Table of Contents

Chapter 1 Background

1.1 The Malé Declaration

The Malé Declaration process was initiated in March 1998 during a policy dialogue organised by United Nations Environment Programme (UNEP) in collaboration with the Stockholm Environment Institute (SEI) with financial support from the Swedish International Development Cooperation Agency (Sida). Senior government officials of the South Asian region and experts on air pollution attended the policy dialogue. The meeting agreed on a draft declaration on air pollution to presented to Environment Ministers during the Seventh meeting of the Governing Council of the South Asia Cooperative Environment Programme(SACEP).

The Seventh meeting of SACEP, held in April 1998 in Malé, the Republic of Maldives, adopted the declaration naming it the "Malé Declaration on Control and Prevention of Air Pollution and its likely Transboundary Effects for South Asia".The Malé Declaration stated the need for countries to carry forward, or initiate, studies and programmes on air pollution in each country of South Asia. The first stage in this process is to document current knowledge and information/institutional capacity in each nation relevant to air pollution issues. To this end it was agreed that baseline studies would be developed. Gaps in the current status of knowledge and capacity would become apparent and national action plans to fill these gaps could then be implemented, creating a solid scientific basis for the policy process. Implementation of the action plan will put in place expertise, equipment and information for quantitative monitoring, analysis and policy recommendations for eventual prevention of air pollution.

1.2 Implementation of the Malé Declaration

The implementation of the Malé Declaration was envisaged to be in separate phases. Phase I started with the establishment of a network of organisations to implement the Declaration. The air quality baseline studies were based on data collected from different agencies in the countries relating to the structure, modalities, regulations, institutions and capacities available, to address the problems of local air pollution. The baseline studies led to the formulation of National Action Plans by NFPs and NIAs, indicating requirements in terms of monitoring equipment and capacity building for the measurement and analysis of air quality data.

The implementation plan for Phase II was to put in place expertise, equipment and information for quantitative measurement and monitoring, analysis and policy recommendations for eventual prevention/control of air pollution.

The sixth session of the inter-governmental network meeting, which was held in Teheran, Iran in October 2004, adopted the plan of implementation for Phase III. The general objective of Phase III was to continue to promote the scientific base for prevention and control of transboundary air pollution in South Asia, and to encourage and facilitate coordinated interventions of all the stakeholders on transboundary air pollution at the national and regional level. One of the focuses of Phase III is to enhance the analytical and impact assessment capabilities at the national level through integration of findings from the monitoring stations, local pollution prevention studies and conducted impact assessment studies.

1.3 The Crop Impact Assessment

Ground level ozone (O_3) is arguably the most important atmospheric pollutant causing damage to agricultural productivity across the globe. As part of Phase III, capacity building activities on assessing O_3 impacts on crop included regional-level trainings and case studies at the national level. Two training workshops and 5 field experiments are completed. The level of capacity to assess crop impacts from air pollution has been substantially increased in the participating countries of the Malé Declaration through successful training workshops held in Dhaka/Mymensingh, Bangladesh (2007) and Bangkok, Thailand (2008). At these training workshops scientists, representing all Malé Declaration countries, were trained in the clover-clone and EDU experimental methods. Filed experiments were conducted in Bangladesh, India, Nepal, Pakistan and Sri Lanka. The crop impact assessments carried out in the participating countries have, for the first time, allowed co-ordinated and standardised assessments of field based evidence of O_3 impacts to be conducted. The clover-clone method has offered the opportunity to perform direct comparisons of O_3 impacts within and between regions including Europe, North America, and southern Africa. In contrast, the EDU experiment has provided the opportunity to quantitatively assess the impact of O_3 on the yield of important local crops. The results from these field campaigns clearly indicate that O3 is causing significant impacts to crops in the participating countries of the Malé Declaration. This report presents the preliminary results of the crop impact assessment studies in Bangladesh, Nepal, Pakistan, and Sri Lanka. The report will be further elaborated and soci-economic impacts will be evaluated when all the results from the participating countries are available.

Chapter 2 Assessment Methodology

The crop impact activities carried out in Phase III consisted of i) a provisional modeling-based assessment of risks posed to crops by the air pollutant ground level ozone (O_3), ii) a bio-monitoring campaign using white clover , iii) a chemical protectant study using a variety of crops and iv) a socio-economic assessment of the impacts of O_3 on crop productivity.

2.1 Provisional risk assessment

The distribution of O_3 concentrations in the south Asian region was modeled using the MATCH model (Engardt, 2008), a photo-chemical model that estimates atmospheric formation and transfer of O_3 according to an inventory of pollutant emissions and prevailing meteorology. To identify areas where crop productivity may be affected by O_3 concentrations, the modeled O_3 concentrations need to be characterized using a suitable O_3 exposure metric that relates concentration to crop damage; receptor information should also be collated to show crop distribution, preferably as a digital map with an associated database describing relevant crop statistics (e.g. yield, cropping intensity, crop production). Overlaying these data will enable the production of maps showing locations where concentrations exceeded critical levels and hence where there may be a risk of yield reductions for sensitive crops.

The provisional risk assessments was performed with the intention of identifying O_3 hot-spot areas across the South Asian region using modelled O_3 concentration and agricultural data (e.g. crop distribution and production statistics, growing season information). These data were combined using provisional Air Quality Guidelines (AQGs) that have been recommended by APCEN (Air Pollution Crop Effect Network, http://www.sei.se/apcen/). The examples of the modelling work described here made use of existing AQGs that have been developed in Europe for crops representing the staple crops of the South Asian region (e.g. wheat, beans) that characterise O_3 according to Accumulated Over Threshold (AOT) concentrations.
The areas across the region where these thresholds are exceeded indicated locations where the agricultural crops may be at risk of damage from O_3, and the magnitude of the exceedance would provide an indication of the spatial distribution of relative risk. The production of maps showing exceedance will form the basis upon which to prioritise future work.

2.2 Bio-monitoring campaign

A bio-monitoring campaign was carried out at selected sites in 5 Malé Declaration countries to “ground truth” the provisional risk assessments (described above) and to provide evidence of the real impacts of ground level O_3 on crop biomass and yield.
The campaign used O_3-sensitive and O_3-resistant white clover genotypes (*Trifolium repens* cv. Regal) and worked on the principle that the difference in plant foliar injury as well as the biomass ratio between the O_3-sensitive (NC-S) and O_3-resistant (NC-R) clover genotypes can be directly related to the prevalent O_3 concentrations during the exposure period. This comparatively resource efficient clover bio-monitoring method benefits from its high standardisation (genetically uniform plants, automated water supply, standardised soil and fertilizer) and was expected to provide information on the spatial extent and magnitude of O_3 impacts across the region in relation to O_3 exposures. The method was initially developed in the humid subtropical climate of North Carolina, U.S.A. (cf. Heagle et al., 1994) and has been used extensively and successfully in Europe and North America for almost two decades (e.g. Harmens et al., 2005); hence the results from application of this method in South Asia will enable a direct comparison of O_3 impacts on white clover grown in different parts of South Asia, as well as in Europe and North America.

Visible foliar injury assessments and destructive harvests were performed every 7 and 28 days, respectively with a total duration of the experiment of 4 months. 4-weekly average O_3 concentrations were monitored with passive samplers provided by IVL, Sweden, while temperature and relative humidity were recorded every 30 minutes with micrometeorological loggers. These additional data were collected to aid interpretation of any damage to the clover caused by O_3.

2.3 Chemical protectant method

The chemical protectant method applied here was expected to quantify the yield loss of staple crops of economic and nutritional importance in the Malé Declaration countries, such as wheat, mung bean and spinach. The chemical protectant ethylenediurea ((N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N-phenylurea), abbreviated EDU) was used for this study.
EDU is an anti-ozonant that has been used successfully in a number of experimental campaigns in Europe, North America and Asia (for a review of EDU studies, see http://www.sei.se/apcen/downloads/EDU_experimental_protocol_Male.pdf), to assess the damage caused by ambient O_3 concentrations on a range of crop growth and physiological parameters (including quantity (e.g. yield) and quality (e.g. nutritional content)). EDU is known to suppress typical O_3-induced phyto-toxic effects, such as foliar injury, biomass reductions and premature senescence (Carnahan et al., 1978).

A standardised protocol (e.g. automated water supply, standardised soil and fertilizer) for the EDU experimental studies was developed within APCEN (http://www.sei.se/apcen/) for application within the Malé Crops project to quantitatively assess the actual yield losses for specific crops due to elevated O_3 concentrations across the South Asian region. In order to secure the protective effect of EDU against O_3-induced crop injury, the EDU has to be applied at appropriate concentrations and at specific intervals over the crop growth period. This information was defined for different crops and included in the standardised protocol.
Visible foliar injury was assessed once every week and a single destructive harvest was carried out at the end of the growing period (depending on the crop, this varied between 8 and 12 weeks). 4-weekly average O_3 concentrations were monitored with passive samplers provided by IVL, Sweden, while temperature and relative humidity were recorded every 30 minutes with micrometeorological loggers. As for the bio-monitoring experiment, these additional data were collected to aid interpretation of any crop damage caused by O_3.

2.4 Socio economic risk assessment

It was important to ensure that the experimental data collected from the EDU experiments was interpreted in relation to other existing stresses that might be present and affecting crop productivity in the region. This also provides an opportunity to place the damage caused by O_3 within the context of other stresses to agriculture. This is not an insignificant task and a full assessment is outside the scope of the current study. However, in order to gain some insight into the impact of other stresses and place O_3 impacts in context, SEI has developed a questionnaire that was sent to all Malé participants. This questionnaire asked for details of some of the key problems affecting agricultural production in the areas surrounding the experimental sites. Such details will include the prevalence of water shortage, nutrient deficiency, pest and disease, acidity, salinity and soil erosion. Details of industrial activities on-going in the local area that might impact on crops (e.g. thermal power plants, smelters, brickworks etc.) were also requested. Finally, participants were asked to provide local crop yield statistics for the crops that had been used in the experiments.

This information was synthesised at SEI to aid interpretation of the experimental data and was considered in relation to the socio-economic framework developed under APCEN in terms of data availability and how to best use such data to inform risk assessments for awareness raising and policy formulation.

2.5 Additional activities

Bhutan sampler study

Since Bhutan was not participating in the experimental crop impact studies but expressed interest in investigating the scale of the O_3 concentration levels in Bhutanese agricultural areas, SEI, in conjunction with the Bhutanese NIA, developed a proposal for an extensive passive sampler campaign to be carried out during one crop growing season in Phase III. This proposal was approved by Sida.

It was suggested to expose passive samplers, provided by IVL Sweden, for the entire growing season (July to September) at approximately 10 sites representing important Bhutanese agricultural areas, most of which are restricted to lower elevations and valleys. The exact location of the sites was selected by the Bhutanese NIA.
This study should give an indication of the spatial and temporal distribution of the O_3 concentrations during the main growing season in Bhutan.

References

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

Engardt, M., 2008. Modelling near-surface ozone over South Asia. Journal of Atmospheric Chemistry 59, 61-80.

Harmens, H., Mills, G., Hayes, F., Williams, P. and the participants of the ICP Vegetation (2005). Air pollution and vegetation. Annual report 2003/2004. ICP Vegetation Coordination Centre, Centre for Ecology and Hydrology, Bangor, UK.
http://icpvegetation.ceh.ac.uk

Heagle, A. S., Miller, J. E., SHERRILL, D.E. 1994. A white clover system to estimate effects of tropospheric ozone on plants. Journal of Environmental Quality 23, 613-621.

Chapter 3 Experiment Results

3.1 Provisional Risk Assessment

The provisional risk assessment aimed at providing both scientists and policy makers with knowledge of the spatial extent of the potential risk posed by ground level O_3 to crop productivity across South Asia. This risk assessment will also aid the interpretation of the experimental evidence collected within the Malé crops project and help target future development and application of methods to assess the impact of O_3 on arable agriculture across the region.

The second APCEN network meeting in Stellenbosch, South Africa, in 2006 identified wheat, rice and mung bean as the most important agricultural crops for South Asia, with wheat and rice being especially important in economic terms, and the pulse mung bean being important nutritionally through its potential as an important source of dietary protein. Since wheat and rice are the main staple crops of South Asia, these have been used as the receptors for the provisional risk assessment. A growing period of 3 months was deemed suitable for these crops, largely representing the sensitive phenological period within the growing season.

As described above, regional risk assessments require that the O_3 concentration to which the crop is exposed be expressed in such a way as to relevant for assessing O_3 induced crop damage. A number of methods of characterizing O_3 concentrations have been developed over recent decades in North America and Europe based on experimental evidence relating O_3 dose to crop response. Two of the most common metrics are i) the daylight (7 to 12 hour) growing season mean O_3 concentration (M7 or M12) developed in North America and ii) the Accumulated Over a Threshold of 40 ppb O_3 concentrations (AOT40) developed in Europe (Mills et al., 2007). These O_3 metrics have been used to define a number of dose-response relationships for a variety of crop species a review and synthesis of which is given by Mills et al. (2007).

Unfortunately, no dose-response relationships have yet been developed for Asian grown crop varieties; therefore, it is necessary to select the most appropriate dose-response relationship for this South Asian provisional risk assessment from those available from European or North American studies. Non-Asian derived dose-response relationships have also been used in other studies in East Asia to assess the regional scale risk posed to agriculture from ground level O_3 (Aunan et al., 2000; Wang and Mauzerall, 2004).

Discussions at the 2nd APCEN workshop led to the recommendation to use the AOT40 metric for two main reasons. Firstly, this metric adds more weight to higher O_3 concentrations, since these are known to have a greater influence in determining crop response the metric provides an improved statistical relationship between dose and response. Secondly, AOT40 relationships lend themselves to deriving critical levels, levels below which crop damage would not be expected to occur, due to the linear nature of the dose-response relationship. In addition, the AOT40 dose-response relationship for European wheat is arguably the most robust of any response function, it is based on 9 different wheat varieties and 52 experimental data points. In comparison, the relationship for rice is based on 6 different cultivars and 32 experimental data points.

Figure 1 shows these AOT40 dose-response relationships for wheat and rice as reported by Mills et al. (2007). The dotted vertical lines represent the statistically significant critical level of 3 ppm.hrs for wheat (Mills, 2004) indicating a 5% yield loss; an equivalent critical level for rice would be 14 ppm.hrs, based on a similar yield loss of 5%. In addition, the mean (dotted vertical lines) South Asian AOT40 values based on O_3 concentration data modelled with MATCH are also shown. According to these AOT40 dose-response functions, an average yield loss of approximately 6% and 2% for Asian-grown wheat and rice respectively could be expected when being exposed to mean ambient South Asian O_3 concentrations.

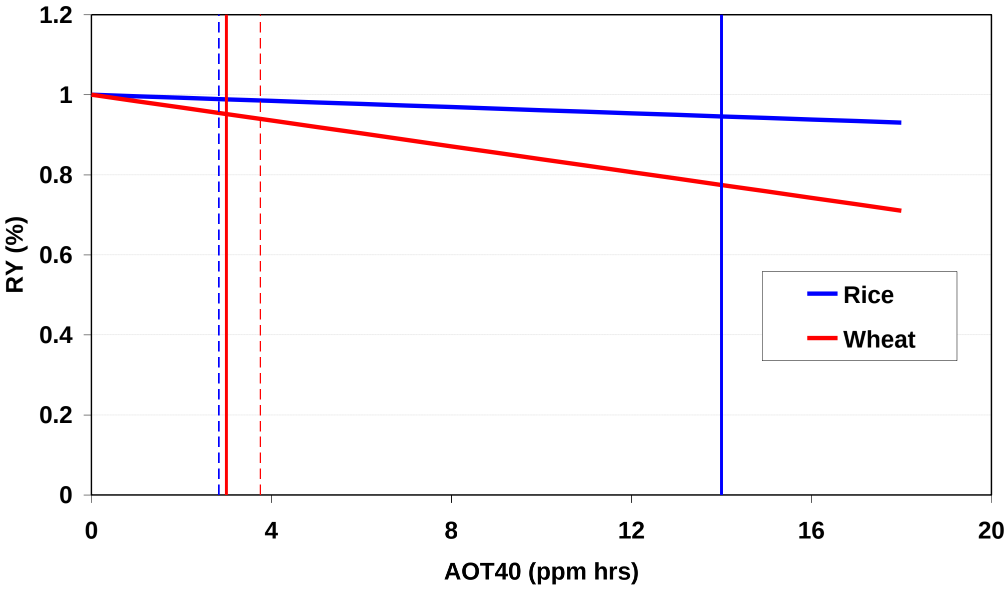


Figure 1 O_3 dose-response relationships for wheat and rice

Figure 1 O_3 dose-response relationships for wheat and rice where O_3 dose is expressed as AOT40 (ppm.hrs) and response as relative yield (%) (Mills et al., 2007). Also shown are the critical level of 3 ppm.hrs for wheat (Mapping Manual, 2004) indicating a 5% yield loss (solid vertical red line) and the respective suggested critical level for rice of 14 ppm.hrs also indicating a 5% yield loss (solid vertical blue line), as well as the mean (dotted vertical lines) South Asian AOT40 values based on O_3 concentration data modelled with MATCH. Note the higher AOT40 exposures for wheat as compared to rice which is determined by the timing of the crop growing season in relation to the seasonal O_3 profile.

Provision of the MATCH modelled O_3 concentration data offers the opportunity to assess the spatial variation in O_3 risk across South Asia through estimation of MATCH AOT40 values for both wheat and rice and reference to the AOT40 dose response relationships. The MATCH model is an off-line Eulerian dispersion model driven by meteorological data from the ECMWF (European Centre for Medium-Range Weather Forecasts) re-analysis. Anthropogenic emissions of SOX, NOX, NHX, NMVOC (non-methane volatile organic compounds) and CO are taken from Streets et al. (2003), biogenic VOC (volatile organic compounds) emission estimates come from Guenther et al. (1995). MATCH (Langner et al., 1998; Robertson et al., 1999) uses a photochemical scheme including approximately 60 chemical species, based on Simpson et al. (1993). The horizontal resolution in MATCH is 50 km, the vertical resolution increases from 20 m near the surface to 400 m at 5 km above surface. MATCH modelled O_3 concentrations were expressed as three month AOT40 values at 3 m above the surface during three month periods of the year 2000 that represented the different wheat and rice crop growth periods.

The results of these simulations are shown in Figure 2 and 3, with Figure 2 representing the main growing season for wheat (March to May) and Figure 3 representing the main growing season for rice (September to November) in South Asia. Both figures show elevated O_3 concentrations extend across the Indo-Gangetic plain, which is the most important agricultural region in South Asia. O_3 concentrations are projected to be higher during the wheat as compared to the rice growing season. This is due to the seasonal profile of O_3 concentrations tending to be elevated during the spring and summer months when solar radiation is greatest; the rice growing season occurs during the south-west monsoon when the heavy rains will "wash out" O_3 from the atmosphere and low solar radiation will reduce the photo-chemical production of O_3. Since the experimental evidence (indicated by the AOT40 dose-response relationships) also suggests that wheat is more sensitive to O_3 than rice, the combination of high O_3 exposures and increased O_3 sensitivity suggest that wheat may well experience larger O_3 induced yield losses than rice across the South Asian region.

Figure 2 for wheat should be viewed in relation to the 3 ppm.hrs critical level, as such any of the coloured areas on the map are areas where wheat crop yields would potentially be at risk from O_3. The figure shows AOT40 concentrations reaching as high as 18 ppm.hrs, this would equate to yield losses of up to 30%. By contrast, Figure 3 for rice should be considered in relation to the higher critical level of 14 ppm.hrs. The map shows that this value is only very rarely exceeded over small parts of South Asia, as such the threat of O_3 induced yield losses to rice suggested by the AOT40 rice relationship be negligible.

However, a recent synthesis of Asian dose-response data performed by Emberson et al. (submitted) suggest that North American and European dose-response relationships may actually underestimate the sensitivity of Asian varieties grown under local conditions. Unfortunately, the lack of standardised experimental procedures do not allow dose-response relationships to be derived from this Asian data but this study does emphasise the need for the establishment of such relationships specifically for Asian conditions. Until such data exist, it is useful to use European and North America relationships to identify the locations and times of year when air pollutants, in particular surface O_3 concentrations, may at least be most likely to impact on agricultural productivity in the region. However, care should be taken when using these non-Asian relationships to infer the **magnitude** of crop yield losses.

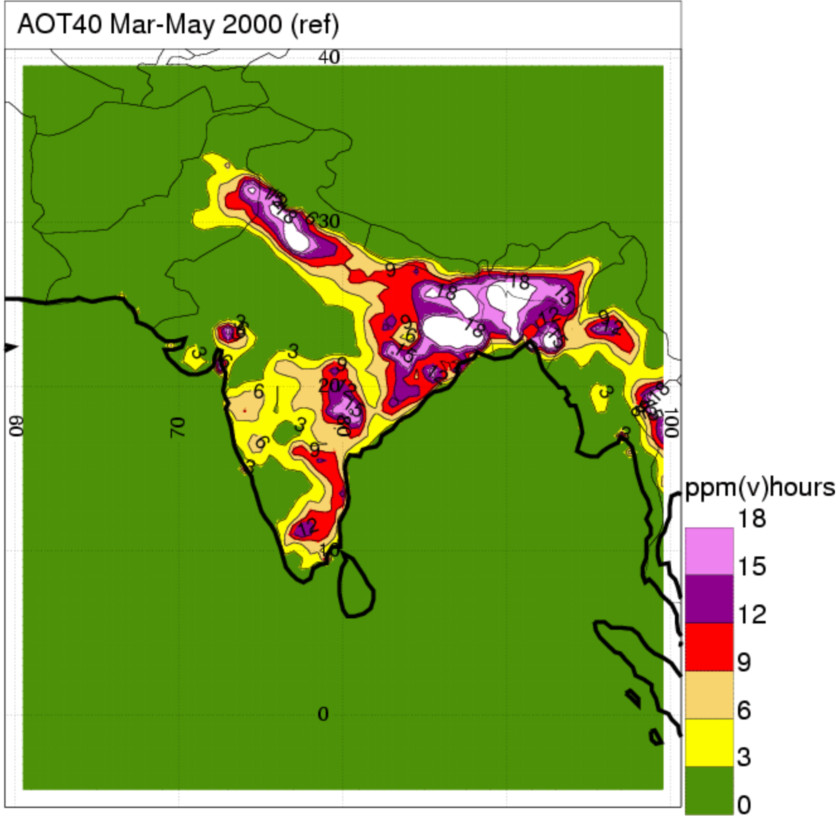


Figure 2 AOT40 simulations for South Asia for March to May 2000, indicating the accumulated O_3 exposure (AOT40) during the wheat crop growing season, calculated using the MATCH model (Engardt, 2008).

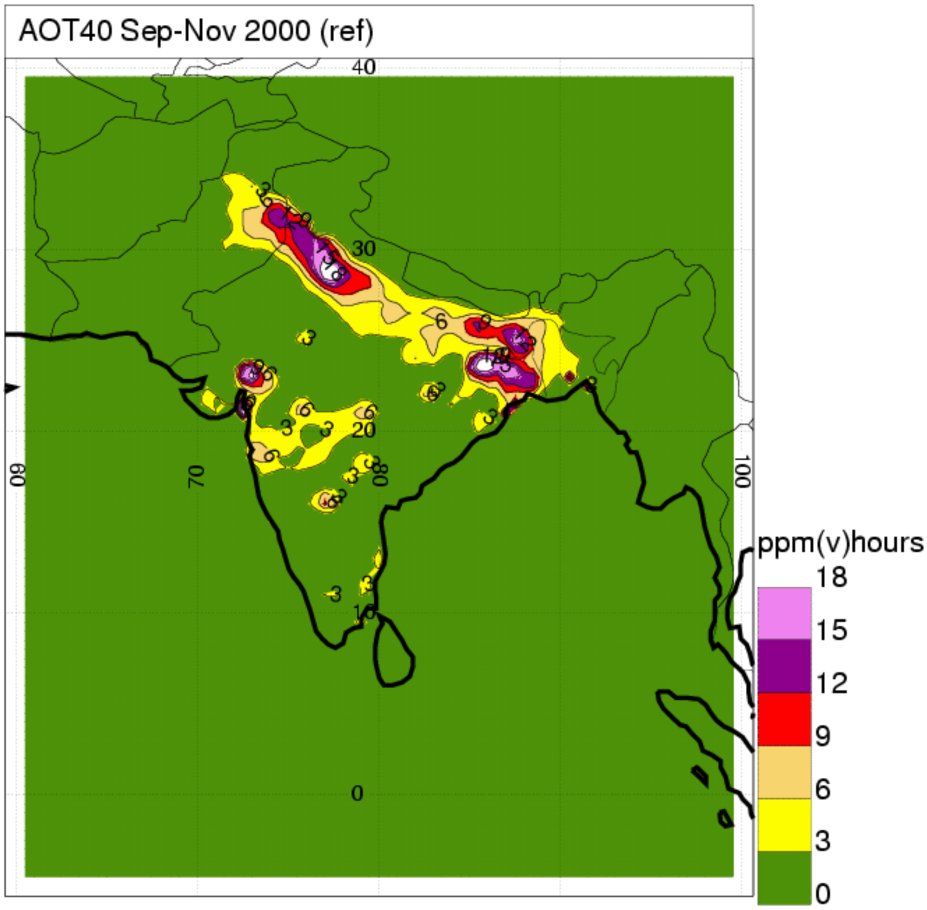


Figure 3 AOT40 simulations for South Asia for September to November 2000, indicating the accumulated O_3 exposure (AOT40) during the rice crop growing season, calculated using the MATCH model (Engardt, 2008).

Supplementary information (e.g. crop distribution statistics, growing season information) is currently being collected from the NIAs and network members with the help of a questionnaire. This information, in conjunction with provisional critical levels that have been recommended by the APCEN, will enable this provisional assessment of O_3 risk to agricultural crops across South Asia to be placed within the context of local agricultural productivity and additional environmental stresses. This work will be completed by end of Phase III.

References

Aunan, K., Berntsen, T.K., Seip, H.M., 2000. Surface ozone in China and its possible impact on agricultural crop yields. Ambio 29, 294-301.

Emberson, L.D., Büker, P., Ashmore, M.R., Mills, G., Jackson, L., Agrawal, M., Atikuzzaman, M.D., Cinderby, S., Engardt, M., Jamir, C., Kobayashi, K., Oanh, K., Quadir, Q.F., Wahid, A. (submitted to Atmospheric Environment). Dose-response relationships derived in North America underestimate the effects of ozone (O3) on crop yields in Asia.

Engardt, M., 2008. Modelling near-surface ozone over South Asia. Journal of Atmospheric Chemistry 59, 61-80.

Guenther, A., Hewitt, C. N., Erickson, D., Fall, R., Geron, C., Graedel, T., Harley, P., Klinger, L., Lerdau, M., McKay, W. A., Pierce, T., Scholes, B., Steinbrecher, R., Tallamraju, R., Taylor, J., Zimmerman, P., 1995. A global model of natural volatile organic compound emissions. Journal of Geophysical Research 100, 8873-8892.

Langner, J., Bergström, R., Pleijel, K., 1998. European scale modeling of sulfur, oxidised nitrogen and photochemical oxidants. Model development and evaluation for the 1994 growing season. Swedish Meteorological and Hydrological Institute, RMK No. 82, Norköpping, Sweden.

Mills, G., 2004. Mapping critical levels for vegetation. In: UNECE Convention on Long-range Transboundary Air Pollution. Manual on Methodologies and Criteria for Mapping Critical Loads and Levels and Air Pollution Effects, Risks and Trends. Constantly updated version available at: <www.oekodata.com/icpmapping>.

Mills, G., Buse, A., Gimeno, B., Bermejo, V., Holland, M., Emberson, L., Pleijel, H., 2007. A synthesis of AOT40-based response functions and critical levels for ozone for agricultural and horticultural crops. Atmospheric Environment 41, 2630-2643.

Wang X., Mauzerall D.L., 2004. Characterizing distributions of surface ozone and its impact on grain production in China, Japan and South Korea: 1990 and 2020. Atmospheric Environment 38, 4383-4402.

Robertson, L., Langner, J., Engardt, M., 1999. An Eulerian limited-area atmospheric transport model. Journal of Applied Meteorology 38, 190-210.

Simpson, D., Anderss-Skjöld, Y., Jenkin, M.E., 1993. Updating the chemical scheme for the EMEP MSC-W oxidant model: current status. EMEP MSC-W Note 2/93, Oslo, Norway.

3.2 Experimental Campaigns

3.2.1 Bangladesh: Bio-Monitoring Study on Tropospheric Ozone Using White Clover at Bangladesh Agricultural University

To our knowledge, O_3 impact assessments on crops have so far not been performed in Bangladesh despite the fact that a large number of crops, vegetables and spices are widely cultivated throughout the year. Hence, the present work on a "Bio-Monitoring Study on Tropospheric Ozone Using White Clover at Bangladesh Agricultural University" will be helpful as a first guideline to evaluate the impacts of ozone (O_3) on selected crops. This will hopefully help to identify crops and cultivars that are less O_3-sensitive than those used to date. A 4 months pot experiment was conducted at the field site of the Department of Environmental Science, Bangladesh Agricultural University, Mymensingh to assess the visual O_3 injury in white clover crops (*Trifolium repens* cv. Regal) in 2007 and 2008. Two genotypes of white clover were used, of which one was O_3-sensitive (NC-S) and the other was O_3-resistant (NC-R).

3.2.1.1 Ozone Concentration and Metrological Condition

The 28-day mean O3 concentrations recorded with passive O3 samplers during the first experiment from May to August 2007 are shown in Table 1, together with the prevailing climatic conditions. The 28-day intervals in Table 1 represent the four white clover exposure periods.

The O3 concentrations declined over the course of the first 3 months (April to August) of exposure. During the same period, air temperature and relative humidity increased, while the sunshine hours decreased, clearly indicating the onset of the monsoon season from June on (see also Appendix 3.2.1 Figure 1).

Table 1. 28-day mean O_3 concentrations (recorded with passive samplers), temperature, relative humidity, sunshine hours and 28-day rainfall sum representing the four white clover exposure periods in Year 1 (2007) at BAU, Mymensingh

White clover exposure period	O_3 concentration (ppb)	Air temperature (°C)	Relative humidity (%)	Sunshine hours (hrs)	Rainfall (mm)
13 May - 10 June	34.84	28.19	84.38	5.00	476.00
11 June - 8 July	26.04	29.06	86.29	4.60	525.40
9 July - 5 Aug.	22.21	28.27	90.46	2.70	712.60
6 Aug. - 2 Sep.	-	38.64	88.32	3.96	297.70

Table 2. 28-day mean O3 concentrations (recorded with passive samplers), temperature, relative humidity, sunshine hours and 28-day rainfall sum representing the four white clover exposure periods in Year 2 (2007/08) at BAU, Mymensingh

White clover exposure period	O_3 concentration (ppb)	Air temperature (°C)	Relative humidity (%)	Sunshine hours (hrs)	Rainfall (mm)
6 Nov. - 4 Dec.	-	23.36	85.38	7.38	67.00
5 Dec. - 1	16.88	25.23	83.32	6.62	0.00

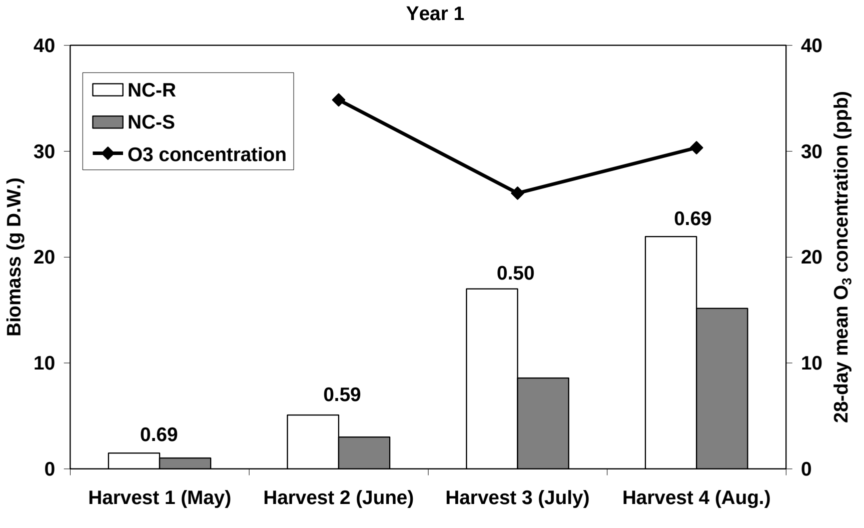
Jan.					
2 Jan. - 29 Jan.	19.66	18.47	83.82	13.65	30.60
30 Jan. - 26 Feb.	-	18.83	75.82	8.02	0.00

The second experiment started just after the monsoon in November and was terminated at the end of February. The 28-day mean O_3 concentrations recorded with passive O_3 samplers during the exposure period 2 and 3 (December to January) as well as the 28-day average climatic conditions of all four exposure periods are presented in Table 2. In comparison to the first experiment, mean O_3 concentrations, mean air temperature and the rainfall sum were lower, whereas the average sunshine hours were increased. The differences between experiment 1 and 2 in terms of the physical and pollution climate might be attributed to differing main wind directions during these two 4-months exposure periods.

3.2.1.2 Assessment on Visible Injury, Weekly Injury and Harvest

During the first experimental period, visual injury symptoms appeared on the leaves of the O_3-sensitive clover genotype (NC-S), classified as very slightly (occurrence of first O_3 injury) to slightly (1-5% of injured leaves) injury with 50%, 55%, 45% and 55% O_3-injured leaves (Appendix 3.2.1 Table 2). The resistant clover genotype showed no visual injury symptoms.

During the second experimental period, visual injury symptoms appeared on the leaves of the O_3-sensitive clover genotype (NC-S), classified as very slightly (occurrence of first O_3 injury) to slightly (1-5% of injured leaves) injury with 45%, 55% and 50% O_3-injured leaves for the first, second and third harvest, respectively (Appendix 3.2.1 Table 4).



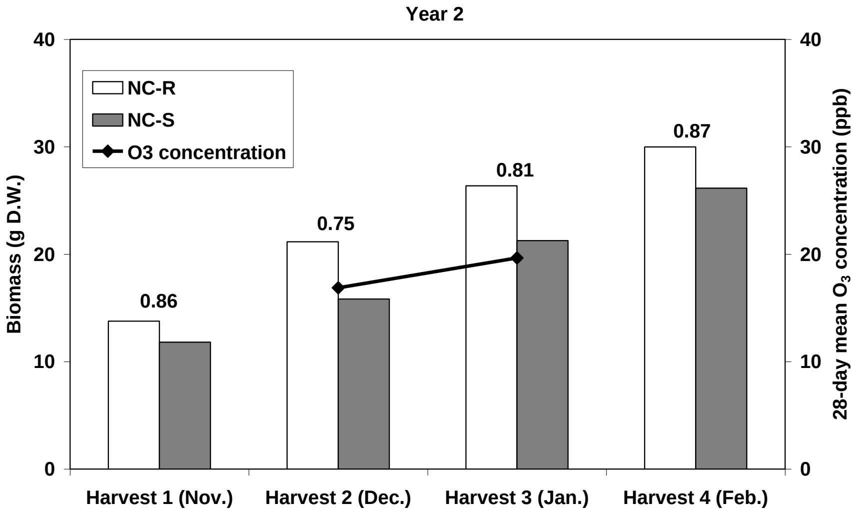


Figure 4 Average biomass (g dry weight) of the NC-S and NC-R white clover genotypes at four harvests of the first (top graph) and second (bottom graph) experimental period. Also shown are the NC-S/NC-R ratios (numbers above bars) and the 28-day average O_3 concentrations where available.

Figure 4 shows the average biomass (g dry weight) of the NC-S and NC-R white clover genotypes for all four harvests of the first and second experimental period. The O_3-resistant genotype always had a higher average biomass, mirrored in NC-S/NC-R ratios of between 0.5 (third harvest of first experiment) and 0.87 (fourth harvest of second experiment) (see also Appendix 3.2.1 Table 1 and 3).

The biomass for both genotypes continually increased over the course of both exposure periods and the exposure of the clover genotypes during the second experimental period (November to February) resulted at all harvests in higher average biomasses as compared to the clover biomasses recorded at the harvests of the first experimental period (May to August). Since growing conditions are believed to have been better during the first experimental period, the higher average biomass of the second experimental period might be related to a further establishment and better adaptation of the clover plants to the prevailing climate conditions in Mymensingh (especially when keeping in mind the general difficulties of establishing the clover plants in Malé Declaration countries).

There was an obvious negative correlation between the NC-S/NC-R ratio and the 28-day average O_3 concentration as measured by passive sampling (R^2 = 0.49, also see Appendix 3.2.1, Figure 2), i.e. higher O_3 concentrations resulted in smaller NC-S/NC-R ratios.

3.2.1.3 Conclusions

The white clover bio-monitoring system was successfully established at the site in Mymensingh. The prevailing O_3 concentrations at the site led to a decrease in biomass of the O_3-sensitive clover genotype which indicates that sensitive crop cultivars might be at risk in the Mymensingh area. Given the projected increases in O_3 concentrations for the near future in the South Asian region, this result demonstrates that detailed risk assessments have to be carried out to be able to quantify the impact O_3 might pose to crops in Bangladesh.

These risk assessments require information about the dose-response relationships of economically and nutritionally important crops; hence, chemical protectant studies with local crop species and cultivars have been proposed for the near future.

Reference

Agrawal, M., 2003. Air pollution impact on vegetation in India. In: Emberson, L.D., Ashmore, M.R., Murray, F. (Eds.), Air Pollution Impacts on Crops and Forests: a Global Assessment. Imperial College Press, London, U.K., pp. 165-187.

Bambawale, O.M., 1989. Control of ozone injury on potato. Indian Phytopathology 42, 509-513.

Brunschoen-Harti, S., Fangmeier, A., Jäger, H.-J., 1995a. Influence of ozone and ethylenediurea (EDU) on growth and yield of bean (*Phaseolus vulgaris* L.) in open-top chambers. Environmental Pollution 90, 89-94.

Brunschoen-Harti, S., Fangmeier, A., Jäger, H.-J., 1995b. Effects of ethylenediurea and ozone on the antioxidative systems in beans (*Phaseolus vulgaris* L.). Environmental Pollution 90, 95-103.

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

Chawla, H.S. 2005. Introduction to plant Biotechnology. Oxford & IBH publishing Co. Pvt. Ltd. NewDelhi. Pp. 396-427.

Eckardt, N.A., Pell, E.J., 1996. Effects of ethylenediurea (EDU) on ozone-induced acceleration of foliar senescence in potato (*Solanum tuberosum* L.). Environmental Pollution 92, 299-306.

Elagoez, V., Manning, W.J., 2005. Factors affecting the effects of EDU on growth and yield of field-grown bush beans (*Phaseolus vulgaris* L.), with varying degrees of sensitivity to ozone. Environmental Pollution 136, 385-395.

Emberson, L.D., Ashmore, M.R., Murray, F. (Eds.), 2003. Air Pollution Impacts on Crops and Forests: a Global Assessment. Imperial College Press, London.

ENVIRON, 2006. CAMx, comprehensive air quality model with extensions. User's Guide, Version 4.30, ENVIRON International Corporation, Novato, California, U.S.A. http://www.camx.com/

Fleming, G., van der Merwe, M., 2002. Spatial disaggregation of greenhouse gas emissions inventory data for Africa south of the equator, http://gis.esri.com/library/userconf/proc00/professional /papers/ PAP896/p896.htm.

Fuhrer, J., Skärby, L., Ashmore, M.R., 1997. Critical levels for ozone effects on vegetation in Europe. Environmental Pollution 97, 91-106.

Fuhrer, J., Booker, F., 2003. Ecological issues related to ozone: agricultural issues. Environment International 29, 141-154.

Gatta, L., Mancino, L., Federico, R., 1997. Translocation and persistence of EDU (ethylenediurea) in plants: the relationship with its role in ozone damage. Environmental Pollution 96, 445-448.

Godzik, B., Manning, W.J., 1998. Relative effectiveness of ethylenediurea, and constituent amounts of urea and phenylurea in ethylenediurea, in prevention of ozone injury to tobacco. Environmental Pollution 103, 1-6.

Grandjean, A., 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. Physiologia Plantarum 77, 389–394.

Grantz, D.A., Yang, S.Y., 2000. Ozone impacts on allometry and root hydraulic conductance are not mediated by source limitation or developmental age. Journal of Experimental Botany 51, 919–927.

Harmens, H., Mills, G., Hayes, F., Williams, P. and the participants of the ICP Vegetation (2005). Air pollution and vegetation. Annual report 2003/2004. ICP Vegetation Coordination Centre, Centre for Ecology and Hydrology, Bangor, UK. http://icpvegetation.ceh.ac.uk

Harmens, H., Mills, G., Emberson, L.D., Ashmore, M.R., 2005. Implications of climate change for the stomatal flux of ozone: a case study for winter wheat. Environmental Pollution.

Hassan, I.A., 2006. Physiological and biochemical response of potato (*Solanum tuberosum* L. cv. Kara) to O_3 and antioxidant chemicals: possible roles of antioxidant enzymes. Annals of Applied Biology 148, 197-206.

Hassan, I.A., Ashmore, M.R., Bell, J.N.B., 1995. Effect of ozone on radish and turnip under Egyptian field conditions. Environmental Pollution 89, 107-114.

Heagle, A.S., 1989. Ozone and crop yield. Annual Review of Phytopathology 27, 397-423.

Heagle, A.S., Miller, J.E., Sherrill, D.E., 1994. A white clover system to estimate effects of tropospheric ozone on plants. Journal of Environmental Quality 23, 613–621.

Islam M.T.; Sattar, M. A.; Baten, M. A.; Salam, M. A.; Hashmi, Q. S. I.; Ali, M. S.; Bueker, P; Emberson L. and Iyngararasan, M. 2007. Assessing the Impacts of Tropospheric Ozone on Crop Losses in Bangladesh. Regional Workshop proceedings. August 2007, Dhaka

Lehnherr, B., Grandjean, A., Mächler, F., Fuhrer, J., 1997. The effect of ozone in ambient air on ribulose bisphophate carboxylase/oxygenase activity decreases photosynthesis and grain yield in wheat. Journal of Plant Physiology 130, 189–200.

McCarthy, J.J., Canziani, O.F., Leary, N.A., Dokken, D.J., White, K.S. (Eds.), 2001. Climate Change 2001: Impacts, Adaptation, and Vulnerability. Intergovernmental Panel on Climate Change, Working Group II. Cambridge University Press, Cambridge, U.K.

McKee, I. F., Bullimore, J. F., Long, S. P., 1997. Will elevated CO_2 concentrations protect the yield of wheat from O_3 damage? Plant Cell and Environment 20, 77–84.

Miller, J.E., Pursley, W.A., Heagle, A.S., 1994. Effects of ethylenediurea on snap bean at a range of ozone concentrations. Journal of Environmental Quality 23, 1082-1089.

Regner-Joosten, K., Manderscheid, R., Bergmann, E., Bahadir, M., Weigel, H.J., 1994. HPLC method to study the uptake of and partitioning of the antiozonant EDU in bean plants. Angewandte Botanik 68, 151-155.

Sanz, J., Muntifering, R.B., Bermejo, V., Gimeno, B.S., Elvira, S., 2005. Ozone and increased nitrogen supply effects on the yield and nutritive quality of *Trifolium subterraneum*. Atmospheric Environment 39, 5899-5907.

Tiwari, S., Agrawal, M., Manning, W.J., 2005. Assessing the impact of ambient ozone and growth and productivity of two cultivars of wheat in India using three rates of application of ethylenediurea (EDU). Environmental Pollution 138, 153-160.

van Tienhoven, A.M., Zunckel, M., Emberson, L.D., Koosailee, A., Otter, L., 2006. Preliminary assessment of risk of ozone impacts to maize (*Zea mays*) in southern Africa. Environmental Pollution 140, 220-230.

Varshney, C.K., Rout, C., 1998. Ethylene diurea (EDU) protection against ozone injury in tomato plants at Delhi. Bulletin of Environmental Contamination and Toxicology 61, 188-193.

Wahid, A., 2003. Air pollution impacts on vegetation in Pakistan. In: Emberson, L.D., Ashmore, M.R., Murray, F. (Eds.), Air Pollution Impacts on Crops and Forests: a Global Assessment. Imperial College Press, London, U.K., pp. 189-213.

Wahid, A., Milne, E., Shamsi, S.R.A., Ashmore, M.R., Marshall, F.M., 2001. Effects of oxidants on soybean growth and yield in the Pakistan Punjab. Environmental Pollution 113, 271-280.

Weidensaul, T.C. 1980. N-[2-(2-oxo-1-imidazolidinyl) ethyl]-N'-phenylurea as a protectant against ozone injury to laboratory fumigated pinto bean plants. Phytopathology 70, 42-45.

Zunckel, M., Koosailee, A., Yarwood, G., Maure, G., Venjonoka, K., van Tienhoven, A.M., Otter, L., 2006. Modelled surface ozone over southern Africa during the Cross Border Air Pollution Impact Assessment project. Environmental Modelling & Software 21, 911-924.

3.2.2 Nepal: Assessing the Impact of Ambient Ozone on Growth and Yield of Mungbean with the use of Ethylendiurea (EDU) at Rampur, Chitwan

Mungbean is a short duration crop which plays a vital role to supplement protein in the cereal-based low protein diet of the Nepalese people. Besides, it can be used as a green manuring crop which provides the residual nitrogen (30 kg/ ha/ year) to the succeeding crops like cereals (Tilak and Singh, 1996). The climatic condition of the Terai region (lower and mid hills in Nepal) are favorable for mungbean cultivation (NARC, 2006). However, the present productivity of mungbean is not satisfactory. Various studies show that concentration of various pollutants have crossed the WHO prescribed standard in major cities of Nepal (SEI, 2008, Personnel Communication). Mungbean has been identified as an O_3 susceptible crop by RAPIDC, 2007. No study on the impacts of O_3 on mungbean has so far been conducted in Nepal. Therefore, the present investigation was accomplished to monitor the temporal trends of ambient air pollutants and to assess the biological impact of ambient O_3 on growth and yield of mungbean at Rampur, Chitwan, Nepal.

3.2.2.1 Material and methods

Mungbean seeds were planted in early April 2008 according to the APCEN standardized experiment protocol. The anti-ozonant ethylenediurea(EDU) was supplied as a soil drench on day 7, 17, 27, 37, 47 and 57. Visible leaf injury was assessed on a weekly basis. The final destructive harvest was performed on day 70, i.e. June 16th 2008. The following parameters were assessed at the harvest: number of pods/plant, number of seeds/pod, total seeds/pot, length of fruit or capitulate, 100 seeds weight and total above ground biomass.

3.2.2.2 Ozone Concentration and Metrological Condition

Table 3 summarises the monthly averages of the O_3 concentration, temperature and relative humidity as well as the monthly sum of rainfall at the Rampur site. The passive samplers exposed in May and June are currently being analysed at IVL, Sweden.

Table 3. Monthly averages of O_3 concentration, temperature and relative humidity, and monthly sum of rainfall at the Rampur site. $^+$ = Mean of March 12th to 31st; * = Mean of June 1st to 22nd.

Month	O_3 concentration (ppb)	Air temperature (°C)	Relative humidity (%)	Rainfall (mm)
March	31.0	23.33$^+$	66.43$^+$	33.8
April	36.3	26.77	61.87	40.4
May	analysis awaited	27.96	68.59	118.9
June	analysis awaited	28.25*	72.43*	378.6
Growing season mean	-	27.94	68.30	-

The air temperature and relative humidity increased over the course of the growing season (07/04/08 until 16/06/08) with an average growing season air temperature of 27.94 °C and relative humidity of 68.30 %.

3.2.2.2 Assessment of visible injury, growth and yield parameters

Non-EDU treated plants exposed to ambient air at Rampur showed severe foliar injury, especially during the last week of April and the first week of June (Appendix 3.2.2 Table 1).

Our research shows that various recorded growth attributes were significantly higher for EDU-treated as compared to non-EDU treated plants (Appendix 3.2.2 Table 2). These attributes all increased over the course of the growing season (i.e. with development of consecutive growth stages). Mungbean is an in-determinate crop that shows continuous growth under favourable irrigation and fertiliser management. The crop matures with in 75 days with two to three flushes; however, it can also grow for a longer period.

Table 4. Various yield parameters of non-EDU and EDU treated mungbean plants exposed to ambient air in Rampur, Chitwan region, Nepal. All parameters were recorded at the final harvest on June 16th 2008 after 70 days of exposure.

Characters measured	**EDU**	**Non-EDU**	**% over non-EDU pots**
Pods/plat	45.7	29.55	+54.65
Seeds/pod	6.33	4.65	+36.12
Seeds/plant	273.55	134.8	+102.93
Pod length (cm)	7.55	7.04	+7.24
100 seed weight (g)	2.97	3.88	-23.45
Seed yield (g/pot)	7.72	5.24	+47.32
Biological yield (g/pot)	37.73	20.71	+82.18
Harvest index	0.27	0.32	-15.62

Table 4 presents various yield parameters recorded at the final destructive harvest. All these parameters, with the exception of 100 seed weight and the harvest index, have higher values for EDU-treated as compared to non-EDU treated plants. The higher biomass of EDU-treated mungbean plants is reflected in the larger amount of pods and seeds per pod; however, the seed size was reduced.

3.2.2.3 Discussion

Non-EDU treated plants flowered first and showed an earlier fruiting as compared to EDU-treated plants. This effect can be explained by the vigorous growth of EDU-treated plants which significantly delayed the flowering and fruiting stage.

In general, the EDU seems to have provided good protection to mungbean from elevated O_3 concentrations. The experiment has clearly shown that O_3 concentrations are high enough in the Chitwan region to adversely affect growth and yield of mungbean plants. An economic loss assessment would be desirable since mungbean is an important protein-rich part of the Nepalese diet.

Reference

NARC, 2006. Mungkheti ko prabidhi chinari. FORWARD, NARC, CAZS.

Tilak, K. V. B. R. and Singh, G. 1996. Residual effect of legumes on succeeding cereal crops. Fertilizer News 41:3, 29-35.

3.2.3 Pakistan: Biomonitoring the Impact Tropospheric Ozone in Lahore

Experiments were carried out in an expanded metal enclosure in the Botanical Garden (31°30'N lat., 74°18'E lon. and 680 ft. above sea level) University of the Punjab, Q.A. campus, Lahore. The site was free from trees and about 2km away from main roads on all sides. Metallic enclosures provided adequate protection from birds and rodents etc. In this study, in addition to the use of bio-monitors, suitability of EDU as a soil drench to assess the impacts of ambient ozone (O_3) on commercially important local spinach and mung bean cultivars in a sub-urban area of Lahore was also ascertained. This is the first time that white clover monitors along with diffusive samplers are being used to assess the occurrence of O_3 in Pakistan.

3.2.3.1 Ozone Concentration and Metrological Condition

Daily temperature, especially the minimum night temperature, steadily increased and relative humidity declined during the experimental period (Appendix 3.2.3 Table 1, Figure 1 and Figure 2). Besides, there was a significant increase in ambient O_3 concentration with increase in temperature from April to May. It can be noted from Table 5 that 28-day mean ambient O_3 concentrations increased from 34 ppb in April to 45 ppb in May with rise in air temperature from 27 to 31°C. Data for the month of June is not complete because of termination of experiments in view of end of growth season. Nevertheless, overall seasonal mean O_3 concentration (40.4 ppb) at the experimental site was quite high and well within detection limits.

Table 5 Ozone concentration in the ambient air during experimental period

Site*	Duration	Days	Temp (°C)	O_3 (ppb)
PUBG	Apr 2, 07 to Apr 30, 07	28	27.6	34.0
PUBG	Apr 30, 07 to May 28, 07	28	31.3	44.9
PUBG	May 28, 07 to Jun 6, 07	9	32.5	42.0

*Each O_3 concentration. level is mean of two replicates according to UNEP Protocol based on the analysis of diffusive samplers carried out by IVL, Sweden; * PUBG: Punjab University Botanical Garden, Lahore-Pakistan*

3.2.3.2 Assessment on Visible Injury, Weekly Injury and Harvest

Growth Response of White Clover Biomonitors to Tropospheric Ozone

In two batches of cutting, NC-R cuttings appeared damaged and soft and plants died after some initial growth. On the other hand, NC-S cuttings were firm and healthy, quickly established into young plants which survived and continued to grow. Surviving NC-S plants showed O_3 injury symptoms on some leaves in the form of fine pale yellow specks on the upper leaf surface in the first week of May. However, minute insects (around 1mm diameter) accompanied by fine webbing and eggs were also detected on the lower surface of the affected leaf under a stereo microscope. By the end of May 2007, the surviving plants were shifted to deep natural shade of a tree plantation in the Botanical Garden to protect them from strong sunlight and high temperature of June. Only one of these plants survived and continued to grow producing plenty of lush green, rather weak leafy stolons. No new O_3 injury symptoms were noticed after shifting the plants to deep shade.

However, it should be noted that a further trial of the clover bio-monitoring performed earlier in 2008 was more successful than in 2007. The results will become available very soon, i.e. in time for the final report.

Effectiveness of EDU in Controlling Growth and Yield Losses of Spinach and Mungbean due to Tropospheric Ozone

Experiment was setup as per protocol (Appendix 3.2.3 Table 3). In spinach, O_3-induced initial foliar injury symptoms appeared in non-EDU treatment plants as brown-yellowish flecks on upper leaf surface just after the start of EDU treatment as seen in 33-day old plants. Growth was better and vigorous in EDU-treated plants with fully expanded lush green leaves compared to the non-EDU ones with relatively less developed light green leaves. At the time of final harvest, 65-day-old EDU-treated plants appeared much more highly developed with fully expanded leaves as compared to the non-treated ones (Appendix 3.2.3 Table 4).

Table 6 Effect of EDU treatment on growth of mung bean plants at harvest (± S.E.)

Variety	**Treatment**	**Dry weight of plant components (g)**				
		Stem	**Leaves**	**Pods**	**Seeds**	**Total**
Tall	**EDU-treated**	3.320 ± 0.508 (13%)	3.028 ± 0.489 (5%)	1.797 ± 0.155 (23%)	3.773 ± 0.398 (23%)	11.918 ± 1.363 (15%)
	non-EDU	2.930 ± 0.45	2.880 ± 0.48	1.460 ± 0.09	3.060 ± 0.33	10.330 ± 1.11
Dwarf	**EDU-treated**	0.410 ± 0.072 (-29%)	0.830 ± 0.277 (19%)	0.770 ± 0.263 (22%)	1.500 ± 0.531 (25%)	3.510 ± 1.051 (13%)
	non-EDU	0.580 ± 0.092	0.700 ± 0.129	0.630 ± 0.109	1.200 ± 0.176	3.110 ± 0.466

In tall variety, mean is of 13 and in dwarf of 5 replicates in both EDU and non-EDU treatment plants

Figures in parenthesis gives % increase / decrease relative to non-EDU treatment plants.

For mungbean, the majority of plants turned out to be tall and others as dwarf plants. O_3 injury symptoms as white/silver spots appeared on upper leaf surface in non-EDU treatment plants very early and later turned bronze / necrotic towards crop maturity. Growth was better and vigorous in the plants treated with EDU. Research shows a slight increase in the total number of leaves per plant as a result of EDU treatment especially in the tall variety during weekly assessments V and VI (Appendix 3.2.3 Table 5). The number of O_3 damaged leaves in non-EDU treatment plants was very high at the time of weekly assessments II and III and the extent of injury was assessed as “very heavy injury”.

Growth parameters showed positive influence of EDU application as evidenced by increments in dry weight of stem, leaves, pods, seeds and total biomass of plants compared to their non-EDU counterparts, shown in Table 6. Increase in the dry weight of pods and seeds per plant were much more pronounced than that of the stem and leaves. With regard to yield components, number of filled pods, number of seeds per pod and per plant increased by 30%, 60% and 66% respectively in EDU treated plants as compared to the non-treated ones in the tall variety (Appendix 3.2.3 Table 6). Negative response in the dwarf variety can be attributed to fewer replicates. EDU treatment also increased 100-seed-weight and individual seed weight by 19% and seed weight per plant (all on air-dry weight basis) by 24% compared to non-EDU counterparts in the tall variety (Appendix 3.2.3 Table 7).

3.2.3.3 Discussion

For the clover biomonitoring, there was difficulty in establishing clover cuttings in green-house in the month of March and failure of clover plants to grow under ambient field conditions in April can be attributed to exposure to high temperature. Cuttings got a temperature shock during their import as they remained in transit for more than a week. The shock impact was expressed as decay and decomposition of the apical meristem of the cuttings especially if they were young, soft and packed in excessively moist paper towels. Any mechanical injury during their preparation was another source of damage during transit.

On exposure to field conditions in the month of April, young greenhouse clover plants experienced another high temperature shock leading to their wilting, drying and death. The temperature shock effect can be appreciated from the calendar of phenological events of life-cycle of white clover in relation to climatic conditions (Appendix 3.2.3 Figure 3).Clover grows luxuriantly during cold winter months and starts flowering in warm April. In the present study however, clover plantation in March and field exposure during April could not be avoided due to constraints of the contract; hence establishments and growth of white clover under field conditions could not be achieved. Cool to cold winter from November to March with high humidity and short day length is ideal for growth of white clover (Appendix 3.2.3 Figure 4).

EDU treatments were quite effective in protecting both spinach and mungbean from O_3 leaf injury in the earlier growth stages. However, this protective effect was not so marked in the later growth stages especially in the case of mung bean with only 15% biomass increase in EDU-treated plants compared to 44.5% in spinach (Table 5-7). In terms of the effects on yield, it was the effect of O_3 on the pod size and number of seeds per pod which was a critical factor, although small effects were also found on 100 seed weight and seed number per plant and the number of unfilled pots.

Reference

Agawal, S.B., Singh, A., Agawal, S.B., Bell, J.N.B., and Marshall, F. 2006. The effect of air pollution on the yield and quality of mung bean grown in peri-urban area of Varanasi. *Water Air and Soil Pollution*, 169: 239-254.

Agawal, S.B., Singh, A., and Rathore, D. 2005. Role of ethylenediurea (EDU) in assessing impact of ozone on *Vigna radiata* L. plants in a suburban area of Allahabad (India). *Chemosphere*, 61: 218-228.

Agrawal, M., Singh, B., Rajput, M., Marshall, F., and Bell, J.N.B. 2003. Effect of air pollution on peri-urban agriculture: a case study. *Environmental Pollution*, 126: 323-329.

Ashmore, M.R., marshall, F.M. 1998. Ozone impacts on agriculture: an issue of global concern. Advanced Botany Research, 29: 31-52.

Bortier, K., Dekelver, G., De Temmerman, L., and Ceulemans, R. 2001. Stem injection of *Populus nigra* with EDU to study ozone effect under field conditions. *Environmental Pollution*, 111: 199-208.

Buker, B., Plijel, H.H., and Agrawal, M. 2006. Experimental protocol for monitoring the impact of tropospheric ozone on white clover. Male Decleration Network.http://www.rrcap.unep.org/issues/air/maledec.

Buker, B., Plijel, H.H., and Agrawal, M. 2006. Experimental protocol for quantifying the impact of tropospheric ozone on crops using protective chemicals. Male Decleration Network. http://www.rrcap.unep.org/issues/air/maledec.

Eckardt, N.A., and Pell, E.J. 1996. Effect of ethylenediurea (EDU) on ozone-induced acceleration of foliar senescence in potato (*Solanum tuberosum* L.). *Environmental Pollution*, 92: 299-306.

Fuhrer, J., Skarby L., and Ashmore, M.R. 1997. Critical level of ozone effects on vegetation in Eurpoe. *Environmental Pollution*, 97:91-106.

Fumagalli, I., Mignanego, L., Mills, G. 2003Ozone Biomonetering with clover clones: Yiels loss and carryover effect under high ambient ozone levels in northern Italy. *Agriculture Ecosystem and Environment*, 95: 119-128.

Gillespie, C.V.B., Cardoso-Vilhena, J., Pearson, S., Ollereshaw, J., and Barnes, J. 1998. Mechanisms underlying EDU-induced ozone resistrance. In: Dekok, L.J., Stolen, I. (Eds.), Responses of Plants to Air Pollution. Backhuya Publisher, Leiden, The Netherland.

Godzik, B., and Manning, W.J. 1998. Relative effectiveness of ethylenediurea, and consistent amount of urea and phenylurea in ethylenediurea, in prevention of ozone injury to tobacco. *Environmental Pollution*, 103: 1-6.

Heck, W.W., Taylor, A.C., and Tingey, D.T.: (Eds) 1988. Assessment of Crop Loss from Air Pollution, Elsevier, London.

Jager, H.J., Unsworth, M., Temmerman, L., and Mathy, P.: 1994. 'Effect of Air Pollution on Agricultural Crops in Europe', Air Pollution Report 46. CEC, Brussels.

Kobayashi, K., Okada, M., Nouchi, I. 1995. Effect of ozone on dry matter partitioning and yield of Japanese cultivars of rice (*Oryza sativa* L.). *Agriculture Ecosystem and Environment*, 53: 109-122.

Komala, N., and Ogawa, T. 1991. Diurnal and seasonal variations of the tropospheric ozone in Indonesia. In: Ilyas, M. (Ed.), Ozone Depletion: Implications for the Tropics, University Science Malaysia and the United Nations Environmental Programme (UNEP), pp. 178-188.

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. *Environmental and Experimental Botany*, 38: 199-209.

Manning, W.J., Flagler, R.B., and Frenkel M.A. 2003. Assessing plant response to ambient ozone: growth of ozone sensitive loblolly pine seedling treated with ethylenediurea or sodium erythorbate. *Environmental Pollution*, 126: 73-81.

Pardede, L.S. 1991. Surface ozone measurements at Bandung. In: Ilyas, M. (Ed.), Ozone Depletion: Implications for the Tropics, University Science Malaysia and the United Nations Environmental Programme (UNEP), pp.189-195.

Pleijel, H., Skarby, L., Wallin, G., Sellden, G. 1991. Yield and grain quality of spring wheat (*Triticum aestivum* L.., cv. Drabant) exposed to different concentration of ozone in open-top chamber. *Environmental Pollution*, 69: 151-168.

Sanders, G.E., Colls, J.J., Clark, A.G., Galaup, S., Bebte, J., and Canteul, J.: 1992, '*Phaseolus vulgaris*, and ozone: results from open top chamber experiments in France and England', *Agriculture Ecosystem and Environment*, 38: 31-40.

Tariq, M.N., and Hayat, T.Q.A.S. 1989. Air quality surveillance needs of Pakistan; A Rreport for the Government of Pakistan and the Commission of the European Communities.

Tingey, D.T., Olysk, D.M., Herstorm, A.A., and Lee, E.H. 1993. Effects of ozone on crops. In: McKee, D.J. (Ed.), tropospheric ozone: Human health and agricultural impacts. Lewis Publisher, Boca Raton, U.S.A., pp 175-206.

Wahid, A., Maggs, R., Shamsi, S.R.A., Bell, J.N.B., and Ashmore, M.R. 1995. Air pollution and its impact on wheat yield in the Pakistan Punjab. *Environmental Pollution,* 88: 147-154.

Wahid, A., Milne, E, Shamsi, S.R.A., Ashmore, M.R., and Marshall, F.M. 2001. Effects of oxidants on soybeans growth and yield in the Pakistan Punjab. *Environmental Pollution*, 113, 271-280.

Zheng, Y., Steveuson, K.J., Barroweliffe, R., Chen, S., Wang, H., and Barnes, J.J. 1998. Ozone level in chongqing: a potential threat to crops plants commonly grown in the region. *Environmental Pollution*,99: 299-308.

3.2.4 Sri Lanka : Impacts of Tropospheric Ozone on Mung Bean in Peradeniya

Dr. Anoma Perera, Peradeniya University

The impacts of tropospheric ozone (O_3) on the reduction of the quality and quantity of crop species have not yet been estimated for crops grown in Sri Lanka. The Peradeniya Town, located on the outskirts of Kandy City of Sri Lanka, contains moderate amounts of tropospheric O_3. The main objective of this study is to estimate the impacts of tropospheric O_3 on the growth and yield of Mung bean (*Vigna radiata*) in Peradeniya. The experiment was conducted from 14 November 2007 to 14 February 2008.

3.2.4.1 Ozone Concentration and Metrological Condition

The O_3 concentration in the atmosphere measured during the study period was not very high, especially in late December and early January (Table 7). This might be because the study area received a heavy rainfall before and during the study period (Appendix 3.2.6 Figure 1). Accordingly, the average air temperature was as low at 25^{o}C (Appendix 3.2.6 Figure 2) while the average relative humidity was around 80% (Appendix 3.2.6 Figure 3).

Table 7. Monthly mean O_3 concentration, air temperature and relative humidity as recorded in Peradeniya, Sri Lanka.

Duration		O_3 concentration (ppb)	Air temperature (°C)	Relative humidity (%)
from	to			
14th Nov. 2007	14th Dec. 2007	20.6	24.0	79.7
14th Dec. 2007	14th Jan. 2008	13.4	23.1	84.6
14th Jan. 2008	14th Feb. 2008	21.3	-	-

3.2.4.2 Assessment on Visible Injury, Weekly Injury and Harvest

No leaf injuries were observed on non-EDU and EDU treated plants. At initial stages of the experiment, seedlings in both treatments performed equally well so that the number of leaves per plant in EDU treated and non-EDU treatments were not significantly different ($p>0.05$). After about 30 days, EDU treated plants performed slightly better despite there is no statistically significant difference between the two treatments. After about 36 days, almost all plants bloomed and produced pods. The number of pods per plant was slightly higher in the EDU treated plants despite that it is not statistically significant due to the high variation among individuals (Appendix 3.2.6 Figure 4). However, the number of seeds per pod in two experimental treatments were not significantly different ($p>0.05$) (Appendix 3.2.6 Figure 5). The number of seeds per plant in EDU treated Mung plants were slightly higher than non treated Mung plants despite that that this difference is not statistically significant (Appendix 3.2.6 Figure 6). However, in contrast, the dry weight of seeds in plants of the EDU treatment was slightly lower than the Non EDU treatment (Appendix 3.2.6 Figure 7) despite the fraction of immature pods in the EDU treatment was comparatively higher than Non EDU treatment (Appendix 3.2.6 Figure 8).

Therefore, research shows the O_3 free environment has improved the number of pods per plant as well as the number of seeds per plant despite that these differences were not statistically significant. Whilst on the other hand, research shows that the seed biomass is comparatively lower in the EDU treated plants.

3.2.4.3 Discussion

The non-significant difference between the parameters measured in EDU treated and Non EDU Mung plants may be due to 1) heavy rainfall, low air temperature and high atmospheric humidity during the study period, which themselves result in a low atmospheric O_3 concentration that hinders these measurements; 2) the applied EDU may have also washed out by the rain, especially there were rains immediately after the EDU application; 3) O_3 induces early senescence, the pods of Non EDU plants became mature earlier than the EDU treated plants. There were many immature pods in EDU treated plants by the day of harvesting. In general, the immature seeds contain more moisture and therefore, the dry weight is lower than mature seeds. And thus the seed weight of EDU treated plants was lower; 4) a caterpillar attack when the Mung plants were about 59 days old, is also responsible for reduction of number of seeds and seed weight in EDU treated plants, which eventually result in the number of seeds per plant and the seed dry weight of the EDU treated plants were lower. As O_3 induces early senescence, the well-matured brown colour pods (more than 70%) in Non-EDU plants were not liable to get attacked. Whilst most of the immature pods were in EDU treated plants, the attack by caterpillars thus results in loss of seeds.

References

Theivathavapalan, J. 2006. *Biomonitoring of tropospheric ozone pollution in Kandy area*. M.Sc. dissertation, University of Peradeniya, Sri Lanka.

Experimental Protocol for quantifying the impact of tropospheric ozone on crops using protective chemicals 2007. International Cooperative Programme on effects of air pollution on natural vegetation and crops.

Chapter 4: Synthesis of Results

This chapter can only be completed once all results have been received. However, Table 8 gives already an overview of the success of the chemical protectant and bio-monitoring study in Malé Declaration countries during the three years of Phase III.

Table 8. Overview of success of crop impact studies carried out in various Malé Declaration countries

Country	India	Pakistan	Bangladesh	Nepal	Sri Lanka
Malé member	Prof. Agrawal	Prof. Shamsi	Prof. Sattar, Mr. Islam	Prof. Lal Amgain	Dr. A. Perera
Location	Varanasi	Lahore	Mymensingh	Rampur	Paradeniya
Clover-clone bio-monitoring	✕	2007 2008	2007 2008	✕	✕
EDU Chemical protectant	2006-2008 wheat, mung bean, spinach, potato	2007-2008 mung bean, spinach	2008 various crops	2008 mung bean	2007-2008 mung bean

Appendix

3.2.1 Bangladesh

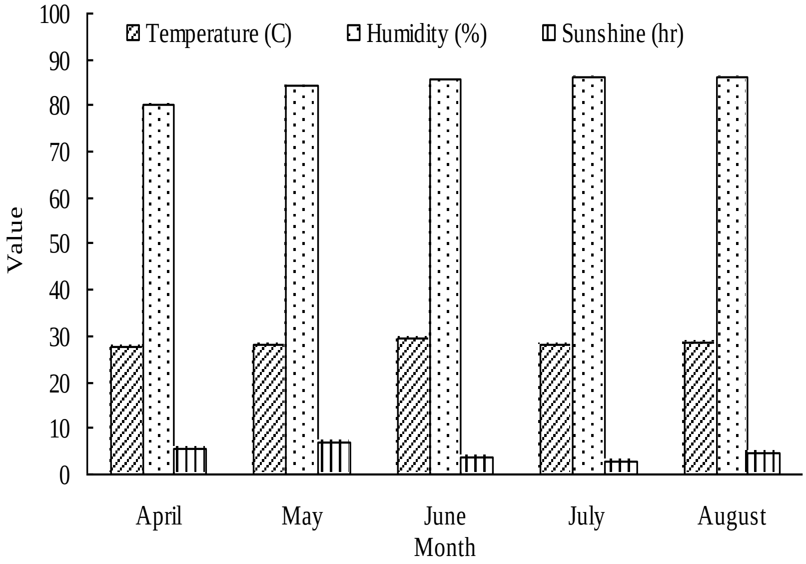


Figure 1 Meteorological data in the experimental fields

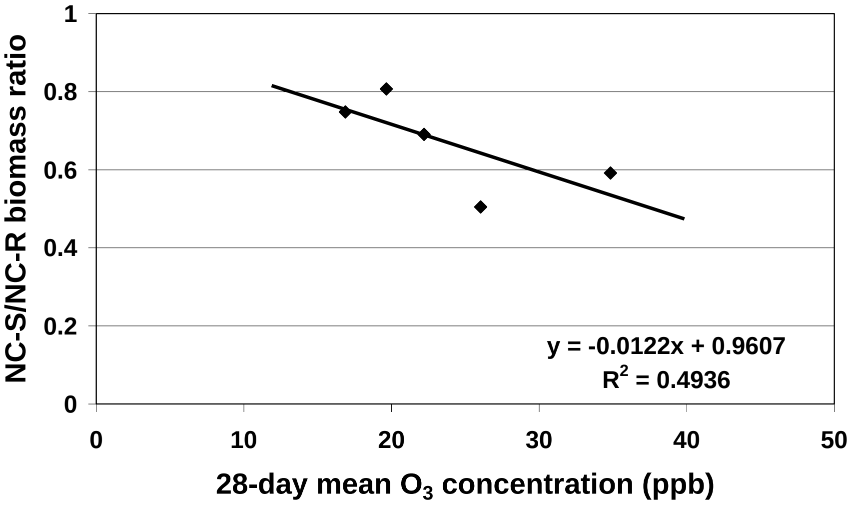


Figure 2 Effects of tropospheric ozone on biomass ratio of white clover

Table 1. Dry weight and biomass ratio of clover plant in different harvesting period in first year

Harvesting period	Plant biomass (g/plant)				BM (NC-S/NC-R)
	Ozone sensitive (NC-S)		Ozone resistant (NC-R)		
	Range	Mean ± SE	Range	Mean ± SE	
1st harvest	0.31 - 2.70	0.980± 1.859	0.28 – 4.50	1.387± 3.906	0.71
2nd harvest	0.33 – 8.30	3.007 ± 2.832	1.03 - 16.68	5.207± 5.981	0.58
3rd harvest	4.70 – 16.40	8.992 ± 11.20	8.10 - 21.18	15.932 ± 3.93	0.56
4th harvest	8.47 - 20.45	12.342 ± 12.21	11.25 - 27.56	22.346 ± 4.23	0.55

BM = Biomass ratio (NC-S/NC-R)

Table 2. Visual in jury in different harvesting period

Harvesting period	Ozone injured plant (NC-S)	Very slight injury	Slight injury
Ist harvest	50%	60%	40%
2nd harvest	55%	60 %	40%
3rd harvest	45%	40%	60%
4th harvest	55%	60%	40%

Very slight injury = Occurrences of first symptom
Slight injury = 1-5 % of a injured leaves in NC-S

Table 3. Dry weight and biomass ratio of clover plant in different harvesting period at first year

Harvesting period	Plant biomass (g/plant)				BM (NC-S/NC-R)
	Ozone sensitive (NC-S)		Ozone resistant (NC-R)		
	Range	Mean ± SE	Range	Mean ± SE	
Ist harvest	3.27 - 19. 32	5.29 ± 1.025	6.72 – 19.87	9.81± 2.164	0. 54
2nd harvest	9.54 – 23.78	10.63 ± 1.732	13.65 – 33.24	20.47± 4.263	0. 51
3rd harvest	10.3 - 35.9	14.16 ± 6.324	21.1 - 41.3	29.52 ± 2.012	0. 50

Table 4. Visual in jury in different harvesting period

Harvesting period	Ozone injured plant (NC-S)	Very slight injury	Slight injury
Ist harvest	45%	50%	50%
2nd harvest	55 %	55 %	45%
3rd harvest	50%	60%	40%

Very slight injury = Occurrences of first symptom
Slight injury = 1-5 % of a injured leaves in NC-S

3.2.2 Nepal

Table 1. Ozone injury assessment in mungbean under EDU and non-EDU pots grown during spring, 2008 at Rampur Chitwan Nepal

Characters measured	Treatments	14 DAS	20 DAS	30 DAS	40 DAS	50 DAS	60 DAS	70 DAS (at harvest)
No. of leaves injured/pot	EDU	4	20	15	96	74	82	84
	Non-EDU	10	76	28	169	161	193	156
Total no. of leaves/pot	EDU	95	205	358	553	793	739	998
	Non-EDU	82	217	364	636	586	537	704
% leaves affected	EDU	4.21	9.76	4.19	17.36	9.33	11.10	8.42
	Non-EDU	12.20	35.02	7.69	26.57	27.47	35.94	22.16

Table 2. Various growth attributes of mungbean over a period of time under EDU and non-EDU pots grown under Rampur condition of Chitwan, Nepal

Characters measured	Treatments	14 DAS	20 DAS	30 DAS	40 DAS	50 DAS	60 DAS	70 DAS (at harvest)
No. of leaves/plant	EDU	4.75	10.25	17.90	27.65	39.65	36.95	49.9
	Non-EDU	4.10	10.85	18.2	31.8	29.3	26.85	35.2
Leaf area/plant	EDU	-	-	129.60	292.26	436.15	428.62	723.44
	Non-EDU	-	-	91.91	246.45	221.32	215.06	442.61
SPAD reading/trifoliate leaf	EDU	-	-	46.64	52.06	51.64	51.12	56.18
	Non-EDU	-	-	39.18	50.31	48.57	48.60	48.78
No. of Branches/plant	EDU	-	0.70	2.5	2.25	2.35	2.30	4.30
	Non-EDU	-	0.15	2.5	2.60	2.3	2.4	3.50
Plant height (cm)	EDU	-	11.23	22.39	33.53	41.92	44.10	42.78
	Non-EDU	-	11.	22.84	30.87	36.44	37.60	37.97

			75					
Root length (cm)	EDU	–	–	–	–	–	–	17.62
	Non-EDU	–	–	–	–	–	–	15.22
Root volume (mm3)	EDU	–	–	–	–	–	–	5.96
	Non-EDU	–	–	–	–	–	–	4.35
Effective nodules	EDU	–	–	–	–	–	–	32.95
	Non-EDU	–	–	–	–	–	–	27.15
75% flowering date	EDU	–	–	–	–	–	–	37.2
	Non-EDU	–	–	–	–	–	–	36.0
75% fruiting date	EDU	–	–	–	–	–	–	50.65
	Non-EDU	–	–	–	–	–	–	48.8

3.2.3 Pakistan

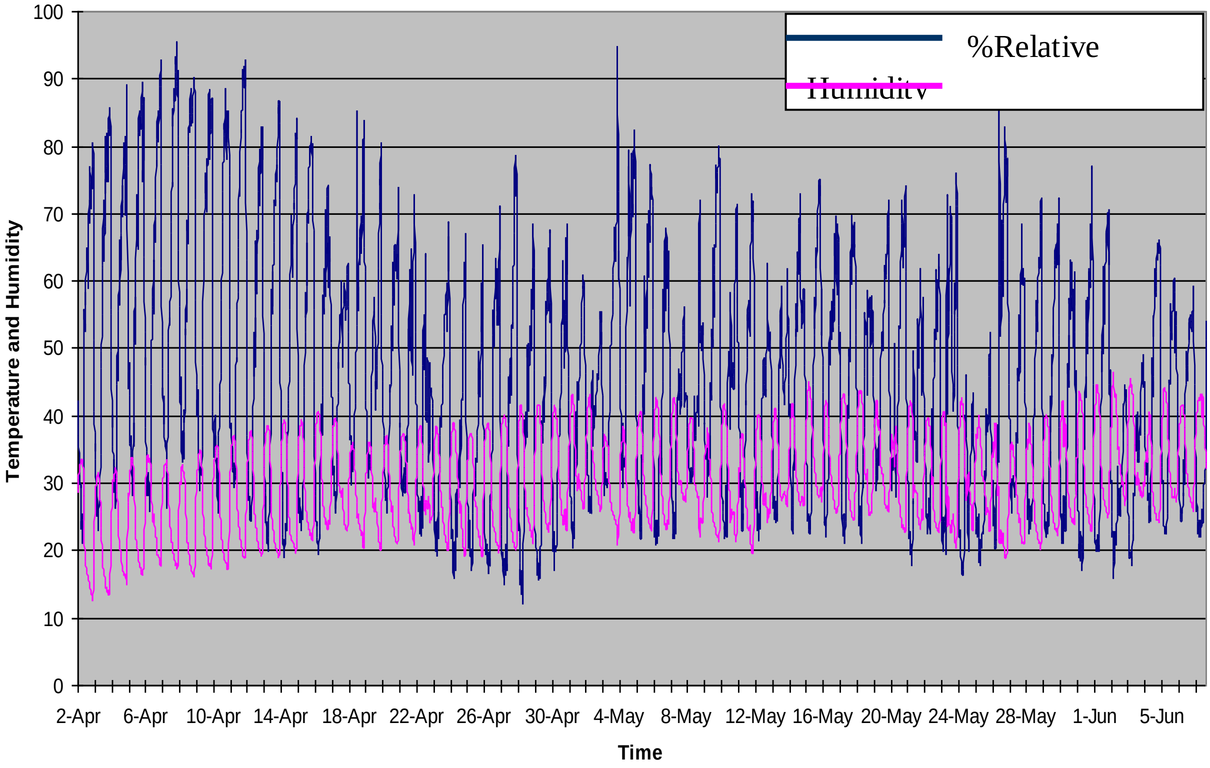
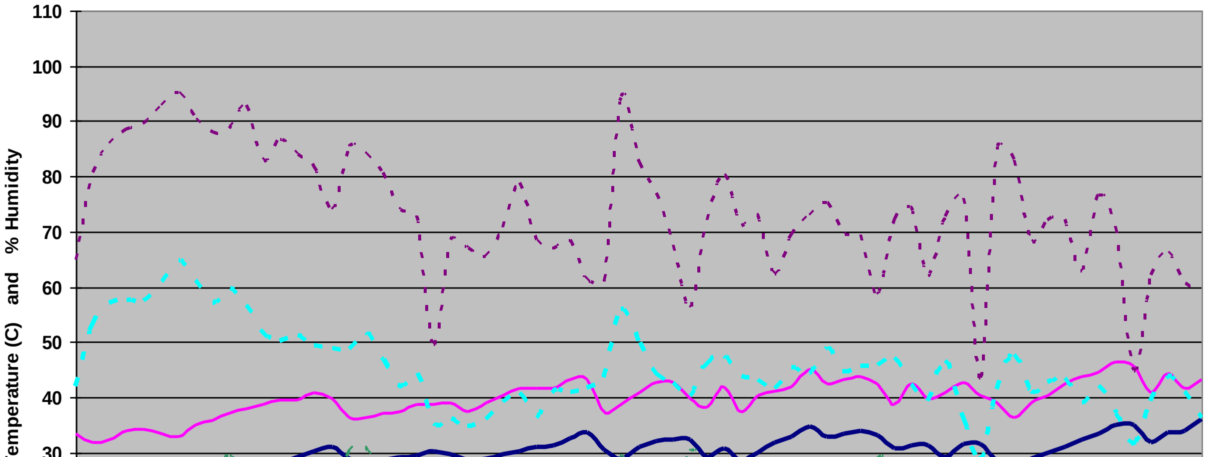


Figure 1. Temperature and % relative humidity vs time (every 30 min) at the experimental site (Actual Tinytag Data)



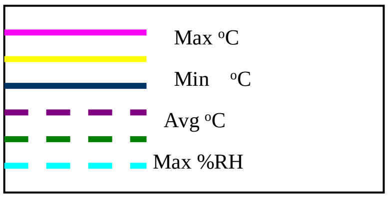
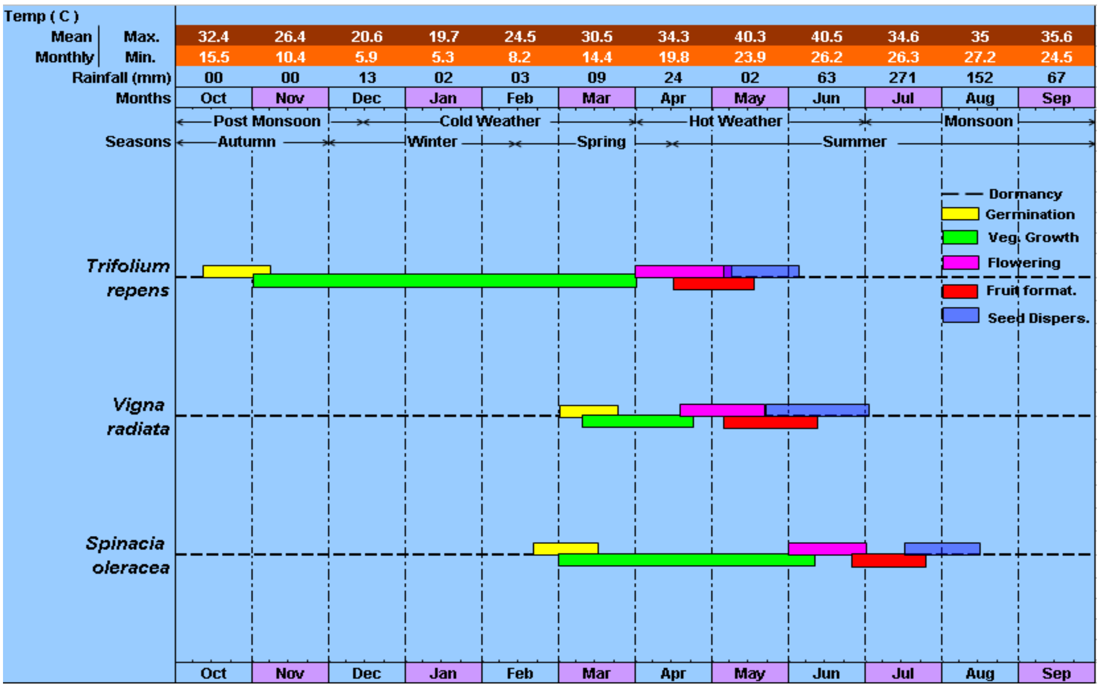


Figure 2.Temp and %RH (daily Max, min and Avg) vs Time at the experimental site (values calculated from Tinytag data)



Temp (C)		Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep
Mean Monthly	Max.	32.4	26.4	20.6	19.7	24.5	30.5	34.3	40.3	40.5	34.6	35	35.6
	Min.	15.5	10.4	5.9	5.3	8.2	14.4	19.8	23.9	26.2	26.3	27.2	24.5
Rainfall (mm)		00	00	13	02	03	09	24	02	63	271	152	67

Figure 3. Calender of phenollogical events of lifecycles of Trifolium repens, Vigna radiata and Spinicia oleracia in relation to climatic conditions in Lahore-Pakistan, Punjab.

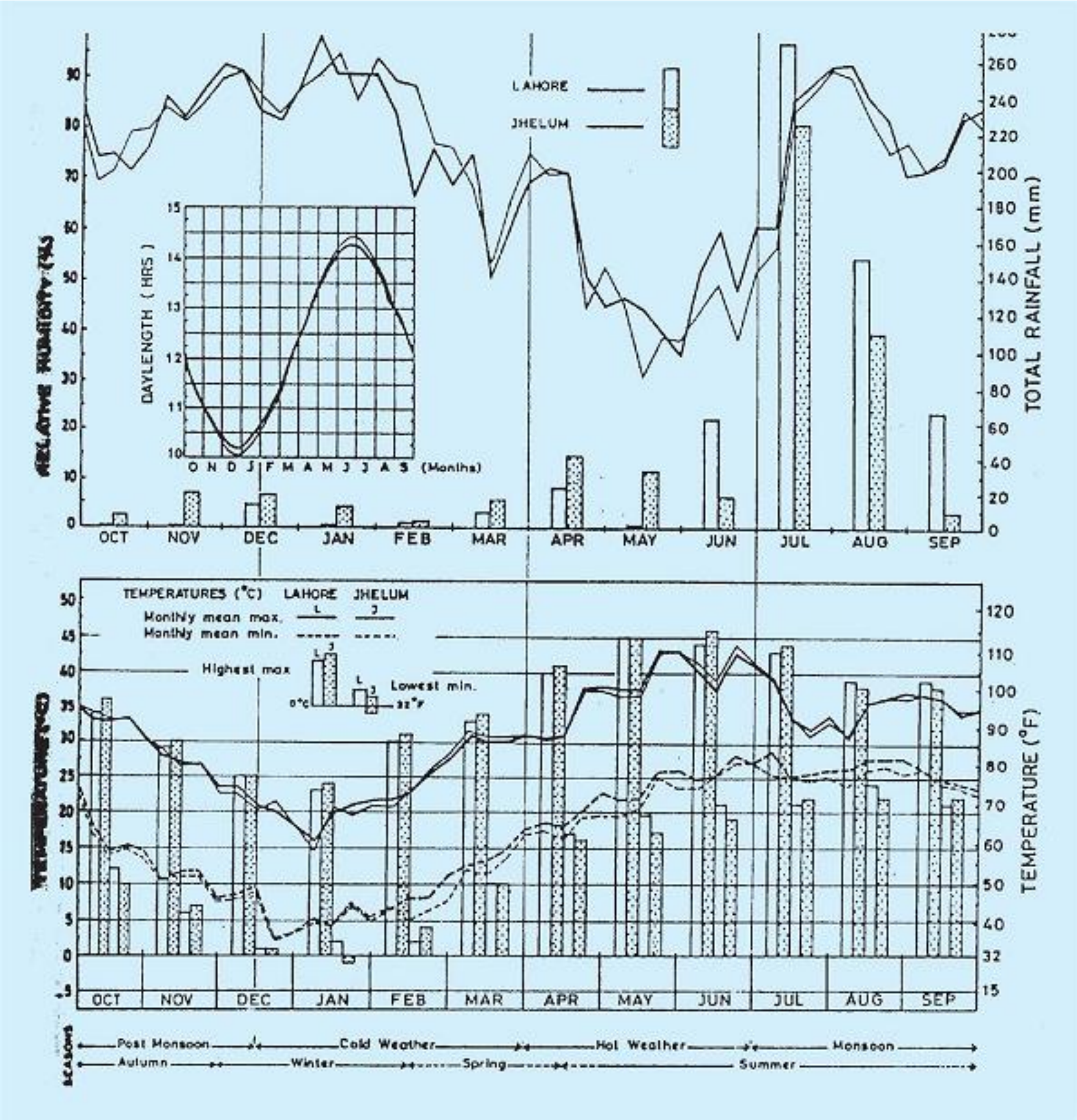


Figure 4 . Climatic diagram on temperature, %RH, rainfall and day-length at Lahore and Jehlum, Pakistan, Punjab.

Table 1. Ambient temperature and relative humidity at the site during experimental period

Site*	**Duration**	**Days**	**Avg Temp (C)**	**Avg % RH**
PUBG	Apr 2, 07 to Apr 30, 07	28	27.6	49.2
PUBG	Apr 30, 07 to May 28, 07	28	31.3	43.7
PUBG	May 28, 07 to Jun 6, 07	9	32.5	40.1

Each Avg temp. and %RH value is mean of 1344 readings for the first two months and 432 readings for the month of June

** PUBG: Punjab University Botanical Garden, Lahore-Pakistan*

Table 3. Major events / activities in spinach and mung bean EDU experiments in chronological order

Activity / Events		Date	Plant Age (DAS*)
A **Sowing / Germination**	Seed sowing **I**	24-Mar-07	0
	Germination, seedling decay and death	2-Apr-07	9
	Seed sowing **II**	2-Apr-07	0
	Germination	16-Apr-07	14
	Thinning / Selection	22-Apr-07	20
B **Treatment**	EDU Treatment **I**	29-Apr-07	27
	EDU Treatment **II**	9-May-07	37
	EDU Treatment **III** (spinach only)	19-May-07	47
	EDU Treatment **IV** (due on 29.5.07: delayed by 4 days)	2-Jun-07	61
C **Weekly leaf injury assessment**	Assessment No **I**	29-Apr-07	27
	Assessment No **II**	6-May-07	34
	Assessment No **III**	13-May-07	41
	Assessment No **IV**	20-May-07	48
	Assessment No **V**	27-May-07	55
	Assessment No **VI**	3-Jun-07	62
D **Harvesting**	Final destructive harvest of above ground plant parts	6-Jun-07	65

** Days after sowing*

Table 4. Effect of EDU treatment* on the extent of leaf injury in spinach plants with time (Mean of 17 replicates + S.E.)

S. No.	**Assesments Date/ Plant Age**	**Treatment**	**No. of Leaves/plant**		**% Damage**	**Injury Scale**
			Total	**Damaged**		
I	29-Apr-07	EDU treated	2.95	0.10	3.39%	1
	27 DAS**	non-EDU	3.20	0.15	4.69%	1
II	6-May-07	EDU treated	11.15	0.70	6.28%	2
	34 DAS**	non-EDU	10.10	3.40	33.66%	3
III	13-May-07	EDU treated	18.95	1.05	5.54%	2
	41 DAS**	non-EDU	18.05	4.85	26.87%	3
IV	20-May-07	EDU treated	29.63	1.32	4.45%	1
	48 DAS**	non-EDU	27.65	7.40	26.76%	3
V	27-May-07	EDU treated	43.78	1.72	3.93%	1
	55 DAS**	non-EDU	37.47	11.94	31.87%	3
VI	3-Jun-07	EDU treated	65.65	7.29	11.10%	2
	62 DAS**	non-EDU	49.41	14.00	28.33%	3

** Three regular EDU treatments applied at ten day intervals and a delayed 4th on 2.6.07*

*** Days after sowing*

Table 5. Effect of EDU treatment* on the extent of leaf injury in mung bean plants with time (+ S.E.)

Variety:		TALL				
Assesment Date/ Plant Age		Treatment	No. of Leaves/plant		% Damage	Injury Scale
			Total	Damaged		
I	29-Apr-07 27 DAS*	EDU treated non-EDU	1.44 1.38	0.00 0.00	0.00% 0.00%	0 0
II	6-May-07 34 DAS*	EDU treated non-EDU	3.31 3.31	0.13 2.54	3.93% 76.74%	1 4
III	13-May-07 41 DAS*	EDU treated non-EDU	5.62 5.70	0.25 3.70	4.45% 64.91%	1 4
IV	20-May-07 48 DAS*	EDU treated non-EDU	6.99 7.46	2.25 4.95	32.19% 66.35%	3 4
V	27-May-07 55 DAS*	EDU treated non-EDU	8.40 9.50	5.12 7.40	60.95% 77.89%	4 4
VI	3-Jun-07 62 DAS*	EDU treated non-EDU	9.44 10.46	7.25 9.77	76.80% 93.40%	4 4
		DWARF				
I	29-Apr-07 27 DAS*	EDU treated non-EDU	1.00 1.17	0.00 0.00	0.00% 0.00%	0 0
II	6-May-07 34 DAS*	EDU treated non-EDU	2.00 2.67	0.00 2.17	0.00% 81.27%	0 4
III	13-May-07 41 DAS*	EDU treated non-EDU	3.50 4.30	0.00 3.20	0.00% 74.42%	0 4
IV	20-May-07 48 DAS*	EDU treated non-EDU	4.75 5.67	1.00 4.00	21.05% 70.55%	2 4
V	27-May-07 55 DAS*	EDU treated non-EDU	5.00 6.00	3.75 5.30	75.00% 88.33%	4 4
VI	3-Jun-07 62 DAS*	EDU treated non-EDU	5.25 6.30	4.50 6.00	85.71% 95.24%	4 4

** Two regular EDU treatments applied on 29.4.07 and 9.5.07 and a delayed third on 2.6.07*
1. In tall variety, all weekly assessment means are of 13 replicates in both the treatments except for 15 replicates in EDU treated plants of I - IV assessments; 2. In dwarf variety, all weekly assessment means are of 5 replicates in both the treatments; In tall variety, mean is of 13 and in dwarf of 5 replicates in both EDU and non-EDU treatment plants; Figures in parenthesis gives % increase / decrease relative to non-EDU treatment plants.

Table 6. Effect of EDU treatment on yield components (reproductive growth) of mung bean plants at harvest (+ S.E.)

Variety	Treatment	Number of pods per plant			Number of seeds	
		Filled	Un filled	Total	per pod	per plant
Tall	**EDU-treated**	11.150 ± 1.19 (30%)	1.000 ± 0.45	12.15 ± 0.99	13.000 ± 0.39 (60%)	144.95 ± 9.87 (66%)
	non-EDU	10.850 ± 0.93	2.310 ± 0.62	13.080 ± 0.71	8.130 ± 0.42	87.310 ± 8.2
Dwarf	**EDU-treated**	4.800 ± 1.16 (-20%)	1.200 ± 0.37	6.000 ± 0.89	5.790 ± 0.57 (-5%)	27.200 ± 5.69 (-21%)
	non-EDU	6.000 ± 1.1	1.200 ± 0.58	7.200 ± 1.46	6.110 ± 0.47	35.200 ± 5.6

In tall variety, mean is of 13 and in dwarf of 5 replicates in both EDU and non-EDU treatment plants

Table 7. Effect of EDU treatment on seed yield* of Tall mung bean variety

	100 seed weight (gm)	**weight/seed (mg)**	**seed weight/plant (gm)**
EDU-treated	4.446 (19%)	44.46 (19%)	4.043 (24%)
non-EDU	3.743	37.34	3.275

**Based on fresh (air dry) weight of the seeds*
Figures in Parenthesis shows % increase relative to non-EDU treatment

3.2.4 Sri Lanka

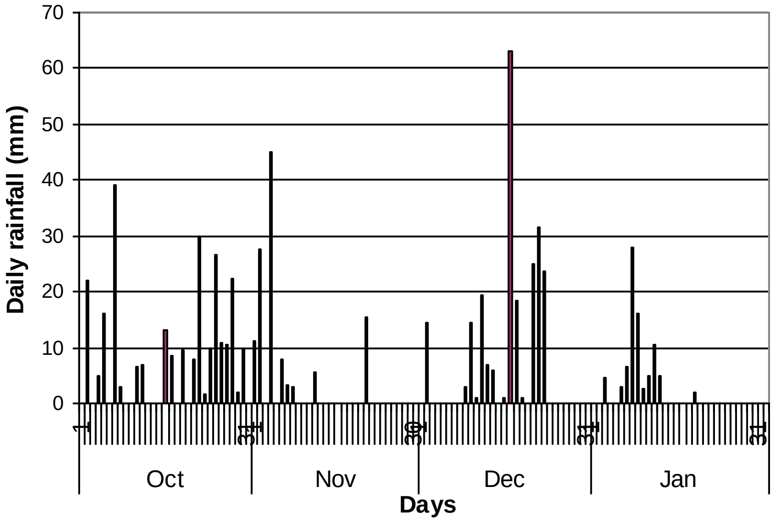


Figure 1. Daily rain fall during the sampling period

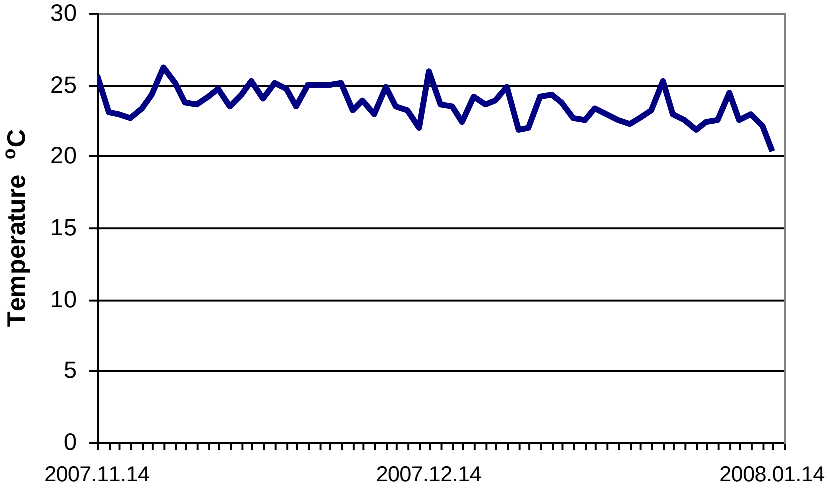


Figure 2. Average daily temperature of the study site

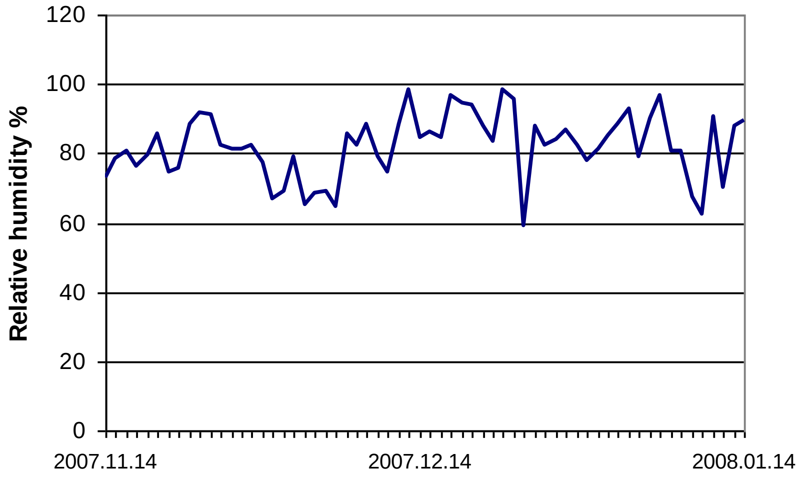
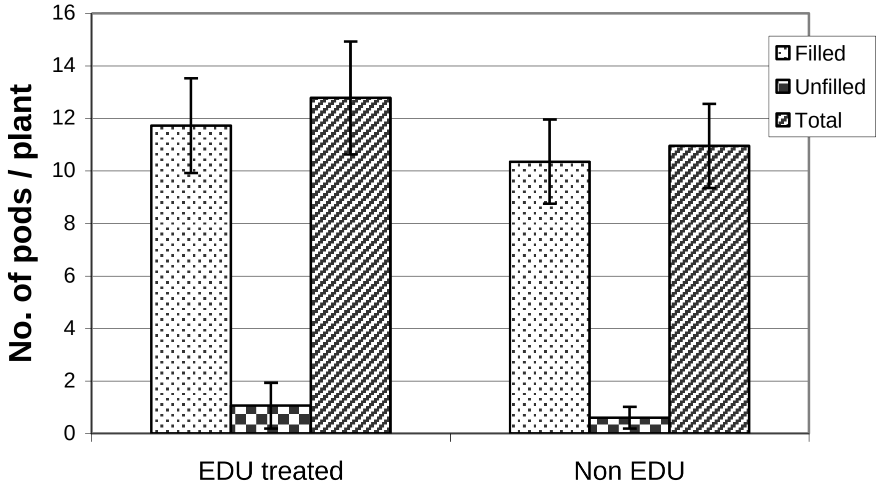


Figure 3. Average relative humidity of the study site



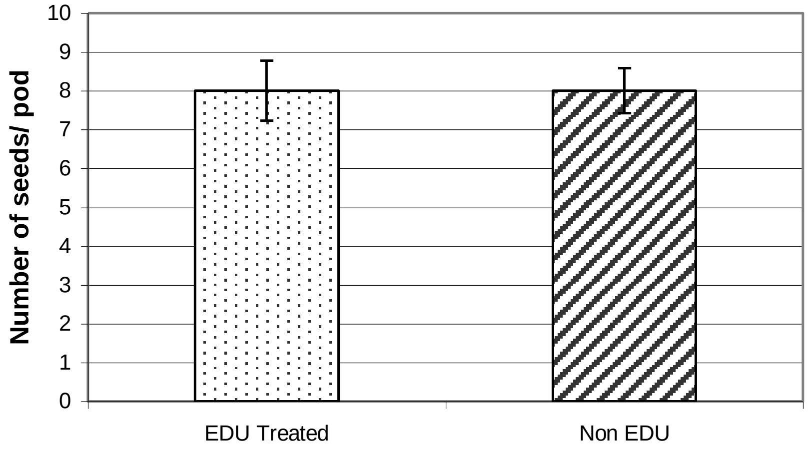


Figure 5. Number of seeds per filled pod

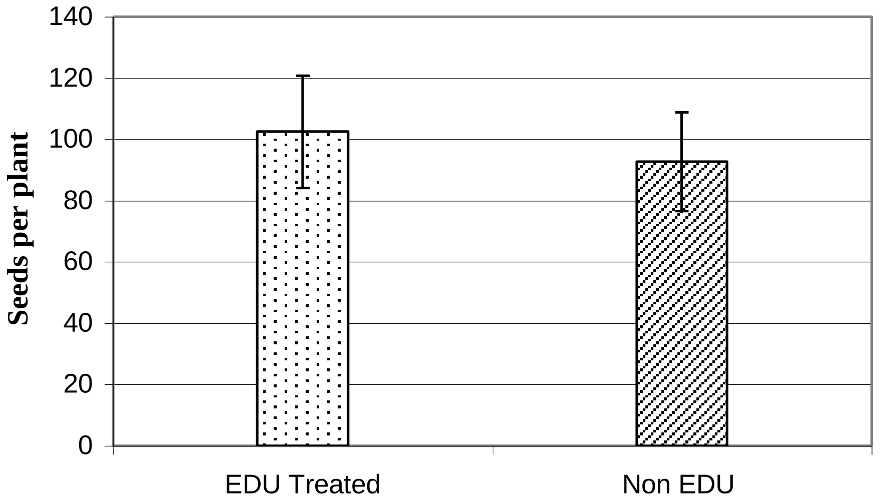


Figure 6. Number of seeds in filled pods per plant

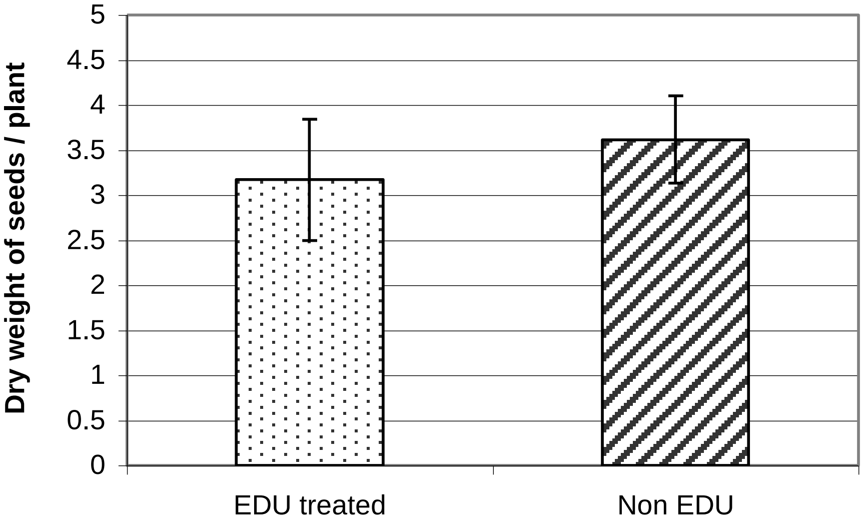


Figure 7. Seed dry weight per plant with 95% confidence limits

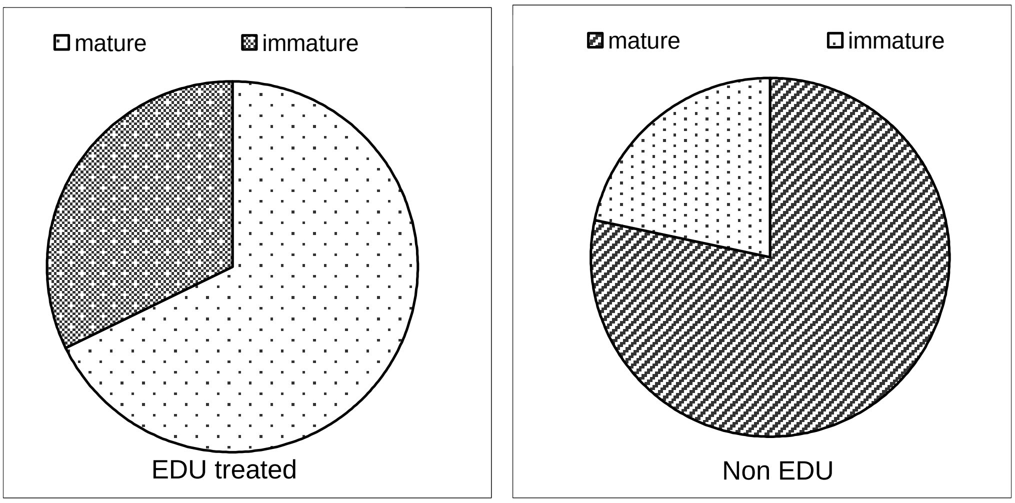


Figure 8. Maturity status of filled pods per plant