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Ozone-induced effects on leaves in African crop species.

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Abstract

Tropospheric (ground-level) ozone is a harmful phytotoxic pollutant, and can have a negative impact on crop yield and quality in sensitive species. Ozone can also induce visible symptoms on leaves, appearing as tiny spots (stipples) between the veins on the upper leaf surface. There is little measured data on ozone concentrations in Africa and it can be labour-intensive and expensive to determine the direct impact of ozone on crop yield in the field. The identification of visible ozone symptoms is an easier, low cost method of determining if a crop species is being negatively affected by ozone pollution, potentially resulting in yield loss. In this study, thirteen staple African food crops (including wheat (*Triticum aestivum*), common bean (*Phaseolus vulgaris*), sorghum (*Sorghum bicolor*), pearl millet (*Pennisetum glaucum*) and finger millet (*Eleusine coracana*)) were exposed to an episodic ozone regime in a solardome system to monitor visible ozone symptoms. A more detailed examination of the progression of ozone symptoms with time was carried out for cultivars of *P. vulgaris* and *T. aestivum*, which showed early leaf loss (*P. vulgaris*) and an increased rate of senescence (*T. aestivum*) in response to ozone exposure. All of the crops tested showed visible ozone symptoms on their leaves in at least one cultivar, and ozone sensitivity varied between cultivars of the same crop. A guide to assist with identification of visible ozone symptoms (including photographs and a description of symptoms for each species) is presented.

Capsule

Thirteen African crop species exposed to an episodic ozone regime showed ozone-induced effects on leaves in at least one cultivar, including visible ozone symptoms, early leaf loss and accelerated senescence.

Keywords

Ozone, visible leaf symptoms, African crops, cultivars, early leaf senescence

Introduction

Tropospheric (or ground-level) ozone (O_3) is a secondary air pollutant, formed from reactions between anthropogenic and biogenic emissions of pre-cursor gases including nitrogen oxides, carbon monoxide, methane and non-methane volatile organic compounds in the presence of sunlight (Simpson et al., 2014). While ozone concentrations have begun to level out in Europe and decline in North America, levels in rapidly developing regions such as East Asia continue to rise, (Mills et al., 2018a). Ozone concentrations are regularly monitored via a network of air quality monitoring stations across South Africa (http://saaqis.environment.gov.za/). High ozone concentrations are observed in many areas within the interior of South Africa, which exceed the South African standard ozone limit (Laban et al., 2018). While there is little measured ozone data for other parts of Africa, model simulations suggest ozone increased over the last four decades in central Africa (Ziemke et al., 2019). Ozone is a transboundary pollutant, for example, dry season ozone concentrations in Rwanda are increased by transport of precursor gases from biomass burning in northern and southern Africa (DeWitt et al., 2019). Ozone concentrations are predicted to continue to increase in developing regions in the future (Turnock et al., 2018) unless precursor emissions are further controlled.

Ozone is a phytotoxic pollutant, entering plant leaves via the stomatal pores (current understanding reviewed by Emberson et al., 2018). Ozone dissolves in the apoplastic fluid of cells to form ‘reactive oxygen species’ (ROS) (Fiscus et al., 2005). Plants have some ability to detoxify these harmful reactive biomolecules with antioxidants (for example ascorbic acid, Severino et al., 2007). However, during acute exposure, when ozone concentrations are high (for example, during “ozone episodes”), and ROS exceeds cell detoxification capacity, further ROS production is triggered, leading to a cycle of oxidative cell death (Kangasjärvi et al., 2005). These processes can result in visible symptoms on the leaf surface, including spotting (stipples), mottling, yellowing, bronzing, eventually leading to necrosis. While ozone symptoms can vary slightly between species, symptoms on the leaf surface tend to have a number of typical and distinctive features (Schaub & Calatayud, 2013). Visible symptoms appear on the upper leaf surface, between leaf veins, as tiny yellow, black or purple-red spots. Older leaves show more severe symptoms than younger leaves as the severity is determined by the accumulated stomatal flux of ozone into leaves (age effect). Leaves with severe ozone symptoms can show signs of necrosis, with spots joining to form large patches on the leaf surface. When ozone exposure is chronic, i.e. continued exposure over weeks or months, accelerated senescence (leaf aging) can occur, eventually leading to premature leaf abscission. For example, Pleijel et al. (1997) demonstrate loss of chlorophyll with time similar to normal senescence proceeding faster in ozone treatments for spring wheat (*Triticum aestivum* L.).

The formation of visible ozone symptoms on leaves can have economic consequences for crop production. Visible damage to ozone sensitive leafy crops, including spinach and lettuce, can negatively affect crop quality and therefore market value, with potential financial implications for farmers (Zhao et al., 2011). Ozone pollution can also cause reductions in crop yield, for example the duration of green leaf area after anthesis is an important factor in determining the final grain yield of wheat (Pleijel et al., 1997). The negative effect of ozone on crop yield has been demonstrated experimentally for a number of globally important crop species, including wheat (Pleijel et al., 2018) and rice (*Oryza sativa*) (Shi et al., 2009). Crop species vary in sensitivity to ozone. Using modelled ozone uptake data and flux-effect relationships, Mills et al. (2018b) predicted average annual global yield losses of 12.4%, 7.1%, 6.1% and 4.4% for soybean (*Glycine max*), wheat, maize (*Zea mays*) and rice respectively. Different cultivars of the same crop can also show varying sensitivity to ozone in terms of crop yield, e.g. Chinese rice (Shi et al., 2009) and African wheat cultivars (Hayes et al., 2019). In the absence of crop yield data, biomonitoring can be used to gather data on visible ozone symptoms as an indication of the level of ozone damage to vegetation (Hayes et al., 2007), under the consideration of species-specific sensitivity and microclimatic conditions.

Visible symptoms on plant foliage can be caused by a variety of biotic and abiotic stresses, including ozone, drought, nutrient deficiency, insects, or bacterial and fungal infections (Günthardt-Goerg & Vollenweider, 2007). Visible ozone symptoms can be difficult to identify with certainty in some cases (Bussotti et al., 2003). It is also possible for crops to show visible ozone symptoms without a subsequent negative effect on yield for some cultivars (e.g. rice, Sawada & Kohno, 2009; common beans (*Phaseolus vulgaris*), Hayes et al., 2019).

However, the identification of visible ozone symptoms is a quick and relatively simple way to tell if a plant is potentially being damaged by ozone. The use of biological monitoring enables a low cost method for assessing the impact of ozone on vegetation in the field on a large scale (Francini et al., 2009). There are records of ambient ozone concentrations inducing visible ozone symptoms from all over the world, including records on over 30 crop and 80 semi-natural vegetation species from 16 countries in Europe (Hayes et al., 2007), extensive biomonitoring for forest species in Europe (Schaub & Calatayud, 2013; Schaub et al., 2018) and the USA (Smith, 2012), studies in Brazil (e.g. Moura et al., 2018) and numerous studies from Asia (e.g. Chaudhary et al., 2013, Feng et al., 2014). However, there is little documented for crop species that are important in Africa, particularly which include photo-guides and a detailed description of visible ozone symptoms.

This study will investigate ozone-induced effects on leaves in a range of African staple crops, including a) visible symptoms on leaves in a variety of species; b) demonstrating how visible ozone symptoms and leaf number can change with time over the season using common bean cultivars exposed to differing ozone levels as an example; c) investigating how the rate of senescence varies between different cultivars using African wheat exposed to differing ozone levels as an example.

Materials and Methods

Crop growing

During the summer (~May – Sept.) growing seasons of 2017 - 2019, cultivars of wheat (n = 5 Kenyan cultivars; ‘Kenya Korongo’, ‘Kenya Wren’, ‘Kenya Hawk 12’, ‘Eagle 10’, ‘Njoro BWII’), pearl millet (*Pennisetum glaucum*; n= 4 Kenyan cultivars; ‘Okashana’, ‘Shibe’, ‘ICMV221’, ‘KATPM1’), finger millet (*Eleusine coracana;* n= 6 Kenyan cultivars; ‘GuluE’, ‘P224’, ‘KNE624’, ‘KNE814’, ‘U15’, ‘Okhale’), barley (*Hordeum vulgare*; n = 1; ‘Propino’), sorghum (*Sorghum bicolor*; n =2 cultivars; ‘IS1004’, ‘IS27557’), chickpea (*Cicer arietinum*; n =1, ‘ICC 15333’), peanut (groundnut) (*Arachis hypogaea*; n =2 cultivars; ‘Negrito’, ‘Tennessee Red’), amaranth (*Amaranthus hypochondriacus*; n =1 cultivar; ‘Pygmy Torch’), common bean; n= 4 cultivars in 2017; ‘Black Turtle’, ‘Cannellini’, ‘Orca’, ‘Pinto’; 3 in 2018; ‘Mbombo’, ‘Rajama’, ‘Tiger’), mung bean (*Vigna radiate*; n = 1 unspecified cultivar originating from India), cowpea (*Vigna unguiculata*; n = 6 cultivars; ‘Black-eye’, ‘Blue Goose’, ‘Hog Brains’, ‘Old Timer’, ‘Razorback’, 'Whippoorwill’) and sweet potato (*Ipomoea batatas*; n= 1; ‘Erato Orange’) were grown in solardomes (hemispherical glasshouses of 3 m diameter and 2.1 m height), situated in Abergwyngregyn, near Bangor, North Wales, UK. During late summer of 2019, maize (*Zea mays*, ‘Incredible F1’ cultivar) was also grown as a pilot study (see Supplementary Material, Fig. S1). For details on the sources for seeds used, see the Supplementary Material. Cereal crop seeds were sown in 6.5 litre pots (diameter 21 cm, height 25 cm), beans were planted in 7.5 litre pots (diameter 26 cm, height 21 cm), and sweet potatoes were planted in 25 litre tubs (35 cm, height 37 cm). All pots were filled with John Innes No. 3 soil based compost (J. Arthur Bowers, UK). Plants were kept well-watered for the duration of the growing season. Four replicate pots were used per cultivar for each ozone treatment. To simulate tropical temperature conditions, heated solardomes (with a temperature increase of approximately 7˚C above ambient) were used for all species except for wheat (which was grown in unheated domes, as wheat tends to be grown at higher altitudes and cooler temperatures). Plants were established in the solardomes, under the same temperature, light, humidity and ventilation conditions as during the ozone exposure. Ozone conditions during this establishment phase were those of the ‘low’ ozone treatment. Ozone exposure treatments began when plants reached the vegetative phase (~3-5 weeks after sowing, depending on the crop).

Ozone exposure

During each growing season (2017-2019), plants in the solardomes were exposed to an episodic ozone regime, following a profile that might be experienced in agricultural areas of Sub-Saharan Africa (based on profiles experienced in the Mediterranean area of Europe in the 1990s and 2000s, e.g. Hayes et al., 2007). Due to a lack of observed data, it is difficult to predict ozone concentrations for Africa, however 2019/2020 data from the South African air pollution monitoring network (http://saaqis.environment.gov.za/; accessed 1st June 2020) shows maximum daily ozone concentrations above 100ppb during the crop growing season (for wheat and beans) in states with high crop production (FAOSTAT, 2017). Ozone was generated using an oxygen concentrator (G11, Ozone Industries Ltd ozone generator and Sequal 10, Pure O_2 oxygen concentrator respectively). In all solardomes, ozone was added to charcoal filtered air to give the desired concentrations. Three ozone treatments were used (low, medium and high ozone) (Fig. 1). Mean ozone values for the ambient and heated domes for low, medium and high ozone in 2017, 2018 and 2019 are presented in the Supplementary Material (Table S1). Plants were exposed to ozone for the duration of the growing season (with the exception of pearl millet, which grew very quickly and had to be moved to a greenhouse at ambient ozone concentration with more space after 5 weeks of ozone exposure). Due to the different growth cycle of the different species used, this meant that the ozone exposure length varied between species (Table S2).

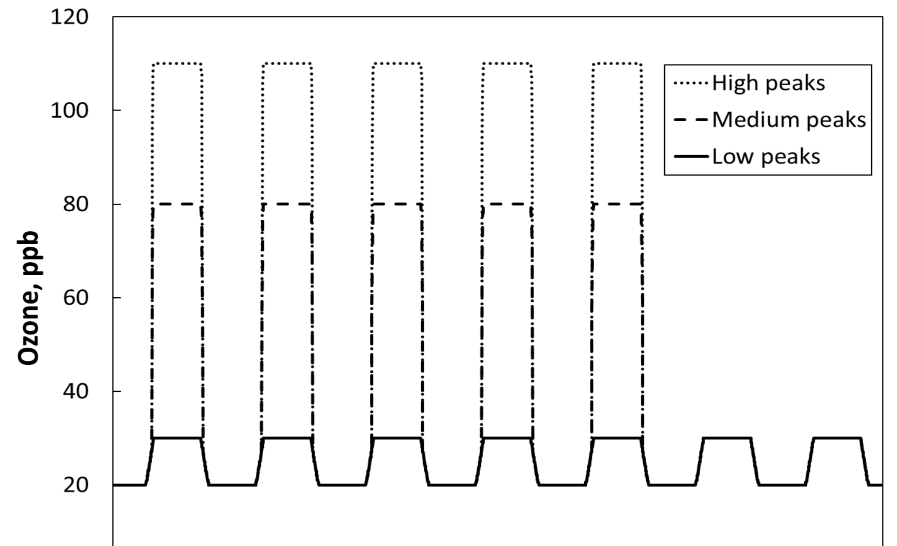


Figure 1. Target diurnal ozone exposure profiles for all years of the experiment. (Target concentrations in the heated and unheated solardomes were the same).

Climatic conditions

The temperature regime used in the heated domes aimed to represent those of African countries such as Kenya, which has daily mean temperature between 20 and 28°C. Temperature and relative humidity were continuously measured (Skye Instruments) in one ambient temperature solardome (2017) and three heated solardomes (2017-2019) (Table S3). Photosynthetically Active Radiation (PAR) was continuously monitored (Skye Instruments) in one ambient temperature dome (Table S3). Climatic conditions and airflow rates were matched between all solardomes, however to minimise any chamber effects, plants and ozone treatments were also moved between solardomes every four weeks. For further details on the methodology, see Hayes et al. (2019).

Assessments of visible ozone symptoms on leaves

Throughout the ozone exposure period, the plants were regularly inspected for visible ozone symptoms on leaves. The following criteria were used to identify ozone symptoms: i) symptoms occurred between leaf veins; ii) older leaves showed more severe symptoms than younger leaves (age effect); and iii) symptoms occurred on the upper leaf surface and tended not to be visible on the lower leaf surface. Weekly assessments were made for wheat, pearl millet, finger millet, mung bean, common bean, cowpea and sweet potato, while ad hoc assessments were made on the other 6 species. Photographs were taken of ozone symptoms for each crop species. During weekly assessments, the presence of leaves in the following categories was recorded: ‘healthy,’ (no ozone symptoms present); ‘mild ozone symptoms’, (<5% of the leaf showing symptoms); ‘moderate ozone symptoms’ (5-25% of the leaf showing symptoms) and ‘severe ozone symptoms’ (>25% of the leaf showing symptoms). More detailed assessments were carried out on common bean and wheat to quantify the extent and progression of visible ozone symptoms on leaves. Detailed assessment for all thirteen crop species was beyond the scope of this study. Leaf counts were carried out on the bean cultivars at weekly intervals. All leaves per plant were counted, and then categorised as above. Changes in the flag leaves of the wheat plants were also recorded weekly, including '% visible ozone symptoms' and '% senescence.' The flag leaf is the final leaf to emerge on a wheat plant. The protection of the flag leaf is important for attaining high grain yield, with early senescence of the flag leaf leading to shorter grain filling duration (Gelang et al., 2000). Four flag leaves were assessed per wheat cultivar, in each ozone treatment.

Statistical analysis

All statistical analyses were run using R (R Core Team, 2018). Following Zuur et al. (2009), top down model selection with examination of Akaike's Information Criterion (AIC) was used to choose the optimal model in each analysis. The model with the lowest AIC is optimal, and models differing in <2, 4-7 and >10 from the top model have substantial, considerably less and no support respectively (Burnham & Anderson, 2002).

Following Hayes et al. (2019), multinomial logistic regression modelling was used to determine if the number of leaves with no, mild, moderate and severe ozone symptoms varied between low, medium and high ozone treatments for four cultivars of common bean. The 'multinom' function from the 'nnet' (Venables & Ripley, 2002) R package was used, with a categorical response variable based on the counts of each ozone symptom category. Model predictor variables were ozone treatment and crop cultivar. Using results from the optimal model, the predicted probabilities of the likelihood of counts being in each category were plotted. Post-hoc testing (using paired t-tests) compared the simulated predicted probability values per cultivar for a specified level of visible ozone symptoms. The analysis was repeated three times using data collected after a) 8, b) 19 and c) 34 days of ozone exposure.

Changes in the total leaf count of common bean cultivars with time (day 8 to day 52 of ozone exposure in 2017) at low, medium and high ozone were analysed using a generalised linear mixed effect model (GLMM) (R package lme4 (Bates et al., 2015)), with a Poisson error distribution and a random effect of pot ID (to control for repeated measures from the same pot). The predictor variables in the model were ozone level (categorical) and 'days of exposure' (continuous) and an interaction term between these variables. A covariate for the initial number of leaves on each plant was also included, to control for the starting size of the plant. Separate model sets were run for each cultivar. For Orca, Pinto and Turtle cultivars, a quadratic term was included for the 'days of exposure' variable. The 'Anova' function from package 'car' (Fox & Weisberg, 2019) was used to obtain p-values for model variables, and differences between ozone levels were assessed using the Wald Test. Model diagnostics including Pearson and Deviance residuals were examined, following Zuur et al. (2009). Where model over-dispersion was detected, an observation level random effect was included in the model.

Using the data for wheat flag leaf assessments done after 7 weeks of ozone exposure, a generalised linear model (GLM) (quasi-binomial error, to deal with over-dispersion) with % senescence on the flag leaf as the response variable and ozone level, cultivar and their interaction as categorical predictors was run. The MuMIn package (Bartoń et al., 2019) was used to calculate quasi-AIC (QAIC), using the dispersion parameter estimated from the global model. The 'Anova' function from package 'car' (Fox & Weisberg, 2019) was used to obtain p-values for model variables. The 'emmeans' package (Lenth, 2019) was used to investigate the differences between cultivars in each ozone treatment.

Results

Visible ozone symptoms

All crop species exposed to elevated ozone showed visible ozone symptoms in at least one cultivar (Fig. 2). Further detail on when symptoms first appeared is given in the Supplementary Material, Table S4. Symptoms varied slightly between crop species, but all showed the distinctive characteristics of ozone exposure, and symptoms worsened with duration and concentration of ozone exposure (Table 1). Some species also showed leaf symptoms that were not due to ozone, for example due to red spider mites (See Supplementary Material, Fig. S2).

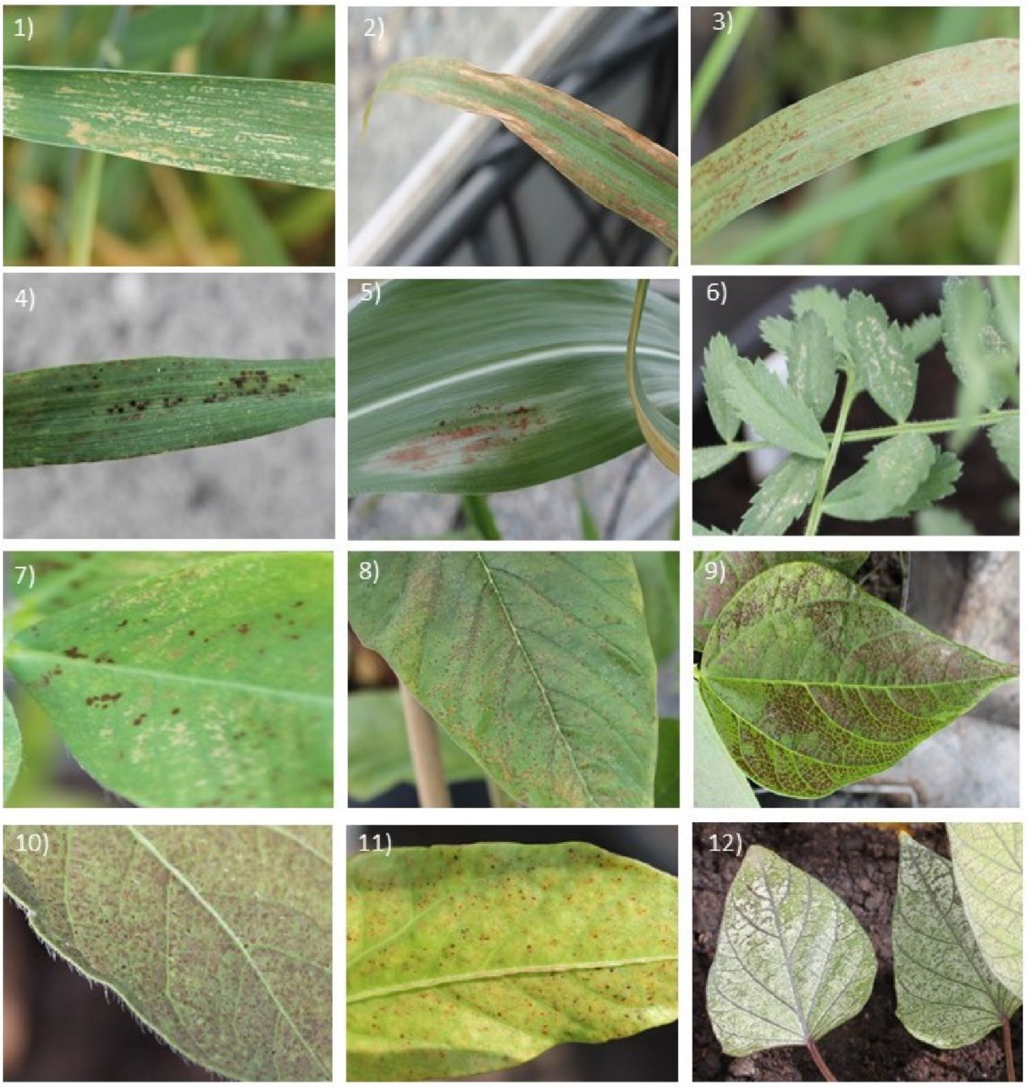


Figure 2. Visible ozone symptoms in African crop species (photographs shown are representative of visible ozone symptoms per crop): 1) Common wheat (*Triticum aestivum*) cv. 'Korongo'; 2) Pearl millet (*Pennisetum glaucum*) cv. 'KATPM1'; 3) Finger millet (*Eleusine coracana*) cv. 'UM15'; 4) Barley (*Hordeum vulgare*) cv. 'Propino'; 5) Sorghum (*Sorghum bicolor*) cv. 'IS1004'; 6) Chickpea (*Cicer arietinum*) cv. 'ICC 15333'; 7) Peanut (*Arachis hypogaea*) cv. 'Negrito'; 8) Amaranth (*Amaranthus hypochondriacus*) cv. 'Pygmy Torch'; 9) Common bean (*Phaseolus vulgaris*) cv. 'Cannellini'; 10) Mung

bean (*Vigna radicate*) cv. unspecified; 11) Cowpea (*Vigna unguiculata*) cv. ‘Old Timer’; 12) Sweet potato (*Ipomoea batatas*) cv. ‘Erato Orange’.

Table 1. Description of visible ozone symptoms for African crop species exposed to elevated ozone levels in solardomes. See the Supplementary Material (Fig. S3) for additional photographs of visible ozone symptoms, and how they change with accumulated stomatal flux of ozone into leaves.

Common name	**Scientific name**	**Symptom description**
Common wheat	*Triticum aestivum*	Pale yellow/white patches on upper leaf surface of older leaves. With accumulation of ozone exposure, patches worsen in severity and join together. Senescence is accelerated, leading to chlorosis and death of cells.
Pearl millet	*Pennisetum glaucum*	Appearance of fine, rusty brown stippling on upper leaf surface. This stippling becomes extensive, across the whole upper leaf surface (on either side of the main leaf vein). Stippled patches eventually become necrotic.
Finger millet	*Eleusine coracana*	Extensive rusty brown stippling on upper leaf surface. With time and continued ozone exposure, necrotic brown patches can appear on older leaves. Brown stippling can also change colour to white (as leaf cells die) later in the season.
Barley	*Hordeum vulgare*	Dark brown/black spotting on upper leaf surface. The spotting begins to join together into patches with time, and as leaf tissue dies, yellow/brown necrotic patches appear.
Sorghum	*Sorghum bicolor*	Rusty brown spotting on upper leaf surface. With time, spots join to form red/brown patches.
Chickpea	*Cicer arietinum*	Fine white stippling on upper leaf surface. With continued ozone exposure, white patches become bigger, with older leaves showing extensive white areas of the leaf.
Peanut (groundnut)	*Arachis hypogaea*	Symptoms first appear as dark brown spotting on the interveinal areas of the upper leaf surface. Pale yellow/white spotting then appears extensively, between the leaf veins, leading to chlorosis and necrotic patches.
Amaranth	*Amaranthus hypochondriacus*	Brown spotting between leaf veins on upper surface of older leaves, which leads to extensive brown/yellow patches. Also chlorosis and early senescence occur.
Common bean	*Phaseolus vulgaris*	Light/dark brown stippling appears gradually. With continued ozone exposure, brown patches join together, leaving the upper surface of older leaves extensively covered. Leaf veins remain green. Patches turn necrotic with time.
Mung bean	*Vigna radiata*	Appearance of dark brown stippling on upper leaf surface of older leaves. With time, upper leaf surface becomes extensively covered.
Cowpea	*Vigna unguiculata*	Brown/reddish brown stipples between veins of upper leaf surface. Gradually join together to cover patches of the leaf surface with continued ozone exposure.
Sweet potato	*Ipomoea batatas*	Extensive white patches on upper leaf surface, between leaf veins. These leaves were found to die and fall from the plant quickly, and newly grown leaves did not show further specific visible symptoms, despite continued ozone exposure. Leaves in the higher ozone treatments showed early senescence throughout the ozone exposure.

Changes in visible leaf symptoms with time

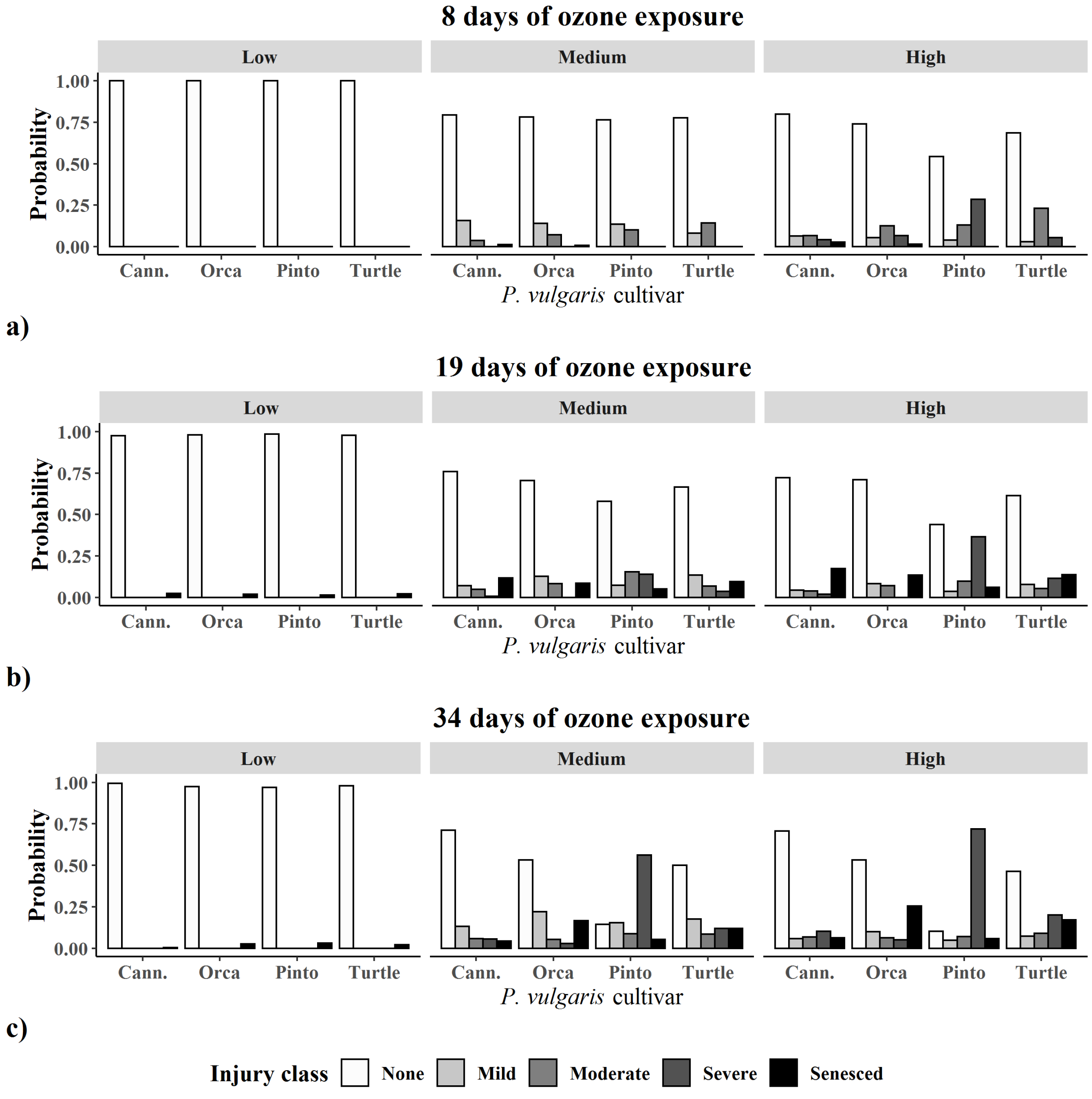


Figure 3. Changes in ozone symptoms with time for leaves of common bean (*Phaseolus vulgaris*) cultivars, showing the model predicted probability of leaves being in the following categories: 'None' = No visible ozone symptoms recorded; 'Mild' = <5% of the leaf showing ozone symptoms; 'Moderate' = 5-25% of the leaf showing ozone symptoms; 'Severe' = >25% of the leaf showing ozone symptoms, after a) 8; b) 19 and c) 34 days of ozone exposure at low, medium and high treatment levels (n = 4 replicates per cultivar per treatment) during the 2017 growing season. "Cann." = 'Cannellini', "Turtle" = 'Black Turtle'.

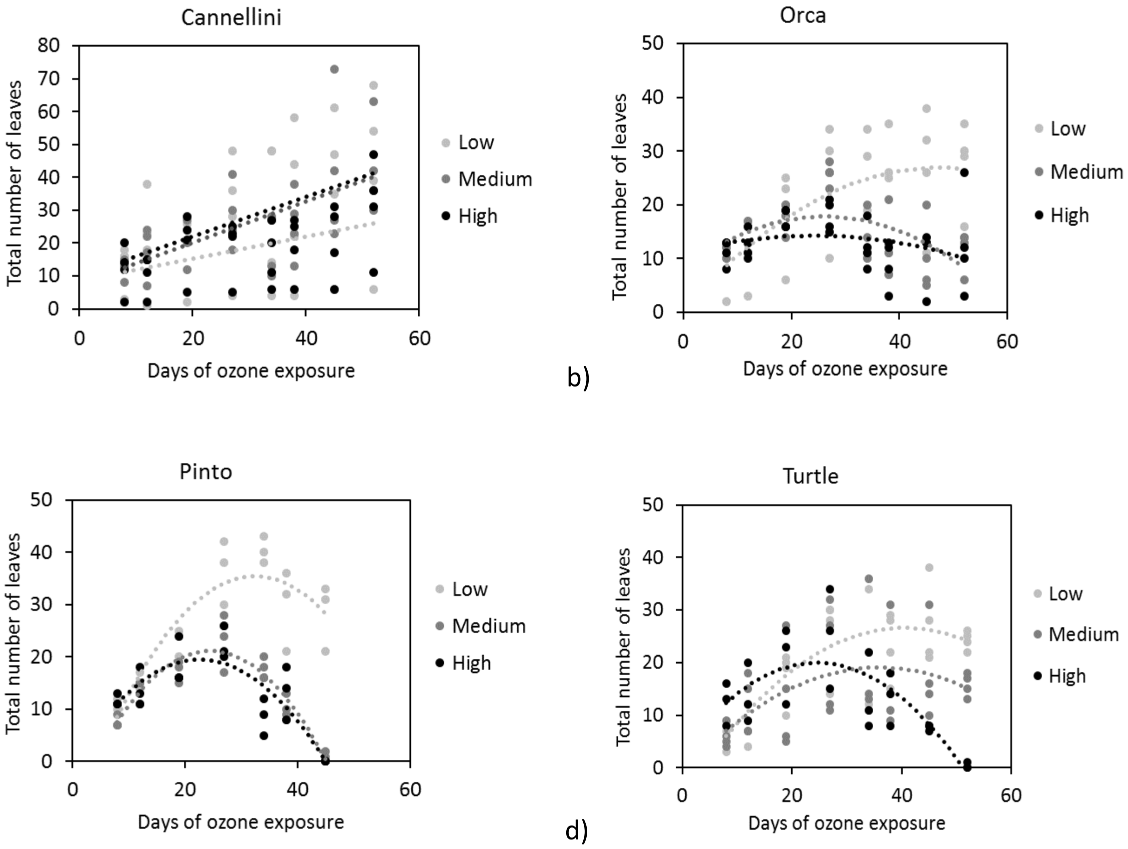
For each of the three time points examined, bean cultivars responded in the same way to the ozone treatments (optimal models did not include an ozone * cultivar interaction). All cultivars showed an increased predicted probability of mild and moderate ozone symptoms in the medium ozone treatment (compared to low ozone), while predicted probabilities of severe symptoms (and senescence for days 19 and 34) increased steadily from low to high ozone. There was, however, a difference in ozone sensitivity between cultivars, for all time points (Fig. 3).

All bean cultivars showed visible ozone symptoms after only 8 days in the medium and high ozone treatments (Fig. 3a). In medium ozone, cultivars behaved similarly. ‘Turtle’ beans had a slightly higher predicted probability of moderate ozone symptoms (‘Turtle’ > ‘Pinto’ > ‘Orca’ > ‘Cannellini’, $p < 0.001$). In the high ozone treatment, ‘Pinto’ beans had the highest predicted probability of severe ozone symptoms (‘Pinto’ > ‘Orca’ > ‘Turtle’ > ‘Cannellini’, $p < 0.001$). ‘Turtle’ beans showed primarily moderate symptoms on leaves, while predictions for ozone symptoms in ‘Orca’ and ‘Cannellini’ were low in all categories. There were no visible ozone symptoms or senesced leaves in the low ozone treatment.

After 19 days, all cultivars began to show leaf senescence (Fig. 3b). There were no ozone symptoms and only very low levels of senescence in the low ozone treatment. In medium ozone, again cultivars were behaving similarly. ‘Pinto’ beans had a higher predicted probability of severe symptoms than the other cultivars (‘Pinto’ > ‘Turtle’ > ‘Cannellini’ > ‘Orca’, $p < 0.001$). In high ozone, there were minor levels of ozone symptoms in the ‘Cannellini’ and ‘Orca’ cultivars, with the ‘No symptoms’ and ‘Senesced’ leaves categories showing the highest predicted probabilities. Predicted probability of severe ozone symptoms was high for ‘Pinto’ beans (30%), while ‘Turtle’ beans showed similar levels of severe symptoms and senesced leaves, but the majority of leaves were predicted to show no ozone symptoms. For the severe ozone symptoms category at high ozone, cultivar sensitivity was in the order ‘Pinto’ > ‘Turtle’ > ‘Cannellini’ > Orca ($p < 0.001$).

After 34 days of ozone exposure, there was no predicted probability of ozone symptoms in the low ozone treatment with the majority of leaves still showing no symptoms and only very low levels of senescence (Fig. 3c). In medium ozone, ‘Pinto’ beans had the highest predicted probability of severe symptoms (>50%) (‘Pinto’ > ‘Turtle’ > ‘Cannellini’ > ‘Orca’, $p < 0.001$). ‘Orca’ beans had the highest predicted probability of senesced leaves ($p < 0.001$) and only mild levels of ozone symptoms, while the probability of counting senesced leaves for ‘Cannellini’ after 34 days was lower than at 19 days. The senesced leaves were dropping from the ‘Cannellini’ plants and new green leaves were growing in their place. In the high ozone, ‘Pinto’ again had the highest predicted probability of severe ozone symptoms (70% leaves) (‘Pinto’ > ‘Turtle’ > ‘Cannellini’ > ‘Orca’, $p < 0.001$). Predictions for the other cultivars suggested that the majority of leaves remained without symptoms. Again, ‘Orca’ beans had the highest predicted probability for senesced leaves.

Changes in total leaf number with time



a) b)

c) d)

Figure 4. Changes in total leaf number with increasing days of ozone exposure (day 8 to day 52 of exposure at low, medium and high ozone, n = 4 replicates per cultivar per ozone treatment) during the growing season for common bean (*Phaseolus vulgaris*) cultivars: a) 'Cannellini'; b) 'Orca'; c) 'Pinto'; d) 'Turtle'.

The effect of ozone exposure on leaf number varied with *P. vulgaris* cultivar (Fig. 4). There was also variation between replicates for the 'Cannellini', 'Orca' and 'Turtle' cultivars, resulting in scatter in the data (Figs. 4a, b &d). This may be due to differences in seed quality or germination rate, which could affect plant size. The covariate for 'Initial leaf number' was not present in the optimal model for any of the cultivars therefore there was no need to control for the starting size of the plants. For 'Cannellini' beans, leaf number steadily increased with time for all ozone treatments (the optimal model included only the 'Days of exposure' variable, $p<0.001$) (Fig. 4a). There was no interaction between ozone treatment and days of exposure ($p > 0.05$), and no effect of ozone on leaf number ($p > 0.05$). After 52 days of ozone exposure, there was no sign of a decrease in leaf number in any of the ozone treatment levels. For the other bean cultivars, leaf count showed a quadratic relationship with time, with leaf number initially increasing as the plant grew new green leaves, and then beginning to decrease again as the plants aged and gradually started to lose older leaves (Figs. 4b – 4d). 'Orca' beans showed no difference in the degree of curvature for the relationship between ozone and days of exposure in the low, medium and high treatments ($p > 0.05$ for O_3 * $Days^2$ interaction). However, there was a difference when comparing the linear slope for the low ozone treatment with the medium and high treatments ($p < 0.001$ for O_3 * Days), as leaf number in the low treatment was still generally increasing after 52 days, while leaf number was gradually declining in the medium and high ozone treatments (Fig. 4b).

'Pinto' and 'Turtle' beans showed an interaction between ozone treatment and the quadratic curves for days of exposure ($p < 0.001$). For the 'Pinto' beans, there was a clear difference between plants in the medium and high ozone treatments, which had lost all of their leaves after 45 days of ozone exposure, compared to plants in the low ozone treatments, which had more leaves during the growing season and started to lose leaves later in the season (Fig. 4c). For 'Turtle' beans, plants in the high ozone treatment started to lose leaves more quickly than plants in the low and medium ozone treatments, and had lost all leaves after 52 days of ozone exposure (Fig 4d). Full model results (including p-values) are given in the Supplementary Material, Table S5.

Senescence of wheat flag leaves

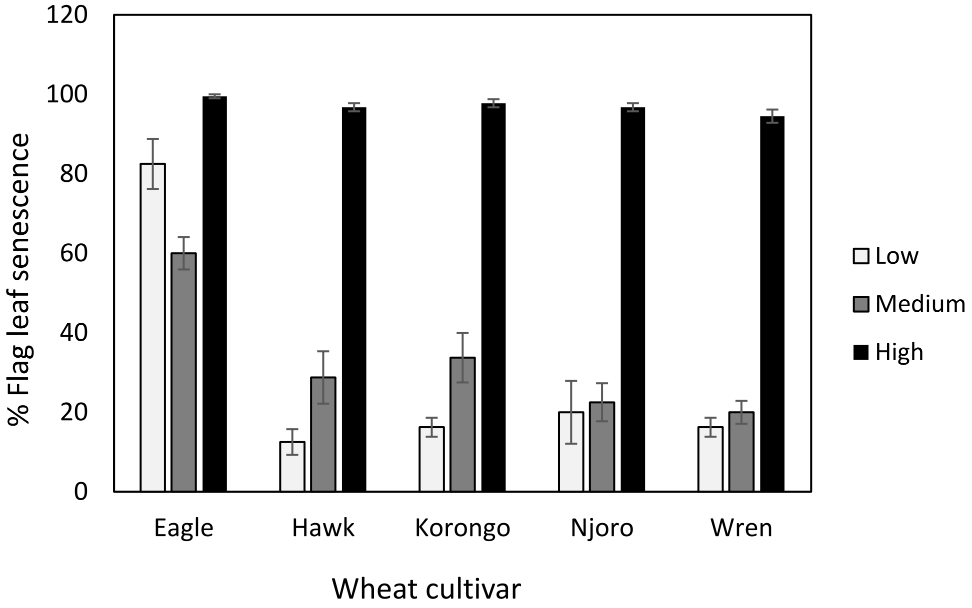


Figure 5. Flag leaf senescence (%) for five African wheat (*T. aestivum*) cultivars after 7 weeks of ozone exposure (low, medium and high), n= 4 replicates per cultivar per ozone treatment. Error bars are ± se.

For all African wheat cultivars, flag leaf senescence was significantly enhanced in the high ozone treatments, compared to the medium and low treatments ($p < 0.001$) (Fig. 5). After 7 weeks of ozone exposure, average flag leaf senescence was 29.5% (± 29% sd), 33.7 % (± 17% sd) and 97% (± 3% sd) for the low, medium and high ozone treatments respectively. There was a difference in cultivar response to ozone, depending on the level of ozone exposure (interaction, $p < 0.01$). In the high ozone treatment, there was no difference in flag leaf senescence between the African wheat cultivars (Table S6), whereas in the low and medium ozone treatments, flag leaf senescence was higher in the Eagle cultivar (average flag leaf senescence of 82.5% (± 12.6% sd) and 60% (± 8.2% sd) respectively) than for the other wheat cultivars (average flag leaf senescence across remaining cultivars 16.3% (± 8.7% sd) and 26.7% (± 11% sd) (Table S6). The Eagle cultivar showed a faster rate of senescence in the lower ozone treatments compared to the other cultivars. Model results (p-values) are given in the Supplementary Material (Table S6).

Discussion

This study presents results on the impact of ozone on the leaves of a variety of African crop species, including visible ozone symptoms, leaf number and leaf senescence. It is important to increase knowledge and awareness of visible ozone symptoms on crops, particularly in countries where it may be difficult/impractical to carry out field experiments on the impact of ozone on crop yield. All of the crop species in this study showed visible ozone symptoms in at least one cultivar under experimental conditions, highlighting that tropical crops might be sensitive to ozone and should be more thoroughly investigated as ambient ozone continues to rise in some areas of the world, including Africa (Ziemke et al., 2019). While specific symptoms (for example, colour of leaf spotting) varied between species, all showed the diagnostic characteristics of visible ozone symptoms, including spotting on the upper leaf surface, between leaf veins, symptoms worsening with time and an age effect. Therefore, using a guide to visible ozone symptoms, it would be possible for crop growers, or local/visiting scientists to search for symptoms in African crops, to provide a first indication that ozone may be causing crop damage, with the potential to decrease crop yield and/or quality.

Ozone impact on yield and thousand-grain weight for the bean and African wheat cultivars has been reported previously (Hayes et al., 2019). ‘Pinto’ beans, the cultivar with the most severe visible ozone symptoms and an early loss of leaves in the medium and high ozone treatments in the current experiment also showed a strong negative effect of ozone on yield and on grain weight. For the other bean cultivars (‘Cannellini’, ‘Turtle’ and ‘Orca’), there was no difference in yield or grain weight between the different ozone treatments. Hayes et al. (2019) also report that, while pearl and finger millets showed visible ozone symptoms, there was no reduction in yield or grain weight due to ozone. As shown in other studies (e.g. Sawada & Kohno, 2009), the acute response to ozone exposure does not necessarily lead to a reduction in crop yield or grain weight. It has been suggested that for some species or genotypes, plants growing under chronic ozone exposure can alter their antioxidant capacity (e.g. Gillespie et al., 2011), and therefore after showing an initial stress response, may be more tolerant to any subsequent stress.

Data on flag leaf senescence for the African wheat cultivars support earlier findings from Pleijel et al. (1997), i.e. higher rates of flag leaf senescence at high ozone. Accelerated senescence of the flag leaf decreases time for grain filling, which can be reflected in the effect of ozone on yield and thousand-grain weight (Gelang et al., 2000). The final grain yield (for 'Korongo' and 'Wren' cultivars) and thousand-grain weight ('Hawk', 'Korongo' and 'Wren') was lower in the high ozone compared to the low ozone treatment (Hayes et al., 2019). Results for the 'Eagle' cultivar suggested an overall faster growth rate compared to the other cultivars. Hayes et al. (2019) show no impact of ozone on total grain yield for the 'Eagle' cultivar, but there was a reduced thousand-grain weight in high compared to low ozone. The 'Hawk' and 'Njoro' cultivars showed accelerated senescence at high ozone in the current study and Hayes et al. (2019) found a decrease in average total yield (by 20 and 29% respectively), however there was no statistically significant difference between total yield in the low and high ozone treatments for these wheat cultivars. This non-significant result may be due to variation between individual plant replicates (particularly in the high ozone treatment). A larger sample size under field conditions is needed to verify the result for these cultivars.

Overall, the occurrence of visible ozone symptoms, early leaf loss and increased rate of senescence can be indicators for a potential negative impact of ozone on yield, but not all plants showing visible ozone symptoms will necessarily show a decrease in yield.

All of the crops studied are important food crops in Africa. In 2017, maize, sweet potato, sorghum and wheat were among the top ten crops produced in Africa (FAOSTAT, 2017). Barley is one of the main staple food crops in the temperate highlands of Sub-Saharan Africa, for example in Ethiopia, (Tigre et al., 2014). Millets, particularly pearl millet, are an important crop in Western and Central Africa (Jukanti et al., 2016). Common beans were the most common legume produced in East Africa in 2017 (FAOSTAT, 2017), providing an important source of dietary protein. For cowpea, 9 of the top 10 global producers in 2017 were in Sub-Saharan Africa (FAOSTAT, 2017). At least 50 tropical countries grow vegetable amaranths and leaves provide some African societies with as much as 25% of their daily protein (National Research Council, 2006). Ethiopia has the highest production of chickpeas in Africa (3% of global production) (FAOSTAT, 2017), where it is grown in rotation with cereals such as wheat. Globally, 7 of the top 10 peanut (groundnut) producers are in Africa (FAOSTAT, 2017), with production mostly for domestic use.

The photographs and descriptions of ozone symptoms from this study are a useful addition to guides or manuals on visible ozone symptoms on crops. However, the following caveats should be considered. Depending on the African country where crops are growing, the ozone concentrations used under the experimental conditions of this study may have been higher or lower than those experienced in field conditions, therefore, it is possible that symptoms observed in field conditions may be less clear or more severe than presented here. Also, for practical reasons, the experiment was carried out in North Wales, UK. Efforts were made to recreate natural conditions, for example, using heated solardomes however conditions in the field will differ and climatic conditions will vary across Africa, with plants facing other stresses, including drought, potentially reducing ozone uptake and thus ozone symptom development.

There are also many other potential causes of leaf damage, including pests (see Supplementary Material, Fig. S2) and diseases, nutrient deficiency and drought (e.g. https://www.plantwise.org) and some of them might be misdiagnosed due to lack of awareness of potential ozone-induced leaf damage. The ICP Vegetation has an online manual describing the procedures that should be followed when recording the presence/absence of ozone symptoms on sensitive species (https://icpvegetation.ceh.ac.uk/get-involved/manuals/ozone-experimental-protocol). Caution should be taken if using early senescence as an indicator for ozone symptoms, and information on the dates when natural senescence would be expected are required (Vollenweider & Günthardt-Goerg, 2005).

If visible ozone symptoms are recorded on a particular crop species, there are actions that a farmer could take to try to mitigate the potential negative impact of ozone on crop yield or quality. Strategic limitation of irrigation could be used to reduce the effect of ozone (Mills et al., 2018b, Harmens et al., 2019). When irrigation is reduced in areas where irrigation is commonly applied, stomata will partially close, therefore reducing the ozone that is taken in by the plant. The results in this current study confirm those highlighting differences in ozone sensitivity between crop cultivars (e.g. Shi et al., 2009). These differences might be influenced by cultivar characteristics including stomatal ozone uptake (Salvatori et al., 2013) or antioxidant capacity (Feng et al., 2016). There is therefore potential for advising crop farmers on which cultivars are more or less sensitive to ozone, and scope for breeding ozone-tolerant cultivars (Mills et al., 2018b), however this needs to be balanced with other favourable characteristics, such as fast growth, high yield and drought tolerance.

Conclusions

All African food crops tested showed symptoms of visible ozone symptoms on leaves. Early leaf loss in common beans and accelerated senescence in African wheat cultivars were found in the high ozone treatments (compared to low ozone). Differences in ozone sensitivity were found between cultivars of the same crop (under experimental conditions). Tropospheric ozone has the potential to reduce crop yield and quality of these important food crops. As it is difficult to assess the direct impact of ozone on crop yield under field conditions where other environmental factors might also affect yield, the recording of visible ozone symptoms is a first indication that there could be a potential negative impact on yield. As there is little known about the exact ozone levels in Africa, it is recommended that surveys are carried out in the field for visible ozone symptoms, alongside the measurement of ozone concentrations using, for example, low cost methods such as diffusion tubes. This will be an important first step towards increasing knowledge on the impact of ozone on crop production in Africa. Ultimately critical levels of ozone for tropical food crops should be developed, preferably using ozone flux to take environmental conditions into account, to more fully quantify the risk to food production.

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