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Ozone-induced effects on leaves in African crop species.

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Supplementary Material

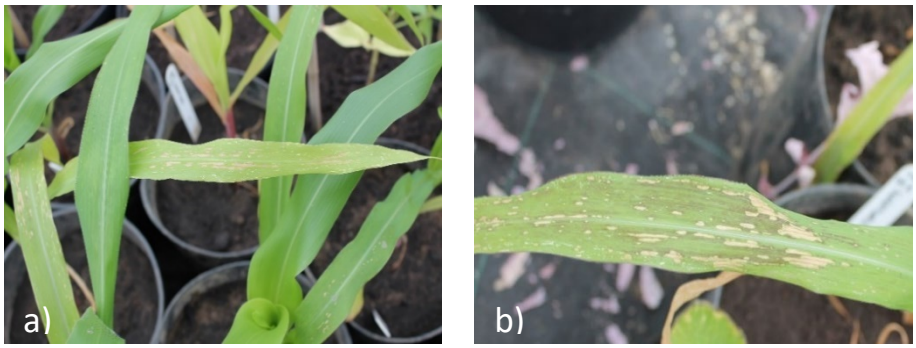

Figure S1. Visible ozone symptoms on maize (*Zea mays*) leaves after an ozone exposure pilot study in late summer 2019.

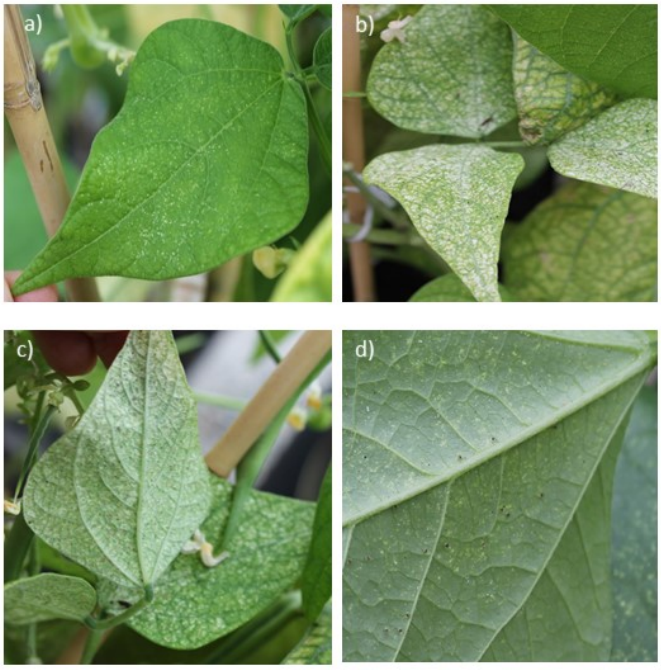

Figure S2: Red spider mite infestation on Common bean (*Phaseolus vulgaris*). White webbing appears on the upper surface of the leaf, which can become extensive in some cases (S2a, b). Unlike leaf symptoms caused by ozone, spider mite symptoms can be found on both surfaces of the leaf, and are not limited to interveinal areas. The dark coloured, pin-head sized mites can be seen crawling on the underside of the leaf (S2c, d).

Figure S3: Progression of visible ozone symptoms in: a) Finger millet (*Eleusine coracana*); b) Chickpea (*Cicer arietinum*); c) Peanut (*Arachis hypogaea*); d) Common wheat (*Triticum aestivum*); e) Pearl millet (*Pennisetum glaucum*).

Sources of seeds used in the experiment

Wheat (*Triticum aestivum)* seeds were obtained from the Kenya Agriculture and Livestock Research Organistion (KALRO; Njoro-Kenya). Finger millet (*Eleusine coracana*) and pearl millet (*Pennisetum glaucum*) seeds were obtained from the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT-Nairobi, Kenya). Widely grown cultivars of common bean (*Phaseolus vulgaris*) ('Black Turtle', 'Cannellini', 'Orca', 'Pinto') were obtained from Jungle Seeds Ltd, UK. (http://www.jungleseeds.co.uk/) The *P. vulgaris* cultivars 'Mbombo', 'Tiger' and 'Rajama,' and the cowpea (*Vigna unguiculata*), amaranth (*Amaranthus hypochondriacus)* and peanut (*Arachis hypogaea*) seeds were obtained from Rareseeds (https://www.rareseeds.com/store/vegetables). Sweet potato (*Ipomoea batatas*), mung bean (*Vigna radiata*) and maize (*Zea mays*) seeds were obtained from Thompson and Morgan, UK (https://www.thompson-morgan.com/). The sorghum (*Sorghum bicolor*) and chickpea (*Cicer arietinum*) seeds were obtained from ICRISAT-India. The barley (*Hordeum vulgare*) was supplied by Syngenta, UK (https://www.syngenta.co.uk/).

Table S1: Growing season mean ozone values (weekly mean and mean daily maximum) for the ambient and heated domes for low, medium and high ozone in 2017, 2018 and 2019. Note that an ozone-temperature interactions study was not carried out. Two temperature regimes are shown because wheat (grown in 2017) was exposed at ambient temperatures to better represent the growing conditions of this crop.

Year: 2017		**Ozone concentration**	
O_3 treatment	Temperature regime	Weekly mean (ppb)	Mean daily max. (ppb)
Low	Ambient	25.6	32.5
Medium	Ambient	40.2	67.3
High	Ambient	45.8	84
Low	Ambient + 7˚C	25.4	33.9
Medium	Ambient + 7˚C	40.6	70.6
High	Ambient + 7˚C	47.8	93
Year: 2018		**Ozone concentration**	
O_3 treatment	Temperature regime	Weekly mean (ppb)	Mean daily max. (ppb)
Low	Ambient + 7˚C	21.6	32.4
Medium	Ambient + 7˚C	37.8	69.8
High	Ambient + 7˚C	44.3	90.4
Year: 2019		**Ozone concentration**	
O_3 treatment	Temperature regime	Weekly mean (ppb)	Mean daily max. (ppb)
Low	Ambient + 7˚C	24.7	32.5
Medium	Ambient + 7˚C	41.3	71.2
High	Ambient + 7˚C	49.2	92.4

Table S2: Ozone exposure start and end dates for 2017, 2018 and 2019 (dd/mm/yyyy). Ozone exposure began when plants reached the vegetative stage (~3-5 weeks after sowing). 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, ozone exposure length varied between species.

Species	Cultivar	Ozone treatment start	Ozone treatment end
Wheat	Korongo	18/05/2017	25/07/2017
	Eagle	18/05/2017	25/07/2017
	Njoro	18/05/2017	25/07/2017
	Hawk	18/05/2017	25/07/2017
	Wren	18/05/2017	25/07/2017
Pearl millet	ICMV 221	24/05/2017	29/06/2017
	KAT PM1	24/05/2017	29/06/2017
	Okashana	24/05/2017	29/06/2017
	Shibe	24/05/2017	29/06/2017
Finger millet	GuluE	1/06/2017	28/09/2017
	KNE624	1/06/2017	28/09/2017
	KNE814	1/06/2017	28/09/2017
	Okhale	1/06/2017	28/09/2017
	P224	1/06/2017	28/09/2017
	U15	1/06/2017	28/09/2017
Mung bean	Unspecified	1/06/2017	5/09/2017
Common bean	Pinto	1/06/2017	31/07/2017
	Orca	1/06/2017	21/08/2017
	Black Turtle	1/06/2017	02/08/2017
	Cannellini	1/06/2017	25/08/2017
	Mbombo	6/06/2018	23/08/2018
	Rajama	6/06/2018	16/08/2018
	Tiger	6/06/2018	16/08/2018
Cowpea	Black-eye	1/06/2017	21/08/2017
	Blue Goose	6/06/2018	23/08/2018
	Hog brains	6/06/2018	23/08/2018
	Old Timer	6/06/2018	16/08/2018
	Razorback	6/06/2018	16/08/2018
	Whippoorwill	6/06/2018	16/08/2018
Chickpea	ICC 15333	19/09/2017	3/11/2017
Amaranth	Pygmy Torch	6/06/2018	30/08/2018
Peanut	Negrito	6/06/2018	23/08/2018
	Tennessee red	6/06/2018	23/08/2018
Sorghum	IS1004	6/06/2018	6/08/2018
	IS27557	6/06/2018	6/08/2018

Barley	Propino	11/06/2019	1/10/2019
Sweet potato	Erato Orange	11/06/2019	1/11/2019
Maize	Incredible F1	27/08/2019	1/11/2019

Table S3: Climatic conditions in the solardomes during the growing season for 2017, 2018 and 2019. Temperature and relative humidity were continuously measured in one ambient temperature solardome (2017) and three heated solardomes (2017, 2018, 2019). Photosynthetically Active Radiation (PAR) was continuously monitored each year in one ambient temperature dome. Seasonal mean ± standard error is presented. Daylight mean temperature and Vapour-Pressure Deficit (VPD) were calculated using hourly values with >200 PAR, and 12 hr mean PAR was calculated using the period 07:00 – 18:00.

3a) 2017

Ozone treatment	**Temperature regime**	**Temperature, daylight mean (°C)**	**VPD, daylight mean (kPa)**	**PAR, 12h mean (mmol m^{-2} s^{-1})**
Low	Ambient	20.3 ± 0.3	0.61 ± 0.1	487 ± 21
Low	Ambient + 7°C	27.2 ± 0.3	1.89 ± 0.1	
Medium	Ambient + 7°C	27.7 ± 0.3	2.05 ± 0.1	
High	Ambient + 7°C	27.3 ± 0.3	1.87 ± 0.1	

3b) 2018

Ozone treatment[1]	**Temperature regime**	**Temperature, daylight mean (°C)**	**VPD, daylight mean (kPa)**	**PAR, 12h mean (mmol m^{-2} s^{-1})[1]**
Low	Ambient			572 ± 18
Low	Ambient + 7°C	30.71 ± 0.14	2.55 ± 0.03	
Medium	Ambient + 7°C	29.62 ± 0.13	2.37 ± 0.03	
High	Ambient + 7°C	29.85 ± 0.14	2.40 ± 0.03	

3c) 2019

Ozone treatment[1]	**Temperature regime**	**Temperature, daylight mean (°C)**	**VPD, daylight mean (kPa)**	**PAR, 12h mean (mmol m^{-2} s^{-1})[1]**
Low	Ambient			465 ± 19
Low	Ambient + 7°C	29.16 ± 0.11	2.22 ± 0.02	
Medium	Ambient + 7°C	28.93 ± 0.12	2.22 ± 0.03	
High	Ambient + 7°C	28.92 ± 0.14	2.32 ± 0.03	

Table S4

Results of regular leaf assessments for crop plants exposed to low, medium and high ozone (O_3) treatments in solardomes. The presence of visible ozone symptoms classed as 'Mild' = <5% of the leaf showing ozone symptoms; 'Moderate' = 5-25% of the leaf showing ozone symptoms and 'Severe' = >25% of the leaf showing ozone symptoms, was recorded for each species.

Species	Development of visible ozone symptoms on leaves
Common wheat (flag leaf)	After 6 days of exposure, mild ozone symptoms seen in all wheat cultivars, in all treatments. Moderate ozone symptoms in 3 of 6 cultivars in medium O_3, and 5 of 6 cultivars in high O_3. Severe ozone symptoms in 1 cultivar in high O_3. After 18 days, moderate ozone symptoms seen in all cultivars in medium and high O_3, and severe ozone symptoms in 4 of 6 cultivars in medium O_3, and all cultivars in high O_3.
Pearl millet	After 7 days of exposure, mild ozone symptoms seen in medium and high O_3 treatments only. After 20 days, all but one cultivar showing severe ozone symptoms in medium and high O_3 treatments. No ozone symptoms in low O_3 treatment (final assessment was after 28 days of exposure).
Finger millet	After 5 days of exposure, mild ozone symptoms in medium O_3, mild and moderate ozone symptoms in high O_3. No ozone symptoms in low O_3. After 12 days, 4 of 6 cultivars showing severe ozone symptoms in medium O_3, and all 6 cultivars showing severe ozone symptoms in high O_3. No ozone symptoms in low O_3 (final assessment was after 21 days of exposure).
Mung bean	After 8 days, mild ozone symptoms in medium O_3 only, after 12 days both medium and high O_3 showing mild ozone symptoms. By 19 days of exposure, medium and high O_3 showing severe ozone symptoms. Mild ozone symptoms appeared in low O_3 treatment after 27 days.
Common bean (2017 cultivars)	After 8 days of exposure, moderate ozone symptoms in medium O_3, and moderate and severe in high O_3. In the medium treatment, after 12 days, severe ozone symptoms seen in 2 cultivars, with all 4 cultivars showing severe ozone symptoms after 34 days. No ozone symptoms in low O_3 treatment recorded during growing season.
Cowpea	Visible ozone symptoms slow to appear. First week of clear ozone symptoms was after 4 weeks of exposure, with symptoms in high O_3 treatment only (mild, moderate and severe, depending on leaf age and cultivar; 2 cultivars with mild and moderate ozone symptoms, 1 with mild, moderate and severe symptoms, 1 with only mild symptoms, and 1 with no symptoms). After 8 weeks of exposure, no ozone symptoms in the low O_3 treatment, mild symptoms in 3 of 5 cultivars in medium O_3.
Chickpea	Ad hoc leaf assessments only.
Amaranth	Ad hoc leaf assessments only.
Peanut	Ad hoc leaf assessments only.
Sorghum	Ad hoc leaf assessments only.
Barley	Ad hoc leaf assessments only.
Sweet potato	After 7 days of exposure, moderate ozone symptoms in both medium and high O_3 treatments.
Maize	Ad hoc leaf assessments only.

Table S5: Model results of the relationship between leaf number and the number of days of ozone exposure, for common bean (*Phaseolus vulgaris*) cultivars exposed to low, medium and high ozone (O_3) treatments. $Days^2$ represents the inclusion of a quadratic term in the model, as the relationship between leaf number and time showed a curved shape (first increasing with time, and then gradually decreasing) for Orca, Pinto and Turtle beans.

5a) Optimal models (chosen using top down selection and Akaike's Information Criterion (AIC)) for each *P. vulgaris* cultivar. The 'X' indicates that the model term was present in the optimal model.

	Model terms				
***P. vulgaris* cultivar**	O_3	Days	Days + $Days^2$	O_3 * Days	O_3*(Days + $Days^2$)
Cannellini		X			
Orca	X		X	X	
Pinto	X		X		X
Turtle	X		X		X

5b) Results (p-values) for the interaction terms in the models for each bean cultivar.

Model	*P. vulgaris* cultivar			
	Cannellini	Orca	Pinto	Turtle
Ozone * Days	0.33	**< 0.0001**	**< 0.0001**	**< 0.0001**
Low vs High O_3	NA	< 0.0001	< 0.0001	< 0.0001
Low vs Medium O_3	NA	< 0.0001	< 0.001	0.019
Medium vs High O_3	NA	0.53	0.16	< 0.0001
Ozone * $Days^2$	NA	0.26	**< 0.0001**	**< 0.0001**
Low vs High O_3	NA	NA	0.002	< 0.0001
Low vs Medium O_3	NA	NA	< 0.0001	0.68
Medium vs High O_3	NA	NA	0.36	< 0.0001

Table S6: Model results (p-values) for investigation of the effect of increased ozone on percentage flag leaf senescence in African wheat (*Triticum aestivum*) cultivars exposed to low, medium and high ozone (O_3) treatments. Values are for post-hoc tests (using the R package 'emmeans') comparing a) African wheat cultivars at low, medium and high ozone; b) ozone treatment response for each African wheat cultivar.

6a)

	p-values		
Contrasts (Wheat varieties)	**Low O_3**	**Medium O_3**	**High O_3**
eagle - hawk	<0.0001	0.0004	0.77
eagle - korongo	<0.0001	0.0053	0.89
eagle - njoro	<0.0001	<0.0001	0.77
eagle - wren	<0.0001	<0.0001	0.53
hawk - korongo	0.96	0.95	0.99
hawk - njoro	0.67	0.88	1
hawk - wren	0.96	0.73	0.95
korongo - njoro	0.97	0.47	0.99
korongo - wren	1	0.34	0.82
njoro - wren	0.97	0.996	0.95

6b)

	p-values				
Contrasts (O_3 treatments)	**Eagle**	**Hawk**	**Korongo**	**Njoro**	**Wren**
high - low	0.040	<0.0001	<0.0001	<0.0001	<0.0001
high - medium	0.004	<0.0001	<0.0001	<0.0001	<0.0001
low - medium	0.004	0.026	0.023	0.91	0.82