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Ozone dose-response relationships for tropical crops reveal potential threat to legume and wheat production, but not to millets

Felicity Hayes*, Harry Harmens, Katrina Sharps, Alan Radbourne

UK Centre for Ecology & Hydrology, Environment Centre Wales, Deiniol Road, Bangor, Gwynedd, LL57 2UW, UK

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ABSTRACT

The tropical-grown crops common bean (*Phaseolus vulgaris*), mung bean (*Vigna radiate*), cowpea (*Vigna unguiculata*), pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*), amaranth (*Amaranthus hypochonriacus*), sorghum (*Sorghum bicolour*) and wheat (*Triticum aestivum*) were exposed to different concentrations of the air pollutant ozone in experimental Solardome facilities. The plants were exposed to ozone treatments for between one and four months, depending on the species. There was a large decrease in yield of protein-rich beans and cowpeas with increasing ozone exposure, partly attributable to a reduction in individual bean/pea weight. Size of individual grains was also reduced with increasing ozone for African varieties of wheat. In contrast, the yield of amaranth, pearl millet and finger millet (all C_4 species) was not sensitive to increasing ozone concentrations and there was some evidence of an increase in weight of individual seedheads with increasing ozone for finger millet. Sorghum did not reach yield, but was not sensitive to ozone based on changes in biomass. Dose-response relationships for these crop species demonstrate that tropospheric ozone pollution could reduce yield of important crops, particularly legumes, in tropical regions such as sub-Saharan Africa.

Introduction

Tropospheric ozone is one of the most important global air pollutants, formed from photochemical production by hydroxyl radical oxidation of molecules including carbon monoxide (CO) and hydrocarbons in the presence of nitrogen oxides (NO_x) [31]. Projections of global emissions show that future ozone concentrations are closely linked to the emissions of precursor molecules [7]. Population growth, increasing urbanisation and the associated increase in transport and industrial infrastructure are anticipated to lead to an increase in ozone pollution, particularly in rapidly developing countries as the production of precursor molecules increases [6]. Ozone precursors, particularly CO, associated with open biomass burning were shown to be a major source of ozone in continental South Africa [23]. Currently, biomass burning is the major contributor of black carbon, organic carbon and smoke aerosols in central Africa [37]. Satellite measurements over the period 1996

* Corresponding author.
E-mail address: fhay@ceh.ac.uk (F. Hayes).

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to 2015 have shown that there is already an increase in surface ozone concentration over central Africa of 2–2.5% per year [24], and the ozone concentrations are anticipated to increase further over coming decades. In regions of low agricultural productivity, emissions of ozone precursors from other sectors are also important and large additions of nitrogen fertiliser across many regions of Africa are expected over the coming decades in order to increase food production [42]. However, this is anticipated to increase soil emissions of NO, and the GEOS-CHEM transport model has been used to show that additions of N based fertiliser of 150 kg ha^{-1} in western Kenya could increase hourly ozone concentrations by up to 2.6 ppb [20].

Detrimental effects of ozone on the growth and yield of crops are well established and information is known about ozone-sensitivity of a range of temperate crops. For example, AOT40-based ozone response functions were developed for 19 temperate crops based on literature synthesis of ozone exposure experiments [28], and a meta-analysis of responses of six crops to ozone showed negative effects on yield even in ambient ozone conditions [10]. The information on crops specific to tropical regions, and for widespread crops growing in tropical conditions is much more limited, but impacts of ozone in current ambient conditions have been shown. In sub-tropical regions of India, ozone-induced visible leaf injury has been shown on crops including rice [36], wheat [14] and soybean [39]. Impacts of ambient ozone on rice have also been shown in the tropical countries of Malaysia [21] and Thailand [2]. Comparatively few ozone exposure studies have been carried out on tropical crop species, with examples including exposure of mung bean (*Vigna radiate* L.), which showed decreased growth and yield with elevated ozone conditions [5]. Exposure of two varieties of cowpea (*Vigna unguiculata* L.) also showed decreased photosynthetic rate, growth and yield with exposure to ozone [40].

Modelling studies have shown yield losses for crops in tropical countries e.g. Africa. For example, global production losses of up to 12% for wheat and up to 16% for soybean due to ambient ozone were shown based on ozone concentration metrics for 2000 [41] and global production losses of 8.5–14% were predicted for soybean, and 3.9–15% for wheat for the year 2000 [3]. However, these studies use dose-response relationships established mostly from European and/or North American data and which may not be representative of other regions [9,11]. In addition, such global modelling studies tend to use a very limited number of species, which are not necessarily the major crops in tropical countries.

It is recognised that risk assessment based on stomatal ozone uptake is preferable to that based on ozone concentration, particularly where meteorological conditions may limit stomatal opening and therefore ozone uptake [25]. This is, therefore, particularly important in tropical regions when a concentration-based analysis could give a very large overestimate of impacts when high temperature are likely to cause stomatal closure. There are a few crops for which information on the response to stomatal ozone uptake is available, including wheat, potato and tomato [29]. In contrast, very little is known about the response of tropical crops to stomatal ozone uptake. Flux-based estimates of global crop losses in particular have so far been based on few species, although these have shown that there is potential for high ozone fluxes to these crops in tropical regions, e.g. for soybean, wheat, maize and rice [30].

In this study, we exposed selected tropical African crop species to ozone in solardomes to test the hypothesis that African crops show a decrease in yield with increasing stomatal ozone uptake. We used a mixture of C_3 and C_4 species (with the product of the first carboxylation reaction being the three-carbon 3-phosphoglyceric acid or the four-carbon oxaloacetate respectively). The response of C_4 crops to ozone has been much less studied that C_3 crops, however, several comparative reviews have indicated reduced sensitivity of C_4 plants to ozone compared to C_3 plants [17,28]. We measured stomatal conductance in order to parameterise a stomatal flux model for each species, and used this data to model stomatal ozone uptake for each crop and treatment combination. We then combined data from different varieties of the same crop to establish dose-response relationships based on stomatal ozone uptake that can be used to improve risk assessment of ozone pollution for tropical agricultural crops.

Materials and methods

Plant material and ozone exposure

Experiments were conducted in solardomes (3 m diameter, 2.1 m height) during 2017 and 2018 at the UK Centre for Ecology & Hydrology (UKCEH) air pollution facility at Abergwyngregyn, North Wales (53.2°N, 4.0°W).

Seeds were planted in pots (6.5 L) filled with John Innes No. 3 compost (J. Arthur Bowers) and were established in solardomes in control conditions for three to five weeks prior to the start of the ozone exposure. Plants were exposed to ozone in heated solardomes (naturally fluctuating ambient temperature +6 ^{0}C) to represent tropical temperature conditions, with the average temperature approximately 25°C. Wheat was exposed in unheated (ambient temperature) solardomes as this tends to be grown in cooler regions. Plants were exposed to a weekly episodic regime with peaks reaching a maximum of 30 ppb, 80 ppb or 110 ppb on five days per week (Fig. 1). There was one solardome per ozone treatment. Therefore, plants within each solardome were moved weekly, and plants and treatments were rotated between solardomes every 4 weeks. The solardomes were ventilated with charcoal filtered air injected with controlled levels of ozone. Ozone was generated from oxygen concentrated from air (Sequal 10) using an ozone generator (G11; Dryden Aqua) and distributed to each solardome via PTFE tubing. Ozone was delivered to each solardome using solenoid valves (Kinesis, UK) using a pulse-width modulation system controlled by computer software (Labview version 2012, Austin, TX, USA). The ozone concentration in each solardome was measured every 30 min using two ozone analysers (Model API 400A, Envirotech, St Albans, UK; Model 49i, Thermo Scientific, MA, USA) of matched calibration. Air temperature, photosynthetically active radiation (PAR) and relative humidity were

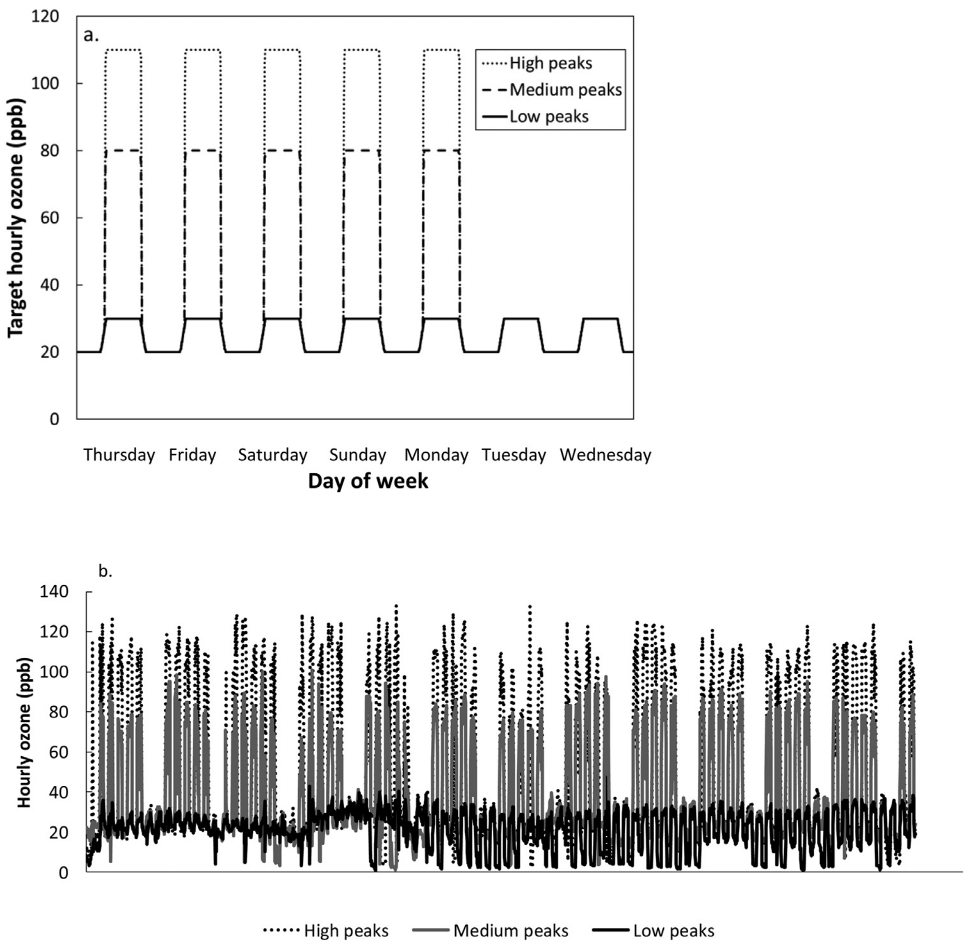

Fig. 1. a) Hourly target ozone concentrations over the course of each week for each ozone treatment in all years, with profile maximum ozone concentrations between 09:00 and 19:00 each day, and b) achieved hourly ozone concentrations over the main experimental period in 2018 (6th June to 31st August, heated domes only) as an example. 2017 data were similar.

also continuously monitored (Supplementary information Table S1). Plants were watered daily or as required, to maintain soil moisture content near field capacity.

In 2017, the crops used were common bean (*Phaseolus vulgaris* cv 'pinto', 'orca', 'black turtle', 'cannellini'), cowpea (*Vigna unguiculata* cv 'blackeye'), mung bean (*Vigna radiata*), pearl millet (*Pennisetum glaucum*), finger millet (*Eleusine coracana*) and wheat (*Triticum aestivum*). Details of the experiments using *P. vulgaris, P. glaucum, E. coracana* and *T. aestivum* in the 2017 experiment are given in Hayes et al. [15]. Varieties of amaranth (*Amaranthus hypochonriacus* cv. 'Pygmy Torch'), sorghum (*Sorghum bicolour* cv IS1004, IS27557), cowpea (*Vigna unguiculata* cv 'Blue goose', 'Hog brains', 'Old timer', 'Razorback', 'Whippoorwill') and bean (*Phaseolus vulgaris* cv 'Mbombo', 'Rajama', 'Tiger') were exposed to ozone in 2018. A summary of the exposure dates of all species is given in Table 1. There were 4 pots per treatments for each species/variety, with the number of plants per pot varying according to the species (*Phaseolus vulgaris 1; Vigna unguiculata 1; Vigna radiata 1; Pennisetum glaucum 2; Eleusine coracana 4; Tricitum aestivum 9; Amaranthus hypochondriacus 4; Sorghum bicolour 2).*

Yield measurements

All cowpea and bean pods were harvested once ripened and dried on the plant. Total pod number and weight, plus bean/pea number and weight were recorded per pot. Seed heads of amaranth, pearl millet and finger millet were harvested, although amaranth had not produced ripe seed at the time of harvest. Sorghum also did not mature to harvest before the autumn weather began, and in this case the whole plant above the soil surface was harvested and weighed for total biomass as flower/seed heads were not present. Wheat ears were removed from the plant when the grains were ripe. Grains were removed from the ears using a hand-held thresher (Minibatt+, Reichhardt, Germany).

Table 1
Summary of the ozone exposure dates of all species (format dd/mm/yyyy).

Species	Variety	Ozone treatment start	Ozone treatment end
Bean	Pinto	01/06/2017	31/07/2017
	Orca	01/06/2017	21/08/2017
	Black Turtle	01/06/2017	02/08/2017
	Cannellini	01/06/2017	25/08/2017
	[A]Mbombo	06/06/2018	23/08/2018
	Rajama	06/06/2018	16/08/2018
	Tiger	06/06/2018	16/08/2018
Cowpea	[A]Black-eye	01/06/2017	21/08/2017
	Blue Goose	06/06/2018	23/08/2018
	Hog brains	06/06/2018	23/08/2018
	Old Timer	06/06/2018	16/08/2018
	Razorback	06/06/2018	16/08/2018
	Whippoorwill	06/06/2018	16/08/2018
Pearl Millet	[A]ICMV 221	24/05/2017	29/06/2017
	[A]KAT PM1	24/05/2017	29/06/2017
	[A]Okashana	24/05/2017	29/06/2017
	[A]Shibe	24/05/2017	29/06/2017
Finger Millet	[A]GuluE	01/06/2017	28/09/2017
	[A]KNE624	01/06/2017	28/09/2017
	[A]KNE814	01/06/2017	28/09/2017
	[A]Okhale	01/06/2017	28/09/2017
	[A]P224	01/06/2017	28/09/2017
	[A]U15	01/06/2017	28/09/2017
Amaranth	Pygmy Torch	06/06/2018	30/08/2018
Sorghum	[A]IS1004	06/06/2018	06/08/2018
	[A]IS27557	06/06/2018	06/08/2018
Wheat	[A]Korongo	18/05/2017	25/07/2017
	[A]Eagle	18/05/2017	25/07/2017
	[A]Njoro	18/05/2017	25/07/2017
	[A]Hawk	18/05/2017	25/07/2017
	[A]Wren	18/05/2017	25/07/2017

[A] indicates an African variety. Additional varieties of some crops were used due to availability/import restrictions.

Ozone stomatal flux calculations

Stomatal conductance measurements were made on amaranth (130 measurements), sorghum (276 measurements), cowpea (626 measurements) beans (333 measurements), pearl millet (1000 measurements), finger millet (1406 measurements) and wheat (255 measurements) during the course of the ozone exposure using a porometer (AP4, Delta-T), with corresponding measurements of soil moisture using a hand-held portable theta probe (ML2x probe attached to HH2 Moisture metre, Delta-T, UK). These measurements were made between 15th June 2017 and 1st August 2017, and 19th June 2018 and 9th August 2018 over a range of times and weather conditions, and were used to parameterise a stomatal flux model for each of the species based on that described by Emberson et al. [8]. Measurements were made on fully expanded leaves near the top of the canopy, and with some paired abaxial:adaxial measurements to allow stomatal conductance to be expressed on a projected leaf area basis. For the parameterisations for the modification of stomatal conductance by light, temperature, VPD and soil water potential (f_{light}, f_{temp}, f_{VPD} and f_{SWP}) respectively, the x-axis was subdivided into segments and for each segment the 90th centile for relative stomatal conductance was calculated. A physiologically relevant curve, as described in Emberson et al. [8] was then fitted to these data points, with curves of the same shape used for C_3 and C_4 species. The values of the constants calculated for these parameterisations are shown in Table S2, and the fitted plots in Figures S6-S12. For wheat, the measured values showed a good fit to the established parameterisations for wheat grown in Mediterranean conditions [13,25] and therefore the Mediterranean wheat parameterisation was used in subsequent calculations in this case. Stomatal ozone uptake above a threshold of 6 nmol m^{-2} PLA s^{-1} (POD_6) for each species was calculated using the stomatal flux model parameterisation for each species, POD_6SPEC, using version 3.03 of the DO3SE model ([8,25], https://www.sei-international.org/do3se). The DO_3SE model uses hourly values of ozone and meteorological conditions to estimate instantaneous stomatal ozone uptake. For this study, soil moisture was assumed to be non-limiting throughout the ozone exposure period as plants were frequently irrigated, although there were some occasions when soil moisture declined prior to watering. POD_6SPEC was summed over the ozone exposure period for each species in each treatment.

For each species/cultivar, the relative yield was calculated by the absolute yield per species, divided by the y-axis intercept of the linear relationship between absolute yield and POD_6SPEC. Following the methodology of Fuhrer [12], straight line extrapolation was used to estimate the yield at zero flux (y-axis intercept) from the measured yield and stomatal flux values for each crop species. Actual yield was then divided by the yield at zero flux to calculate relative yield.

Statistical methodology

All statistical analysis was carried out using R [34]. For the relative yield and grain weight data, all crop cultivars tended to show a similar response to low POD_6SPEC values, with relative values close to 1. However, the plant response tended to vary more between cultivars as POD_6SPEC values increased. This resulted in the violation of the homogeneity of variance assumption, one of the most important assumptions of linear regression modelling. Following Zuur et al., [46], generalised least squares (gls) models were fitted using the R nlme package [33]. The models allow for the inclusion of variance structures, incorporating the heterogeneity in the data into the models. There are a number of different variance structures that can be used, for example the fixed variance structure, the power of the covariate variance structure and the exponential variance structure. All of these structures allow for an increase (or decrease) in residual variation in relative yield along a continuous variance covariate, in this case POD_6SPEC. The optimal model structure was selected by examining the Akaike Information Criterion (AIC) of each model and choosing the model with the lowest value. Models differing in 2–7 AIC units from the top model have little empirical support [4]. Graphical model validation was done using plots of the standardised residuals, to ensure that there was no longer any evidence of heterogeneity. The final effect of POD_6SPEC on relative yield (and 1000 grain weight) was then determined using a Likelihood Ratio Test (LRT) (which gives the AIC value for the model with and without POD_6SPEC, and also a p-value for the POD_6SPEC fixed effect). As plants and treatments were regularly rotated between solardomes, a random effect of solardome was not included in the models.

For wheat, the flux effect relationship for relative yield of the African varieties was compared with that for the older wheat varieties presented in the Modelling and Mapping Manual (MM; [25]). The difference between the flux effect relationship for the African and MM wheat varieties was tested by investigating if there was a difference in the slopes of the two relationships. Gls models including stomatal flux as a continuous predictor, the categorical predictor 'variety' (African or MM) and their interaction were run. If the flux effect relationships (i.e. the slopes) differed between the two datasets, the interaction term would be statistically significant (p-values for model terms were determined using LRTs). This process was repeated to investigate any differences in the flux effect relationship for relative grain weight.

Results

The flux-effect relationships for bean and cowpea were similar, and as both are legumes and with similar growth habitat and growth requirements the data were combined. There was a large decrease in yield with increasing ozone flux (Fig. 2a; $p<0.001$), which was largely due to a decrease in individual bean/pea weight, as indicated by the 1000-bean weight (Fig. 2b; $p = 0.01$), with no ozone effect on the total number of beans/peas (data not presented). For both bean and cowpea there was a wide variation in the response to ozone of the different cultivars, apparent as large 'scatter' in the dose-response relationships for both total yield and 1000-bean weight. Individual flux-effect relationships for bean and cowpea are given in the Supplementary Material (Figures S1 & S2).

The C4 species generally showed an increase in yield or growth with increasing ozone flux. For finger millet there was an increase in total yield with increasing ozone flux (Fig. 3a; $p<0.001$). The average weight of individual seedheads was higher with increasing ozone flux (Fig. 3b; $p = 0.03$). However, there was no significant response of total yield to ozone flux for pearl millet, which had a much shorter duration of ozone exposure during the vegetative phase only (Figure Supplementary S3; $p = 0.05$).

There was a slight increase in total yield (based on seedhead weight) for amaranth, although this was based on only three datapoints and not statistically significant (Figure Supplementary S4; $p = 0.06$). While sorghum did not reach yield during the course of the experiments, the data suggested a slight increase in total biomass with increasing ozone flux, however the combined response of biomass for sorghum and amaranth showed no significant trend (Fig. 4; $p = 0.055$).

African varieties of wheat showed no significant effect of increasing ozone flux on total yield (Fig. 5a; $p = 0.92$) and a decrease in 1000-grain weight (Fig. 5b; $p = 0.03$). As with bean and cowpea, there was a large variation in the response to ozone of the different cultivars, evident as large 'scatter' in the dose-response relationships, particularly for wheat yield (Also see Supplementary Material, Fig. S5). Comparison of the response of the African wheat varieties in this study to the flux-effect relationship derived using European varieties used within the LRTAP Convention [25] suggested that African wheat was less sensitive to ozone flux compared to European wheat in terms of yield ($p = 0.02$ for slope difference), however there was no difference in the slopes for the effect of ozone on 1000-grain weight ($p = 0.59$) (Fig. 5).

Discussion

This study allows improved risk assessment for the impact of ozone pollution on growth of tropical crops, with the results based on re-creating tropical conditions (in terms of temperature and humidity) in ozone-exposure facilities in the UK to enable ozone-sensitivity of crops to be experimentally tested in controlled conditions. The results indicate that there is a risk to protein consumption for subsistence farmers in tropical regions e.g. Africa and India, since the yield of protein-rich beans and cowpeas was reduced by ozone pollution, but the yield of carbohydrate-rich millets was not affected within the concentration range used in this study. In some parts of these regions, diet is already cereal-dominated, with below recommended levels of protein-rich foods such as pulses [43] therefore, if ozone concentrations increase this discrepancy could worsen. Further studies are needed to investigate the impact of ozone pollution on a greater range of tropical crops,

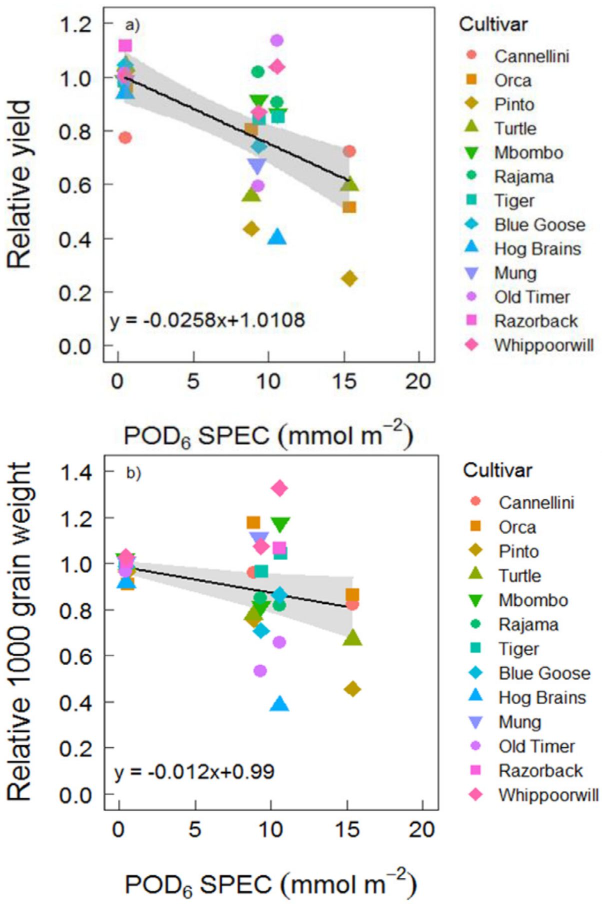

Fig. 2. Ozone flux-effect relationship for a) bean and cowpea yield (based on g/pot) and b) 1000-grain weight ($p<0.001$ and $p = 0.01$). The accumulation time was the ozone exposure duration. Black lines show model fitted values with the 95% confidence interval presented as a shaded bar.

preferably under different climatic conditions and in a range of countries, as current knowledge is very limited. In addition to food crops particularly for subsistence agriculture, it is important to consider potential impacts of ozone pollution on commercial leafy vegetable crops where the monetary value would be reduced if the leaves showed ozone injury symptoms such as extensive yellow mottling, as has been shown for Chinese horticultural crops [45] and giving reduced marketable yield of lettuce in southern Europe [26]. Some horticultural crops grown in Africa are the same as those grown in other parts of the world. Other indirect impacts of ozone in these regions should also be considered, for example, reduced crop cover can increase soil erosion or give opportunities for weed growth, as rates of soil erosion and weed growth both increase when the crop canopy cover is low [27]. There could also be reduced soil fertility as a consequence of decreased nitrogen fixation by legumes, which is particularly important in intercropping and mixed cropping systems [19].

In terms of the data collected, it would be useful to gather further data at different levels of POD_6SPEC to provide more confidence in the ozone-dose response relationships for each species (particularly for African wheat). Variation in response of cultivars to ozone (the 'scatter' in the regression lines) indicates the scope for selecting ozone-resistant varieties in regions at risk from impacts of ozone pollution. It is possible that the large scatter is because historically ozone pollution has been low in the region [18] and, therefore, ozone tolerance has not inadvertently been selected for when selecting new varieties, which can occur if varieties that perform well in ambient conditions are used. In future ozone conditions it may become necessary to select ozone-tolerant varieties in order to minimise production losses, and ozone tolerance should be considered alongside the other stresses [30] that reduce crop yields in tropical agricultural regions. In the current study information from all cultivars was used to generate a flux-effect relationship to indicate the sensitivity to ozone of the crop, however, to set critical levels of ozone for African wheat and tropical crops this would need to be based on the most sensitive cultivars only [25].

This study has shown that for 1000-grain weight, Kenyan varieties of wheat are as sensitive to ozone flux as European varieties, which are considered to be sensitive to ozone pollution [25,29]. This is concerning for wheat production in tropical regions, where predictions using the response of European varieties have shown yield losses due to ozone of >10% in some parts of Sub-Saharan Africa, and >17.5% in some parts of south-east Asia [30]. The relationship for wheat yield of the Kenyan

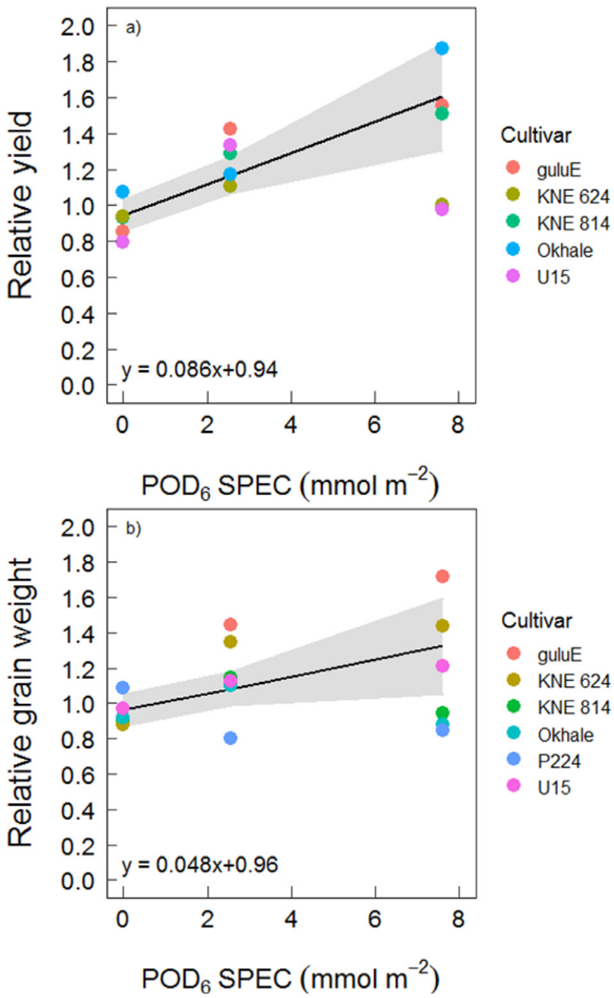

Fig. 3. Ozone flux-effect relationship for a) finger millet yield and b) individual seedhead weight (based on g/pot; $p<0.001$, $p = 0.03$). The accumulation time was the ozone exposure duration. Black lines show model fitted values with the 95% confidence interval presented as a shaded bar.

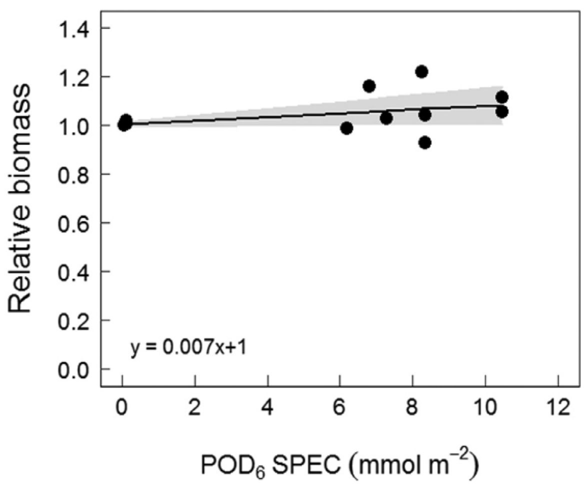

Fig. 4. Ozone flux-effect relationship for sorghum and amaranth biomass (based on g/pot; p = *n*.s.). The accumulation time was the ozone exposure duration. The black line shows model fitted values with the 95% confidence interval presented as a shaded bar.

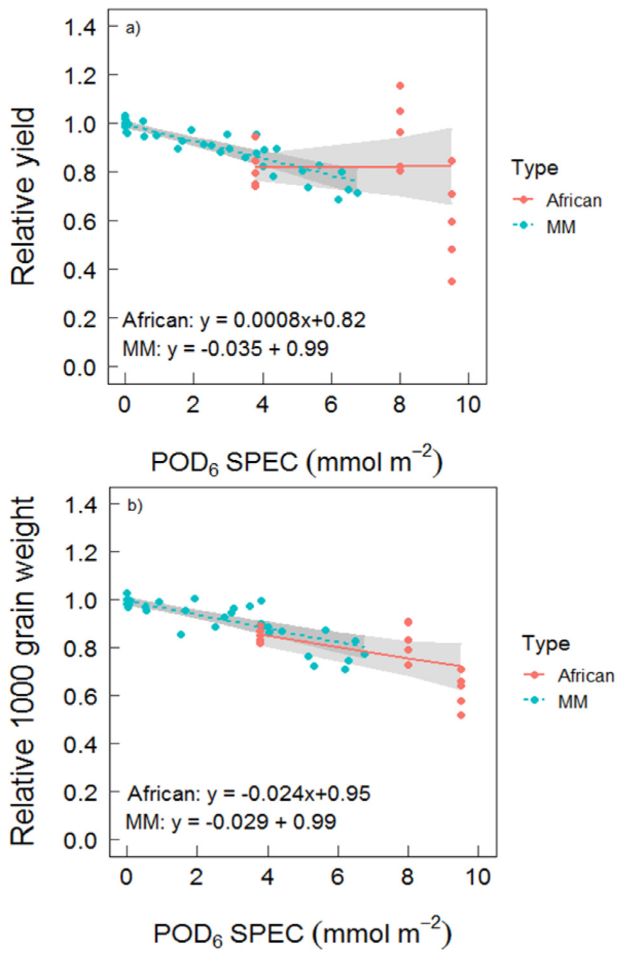

Fig. 5. Ozone flux-effect relationship for wheat yield (based on g/pot) and 1000-grain weight. The accumulation time was the ozone exposure duration. Coloured lines show model fitted values for the two groups of wheat variety and shaded bars are the 95% confidence intervals.

varieties showed a lot of scatter, which may be related to the size of the pots used and a large influence on yield to the number of ears that developed in each pot. In addition, the maximum stomatal conductance of the varieties used in this study was very variable [15]. It would be important to determine whether the relationship between ozone flux and yield in wheat is linear (as is the case for European wheat) or non-linear. The life cycle of these modern Kenyan wheat varieties used was short compared to European wheat, with sowing to harvest in less than three months, implying that these cultivars have rapid photosynthesis and stomatal opening when environmental conditions are favourable, which may also give vulnerability to ozone if concentrations are high. These Kenyan varieties of wheat showed decreased stomatal conductance and decreased grain number per ear with increasing ozone [15].

In the current study, bean and cowpea were also shown to be sensitive to ozone pollution, which is in agreement with previous findings such as Chaudhary and Agrawal [5], using mung beans in open-top chambers in India, and Agrawal et al. [1] in ambient air conditions in India. As beans and cowpeas are the main source of protein for many rural communities relying on subsistence farms [35] it may become necessary for a larger proportion of the farm area to be planted with beans and cowpea in order to compensate for increased production losses due to ozone pollution in the future.

In contrast, the C_4 plants used in this study (pearl millet, finger millet, sorghum and amaranth) showed increased growth/yield with increased ozone exposure in the range of ozone concentrations used. Finger millet and pearl millet had shown visible leaf injury at these ozone concentrations, indicating some sensitivity to ozone, although antioxidant pathways may have been upregulated in response as no decrease in chlorophyll content or photosynthesis was observed [15]. Some C_4 weed plants, such as *Amaranthus palmeri*, have previously been shown to be resistant to ozone pollution [32], which can cause losses for commercial crops when competing with such weeds. Fewer studies have been carried out on the impacts of ozone on C_4 crop plants, however, pearl millet is known to be resistant to a range of biotic and abiotic stresses [38]. The mechanism of tolerance of C_4 plants is unclear, although it has been suggested that there may be crosstalk between abiotic stress response and upregulation of auxin, and this has been shown in sorghum in response to drought and salt stress [44].

There is a need to know whether the growth stimulation in response to ozone for these C_4 plants gives increased vulnerability to other stresses. It is also important to know whether these effects still occur in field conditions, when drought conditions could reinforce stomatal closure and protect the plants from ozone stress. As C_4 plants evolved primarily in the tropics they are particularly well adapted to some abiotic stresses such as high temperature drought and high light intensity, which can cause production of reactive oxygen species. In addition, in these conditions C_4 plants can attain high photosynthetic rates but with reduced stomatal opening, which may limit pollutant uptake compared to C_3 plants in similar conditions.

Previous studies have shown that for drought stress in pearl millet, an effect on yield is most apparent if the stress is after flowering, as phenotypic plasticity can be used to delay flowering in unfavourable conditions [22]. In this study, pearl millet was only exposed to ozone in the vegetative phase, therefore, although no significant effects of ozone on yield were observed there could be an effect of ozone on yield if the stress was imposed during and/or after flowering. It is also important to test whether timing of flowering is altered by ozone in field conditions, as such delays, if prolonged, could delay harvests that need to be completed before a change in season.

This study highlights the potential impact of ozone pollution on production of legumes and is therefore a threat to the nutrition of those people in rural areas relying on subsistence agriculture. Further studies to identify whether there is an impact on crop quality are needed. It is important to validate these findings in field conditions in these regions to better predict the magnitude and location of impacts, for example, in the current study all plants were well-watered and there could be differences in sensitivity to ozone according to water availability particularly in rain-fed conditions, as water deficit could lead to stomatal closure and reduced ozone uptake. Further information would be required in order to enable farmers to adapt their agricultural practices in response to the changing pollution climate, including for example a change in choice of the crop species grown or the variety used and a change in irrigation regime if irrigation is applied [16,30].

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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