: 6191–6205.

Xu, J., Ma, J.Z., Zhang, X.L., Xu, X.B., Xu, X.F., Lin, W.L., Wang, Y., Meng, W., Ma, Z.Q. (2011) Measurments of ozone and its precursors in Beijing during summertime: impact of urban plumes on ozone pollution in downwind rural areas. Atmospheric Chemistry and Physics ll:12241-12252.

Zuur, A.F., Ieno, E.N., Walker, N.J., Saveliev, A.A. & Smith, G.M. (2009) Mixed effects models and extensions in ecology with R. Springer, New York.

Table 1: Summary of experimental timings for the crops used.

Crop	*T. aestivum*	*E. coracana*	*P. glaucum*	*P. vulgaris*
Unheated/heated (ambient temperature + 7°C)	unheated	heated	heated	heated
Number of cultivars	5 Kenyan + 1 UK	6	4	4
Number of plants per pot	9	4	2	1
Start of ozone treatment	18th May	1st June	24th May	1st June
End of ozone treatment†	25th July	26th October	29th June (moved to glasshouse as plants became too big for solardomes)	Pinto: 26th July Turtle: 2nd August Orca: 21st August Cannellini: 25th August
Nutrient addition	25th May, 80 kg N/ha; 16th June, 80 kg N/ha	16th June, 80 kg N/ha; 8th August, 80 kg N/ha	25th May, 80 kg N/ha; 22nd June, 80 kg N/ha;	22nd June, 40 kg N/ha; 14th July, 40 kg N/ha;
Dates of A_{sat} measurements	28th June, 5th July, 11th July,	12th July, 26th-27th July, 2nd August	4th August, 14th-15th August	6th July, 4th August, 8th August
Harvest of yield	25th July-1st August	28th September – 26th October	5th September	Pinto: 26th July Turtle: 2nd August Orca: 21st August Cannellini: 25th August

† The exposure length varied for the different bean varieties due to the differences in growing season length of the varieties used.

Table 2: Ozone concentrations and climatic conditions for the treatments over the experimental period**.**

Ozone treatment	Temperature regime	Ozone, weekly mean (ppb)	Ozone, mean daily max. (ppb)	Temperature, daylight mean (˚C)	VPD, daylight mean (kPa)	PAR, 12h mean (mmol/m^2/s)
Low	Ambient	25.6±0.3	32.5±1.0	20.3±0.3	0.61±0.1	487±21
Medium	Ambient	40.2±1.3	67.3±2.8	20.3±0.3	0.61±0.1	487±21
High	Ambient	45.8±2.1	84.0±4.5	20.3±0.3	0.61±0.1	487±21

Low	Ambient + 7˚C	25.4±0.5	33.9±0.7	27.2±0.3	1.89±0.1	487±21
Medium	Ambient + 7˚C	40.6±1.3	70.6±2.7	27.7±0.3	2.05±0.1	487±21
High	Ambient + 7˚C	47.8±2.0	93.0±4.3	27.3±0.3	1.87±0.1	487±21

Table 3: Calculated g_{max} for the crop type and cultivars tested.

	T. aestivum	*E. coracana*	*P. glaucum*	*P. vulgaris*
G_{max} (mmol O_3 m^{-2} PLA s^{-1})	Mean: 440 Eagle 10: 345 Hawk 12: 530 Korongo: 375 Njoro BWII: 530 Wren: 425	Mean: 110 GuluE: 140 KNE624: 100 KNE814: 125 Okhale: 110 P224: 90 U15: 110	Mean: 350 ICMV221: 370 KATPM1: 310 Okashana: 325 Shibe: 355	Mean: 340 Pinto: 365 Turtle: 400 Orca: 295 Cannellini: 300

Figure Legends:

Figure 1: A) Weekly target diurnal ozone concentrations for the treatments used. Note that the target concentrations in heated and unheated solardomes were the same, and B) Achieved ozone concentrations for a 'typical' day (8th June, 2017).

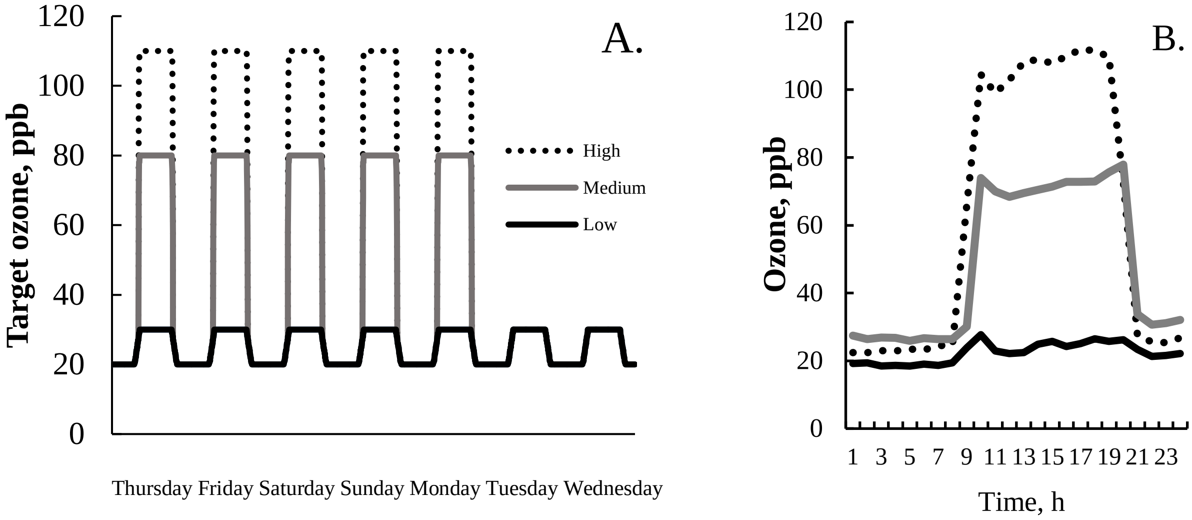
Figure 2: Predicted probabilities of leaf injury being present: mild (<5% of leaf affected), moderate (5-25%) or severe (>25%) after 2 weeks of exposure to low, medium and high ozone for *T. aestivum* (wheat), *E. coracana* (finger millet), *P. glaucum* (pearl millet) and *P. vulgaris* (common bean). Wheat cultivar Kor. = Korongo. Bean cultivar Can. = Cannellini.

Figure 3. Relationship between CCI (Chlorophyll Content Index) and A_{sat} (µmol m^{2} s-^{1}) at low, medium and high ozone levels for a) *T. aestivum*, b) *E. coracana*, c) *P. glaucum* and d) *P. vulgaris*. Plotted lines use model fitted values.

Figure 4. The effect of increasing stomatal conductance ($g_{s;}$ mol H_2O m^{-2} PLA s^{-1}) on A_{sat} (µmol m^{-2} s^{-1}) for a) *T. aestivum*, b) *E. coracana*, c) *P. glaucum* and d) *P. vulgaris*. Measurements at different ozone treatments are represented by different symbols for *E. coracana* and *P. glaucum*. Plotted lines use model fitted values.

Figure 5. The effect of increasing ozone levels (low, medium and high treatments) on total yield for different cultivars of a) *T. aestivum*, b) *E. coracana*, c) *P. glaucum* and d) *P. vulgaris*. Error bars are ± se.

Figure 6. The effect of increasing ozone levels on grain/seed weight for different cultivars of *T. aestivum* and *P. vulgaris* (a & d) and seed head weight (b & c) for *E. coracana* and *P. glaucum*. Error bars are ± se.



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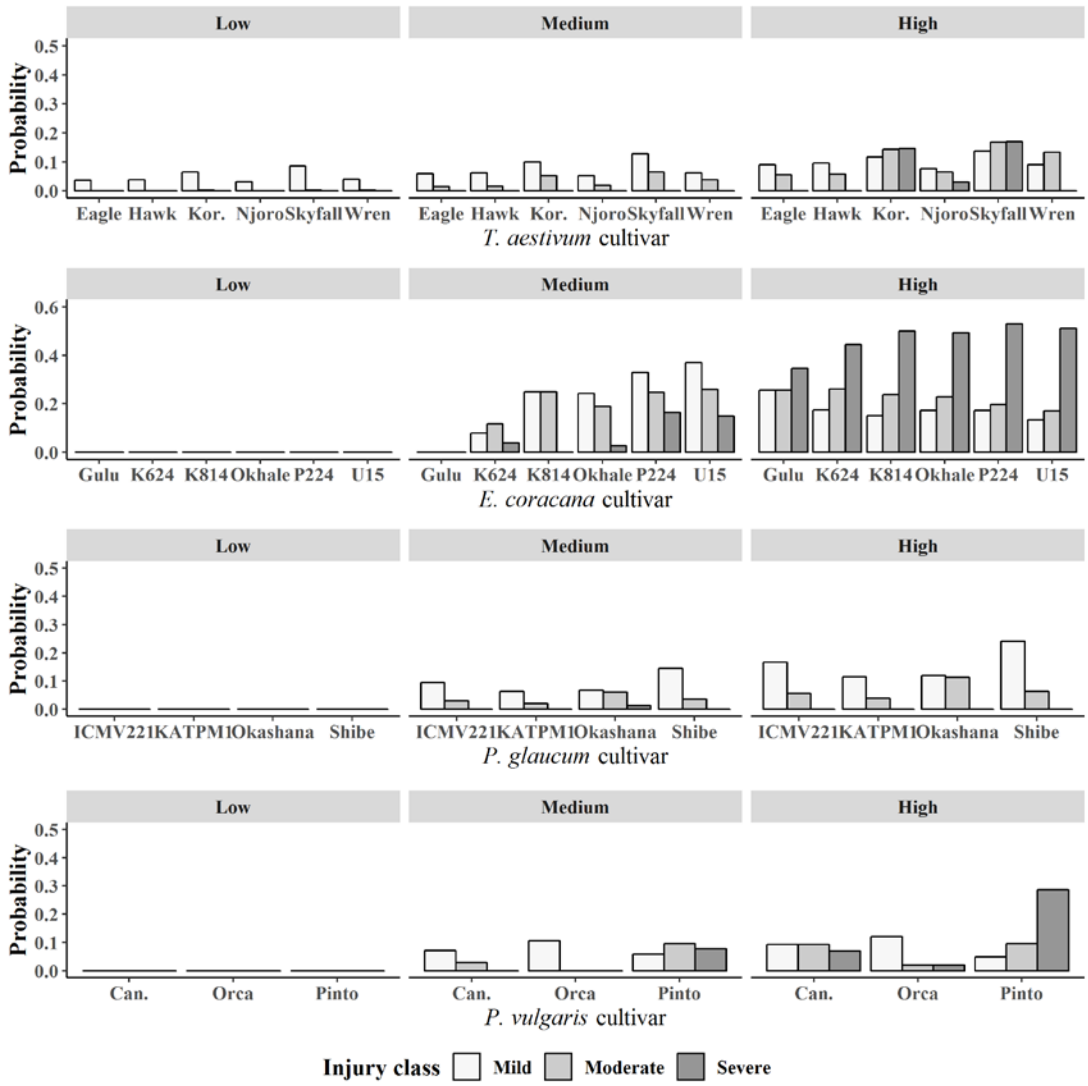

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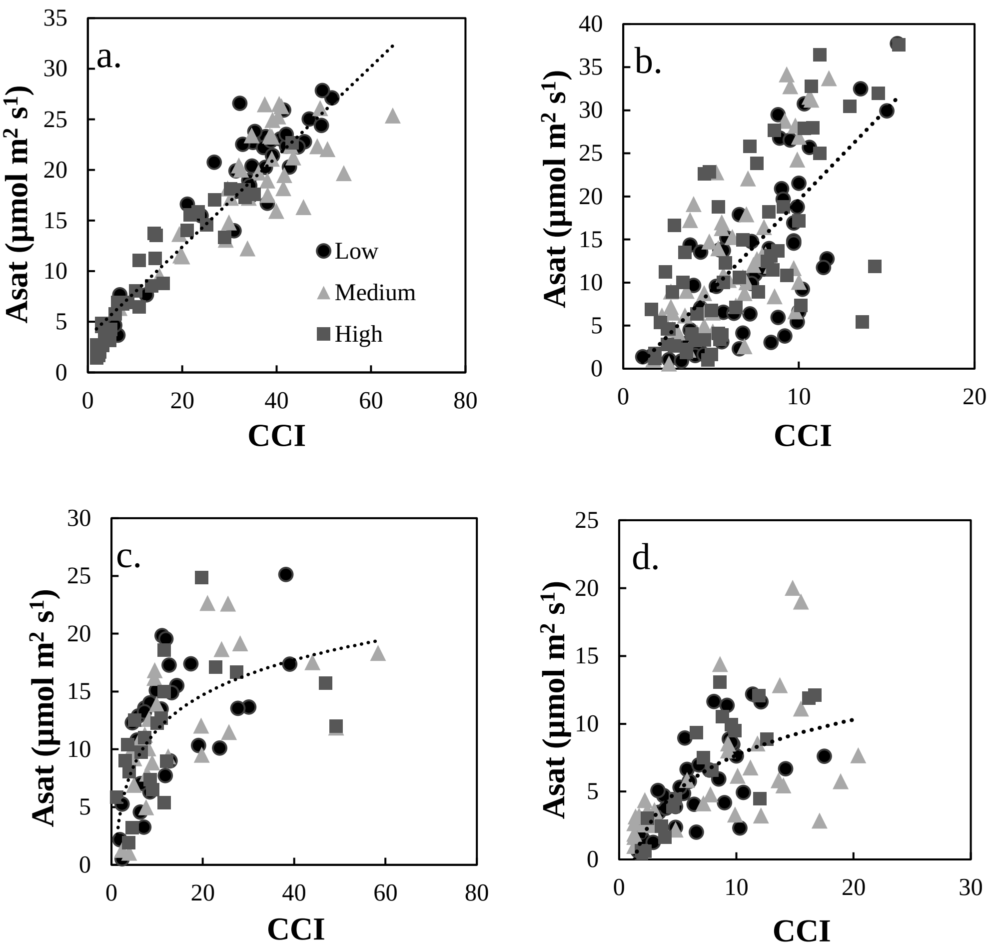

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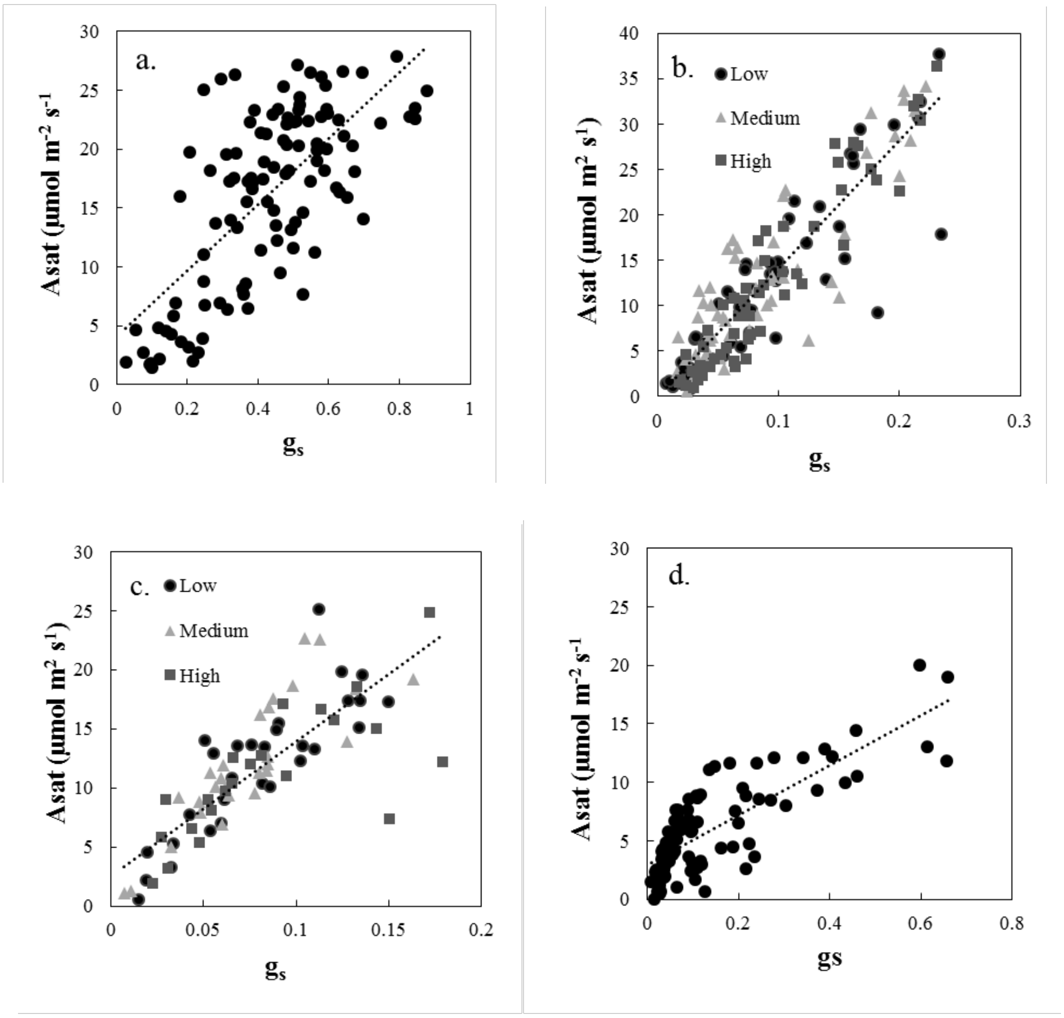

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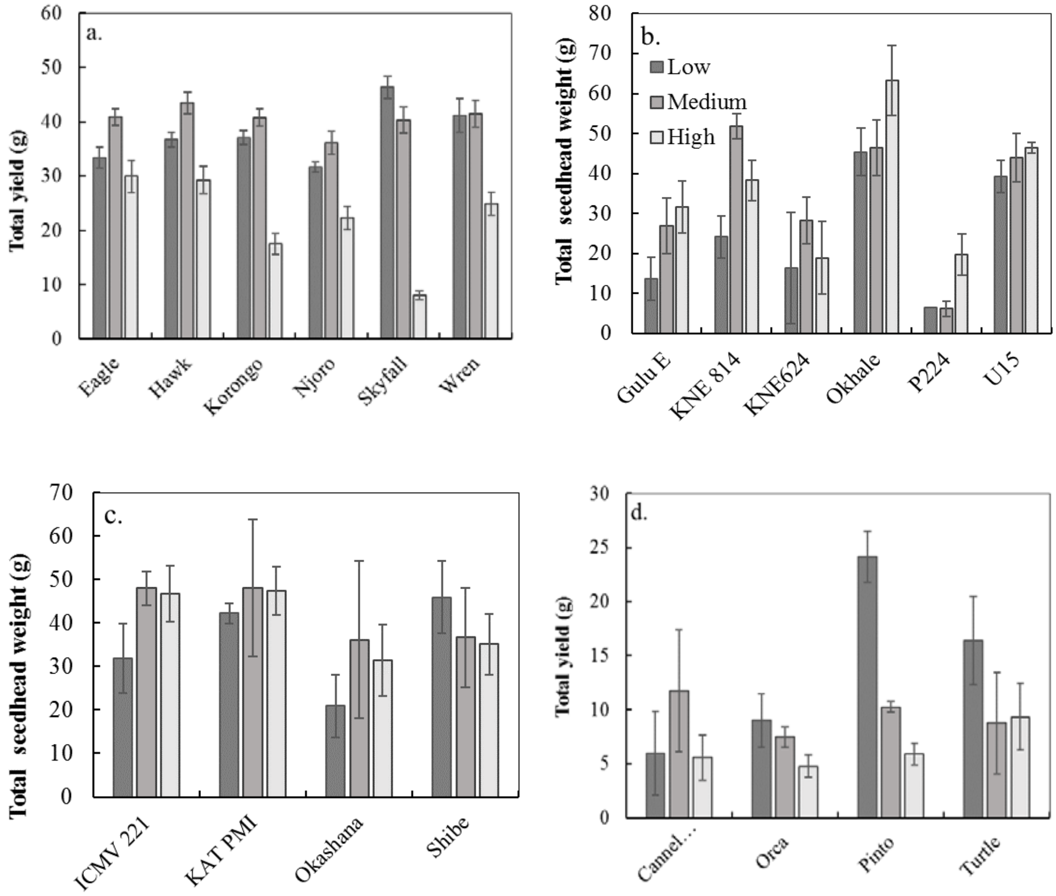

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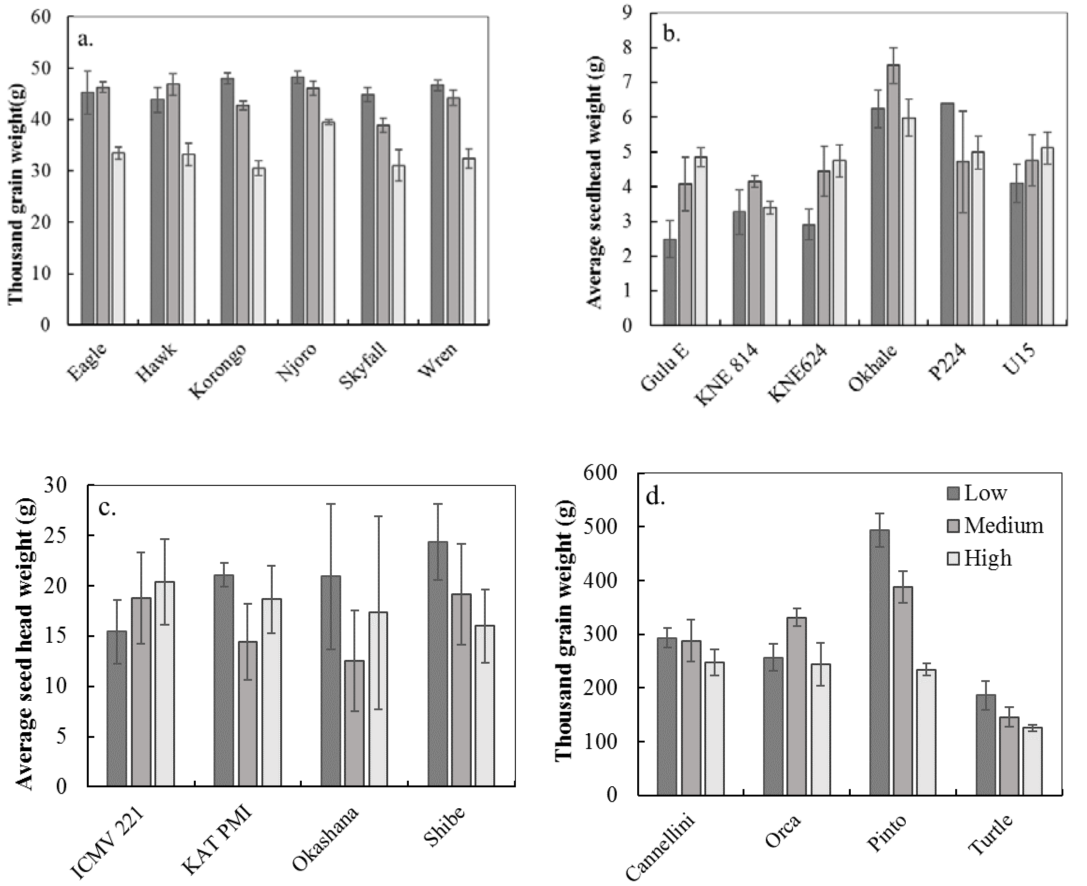

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