Journal of Agriculture and Value Addition
June 2022, Vol. 5(1): 33-44
https://dx.doi.org/10.4038/java.v5i1.33

RESEARCH ARTICLE

Assessing the impact of tropospheric ozone pollution on grain yield of mung bean [*Vigna radiata* (L.) R. Wilczek] using ethylenediurea: A case study in Peradeniya, Sri Lanka

G.A.D. Perera[a]*, S.M. Wijesooriya[a], R.M.U.K. Gunarathne[a], L. Emberson[b], P. Büker[b]

[a]*Department of Botany, University of Peradeniya, Peradeniya, 20400, Sri Lanka*
[b]*Stockholm Environmental Institute, University of York, York, YO10 5NG, UK*

Submitted: February 25, 2022; Revised: May 17, 2022; Accepted: May 27, 2022
*Correspondence: anoma29@gmail.com, ORCID: http://orcid.org/0000-0001-9030-0135

ABSTRACT

Tropospheric ozone (O_3) is well known to reducing the yield of ozone-sensitive crop varieties. The present study aimed to detect the impact of O_3 pollution in Peradeniya, Sri Lanka on the grain yield of a local variety of mung bean [Vigna radiata (L.) R. Wilczek], using the antiozonant ethylenediurea (EDU) protocol. Mung bean plants grown in pots were exposed to ambient O_3 and O_3 free conditions (by adding recommended doses of EDU) in a complete randomized experimental design. Ozone sensitivity was assessed by recording the number of pods and seeds, the dry weight of pods and seeds and the shoot dry weight. The same experiment was repeated on the same site to verify the results. Tropospheric O_3 concentration, daily rainfall, relative humidity (RH) and air temperature in the study location were recorded for the both study periods. The tested local mung bean variety appears to be sensitive to ambient O_3 levels. Further, the O_3 impact on crop yield loss appeared to be varying with the prevailing climatic conditions, especially the distribution of rainfall. Under rather dry climatic conditions that occurred towards the flowering and fruiting periods seemed causing a reduction in the number of pods and seeds of the crop with a yield loss of about 21%. This study further proved that the EDU protocol can be used to demonstrate yield loss of mung bean due to O_3 pollution. As O_3 impacts are better envisaged under dry climatic conditions, we suggest using the EDU protocol to identify O_3 pollution in dry agro-climatic regions of the intermediate and dry zones of the country and to detect O_3 sensitive crop species that grow in these dry regions.

***Keywords**: Ambient O_3, crop yield, dry and intermediate zones, EDU, O_3 sensitivity*

INTRODUCTION

Primary pollutants such as sulfur dioxide, oxides of nitrogen and volatile organic compounds that are emitted from anthropogenic or biogenic sources may be oxidized by ambient free radicals and subsequently transferred into sulfates, nitrates, particulate organic matter and ozone (Lu *et al.*, 2019). Fast emission of primary pollutants in highly industrialized city areas and their vigorous oxidation would result in serious air pollution *in situ* (Lu *et al.*, 2019). These primary pollutants also move with air currents to surrounding areas other than their points of origin and subsequently oxidize into form secondary pollutants like ozone (O_3) creating high O_3 concentrations in rural or agricultural areas (Heck *et al.*, 1984).

Tropospheric O_3 is a 'greenhouse' gas that contributes to current accelerated global warming (Lu *et al.*, 2019) and the ground level O_3 is among the widespread phytotoxic pollutants that is frequently reported as exceeding the World Health Organization (WHO) air quality guidelines for agricultural crops in many regions of the world (Emberson *et al.*, 2010). Especially, tropospheric O_3 is found to be a major atmospheric pollutant in South and Southeast Asia (Emberson *et al.*, 2010), including India (Emberson *et al.*, 2010; Dhevagi *et al.*, 2021), Nepal and Pakistan (Emberson *et al.*, 2010). Further, rising of mean surface O_3 concentrations of 7.2 ppb is predicted for South Asia by 2030 (Dentener *et al.*, 2006). Therefore, identifying the high O_3 threat areas, O_3 sensitive crop species or cultivars and areas suitable for cultivating O_3 sensitive crop varieties may be very beneficial in combating against the foreseen food scarcity. As the chemical methods of detecting O_3 pollution is rather costly it is better to investigate suitable biomonitoring methods to detect O_3 pollution in developing countries like Sri Lanka (Perera, 2011).

Increased, O_3 concentration results in foliar injury and biomass reduction in sensitive crop species such as wheat, beans, and potatoes (Agrawal *et al.*, 2003, 2006), spinach and lettuce (Emberson *et al.*, 2003), rice (Fuhrer *et al.*, 1997; Fuhrer and Booker, 2003; Agrawal *et al.*, 2003, 2006), soybean and cotton (Fuhrer *et al.*, 1997; Fuhrer and Booker, 2003). Moreover, six Indian cultivars of mung bean [*Vigna radiata* (L.) R. Wilczek] (HUM-1, HUM-2, HUM-6, HUM-23, HUM-24, and HUM-26) were reported sensitive to elevated levels of O_3 (Chaudhary *et al.*, 2013). Yield losses have been attributed to the reduced photosynthetic rate (Lehnherr *et al.*, 1997) and related carbon allocation (Grantz and Yang, 2000) or due to the indirect effect of accelerated leaf senescence (Grandjean and Fuhrer, 1989). Also, the nitrogen content of grains and tubers and the nutritive quality of forage crops are found to be declined with increasing tropospheric O_3 contents (Pleijel *et al.*, 1999; Vorne *et al.*, 2002; Sanz *et al.*, 2005).

Biomonitoring of O_3 pollution is now widely practiced across the South and Southeast Asia using the synthetic chemical, ethylenediurea (*N*-[2-(2-oxo-1-imidazolidinyl) ethyl]-*N*-phenylurea) (EDU) (Emberson *et al.*, 2010). EDU is identified as protecting plants from phytotoxic effects of tropospheric O_3 (Salvatori *et al.*, 2017; Nigar *et al.*, 2021), by increasing the plant resistance to ozone (Carnahan *et al.*, 1978). However, EDU is a research chemical which is not commercially available (Carnahan *et al.*, 1978). It is only used as an experimental tool to determine the location and magnitude of crop losses due to O_3. EDU-treated plants showed increased shoot and root length, leaf area, absolute growth rate, relative growth rate and net primary productivity, and can be used effectively to monitor the loss of growth and yield of crops in areas experiencing elevated concentrations of O_3. EDU lessens the negative impacts of O_3 by enhancing antioxidant activities (Salvatori *et al*, 2017; Gupta *et al.*, 2018) and antioxidative enzymes (Gupta *et al.*, 2018) and by holding chlorophylls and, maintaining glutathione reductase and reduced glutathione levels during

O_3 exposure (Lee *et al.*, 1997). However, the effects of EDU treatment may vary with species (Singh *et al.*, 2018) and with cultivars (Singh and Agrawal, 2009).

High air quality index (AQI) values have been reported from Colombo and Kandy city areas of Sri Lanka (Premasiri *et al.*, 2012, Ileperuma, 2020), and these values often exceed the safe limits (Ileperuma, 2020). One-hour ambient O_3 levels in abovementioned areas are recorded as 200 µg m^{-3}. However, neither the ambient ozone levels in the peripheral areas of these cities nor the eight-hour ambient O_3 levels during the peak time/drought periods are available and therefore, it is difficult to understand the O_3 pollution levels in adjacent rural areas. However, biomonitoring of tropospheric O_3 in Kandy and Peradeniya areas had been detected using ozone sensitive (*Bel W3*) and ozone resistant (*Bel B*) varieties of tobacco (*Nicotiana tabacum* L.) during a period where dry climatic conditions prevailed (May-August 2004). Theivathavapalan (2004) revealed moderate level O_3 pollution at Peradeniya. However, elevated O_3 levels causing leaf injuries in tobacco had occurred within the Kandy city, during the study period but the impacts of ambient O_3 on other agricultural crops have not been examined so far. The present study was carried out to detect O_3 pollution in Sri Lanka, as a part of an O_3 biomonitoring experiment to model ambient ozone pollution in parts of Asia and South Africa, using the EDU experimental protocol (Büker *et al.*, 2004). However, the present paper only aimed to demonstrate the ambient O_3 impacts on the yield of mung bean that was grown in Peradeniya, Sri Lanka using the antiozonant EDU.

MATERIALS AND METHODS

This biomonitoring experiment was conducted at Peradeniya, Sri Lanka at about 4 km away from the heavily polluted Kandy city. The experimental setup was established in an open ground on the premises of the Department of Botany, University of Peradeniya (7.259° N, 80.596° E). A locally growing mung bean [*Vigna radiata* (L.) cultivar R. Wilczek] was used as the test species. The EDU chemical protectant protocol given by Büker *et al.* (2004) was used to assess the yield of mung bean under O_3 free (EDU treatment) and ambient O_3 conditions (control experiment), as briefly described below.

Forty-four pots were filled with the prepared soil mixture (garden soil:sand:mould in 3:2:1 ratio) while keeping three glass fiber wicks per pot in a way that the one end of each of the glass fiber wicks come out from the bottom of the pot through holes. Thirty grams of NPK fertilizer [$12N:6P_2O_5:12K_2O$ (+MgO, S)] were added to each pot when filling the soil mixture into them. All these pots were kept on buckets filled with water and allowed glass fiber wicks to absorb water and moisten the soil in the pot. These were arranged in an open place in a protective cage covered with wire mesh (Plate 1) in order to protect from animals like toque macaque.

Plate 1: Experimental site with 44 pots before commencing experiment. Tiny Tag® air temperature and humidity data logger was hung 1.5 m above the ground level, in an open PVC cylinder which was painted with black and white paints (center of the image), in order to protect the probe form direct sun light.

Three seeds of mung bean were kept initially in each pot, after soaking in distilled water overnight allowing germination. After a week, these were thinned out, leaving the healthiest seedling in the pot to remain (Plate 2 a and b).

Plate 2: Germinated mung bean seedlings in a pot (a) before thinning and (b) after thinning.

Once the seedlings were seven days old, the 44 pots with healthy mung seedlings were divided into two groups on random basis, in line with complete randomized design (CRD) (Plate 3). One set (22 pots) were treated with the recommended doses of antiozonant EDU, over a period of 90 days (EDU treatment).

Plate 3: EDU treated and non-EDU mung bean plants arranged in a complete randomized design. Pots with EDU treated plants are marked with a red label.

Thereafter, the recommended amounts of freshly prepared 400 ppm EDU solution, as given in Table 1, were added to each pot in the EDU treatment at regular time intervals. A similar volume of distilled water was added to the seedlings in the rest of the 22 pots that grew under ambient O_3 conditions (control experiment). Both EDU and distilled water were added during the daytime, when no rains occurred.

Table 1: Details of the EDU treatment given in each study period.

Age of seedlings (Days after germination)	Concentration of the EDU solution (ppm)	Volume of EDU added (mL)
7	400	100
17	400	100
27	400	150
37	400	150
47	400	200
57	400	200

Air temperature and atmospheric humidity were detected from the beginning of the experiment every half an hour using a TINYTAG® Datalogger (Plate 1). The rainfall data were collected from the Tea Research Institute at Hantana which is situated about 5 km North of the study site. In order to compare the results of biomonitoring experiments with the actual O_3 concentrations, the tropospheric O_3 content was trapped 3 m above the ground level by keeping two IVL® diffusive samplers.

Exposed passive samplers were collected every 28 d intervals and sent to Stockholm Environmental Institute, UK for analysis. The experiment was conducted from October 14, 2007 to January 15, 2008 (Study period 1) and repeated once again from June 04, 2008 to September 04, 2008 (Study period 2), targeting a wet period and rather a dry period, during the project duration.

Number of pods per plant, the dry weight of pods per plant, number of seeds per plant, the weight of seeds and the shoot dry weight of the EDU treated and non-EDU (control experiment) plants were analyzed by performing 2-sample t tests, using the Minitab® statistical package. Average yield loss was calculated by deducting the average dry weight of mung bean seeds in the control experiment from that in the EDU treatment and given as a fraction to the average dry weight of seeds of the plants in EDU treatment.

RESULTS AND DISCUSSION

As indicated in Table 2, the dry weight (g) of pods, seeds and the shoot of mung bean in both EDU and non-EDU (control) treatments in the first study period were more or less the same (*P*>0.10). In contrast, EDU treated plants that grew in the second experiment (Study period 2) showed a significantly (*P*≤0.10) higher number of pods and seeds per plant though these results were statistically significant only at $\alpha=0.1$ probability level (Table 2). Yield loss (calculated based on the dry weight of seeds) during the second study period due to O_3 pollution was found to be 21% but no yield reduction was observed during the first study period. However, O_3 leaf injuries were observed on mung bean plants in any of the occasions. Caterpillar attacks appeared to be higher during heavy rains, but these pests were removed manually, once detected.

Table 2: Average yield of mung bean in EDU treated and untreated plants in the two study periods.

Parameter	Experiment (study period)	EDU plants	Non-EDU plants	Probability
Number of pods/plant	1	12.77±4.65	10.95±3.64	0.191
	2	11.45±5.38	9.38±3.38	0.075*
Number of seeds/plant	1	102±39.6	93±36.6	0.438
	2	98±53.8	74±34.6	0.1*
Dry weight of pods/plant (g)	1	4.46±0.8	4.97±1.2	0.438
	2	7.81±1.1	6.32±1.6	0.161
Dry weight of seeds/plant (g)	1	3.16±1.46	3.6±1.11	0.363
	2	5.59±3.01	4.41±1.84	0.133
Average shoot biomass (g)	1	5.97±1.77	6.05±1.55	0.913
	2	7.96±3.33	7.33±2.36	0.497

*Significant at 0.1 level

As shown in Table 3, the study area experienced a heavy rainfall in both study periods, and there was no significant (*P*>0.05) difference between the monthly rainfall received during the two study periods (two sample t test, *P*=0.587).

However, during the first study period, heavy rains occurred at the beginning and towards the end of the study period but in the second study period, heavy rains occurred only during the middle parts of the experiment (in the month July, 2008). However, toward the fruit maturing stage, the monthly rainfall was very low (Table 3). RH was also high (above 80%) during both study periods and there was no significant (P>0.05) difference between the average RH between the two study periods (two sample t test, P=0.707). The average air temperature was low and varied from 23-25 °C (Table 3) (two sample t test, P=0.101). However, during the latter part of the second study period, the RH was comparatively lower while the average air temperature was numerically higher, indicating rather dry climatic conditions. However, the average ambient O_3 concentrations did not vary significantly (P>0.05) between the two study periods (two sample t test, P=0.810); but, it fluctuated from 31.3-42.5 µg m^{-3} throughout the measured periods in both seasons.

Table 3: Weather and tropospheric O_3 concentration at Peradeniya during the two study periods.

Study period	Passive sampler exposure period	Average temperature (°C)	Total rainfall (mm)	Average RH (%)	O_3 concentration (µg m^{-3})*
1	14.10.2007 – 10.11.2007	24	357.0	87	42
	11.11.2007 – 08.12.2007	24	60.0	80	40
	09.12.2007 – 05.01.2008	23	209.0	85	31
2	04.06.2008 – 02.07.2008	25	86.3	84	32
	02.07.2008 – 30.07.2008	24	290.4	84	45
	31.07.2008 – 27.08.2008	25	40.0	81	40

*at standard temperature and pressure

The present study reveals that the ambient O_3 pollution in Peradeniya, Sri Lanka during some time periods of the year is high enough to cause yield reduction of mung bean up to 21% and it agrees with Emberson *et al.* (2010), Chaudhary *et al.* (2013) and Chaudhary and Agrawal (2015), and that mung bean is an O_3 sensitive crop plant. Such pollution conditions might also affect to other O_3 sensitive crop species of the country. Air pollutants, especially the phytotoxic O_3 levels are found to be responsible for yield reduction in many other crop species including rice (Fuhrer *et al.*, 1997; Fuhrer and Booker, 2003; Agrawal *et al.*, 2003; 2006), beans and potato (Agrawal *et al.*, 2003; 2006), spinach and lettuce (Emberson *et al.*, 2003), and soybean (Fuhrer *et al.*, 1997; Fuhrer and Booker, 2003). During the period from 2012 to 2017, rice and black gram yields in Sri Lanka have shown negative growth increments while many other crops except sorghum have shown a low yield increase (Annon., 2017). It is very likely that among other factors, the elevated ambient O_3 levels could have played a significant role in the reduction of yield in these crop species in Sri Lanka. Therefore, O_3 impacts on all Sri Lankan crop varieties must be examined as a national requirement in order to take actions for selecting O_3 resistant crop varieties and to prevent yield reductions. The present study also demonstrated that the EDU protocol can be successfully used in detecting the impacts of tropospheric O_3 pollution on the reduction of the yield of mung bean.

Among the factors that affect the ozone levels in the troposphere, the climate plays a major role (Emberson *et al.*, 2010). It was obvious that low O_3 impacts on mung bean plants occurred during the first growing period, which was conducted during the major rainy season with heavy monsoonal rains, especially during initial stages and during fruiting and pod maturity stages. A similar observation has been made by Theivathavapalan (2004) that the ozone leaf lesions initiated on tobacco leaves disappeared with the onset of rains. This may be due to hindrance of the movement of O_3 precursors by raindrops or due to the wash away of O_3 precursors or produced ambient O_3 by rains.

Although the second experiment was planned to be conducted when rather dry climatic conditions prevails (June-September) in the study area, unexpected heavy rains occurred during this study periods as well. Being an oceanic island, Sri Lanka frequently experiences inter-monsoonal rains on unexpected occasions which may affect either the transport of O_3 precursors from the point of origin or prevent the accumulation of O_3 in the troposphere. However, toward the end of the experiment, dry conditions prevailed again [low monthly rainfall (40 mm), comparatively low monthly average RH (81%) and a high monthly average air temperature (25 °C)] and this may be the reason for the observed 21% yield reduction of mung bean during the second study period. In some other parts of South Asia where severe dry environmental conditions prevail for the most parts of the year, the yield reduction appears to be much higher. For instance, the yield loss of mung bean that estimated in Pakistan, India and Nepal from 2006 to 2008 has been varied from 32-64% and these have well correlated with tropospheric O_3 concentrations in respective study sites (Emberson *et al.*, 2010). Therefore, similar studies should be continued further in the intermediate and dry climatic regions of Sri Lanka where the rainfall is seasonally distributed with two clear drought periods (Perera, 1998). In these areas, the majority of people are subsistence farmers and engaged in cultivating paddy and vegetables in permanent and swidden farmlands (Perera, 1998) and therefore, they should be enlightened with novel pollution threats and introduce them with O_3 resistant crop varieties. Therefore, low cost O_3 biomonitoring experiments should be conducted in such dry regions of the country which enables to understand the tropospheric ozone effects on the crops cultivated in these areas.

Further, no leaf injuries due to the toxic effects of the pollutant were observed on mung plants in both study periods during the present study. As Emberson (2010) explained, either the exposure to moderate ozone concentrations (~78 $\mu g\ m^{-3}$) for a longer period or the exposure to an acute O_3 concentration for a shorter period is required for the development of leaf injuries and drastic yield losses. In the present experiment, the monthly average O_3 concentrations were more or less the same during the two study periods. It varied from 31-42 $\mu g\ m^{-3}$ in the first season and from 32-45 $\mu g\ m^{-3}$ during the second season. Thus, the monthly average O_3 concentration in the study site during the both study period can be considered as not very high so as to create severe impact on mung plants. However, loss of yield of mung bean was observed only during the second season and this implies that the distribution of rainfall and accompanied comparatively low RH and high air temperature in the second study period may have caused to reduce the yield of non-EDU mung bean plants. As we have used passive samplers which give the accumulated amount of O_3 over 28 d to monitor monthly average ozone levels, we were not able to find out the peak times or durations with risky ozone levels.

Moreover, delayed senescence in EDU treated plants was observed during the present study. EDU is a systemic antioxidant (Singh *et al.*, 2015), and once applied, EDU is transfered to the leaves of plants (Agathokleous *et al.,* 2016). High doses of EDU may act as a nitrogen fertilizer too and cause to increase the leaf N content. But such high doses of EDU may not cause any toxic effect on fast growing plants (Agathokleous *et al.,* 2016). During the present study, we have not used high doses of EDU instead used only the permitted doses recommended for O_3 biomonitoring experiments using mung bean (Büker *et al.*, 2004). Although the number of pods and seeds in EDU treated plants were slightly higher ($P \leq 0.1$) during the second (rather dry season) compared to non-EDU plants, the dry weights of pods and seeds were not significantly ($P>0.1$) different between the two plant groups. This may be due to the inherent nature of the experimental protocol. As Grandjean and Fuhrer (1989) stated, O_3 accelerates leaf senescence and as a result pods in non-EDU plants get mature before the pods in EDU treated plants. Here, harvesting was done once 50% of the pods became mature and turned into a brown colour irrespective of the experimental treatment. Therefore, by the time of harvesting, the majority of pods in EDU treated plants were at the pod filling stage and this might resulted a lower dry weight for pods and seeds in EDU treated plants. Therefore, we propose to consider harvesting of pods in the two treatments (EDU and non-EDU plants) individually, once 50% of the pods in each treatment become mature and turned brown.

The use of chemical methods and sophisticated equipment to detect atmospheric ozone concentration may not be appropriate for rural agricultural areas in low- and middle-income countries in the world (Perera, 2011), as these methods are more expensive. Therefore, the use of crop species that cultivated in the area itself to detect O_3 pollution is a better alternative. Results of biomonitoring experiments directly provide evidence for the reduction of crop yield due to atmospheric pollution and therefore, such methods are practically useful in establishing the distribution of ozone in areas of interest (Mulgrew and Williams, 2000).

CONCLUSIONS

The tested local mung bean variety appears to be sensitive to ambient tropospheric O_3 at Peradeniya during some time periods, and therefore, it can be used to identify sites with O_3 pollution impacts. However, O_3 impacts on crop yield loss appear to be fluctuated with the prevailing climatic conditions, and it is low or negligible in time periods with substantial rainfall. As O_3 impacts may better envisage under dry climatic conditions, we suggest to use the EDU protocol to identify O_3 pollution in dry agro-climatic regions of the intermediate and dry zones of the country and to detect O_3 sensitive crop species that grow in these regions.

ACKNOWLEDGEMENT

Funds to conduct this experiment were provided by the United Nations Environment Programme (UNEP), Thailand through the Ministry of Environment, Sri Lanka. Rainfall data for the study period was provided by the Tea Research Institute, Hantana, Sri Lanka. Mr. Kosala Samarasinghe, Mr. Chaminda Sampath and Mr. P. Nandalal are acknowledged for their involvement in establishing the shade house and Ms. R. Vansapura for the assistance extended in maintaining the grown plants during the first study period.

REFERENCES

Agathokleous, E., Paoletti, E., Saitanis, C.J., Manning, W.J., Shi, C. and Koike, T. (2016). High doses of ethylene diurea (EDU) are not toxic to willow and act as nitrogen fertilizer. Sci. Total Environ., 566-567: 841-850. Doi: 10.1016/j.scitotenv.2016.05.122.

Agrawal, M., Rajput, M. and Singh, R.K. (2003). Use of Ethylenediurea to assess the effects of ambient ozone on *Vigna radiata*. Biotronics. 32: 35-48.

Agrawal, M., Singh, B., Agrawal, S.B., Bell, J.N.B. and Marshall, F. (2006). The effect of air pollution on yield and quality of mung bean grown in peri-urban areas of Varanasi. Wat. Air and Soil Poll., 169: 239-254.

Annon. (2017). Overview: National strategic review of food security and nutrition, towards zero hunger. World Food Programme (WFP), Country Report, Colombo, Sri Lanka. 34pp. https://www.wfp.org/publications/2017-sri-lanka-national-strategic-review-food-security-nutrition, Retrieved on May 14, 2022.

Büker, P., Pleijel, H. and Agrawal, M. (2004). Experimental protocol for quantifying the impact of tropospheric ozone on crops using protective chemicals. Report prepared for the Male Decleration Network, Thailand. Pp. 7.

Carnahan, J.E., Jenner, E.L. and Wat, E.K.W. (1978). Prevention of ozone injury to plants by a new protectant chemical. Phytopathology, 68:1225-1229.

Chaudhary, N. and Agrawal, S.B. (2015). The role of elevated ozone on growth, yield and seed quality amongst six cultivars of mung bean. Ecotoxicol. Environ. Saf., 111: 286-294. Doi: 10.1016/j.ecoenv.2014.09.018.

Chaudhary, N., Singh, S., Agrawal, S. and Agrawal, M. (2013). Assessment of six Indian cultivars of mung bean against ozone by using foliar injury index and changes in carbon assimilation, gas exchange, chlorophyll fluorescence and photosynthetic pigments. Environ. Monit. Assess., 185: 7793–7807. doi:10.1007/s10661-013-3136-0

Dentener, F., Stevenson, D. and Ellingsen, K. (2006). The global atmospheric environment for the next generation, Environ. Sci. & Technol., 40: 3586-3594. Doi:10.1021/es0523845.

Dhevagi, P., Ramya, A., Priyatharshini, S. and Poornima, R. (2021). Effect of Elevated Tropospheric Ozone on *Vigna mungo* L. Varieties. Ozone: Sci. Eng., 2021: 1-21. DOI: 10.1080/01919512.2021.2009332.

Emberson, L.D., Ashmore, M.R. and Murray, F. (Eds). (2003). Air pollution impacts on crops and forests: a global Assessment. Imperial College Press, London. 355pp.

Emberson, L.D., Büker, P., Engardt, M. et al. (2010). Assessing ground-level ozone (O_3) impacts to crops in parts of Asia and Southern Africa. In: Air Pollution: health and environmental impacts. (Gurjar, B.R., Molina, L.T and Chandra Ojha, C.S.P. Eds.). CRC press, USA. 421-445.

Fuhrer, J. and Booker, F. (2003). Ecological issues related to ozone: Agricultural issues. Environ. Int., 29: 141-154. Doi:10.1016/S0160-4120(02)00157-5

Fuhrer, J., Skärby, L. and Ashmore, M.R. (1997). Critical levels for ozone effects on vegetation in Europe. Environ. Pollut., 97: 91-106. Doi:10.1016/S0269-7491(97)00067-5 ·

Grandjean, A. and Fuhrer, J. (1989). Growth and leaf senescence in spring wheat (*Triticum aestivum* L. cv. Albis) grown at different ozone concentrations in open-top field chambers, Physiol. Plant., 77: 389-394. Doi:10.1111/j.1399-3054.1989.tb05658.x

Grantz, D.A. and Yang, S.Y. (2000). Ozone impact on allometry and root hydraulic conductance are not mediated by source limitation or developmental age, J. Exp. Bot., 51: 919-927. Doi:10.1093/jexbot/51.346.919

Gupta, S., Sharma, M., Majumder, B., Maurya, V. and Pandey, V. (2018). Impact of Ethylene diurea (EDU) on growth, yield and proteome of two winter wheat varieties under high ambient ozone phytotoxicity. Chemosphere, 196: 161-173. Doi:10.1016/j.chemosphere.2017.12.150.

Heck, W.W., Cure, W.W. Rawlings, J. O. Zaragoza, L.J. Heagle, A.S. Heggestad, H.E. Kohut, R.J., Kress, L.W. and Temple, P.J. (1984). Assessing impacts of ozone on agricultural crops, II, Crop yield functions and alternative exposure statistics, J. Air Pollut. Control Assoc., 34: 810-817.

Ileperuma, O.A. (2020). Review of air pollution studies in Sri Lanka. Ceylon J. Sci., 49(3): 2020: 225-238. DOI: http://doi.org/10.4038/cjs.v49i3.7773

Lee, E.H., Upadhyaya, A., Agrawal, M. and Rowland, R.A. (1997). Mechanisms of ethylenediurea (EDU) induced ozone protection: Reexamination of free radical scavenger systems in snap bean exposed to O_3, Environ. Exp. Bot., 38(2): 199-209. Doi:10.1016/S0098-8472(97)00016-6

Lehnherr, B., Grandjean, A., Mächler, F. and Fuhrer, J. (1997). The effect of ozone in ambient air on ribulose biphosphate carboxylase/oxygenase activity decreases photosynthesis and grain yield in wheat, J. Plant Physiol., 130: 189-200.

Lu, K., Guo, S., Tan, Z., Wang, H., Shang, D., Liu, Y., Li, X., Wu, Z., Hu, M., and Zhang, Y. (2019). Exploring atmospheric free-radical chemistry in China: the self-cleansing capacity and the formation of secondary air pollution, Natl. Sci. Review, 6(3): 579–594. Doi:10.1093/nsr/nwy073

Mulgrew, A. and Williams, P. (2000). Biomonitoring of air quality using plants. Air Hygiene Report No. 10, WHO collaborating Centre for Air Quality Management and Air pollution, Berlin, Germany. 165pp.

Nigar, S., Nazneen, S., Khan, S., Ali, N. and Sarwar, T. (2021). Response of Vigna radiata L. (Mung Bean) to Ozone Phytotoxicity Using Ethylenediurea and Magnesium Nitrate. J. Plant Growth Regul., 2021: 1-13.

Perera, A. (2011). Biomonitoring: a low-cost method for detecting air quality. Zoologica Zeylanica, 2: 7-10.

Perera, G.A.D. (1998). Regeneration and succession following shifting cultivation of dry tropical deciduous forests of Sri Lanka. Doctor of Philosophy (D.Phil.) Thesis, University of Oxford, Oxford, UK.

Pleijel, H., Mortensen, L., Fuhrer, J., Ojanperä, K. and Danielsson, H. (1999). Grain protein accumulation in relation to grain yield of spring wheat (*Triticum aestivum* L.) grown in open-top chambers with different concentrations of ozone, carbon dioxide and water availability. Agric Ecosyst and Environ., 72: 265-270. Doi:10.1016/S0167-8809(98)00185-6

Premasiri, H.D.S., Samarasinghe, L.H.K. and Lakmali, K.M.N. (2012) Population exposure risk assessment to air pollution in Kandy city area. Proceedings of the National Building Research Organization (NBRO) symposium on sustainable disaster resilient technology, Colombo, Sri Lanka.

Salvatori, E., Fusaro, L. and Manes, F. (2017). Effects of the Antiozonant Ethylene diurea (EDU) on *Fraxinus ornus* L.: The role of drought. Forests, 8(9): 1-17. Doi:10.3390/f8090320.

Sanz, J., Muntifering, R.B., Bermejo, V., Gimeno, B.S. and Elvira, S. (2005). Ozone and increased nitrogen supply effects on the yield and nutritive quality of *Trifolium subterraneum*, Atmos. Environ., 39: 5899-5907. Doi:10.1016/j.atmosenv.2005.06.022.

Singh, A.A., Singh, S., Agrawal, M. and Agrawal, S.B. (2015) Assessment of Ethylene Diurea-induced protection in plants against ozone phytotoxicity. Rev Environ Contam T., 233, 129-184. Doi:10.1007/978-3-319-10479-9_4.

Singh, S. and Agrawal, S.B. (2009). Use of ethylene diurea (EDU) in assessing the impact of ozone on growth and productivity of five cultivars of Indian wheat (*Triticum aestivum* L.). Environ. Monit. Assess., 159(1-4): 125-14. Doi.org/10.1007/s10661-008-0617-7

Singh, S., Singh, P., Agrawal, S.B. and Agrawal, M. (2018). Use of Ethylenediurea (EDU) in identifying indicator cultivars of Indian clover against ambient ozone. Ecotoxicol. Environ. Saf., 147: 1046-1055. Doi:10.1016/j.ecoenv.2017.09.068.

Theivathavapalan, J. (2004). Biomonitoring of ozone pollution in the Kandy District. MSc dissertation, University of Peradeniya, Sri Lanka.

Vorne, V., Ojanperä, K. and De Temmerman, L. (2002). Effects of elevated carbon dioxide and ozone on potato tuber quality in the European multiple-site experiment CHIP project. Eur J Agron., 17: 269-381. Doi:10.1016/S1161-0301(02)00072-2.