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# Effects of ozone on crops in north-west Pakistan

Muhammad Nauman Ahmad <sup>a,b</sup>, Patrick Büker<sup>c</sup>, Sofia Khalid<sup>d</sup>, Leon Van Den Berg<sup>e</sup>, Hamid Ullah Shah<sup>b</sup>, Abdul Wahid<sup>f</sup>, Lisa Emberson<sup>c</sup>, Sally A. Power<sup>d</sup>, Mike Ashmore<sup>c,\*</sup>

<sup>a</sup> Environment Department, University of York, York YO10 5DD, UK

<sup>b</sup> Agricultural Chemistry Department, Khyber Pukhtoon Khwah Agricultural University, Peshawar, Pakistan

<sup>c</sup> Stockholm Environment Institute, University of York, York YO10 5DD, UK

<sup>d</sup> Division of Biology, Imperial College London, Silwood Park, Ascot, Berkshire SL5 7PY, UK

<sup>e</sup> Aquatic Ecology & Environmental Biology, Radboud University Nijmegen, 6525 AJ Nijmegen, The Netherlands

<sup>f</sup> Department of Environmental Sciences, Bahauddin Zakariya University, Multan, Pakistan

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# ABSTRACT

Although ozone is well-documented to reduce crop yields in the densely populated Indo-Gangetic Plain, there is little knowledge of its effects in other parts of south Asia. We surveyed crops close to the city of Peshawar, in north-west Pakistan, for visible injury, linking this to passive measurements of ozone concentrations. Foliar injury was found on potato, onion and cotton when mean monthly ozone concentrations exceeded 45 ppb. The symptoms on onion were reproduced in ozone fumigation experiments, which also showed that daytime ozone concentrations of 60 ppb significantly reduce the growth of a major Pakistani onion variety. Aphid infestation on spinach was also reduced at these elevated ozone concentrations. The ozone concentrations measured in April–May in Peshawar, and used in the fumigation experiment, are comparable to those that have been modelled to occur over many parts of south Asia, where ozone may be a significant threat to sensitive crops.

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# 1. Introduction

The increase in urbanization, transport and energy use in south Asia over recent decades has been accompanied by increased NO<sub>x</sub> and VOC emissions (Emberson et al., 2001). These precursor emissions are mainly responsible for the gradual rise of ground level background ozone concentrations that is affecting crop growth and yield in this region and around the northern hemisphere (Emberson et al., 2001; Ashmore, 2005; Van Dingenen et al., 2009). Modelling studies suggest that critical levels of ozone for effects on crop yield are exceeded over much of the sub-continent, with the greatest ozone exposure occurring in the densely populated Indo-Gangetic plain (Mittal et al., 2007; Engardt, 2008; Roy et al., 2009). Experimental studies of the effects of ozone in the Indo-Gangetic plain have primarily involved staple cereal and legume crops, for which substantial yield losses due to ozone have been reported, both in India (e.g. Agrawal et al., 2003, 2005; Tiwari and Agrawal, 2009) and Pakistan (e.g. Wahid et al., 2001; Wahid, 2006a,b).

Hence, ozone poses a significant threat to food production in the Indo-Gangetic plain. However, there is a need to assess the impact

\* Corresponding author. E-mail address: mike.ashmore@york.ac.uk (M. Ashmore). of ozone in less densely populated regions of south Asia, and to assess effects on a wider range of species, including vegetable crops, which often form an important element of local diets. Here we report a study which combined measurements of ozone concentrations and assessment of visible injury on crops at two sites close to Peshawar, a city of 1.5 million people situated on the north-west edge of Pakistan, close to the Afghan border, with a UK fumigation study of two potentially sensitive vegetable crops grown in the region, in order to quantify the risk of ozone-induced yield losses.

Spinach and onion were chosen for the fumigation experiments as they are sensitive to ozone and are important vegetable crops in the Peshawar region. European spinach (*Spinacia oleracea*) has been cited as a sensitive crop to ozone by Sakaki et al. (1983) and Calatayud et al. (2003), while in South Asia, Singh et al. (2005) and Tiwari and Agrawal (2009) showed effects of ozone on the summer spinach variety *Beta vulgaris*, All Green. Negative effects of ozone on onion (*Allium cepa*) have been reported by Temple et al. (1990), McCool et al. (1987) and Engle et al. (1965). Pakistani spinach (*B. vulgaris*) is usually grown in winter, and is one of the most important vegetables in Pakistan, providing a cheap source of nutrients for the lower and middle classes (Waseem et al., 2001). Onion is also an important ingredient of recipes such as soups, sauces and a number of seasonal foods (Khan et al., 2005).

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The objectives of the study were to: (1) assess the ambient ozone concentrations at ground level at two rural sites close to Peshawar, using passive samplers; (2) identify any visible injury symptoms characteristic of ozone damage to crops at these two sites; and (3) determine the effects of controlled concentrations of ozone on two vegetable species using open top chambers (OTCs). We finally compare the ozone concentrations in Peshawar with the modelled distribution of ozone across south Asia, in order to assess the wider risk of ozone damage to vegetable crops in this region.

## 2. Materials and methods

# 2.1. Field studies

#### 2.1.1. Study sites and measurement methods

Peshawar is located at 34°01'N 71°35'E in Pakistan at an elevation of 510 m above sea level. The mean annual rainfall and temperature are 404 mm and 22 °C respectively, with the prevailing wind direction mostly from the south (Ahmad et al., 2012). The field studies were carried out at two sites close to Peshawar city: the Agricultural University (AUP), 8 km south west of the city centre, and Tarnab Research Farm (ARI), 12 km north east of the city centre. The mean ozone concentration over a four week exposure period was measured using passive samplers provided by the Swedish Environmental Research Institute (IVL). Gothenburg, Sweden, which are based on a reaction with nitrite to form nitrate (Manes et al., 2003). Passive samplers were installed at AUP and ARI from February 2008 to June 2008, and from November to December 2008, only at the ARI site. Two replicate samples were kept under a metal plate at each location to avoid direct sunlight and rain. The plates were then attached to wooden poles at 2 m height from the ground. Air temperature and humidity were monitored by the local weather stations at ARI and AUP. At the ARI site, monthly mean hydrogen fluoride concentrations in this period were below the detection limit, while monthly mean sulphur dioxide concentrations ranged from 8 to 23  $\mu$ g m<sup>-3</sup> (Ahmad et al., 2012).

#### 2.1.2. Assessment of visible injuries

Foliar injury surveys were carried out from February to June 2008, and in November and December 2008. Observations on seven different crops growing in nearby fields were carried out at both sites at weekly intervals. The mature leaves of okra (*Abelmoschus esculentus*), cucumber (*Cucumis sativus*), tomato (*Lycopersicon esculentum*), onion (*A. cepa*), potato (*Solanum tuberosum*), cotton (*Gossypium hirsu-tum*) and spinach (*B. vulgaris*), were investigated during the summer survey; spinach (*B. vulgaris*) was examined during the winter survey. For each selected field, injury was surveyed in all four corners and in the middle of the field. The number and size of fields varied at each site. All the crops surveyed for injury were of the same variety at both selected sites. Crop species that had high levels of insect/fungal infestation, or showed significant symptoms of water or nutrient deficiency, were not considered for ozone injury assessments, because of the difficulty of distinguishing this from other symptoms.

The damage by ozone pollution was assessed on the basis of % injury of the projected leaf area of mature fully expanded leaves, as follows: no damage, 0% injury; little damage, <30% injury; significant damage, 30–70% injury; severe damage, <70% injury, in accordance with the ICP-NWPC (1999) experimental protocol.

#### 2.2. Open top chamber experiment

#### 2.2.1. Plant growth and ozone exposure

Seedlings of a Pakistani spinach (*B. vulgaris cv.* Nare Palak) and onion (*A. cepa cv.* Swat-1) variety were exposed to four different target concentrations of ozone in open top chambers (OTC) at Silwood Park, Imperial College London for 4 weeks. The spinach experiment was carried out in June/July 2009 and the onion experiment was conducted in August/September 2009. The seeds were first sown in nursery trays using Westland John Innes No. 2 compost inside an unheated greenhouse at Imperial College, Silwood Park. After germination five, one week old, seedlings were transferred to each of 32 pots (30 cm diameter, 30 cm depth). Pots were filled with Westland John Innes No. 2 compost and irrigated as required. Pots were randomly assigned to ozone treatments within the 16 OTCs, with each chamber containing duplicate pots.

The 16 OTCs (each 2 m tall by 2 m diameter) were divided into four replicates of four ozone treatments, with two replicate pots per chamber. All chambers received charcoal filtered air. Ozone was added to charcoal-filtered air to achieve the following daytime (9am–5pm) target concentrations: zero (control), 30 ppb (low), 60 ppb (medium) and 90 ppb (high). Pots were watered with tap water as required, depending upon environmental conditions. Ozone was produced via an oxygen concentrator (model OXC-01, Bio-Fresh Ltd., Newcastle upon Tyne, U.K.) and ozone generator (model GEN02-03, Bio-Fresh Ltd.). Chamber concentrations were monitored hourly with an ozone monitor (model 202, 2B Technologies Inc., Boulder, Colorado, USA) and automatically adjusted with a CompactRio Logger running

LabView software (National Instruments, Austin, Texas, USA). Pots within the same treatment were moved between replicate chambers on a weekly basis to minimise chamber effects. No pesticide applications were made during the experiment. The plant height and leaf number were recorded on Day 1 of the ozone fumigation and then at weekly intervals. Air temperature and relative humidity were monitored by the local weather station at Silwood Park throughout the experiment. A Pico ADC-16 data logger was used with Pico Log for Windows to record ozone data every minute throughout the exposure period.

Weekly injury assessments, of both ozone (interveinal white/chlorotic stipples on upper epidermis) and insect damage, were made in accordance with the ICP-NWPC (1999) assessment protocol, described above. Plants were harvested after about 4 weeks of fumigation, on Day 32 for spinach and Day 33 for onion. The above-ground biomass of each plant was separated into stem, green leaves and dead leaves, dried at 70  $^\circ$ C for 48 h, and then weighed.

#### 2.2.2. Statistical analysis

Statistical analysis was carried out using SPSS 18.0. The dataset for all parameters was explored for skew, kurtosis and normality. The insect injury parameter, which showed major deviations from a normal distribution, was normalised by using  $\log_{10}$  transformation. One-way ANOVA based on chamber mean values was then used for each of the parameters to determine whether the effect of ozone was significant. Tukey's HSD post hoc test was used for multiple comparisons between treatments at *P* = 0.05.

# 3. Results

# 3.1. Field studies

## 3.1.1. Physical and pollution climate

The 4 week mean ozone concentration recorded by passive samplers (Fig. 1) increased between mid February and June, from 26 to 53 ppb at AUP, and from 25 to 48.5 ppb at ARI. The ozone concentrations were higher at AUP; the overall mean ozone concentration was 38 ppb and 34 ppb for AUP and ARI, respectively (Fig. 1). The mean ozone concentrations in November–December were low compared to May–June, ranging from 12 to 22 ppb. The ozone concentrations tended to increase with atmospheric temperature; monthly mean ozone concentrations of 35–40 ppb and above only occurred with a monthly mean temperature of 25 °C or above.

## 3.1.2. Visible injury

No ozone injury was recorded during February and March. However, injury was observed on potato and onion at the ARI site in May, when the mean ozone concentration was 48.5 ppb (Table 1). Significant injury, in the form of typical black flecking, was observed on mature leaves of potato, while severe injury, in the form of white stippling and necrotic tip burn, was observed on leaves of onion plants. Ozone injury was also found on onion and cotton leaves at the AUP site in May, when the mean ozone concentration was 53 ppb. There was no ozone injury found on cucumber, tomato, spinach or okra during the February to June survey period. No ozone injury was found during the November– December survey.

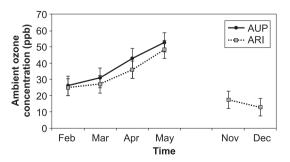


Fig. 1. Ozone concentrations (ppb) at AUP (solid line) and ARI (dashed line). Error bars indicate standard error of the ozone sampler values.

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