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Wheat yield responses to stomatal uptake of ozone: peak vs rising background ozone conditions

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Abstract

Recent decades have seen a changing temporal profile of ground-level ozone (O_3) in Europe. While peaks in O_3 concentrations during summer months have been declining in amplitude, the background concentration has gradually increased as a result of the hemispheric transport of O_3 precursors from other world regions. Ground-level O_3 is known to adversely affect O_3-sensitive vegetation, including reducing the yield of O_3-sensitive crops such as common wheat (*Triticum aestivum* L.). The reduction in wheat yield has been shown to be linearly related to the phytotoxic O_3 dose above a flux threshold of Y (POD_Y) accumulated over a specific period. In the current study, we tested whether the flux-effect relationships for wheat yield and 1000-grain weight were affected by the temporal profile of O_3 exposure. A modern wheat cultivar (Skyfall) was exposed to eight different realistic O_3 profiles repeated weekly: four profiles with increasing background O_3 concentrations (ca. 30 – 60 ppb) including small peaks and four profiles with increasing O_3 peak concentrations (ca. 35 – 110 ppb). Both wheat yield and 1000-grain weight declined linearly with increasing POD_Y. The slope of the flux-effect relationships was not affected significantly by the profile of O_3 exposure. Hence, flux-effect relationships developed for wheat based on exposure to enhanced peak O_3 concentrations are also valid for the changing European O_3 profile with higher background and lower peak concentrations. The current study also shows that the modern wheat cultivar Skyfall is more sensitive to O_3 than European wheat varieties tested for O_3 sensitivity in the 1980s and 1990s.

Introduction

Tropospheric or ground-level ozone (O_3) is a secondary pollutant formed in the atmosphere by solar radiation-driven chemical reactions between O_3 precursor gases, i.e. carbon monoxide (CO), nitrogen oxides (NO_x), methane (CH_4) and non-methane volatile organic compounds (nmVOCs; Monks et al., 2015; Royal Society, 2008). Annual variation in O_3 concentrations depends on geographical location, proximity to sources of O_3 precursors and prevailing meteorological conditions. This variation in concentration is determined by both photochemical and physical processes, including photochemical production and destruction of O_3, hemispheric transport, and removal by deposition at the Earth's surface (Monks et al., 2015). Usually a distinction is made between peak/episodic, hemispheric background and baseline O_3 (Royal Society, 2008). Peak concentrations of O_3 (also known as episodes) occur when high levels of O_3 precursor emissions coincide with meteorological conditions that promote O_3 formation, for example stable, high pressure systems. Hemispheric background O_3 is the remaining concentration when the emissions of anthropogenic O_3 precursors from within a region are excluded. It is the sum of O_3 produced from natural sources of precursors within a region and O_3 imported into the region (derived from all sources). Baseline O_3 is the average measured concentration within a region and is made up of both the anthropogenic emissions produced within the region and the background concentration of O_3.

Ground-level O_3 pollution increased significantly between the end of the 19th and 20th century (Cooper et al., 2014; Marenco et al., 1994). Parrish et al. (2012) reported an approximate doubling of baseline O_3 concentrations between 1950 and 2000 at northern mid-latitudes. Since 2000, however, the rate of increase has slowed, particularly at European sites, to the extent that at present O_3 baseline concentrations are decreasing at some sites in some seasons, especially in the summer (EMEP, 2016). Although measurements at rural O_3 monitoring stations in Europe showed a decline in peak concentrations of O_3 at some (but not all) European sites , there has been a concurrent rise in concentrations in the lower range up to 40 ppb (Simpson et al., 2014; Tørseth et al., 2012). The largest decline in amplitude of peak O_3 episodes has been observed at stations which saw the highest levels of peak O_3 in the early 1990s (Derwent and Hjellbrekke, 2013). Since 1990, a clear downward trend in high summertime O_3 episodes has been confirmed for many EMEP (European Monitoring and Evaluation Programme) rural monitoring stations, whilst the annual mean (baseline) O_3 increased between 1990 and 2001 and began to level off between 2002 and 2012 (EMEP, 2016). The decline in peak O_3 concentrations in Europe in recent decades is the result of the implementation of air pollution abatement policies and the use of cleaner energy in Europe, which has resulted in a decline in emission of O_3 precursors compounds such as NO_x and nmVOCs (EMEP, 2016).

O_3 is known to be toxic for vegetation (Ainsworth et al., 2012; Mills et al., 2011a; Wittig et al., 2009). O_3 enters the leaf through the stomata and triggers a reaction chain involving reactive oxygen species (ROS). Plants have the capacity to detoxify O_3 and ROS but the detoxification capacity is species-specific, with damage occurring when this detoxification capacity is exceeded (Burkey et al., 2006). Recently, it has been shown that impacts of O_3 on vegetation are best correlated with the accumulative stomatal O_3 flux, calculated over a species-specific time period, using a threshold for the stomatal O_3 flux as a surrogate for the O_3 detoxification capacity (Mills et al. 2011a). The accumulative stomatal O_3 flux above an hourly threshold Y has been defined as the Phytotoxic Ozone Dose (POD_Y; Mills et al., 2011b; LRTAP Convention, 2017). A flux-effect relationship has been derived for the crop species common wheat (*Triticum aestivum* L.) based on experimental O_3 exposure studies conducted with five cultivars in four countries. The function uses a wheat-specific parameterisation of the stomatal O_3 flux model DO_3SE (Deposition of O_3 for Stomatal Exchange - http://sei-international.org/do3se; Emberson et al., 2000, 2001) for the flag leaf. For wheat, previous work has shown that the flux threshold Y of 6 nmol m^{-2} projected leaf area s^{-1} (Grünhage et al., 2012) produces the best statistical fit between yield and stomatal flux (Pleijel et al. 2007); the accumulative stomatal O_3 flux is defined as POD_6SPEC (LRTAP Convention, 2017 – Section III.3.5.2). Plant species vary in their sensitivity to O_3, with wheat being an O_3-sensitive crop (Mills et al., 2016). Flux-based critical levels have been defined for a limited number of crop species (LRTAP Convention, 2017). Data for wheat were also used to develop a generic flux-effect relationship for crops for application in large scale modelling, including integrated assessment modelling (IAM), based on a lower O_3 flux threshold Y of 3 nmol m^{-2} projected leaf area s^{-1}, defined as POD_3IAM (LTRAP Convention, 2017 – Section III.3.6). POD_YIAM-based flux models have a simpler form and parameterisation than POD_YSPEC based ones.

Flux-effect relationships for wheat are based on studies conducted between 1987 and 1999 in which the crop was exposed to high O_3 episodes, representing peak O_3 concentrations during the growing season (Grünhage et al., 2012). With the current O_3 temporal profile changing in Europe, we investigated whether O_3 flux-effect relationships based on exposure of vegetation to peak O_3 concentrations are also valid for vegetation exposed to rising background concentrations. We hypothesise that effects on wheat yield are determined by the accumulated stomatal O_3 flux, independent of the temporal profile of O_3 exposure, i.e. background O_3 concentrations or peak O_3 episodes.

Material and Methods

Plant material, experimental site and treatments

The experiment was conducted in 2015 at the Centre for Ecology & Hydrology (CEH) air pollution facility at Abergwyngregyn, North Wales (53.2°N, 4.0°W). On 13th March, wheat (*Triticum aestivum* L., cv. Skyfall) seeds were sown outdoors in containers (0.3 m x 0.3 m x 0.3 m) filled to 25 litres with John Innes No. 3 compost (J. Arthur Bowers). Skyfall is a new, high yielding, bread-making winter wheat variety in the UK and was launched in 2014. Seeds were sown in four rows 7 cm apart with 40 seeds per container, resulting in a seedling density of approximately 260 seedlings per m^2, similar to the recommended field seedling density (AHDB, 2015). Containers were inoculated with soil microbial communities from a nearby wheat field using a soil slurry applied shortly after sowing. Seedlings emerged on 5^{th} April. On 7^{th} May, the containers were randomly distributed between eight hemispherical glasshouses (solardomes; 3 m diameter, 2.1 m height); each dome contained four containers. After an acclimation period in the solardomes, O_3 treatments were started on 15^{th} May. Plants were exposed to O_3 until harvest (11^{th} – 13^{th} August) and each solardome had a different weekly O_3 regime (Figure 1). The O_3 regimes were assigned randomly to the solardomes to minimise the impacts of any potential environmental gradients at the research site. In four solardomes, plants were exposed to varying background O_3 concentrations (low, medium, high, very high) and in the other four solardomes, plants were exposed to varying peak O_3 concentrations (low, medium, high, very high), representing a 5-day O_3 episode per week. The weekly temporal profiles were applied such that pairs of background and peak O_3 treatments represented a similar mean O_3 concentration, e.g. low background and low peak O_3 exposure represented a seasonal 24 hr mean O_3 of 27.0 and 30.3 ppb respectively (Figure 1). The lack of treatment replication in this experiment was due to the limited number of solardomes. However, a previous assessment found that climatic conditions do not vary significantly between the solardomes used in this experiment (Hewitt et al., 2014).

The solardomes were ventilated at a rate of two air changes per minute and charcoal-filtered air was injected with controlled amounts of O_3. O_3 was provided by a G11 O_3 generator (Ozone Industries, UK) equipped with a Sequal 10 oxygen concentrator, (Pure O2, UK). Concentrations were determined by a computer-controlled O_3 injection system (Lab VIEW version 2012, National Instruments, Texas, US). O_3 was distributed to each solardome via PTFE tubing, with the concentration inside each solardome measured for 5 min every 30 minutes using two O_3 analyzers (400a, Enviro Technology Services, Stroud, UK) of matched calibration. In one solardome, ambient air temperature, photosynthetically active radiation (PAR), temperature and relative humidity were continuously monitored by an automatic weather station (Skye Instruments Ltd, Llandrindod Wells, UK). Plants were watered twice a week or as required, to maintain soil moisture content near field capacity.

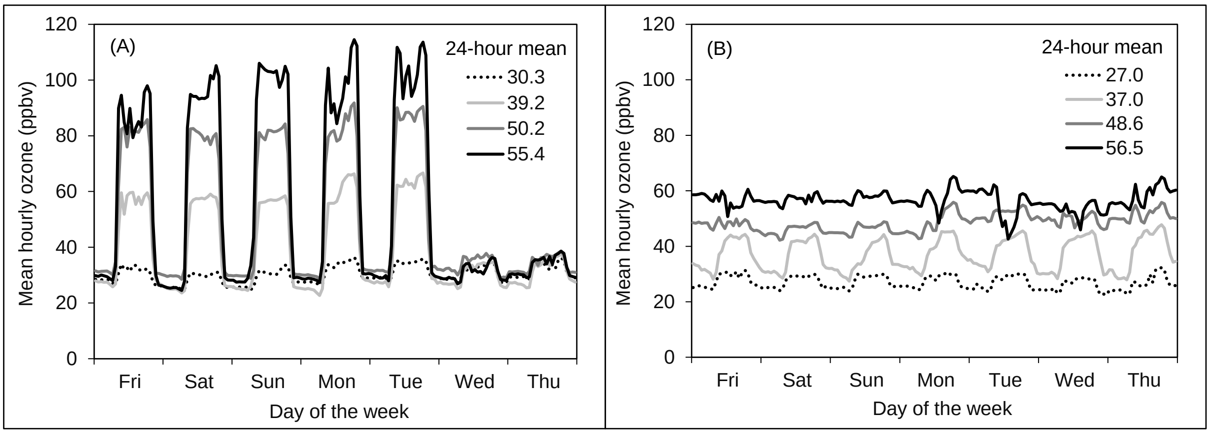

Figure 1. Weekly O_3 exposure temporal profiles for (A) the four peak and (B) the four background profile treatments. Profiles were repeated weekly throughout the experiment (15th May – 13th August 2015). The seasonal 24-hour mean O_3 concentration (ppb) for each treatment is provided in the figure keys.

Yield measurements

The two middle rows of plants per container were harvested, excluding the edge plants at each end of the rows. Ears were threshed and the grains were weighed. In addition, the weight of 100 grains per container was determined to calculate the 1000-grain weight. The relative yield per O_3 treatment was calculated using the method introduced by Fuhrer (1994), i.e. the absolute yield per O_3 treatment was divided by the Y-axis intercept of the linear relationship between absolute yield and POD_6SPEC. This process was also applied to calculate relative 1000-grain weight.

O_3 stomatal flux calculations

O_3 stomatal fluxes were calculated using the DO_3SE model (Deposition of O_3 for Stomatal Exchange, version 3.0.5; https://www.sei-international.org/do3se). O_3 flux was calculated at the flag leaf level according to a method developed by Emberson et al. (2000, 2001). As the plants were well-watered, there was no limitation of soil moisture (f_{SW} = 1) on the stomatal O_3 flux. Hourly monitored air temperature, PAR and relative humidity (for calculating the vapour pressure deficit) were used to calculate impacts of the chamber environment on the stomatal O_3 flux in the DO_3SE model. POD_6SPEC and POD_3IAM were calculated using the stomatal flux model parameterisation for wheat as described in LRTAP Convention, Section

III.3 (2017). Parameterisations of DO_3SE for calculating POD_3IAM are based on the full flux model for wheat (POD_6SPEC), but modifications were made to simplify the full flux model for wheat for application within large-scale regional flux models applied in integrated assessment modelling. The accumulation period for POD_6SPEC was 200 ^oC days before mid-anthesis (midpoint in flowering) to 700 ^oC days after mid-anthesis and the accumulation period for POD_3IAM was 90 days (15th May – 13th August). Resulting flux-effect relationships were compared with those described in LRTAP Convention (2017).

Statistical analyses

To investigate if the effect of O_3 flux on wheat yield (tonnes ha^{-1}) and 1000-grain weight (g) varied between peak and background O_3 profiles, linear models (Gaussian error) including O_3 flux (POD_6SPEC or POD_3IAM) as a continuous predictor and O_3 profile (peak or background) as a categorical predictor were run in R (R core team 2016). As the number of harvested plants per container varied (due to natural variation in germination success of wheat seeds), ‘number of harvested plants per container’ was also included as a covariate in the yield model. To control for any spatial pseudoreplication, the need for the inclusion of a random effect of dome was tested for, and the model with 1000-grain weight as the response variable was run as a linear mixed effects model.

For the linear yield models, single terms were sequentially removed from the global model until only significant variables ($P < 0.05$) remained. For the linear mixed effects 1000-grain weight models, a model set was created using the R package lme4, v1.1-7 (Bates et al. 2015) by sequentially removing single terms from the global model, and model selection was carried out by looking at the change in AIC (Akaike Information Criterion). The model with the lowest AIC value is optimal, with models differing in 2 - 7 AIC units from the top model having little empirical support (Burnham and Anderson, 2002). When the optimal model was selected, P-values were obtained for each term using likelihood ratio tests, following Zuur et al. (2009). For all models, statistical assumptions (normality and even distribution of residuals) were checked using residual plots. Response variables were transformed (square root) where necessary.

To compare the flux effect relationships for the modern wheat variety Skyfall with the older varieties in the Modelling and Mapping Manual (MM; LRTAP Convention, 2017), a linear model (Gaussian error) including O_3 stomatal flux (POD_6SPEC) as a continuous predictor and its interaction with the categorical predictor 'variety' (MM or Skyfall) was run. Following Fuhrer (1994), the true yields were first converted to yields relative to the yield at zero O_3 stomatal flux. The yield at zero O_3 stomatal flux was estimated by straight line extrapolation from measured yields at higher stomatal fluxes. Using this relative yield as the response variable, the intercept is by construction 1 (or very close to 1) for both 'varieties'. Therefore differences between varieties are manifested as differences in the slopes of the lines starting at relative yield 1 for zero O_3 stomatal flux. To test the hypothesis that the slope of the linear response differs between varieties, we have fitted the model 'Relative yield ~ POD_6SPEC + POD_6SPEC:variety'. If the slopes are not different, the interaction term in this relationship is expected not to be significant. This process was repeated using relative 1000-grain weight as the response variable. Statistical assumptions (normality and even distribution of residuals) were checked using residual plots.

Results and discussion

Both wheat yield and 1000-grain weight declined linearly with increasing POD_Y, whether calculated using the full parameterisation (POD_6SPEC) or a simplified parameterisation (POD_3IAM) of DO_3SE ($P < 0.001$ for yield, $P < 0.01$ for grain weight ; Figure 2). The optimal model, for both yield and 1000-grain weight, contained only POD_Y without an interaction with the O_3 temporal profile, i.e. whether wheat was exposed to rising background or enhanced peak O_3 episodes. This supports our hypothesis that the impact of O_3 on wheat yield is determined by the accumulated O_3 stomatal flux above a threshold value, irrespective of the temporal profile of O_3 exposure.

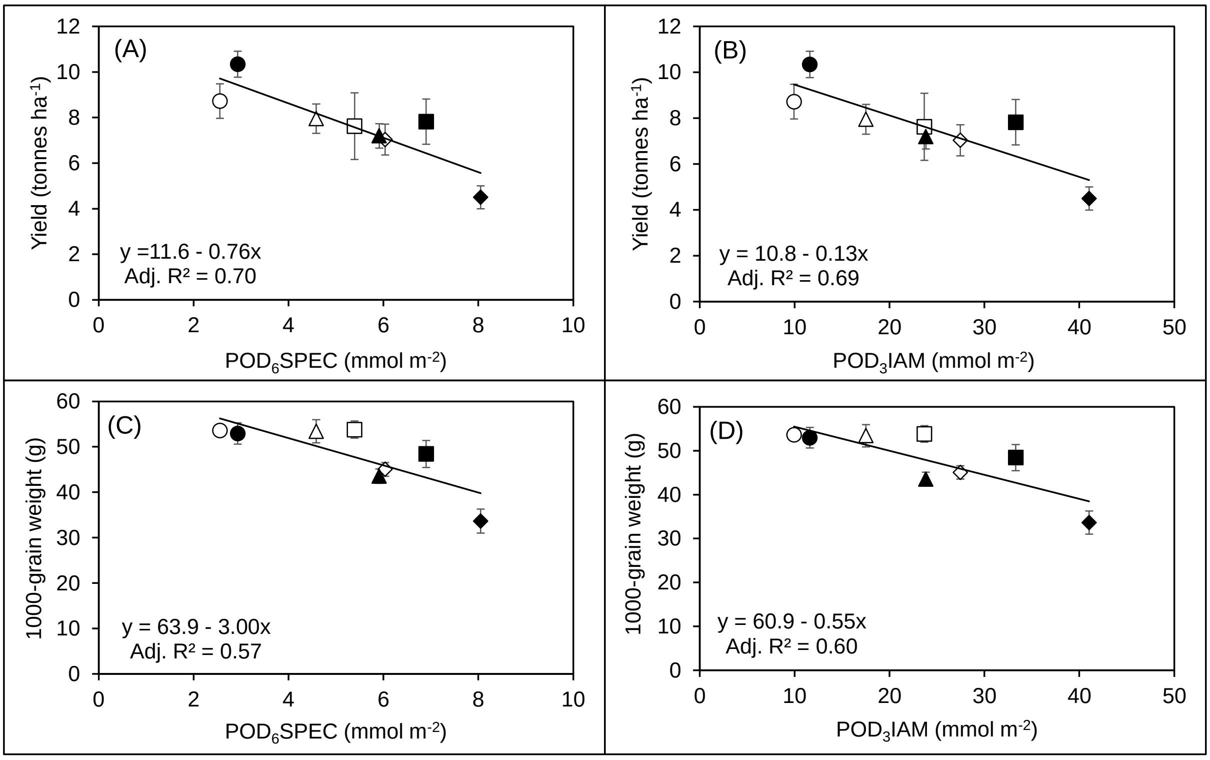

Figure 2. Flux-effect relationships for (A, B) yield and (C, D) 1000-grain weight for wheat using (A, C) POD_6SPEC and (B, D) POD_3IAM as the O_3 stomatal flux metric. Open symbols represent background O_3 and closed symbols represent peak O_3 episodes. Symbols of the same shape represent pairs of similar 24 h mean O_3 concentrations (see Figure 1 for further details).

Flux-effect relationships established under conditions of high O_3 peaks (more frequently occurring in Europe in the past) can therefore also be applied under conditions of rising background O_3. Hence, changes in current and future O_3 temporal profiles would not require an adjustment of the stomatal O_3 flux risk assessment methodology, available flux-effect relationships and associated critical levels (LRTAP Convention, 2017).

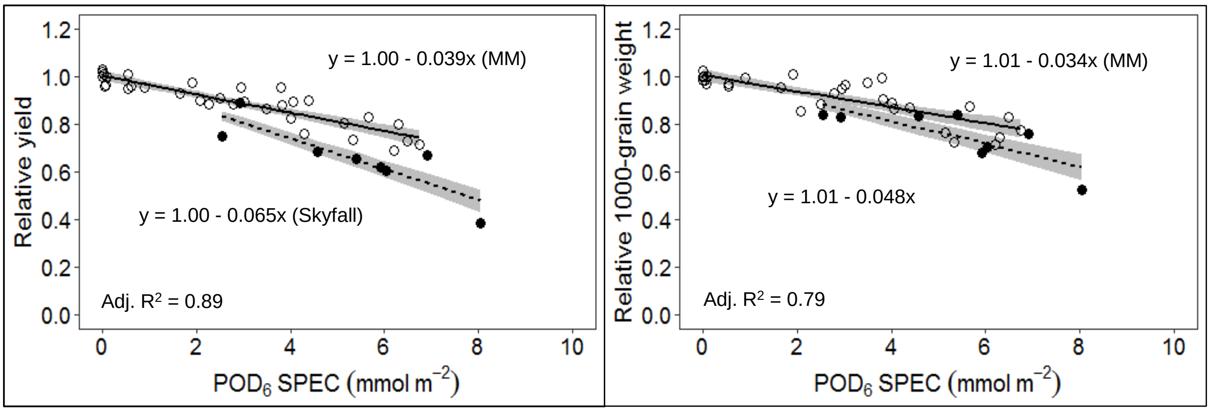

Figure 3. Flux-effect relationships for (A) relative yield and (B) relative 1000-grain weight for wheat using POD_6SPEC as the O_3 stomatal flux metric. Closed circles represent Skyfall data from the current experiment, open circles represent data and flux-effect relationships described in the Modelling and Mapping Manual (MM) of the LRTAP Convention (2017). Adjusted R^2 values are given for the models testing for differences in slope between the two datasets. Lines (dashed for Skyfall and full for MM) are model fitted lines. The 95% confidence interval around each line is shaded in grey.

In comparison with the flux-effect relationships developed by combining data for five wheat varieties exposed to O_3 in open top chambers in the 1980s and 1990s in Belgium, Finland, Italy (only for grain yield) and Sweden (Grünhage et al., 2012; LRTAP Convention, 2017), the modern variety Skyfall is more sensitive to stomatal O_3 flux (POD_6SPEC) than the combined older varieties, with the slope of the relationship for Skyfall being significantly steeper for both grain yield ($P <0.001$) and 1000-grain weight ($P < 0.01$; Figure 3). The same was true for wheat yield when stomatal O_3 flux was expressed as POD_3IAM (data not shown). This is in agreement with previous publications showing that modern wheat varieties bred for high yield might inadvertently have been selected for higher O_3 sensitivity too (Biswas et al., 2008; Pleijel et al., 2006). A similar phenomenon was also observed for soybean, another O_3-sensitive crop species (Osborne et al., 2016). The higher sensitivity in Skyfall might to some extent be driven by an apparently higher maximum stomatal conductance (g_{max}) than the mean g_{max} found in the older wheat varieties (LRTAP Convention, 2017). It might be that agronomic traits targeted by crop breeders are linked to traits associated with high O_3-sensitivity, such as low anti-oxidative capacity and high g_{max} (Biswas et al., 2008; Fiscus et al, 2005). However, it should be noted that from the current study not enough stomatal conductance data are available to develop a completely bespoke parameterisation of the DO_3SE model. More stomatal conductance data should be collected in the future to assess whether a bespoke parameterisation for Skyfall, i.e. different from the parameterisation for wheat described in LRTAP Convention (2017), would be more appropriate.

Conclusion

We have shown that stomatal O_3 flux-effect relationships for wheat yield and 1000-grain weight are not significantly affected by the temporal profile of O_3 exposure. Flux-effect relationships established from experiments where wheat was exposed to peak O_3 episodes are therefore also applicable to wheat exposed to rising background O_3 concentrations. Hence, currently available flux-effect relationships and associated critical levels developed in conditions mimicking past O_3 temporal profiles in Europe (peak episodes) can also be applied to assess the risk of O_3 impacts on wheat in the future, when peak episodes of O_3 are expected to continue to decline due to the implementation of air pollution abatement policies in Europe. This study also adds to existing evidence that that suggests that modern varieties of wheat may be more sensitive to O_3 than wheat varieties used during the 1980s and 1990s. Further research is required to test whether the outcome of this study also applies to other wheat varieties and crop species; and whether the results also apply to varieties grown under different climatic conditions, such as in South-Asia where peak O_3 episodes are currently increasing in frequency and amplitude.

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