Experimental Protocol for quantifying the impact of tropospheric ozone on crops using protective chemicals

Malé Declaration Network
http://www.rrcap.unep.org/issues/air/maledec/
http://www.york.ac.uk/inst/sei/rapidc2/male.html
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Acknowledgement: This protocol is partly based on the experimental protocol used for several years within the ICP Vegetation network (International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops) (cf. Mills et al., 1997) and has been amended for application within Malé countries.

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1. Introduction

In accordance with the overall aim of RAPIDC (Regional Air Pollution in Developing Countries), i.e. "to facilitate the development of agreements and protocols and methods to implement measures which prevent and control air pollution in developing countries", the Swedish International Development Co-operation Agency (Sida) agreed to fund a third RAPIDC project phase with a special focus on assessing the impacts of tropospheric ozone on crops in South Asia and southern Africa.
It has been widely acknowledged that large parts of Asia and Africa experience a distinct increase in air pollution emissions both on a local and regional level due to an increasing population density and industrial development, which leads to higher emissions from household (burning of fossil fuels or wood), transport and industrial activities. Due to its high phytotoxicity and occurrence not only in urban but in particular periurban and rural, agricultural areas, the secondary air pollutant ozone has been identified as a main threat for crop production. In fact, various surveys from different parts of the world have shown that increased ozone concentrations result in foliar injury and biomass reduction of sensitive crop species, such as wheat, rice, beans, spinach and potatoes (e.g. Tingey et al., 1993; Fuhrer et al., 1997; Agrawal et al., 2003, 2006).

In order to assess the biological and economic impacts of increased ozone concentrations on plants and to identify areas at risk in South Asia and southern Africa, a biomonitoring campaign using ozone-sensitive and ozone-resistant white clover genotypes (*Trifolium repens* cv. Regal) and a chemical protectant study using ethylenediurea (EDU) to assess the yield of staple crops was suggested for the third RAPIDC funding phase (2005-2008). The latter study is believed to have the potential to quantitatively assess the yield losses for specific crops resulting from elevated ozone concentrations.

To carry out a chemical protectant study using ethylenediurea (EDU) in South Asia, two countries will be selected by the coordination team of the RAPIDC crop activities. These countries will perform the chemical protectant study alongside the clover biomonitoring campaign in the growing season 2006/07.

Ethylenediurea (EDU) is an ozone chemical protectant that has been used successfully in a number of experimental campaigns in South Asia (cf. Tiwari et al., 2005; Agrawal et al., 2003; Agrawal et al., 2005) to assess the damage caused by ambient ozone concentrations on a range of crop growth and physiological parameters (including quantity (e.g. yield) and quality (e.g. nutritional content)). In fact, a RAPIDC/APCEN EDU-pilot study using mung bean (*Vigna radiata* L.) as a bioindicator was carried out during the summer season 2006 (March-June) in Varanasi, India. Mung bean had been chosen for the pilot study because it is known to be ozone-sensitive and of economic importance in south Asia. EDU (400 ppm) given as soil drench at 10 days-intervals from germination to pod maturity increased the yield of mung bean plants as compared to non-EDU treated plants.

However, the chemical protectant study in South Asia proposed for the coming growing season 2006/07 will not only use mung bean, but also local varieties of tobacco

(*Nicotiana tabacum*), potato (*Solanum tuberosum*) and spinach (*Spinacea olearacea*), since these crops have been identified during the second network meeting of the Air Pollution Crop Effect Network (APCEN) (19 -22 September 2006, South Africa) as being suitable for this study. These crops are of economic importance for the region and are known to be relatively ozone-sensitive.

The physical and chemical climate during the growing season will be recorded using passive samplers and micro-meteorological data loggers. Passive samplers providing four-weekly integrated ozone mean concentrations are cheap, easy to deploy and don't require a power supply and are therefore ideal for this study. The extent of foliar injury and biomass reduction will be related to the four-weekly mean ozone concentrations derived from these passive samplers. Furthermore, micro-meteorological data loggers simultaneously record the parameters air temperature and relative humidity during the growing season.

This experiment, in conjunction with the clover bio-monitoring campaign, will function as a cohesive experimental initiative the participants of the Malé network can identify with. In the long run, participants will be encouraged to add other species and cultivars of high local interest, i.e. representing the respective staple crop, to this experimental initiative. Potential candidates known to be ozone-sensitive and of high economic interest are for example other bean varieties, rice, maize etc.

In conclusion, the aims of the Malé/RAPIDC chemical protectant campaign are:

- to assess the impact of ambient ozone concentrations on a range of growth and physiological parameters of staple crops in Malé countries using the chemical protectant EDU in standardised experiments
- to assess the suitability of EDU for regional risk assessment in South Asia
- to determine the spatial extent and magnitude of air pollution impacts to crop productivity in the Malé countries
- to establish a scientific crop effect community within the Malé network with a special focus on experimental initiatives, such as biomonitoring, chemical protectant and gradient studies
- to give experimental evidence as a basis for a survey on socio-economic implications of crop yield losses related to air pollution

This Experimental Protocol provides the standardised methodology to be adopted by participants in the Malé network for qualitatively and quantitatively assessing the impact of ozone, expressed as foliar injury and biomass reduction, on mung bean, tobacco, potato and spinach using the anti-ozonant ethylenediurea (EDU).

2. OZONE CONCENTRATION MEASUREMENTS (PASSIVE SAMPLERS)

To aid the interpretation of the biomonitoring study results, i.e. to be able to relate the apparent foliar injury and biomass reduction to the pollution climate, it is important to record the ozone concentration at or next to the experimental sites.
Ideally, continuous measurements of ozone should be made at the experimental sites using analyzing methods such as gas-phase chemiluminescent, gas-solid chemiluminescent or UV photometric. However, if continuous analyzers are not available (which will be the case at most sites), the use of passive samplers is recommended. Passive sampling is an inexpensive, reliable and simple ozone measurement technique that doesn't require electricity and is therefore thought to be ideal for use within the RAPIDC biomonitoring activities. The sampling technique is based on molecular diffusion of gases. The gas molecules diffuse into the sampler where they are quantitatively collected on an impregnated filter or an adsorbent material. Hence, this method gives concentration values integrated over time, in this case the mean value of **four-weekly** ozone concentrations. For details on passive samplers, please check Appendix 1.

Instructions for outdoor sampling with ozone passive samplers (as defined by IVL, Sweden)

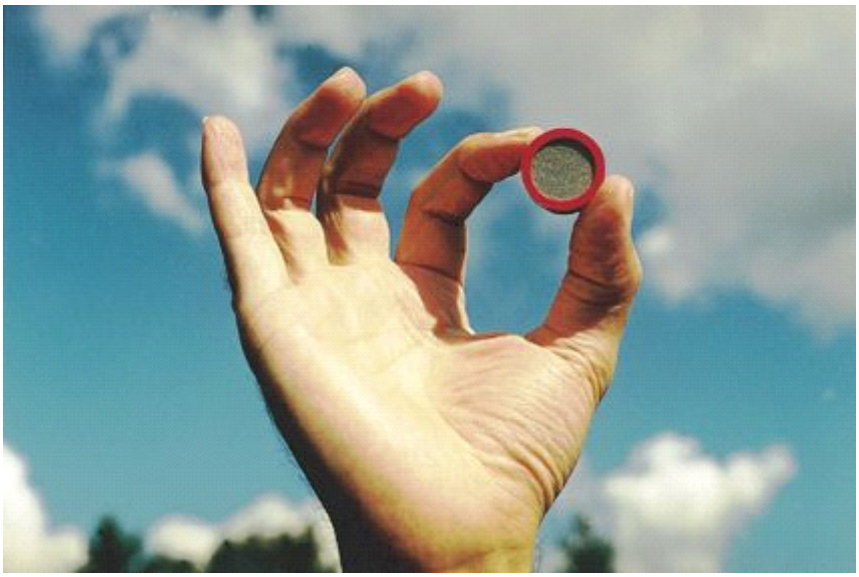


Figure 1 Passive sampler as distributed by IVL

The passive samplers will be distributed by IVL, Sweden (s. address below). For each four-week period, two passive samplers will be exposed to ambient air simultaneously. The passive samplers will arrive in small capped plastic containers sealed in a plastic bag. The containers must only be opened shortly before the start of the passive sampling/exposure, i.e. on the site. **Don't open the container until the supporting**

fixture (see below) is in place and sampling is ready to start! To protect the samplers from rain, the initial delivery of the passive samplers will contain one metal disc for each site with tool holders mounted underneath to support the samplers. The measurement technique relies on good air ventilation around the sampler. Hence, the samplers should be exposed at a height of three meters above ground, which requires a post/pole or a horizontal mounting built from a wall (see Figure 2).

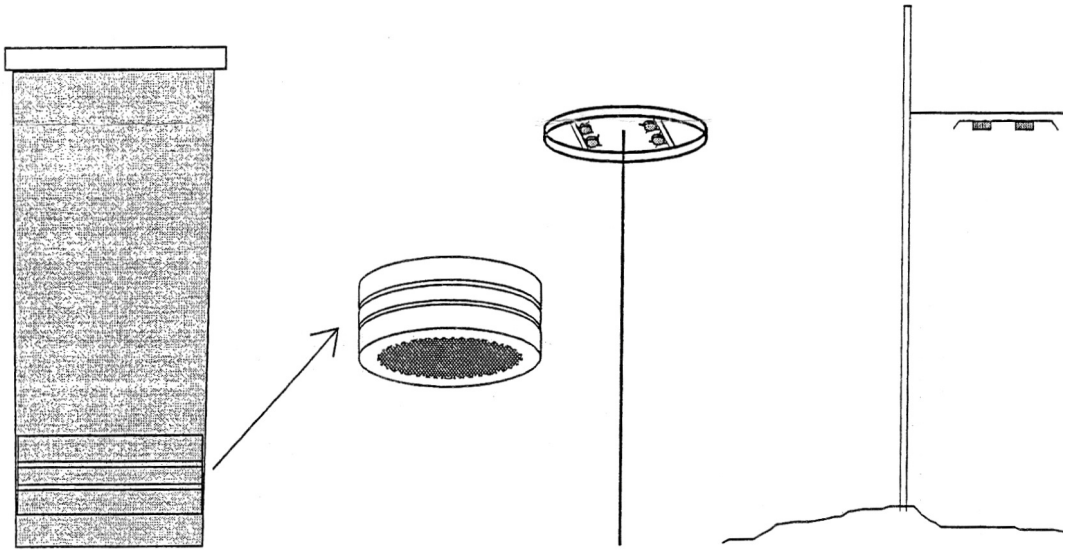


Figure 2 Set-up of passive sampling system (courtesy of IVL)

Step-by-step sampling instructions

a) Carefully remove the samplers from the container and fix firmly to the tool holder with the grey net mesh pointing downwards. Avoid touching the net. The sampler is damaged if the net is pressed into the sampler!
b) Note date, start time and sampler location on the field protocol. Thoroughly check which two samplers (number) were used in a certain time period and at a certain site.
c) At the end of the exposure period (four-weeks), loosen the two samplers carefully and place it in the corresponding plastic container, seal it with a cap, place the container in the plastic bag and seal the plastic bag.
d) Note the date and stop time on the field protocol.
e) Return the sampler to IVL's laboratory in Gothenburg, Sweden, preferably in a cushioned envelope. Mailing address:

IVL Swedish Environmental Research Institute Ltd.
Laboratoriet
Box 5302
S 400 14 Göteborg
Sweden

IVL will contact every participant via email once the samples have been analyzed, which might take four to eight weeks. We would like to ask you to forward their emails to Patrick Büker (pb25@york.ac.uk) in order to pool all data at one place.

Regarding delayed deliveries or other sampling problems which can arise, don't hesitate to contact one of the following persons via telephone (++46-31-7256200, IVL reception) or via fax (++46-31-7256290): Sari Honkala, Karin Sjöberg, Eva Brorström-Lundén or Tina Skårman. Alternatively, you may also contact Patrick Büker (pb25@york.ac.uk). More detailed information on passive samplers are summarised in Appendix 1.

3. RECORDING OF METEOROLOGICAL DATA

To aid the interpretation of the biomonitoring study results, the experimental sites should either be located close to meteorological measurement stations or use micro-met loggers. All sites that are not closely located to meteorological stations will be provided with one Tinytag® data logger which records temperature and relative humidity according to defined time-steps. This time-step should be set to **30 minutes** at all sites.

The logger will come with i) a users' guide (a brief users' guide can also be found in Annex 2 of this protocol), ii) a software installation CD-ROM including a software activation code and iii) an inductive pad to download data to a computer. More detailed information on Tinytag data loggers are provided in Appendix 1.

If any problems occur concerning the installation of the software, the performance of the data loggers, the download of the data etc., please don't hesitate to get in touch with Patrick Büker at SEI-York (pb25@york.ac.uk, ++44-1904-432890).

The microloggers for temperature and relative humidity should be protected against direct sunlight in order to avoid overestimation of temperature and VPD. A simple self-ventilating radiation shield, which can be made of a piece of plastic pipe with approximately 10 cm diameter, will suffice. The Tinytag hangs in the lower part of a tube (see Figure 3), with the upper part of the tube being black and the lower part being either white or protected by a reflective cover (e.g. aluminium foil). The air will warm in the upper black part of the tube and rise, which will enable new air coming in from below. Alternatively, use a Stevenson screen if available or a simple arrangement with a shield made of aluminium foil through which air can pass, but within which the Tinytag is protected against direct sunlight.

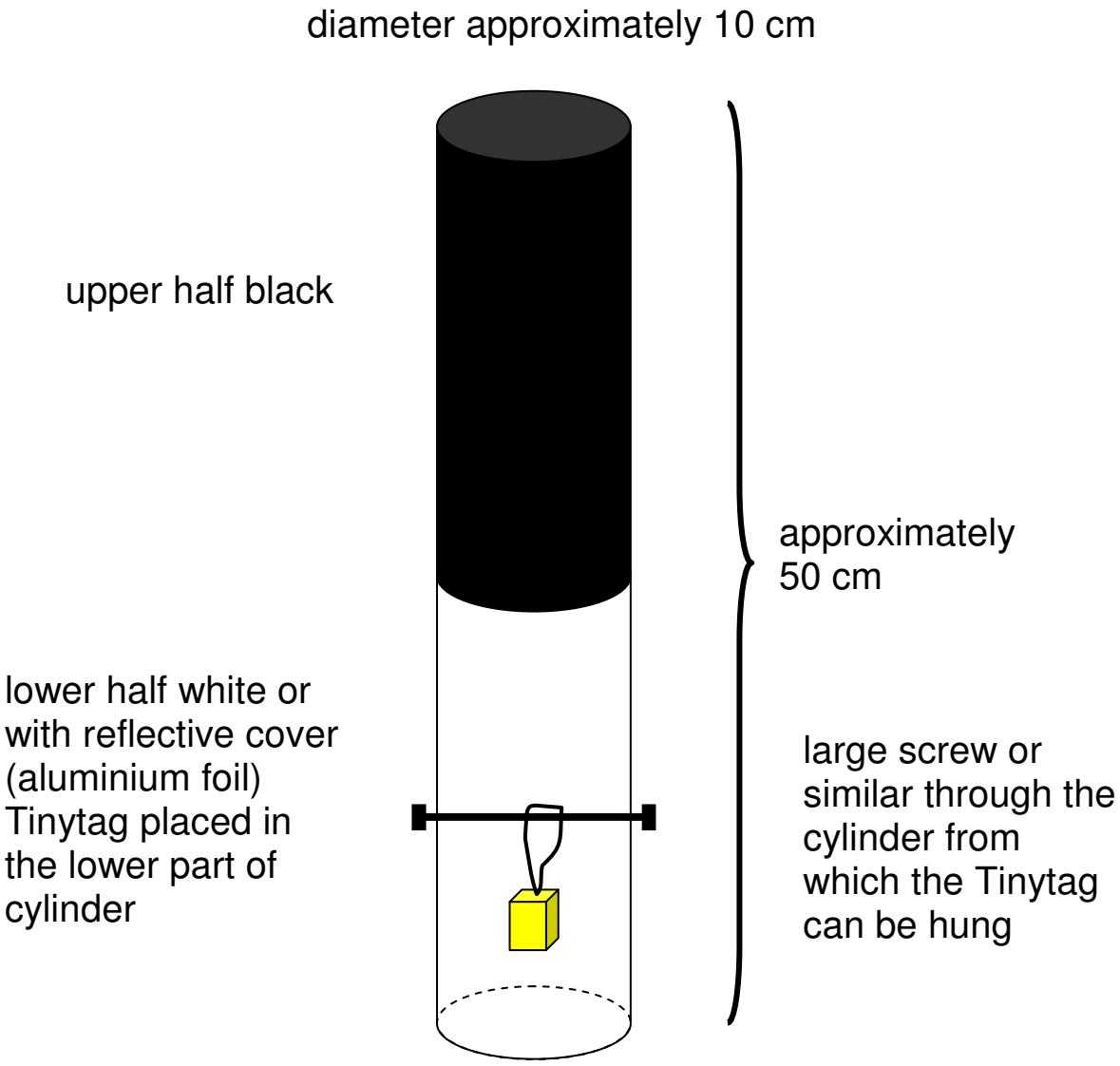


Figure 3 Example of radiation shield to protect Tinytag from direct sunlight (courtesy of Jenny Sundberg)

Since the project budget doesn't allow to provide sites with radiation measuring devices, we would like to encourage you to – if the right equipment is available – measure radiation using already existing instruments. Furthermore, if any of the following parameters are available from a near-by climate monitoring facility, then please add this information to the Excel-spreadsheet described below:

Physical climate
Air temperature (°C)
Relative humidity (%) or vapour pressure deficit (kPa)
Global radiation (Wm^{-2})
Rainfall: daily record of amount of rainfall (mm)
Wind speed ($m\ s^{-1}$) and direction (°)

Pollution Climate
Particulate Matter (PM2.5, PM 10)
Sulphur dioxide (SO_2)
Nitrogen oxides (NOx)

Ideally, the above listed parameters should be measured at 1m height, although data from other measurement heights are acceptable, too.

All meteorological and pollution data should be collated in an Excel spreadsheet as described in Section 4.5.

4. THE CHEMICAL PROTECTANT EHTYLENEDIUREA (EDU)

In order to quantitatively (e.g. yield) and qualitatively (nutritional composition) assess the impact of air pollutants on crops, field studies using chemical protectants have proven to be of high value. These studies use chemicals that have been identified as protecting crops from damage by specific air pollutants.
The antioxidant EDU ((*N*-[2-(2-oxo-1-imidazolidinyl) ethyl]-*N*-phenylurea (abbreviated EDU for ethylenediurea) is the chemical that has most widely been used to examine the effect of elevated ozone concentrations on plant health, as it is know to suppress typical ozone-induced phytotoxic effects, such as foliar injury, biomass reductions and premature senescence. The positive effect of EDU on plant health and productivity was first reported by Carnahan et al. (1978), who showed that EDU applied either to the foliage or the roots of Pinto beans (*Phaseolus vulgaris* L.) significantly reduced leaf injury as compared to unprotected control plants. Ever since, numerous applications of EDU to various plant species of economic importance in both developing and developed countries have shown its strength in effectively reducing detrimental effects of plants' exposure to ozone, which predestines EDU as a tool to quantitatively detect ozone-induced crop losses. Furthermore, several studies have shown that EDU itself doesn't have a toxic effect on plants in the absence of ozone, nor does it function as a pesticide or a nutrient (Miller et al., 1994; Astorino et al., 1995; Brunschön-Harti et al., 1995a,b; Hassan et al., 1995; Godzik & Manning, 1998). However, when applied excessively, negative effects on plant physiology have been reported by Bennett et al. (1978) and Heagle (1989).

Wahid et al. (2001) and Tiwari et al. (2005) used EDU to investigate the effect of ambient ozone concentrations on the yield of pot-grown Pakistani soybean (Glycine max L.) and two Indian field-grown cultivars of wheat (*Triticum aestivum* L.), respectively. In another Asian study, Agrawal et al. (2005) showed that mung bean plants (*Vigna radiata* L.) exposed to ambient ozone concentrations in a suburban area of Allahabad (India) showed significant reductions in plant growth and yield compared to EDU-treated plants of the same cultivar. Similarly, Bambawale (1989) and Varshney & Rout (1998) reported on reduced effects of ozone on several physiological parameters of EDU-treated Indian grown potato and tomato plants, respectively.

For Africa, much less studies using the anti-ozonant EDU have been reported. In an Egyptian study, Hassan et al. (1995) showed that the growth of the economically important crops radish (*Raphanus sativus* L.) and turnip (*Brassica rapa* L.) exposed to controlled levels of ozone in open top chambers (OTC) was increased for EDU-treated plants. Similar effects have also been examined for potato in a second Egyptian study by Hassan (2006). Furthermore, the application of EDU as a foliar spray reduced foliar injury symptoms on the same potato plants.

In Europe and North America, EDU has extensively been used over the last two decades to qualitatively and quantitatively assess the impact of ozone on various crops and tree species. Several studies showed that especially ozone-sensitive bean (*Phaseolus vulgaris* L.) cultivars could be protected from adverse ozone-effects, such as foliar bronzing and yield reductions, by regularly applying EDU throughout the growing season (Brunschön-Harti et al., 1995a; Astorino et al., 1995; Tonneijck & Van Dijck, 1997a; Elagöz & Manning, 2005). EDU also reduced foliar senescence of potato and tobacco plants exposed to ozone in chamber experiments (Eckardt & Pell, 1996; Godzik & Manning, 1998), and increased the biomass of radish exposed to ambient air in Sweden (Pleijel et al., 1999). However, when applied to subterranean clover (*Trifolium subterraneum*), a species know to be ozone-sensitive, exposed to ambient levels of ozone in the Netherlands, EDU had a highly variable effect on biomass but a positive effect on foliar injury (Tonneijck & van Dijck, 1997b; Tonneijck & van Dijck, 2002).

For trees, EDU has shown to minimise foliar injury when infused into trunks of poplar (*Populus nigra*) and European ash (*Fraxinus excelsior*) (Ainsworth et al., 1996; Bortier et al., 2001; Paoletti et al., 2006). However, while Ainsworth et al. (1996) proved that EDU didn't affect leaf gas exchange, height and diameter growth of poplar, Bortier et al. (2001) reported a clear diameter and biomass increment for this species. Manning et al. (2003) also showed an increase in all growth parameters for ozone sensitive seedlings of loblolly pine (*Pinus taeda* L.) after bi-weekly treatments with foliar sprays of EDU. Furthermore, Kuehler & Flagler (1999) suggest that EDU might delay stomatal closure in young loblolly pine seedlings.
Manning (2005) recently published a review on the use of different experimental approaches, including EDU, to establish cause effect relationships for ambient ozone exposure of forest trees.

Table 1 summarises a range of important publications of the last 10 years on the use of EDU in selected Asian and African countries to detect the impact of ambient ozone on plant health and growth. While the studies concentrated mainly on the exposure of plants to ambient air in field experiments, some also included preliminary chamber experiments to determine the concentration of EDU required to protect plants against ozone injury. The results of these dose-response experiments defined the concentration of EDU applied to plants in the subsequent field study.

An EDU concentration of up to 500 ppm is commonly believed to protect most annual crops from ozone injury (cf. Tonneijck & Van Dijck, 1997a; Tiwari et al, 2005; Agawal

et al., 2005; Elagöz & Manning, 2005), however, the exact concentration of EDU has to be carefully chosen and is dependent on the used crop species and the prevalent climate. To also protect newly emerging leaves from ozone, EDU has to be applied repeatedly, with recommended intervals of 10 to 14 days. For crops, EDU is usually applied either as a foliar spray or soil drench.

The exact biochemical mechanism how EDU protects plants from O_3 injury remains largely unknown. When applied as a soil drench, EDU moves systemically in plants (e.g. Carnahan et al., 1978), whereas the movement is translaminar when EDU is applied as a foliar spray (Weidensaul, 1980). Regner-Joosten et al. (1994) and Gatta et al. (1997) showed that EDU persists for at least 10 days in leaves of bean plants, which reflects the typical frequency of EDU applications in field experiments in order to protect newly emerged leaves of crops such as beans and wheat from ozone injury. Gatta et al. (1997) also demonstrated that EDU most likely does not enter the cell, but remains in the apoplast which suggests a direct role of EDU in protection from ozone injury by directly scavenging O_3 and ozone-derived radicals. However, several studies on EDU-induced changes in antioxidants and enzymes revealed an inconsistent picture (Brunschön-Harti et al., 1995b; Gatta et al., 1997; Lee et al., 1997).

Table 1. Details of agricultural species and cultivars for which EDU has been successfully applied in selected Asian and African studies.

Country	Species/ Cultivar	EDU application	EDU conc./mg EDU applied per plant	No. of applications/ application time	Effect of EDU application (reference: non-EDU-treated plants)	Reference
Egypt	radish, turnip; Egyptian cultivars	soil drench	500 ppm; 300 mg	3; 10, 20 and 30 days after seeding	increase in growth of radish and turnip plants exposed to ambient levels of O_3	Hassan et al., 1995
Egypt	potato; Kara	foliar spray	300 ppm; 810 mg	9; 10-day intervals with 1st application 48 days after sowing	reduction of foliar injury and increase in tuber weights of plants exposed to ambient air	Hassan et al., 2006
India	tomato; Pusa Ruby	soil drench	400 ppm; not specified	6 to 7; 12-day intervals	increase in shoot and root length, as well as shoot and root biomass of plants exposed to ambient air	Varshney and Rout, 1998
India	mung bean; Malviya Jyoti	soil drench	500 ppm; 2750 mg	11; weekly intervals with 1st application 1 week after seedling emergence	maintenance of high levels of photosynthetic pigments, soluble protein and ascorbic acid in foliage, as well as growth increment of plants exposed to ambient air	Agawal et al., 2005
India	wheat; Malviya 533 and 234	soil drench	150, 300 and 450 ppm; 270, 540 or 810 mg	11; 10-day intervals with 1st application 10 days after germination	increase in shoot and root length, no. of tiller per plant and total biomass of plants exposed to ambient air	Tiwari et al., 2005
Pakistan	soybean; NARC-1	soil drench	400 ppm; 1200 mg	5; 10-day intervals with 1st application 2 weeks after seedling emergence	increase of see weight per plant exposed to ambient air	Wahid, A., 2001

5. CHEMICAL PROTECTANT STUDY USING LOCAL CROP CULTIVARS

5.1. Experimental aims and outline of experiment

- To assess the impact of ambient ozone concentrations on a range of growth and physiological parameters of staple crops in Malé countries using the chemical protectant EDU
- To assess the suitability of EDU for regional risk assessment in South Asia
- To determine the spatial extent and magnitude of air pollution impacts to crop productivity in the Malé countries

The participating countries will choose easily available local cultivars of mung bean, tobacco, potato and/or spinach. These crops are known to be of economic importance in the region and are relatively ozone-sensitive.
Approx. 40 plants of each cultivar will be grown in ambient air in medium-sized pots filled with a standardised substrate. The identified biomonitoring sites will be equipped with ozone passive samplers and micrometeorological data loggers and will also be used for the clover biomonitoring campaign.
In the following, half of the plants are regularly treated with EDU, whereas the other half functions as untreated control plants. During the growing season, the leaves are regularly checked for visible leaf injury. At the end of the growing season, several harvest parameters dependant on the used crop (e.g. seed and/or total above-ground biomass, number of leaves and pods etc.) are recorded.

Leaf injury, morphological, biomass, climate and ozone data are sent to the coordination team (contact details see below) of the experiment at the end of the growing season using standardised spreadsheets.

5.2. Experimental requirements

Experimental Plot: The site should be situated in an open field in good distance from any pollution point sources (smelters, factories etc.) and at least 200m from main roads and 50m from larger buildings. The plot should be fenced to prevent birds and small mammals from eating the plants. The immediate surroundings of the pots should be kept free from tall growing plants (trees, bushes, tall grasses) to prevent eddy formulation from disturbed wind fields and overshadowing (see Figure 3). However, short vegetation is appreciated to prevent excessive dust formation and mud-splashes on the plants, which might affect the biomass weight. The water supply should be secured.

Pots and wicks: Use approx. 15 litre volume pots with a surface diameter of approx. 30 cm and a height of approx. 30 cm. Each pot should have access to a water reservoir (bucket) as described in section 5.3.2. Fibreglass wick material is supplied by our project partners from CEH Bangor (U.K.).

Soil mixture and fertilizer: A soil mixture likely to work well in most areas is local soil, sand and vermiculite in the ratio 1:1:1. It is essential that participants use a slow release fertilizer such as eight month slow release 'Plantacote pluss 8M' (14N:8P:15K (+ 2 MgO)) or products with a similar N:P:K composition. Approx. 30g of fertilizers are required per pot. **Please inform the biomonitoring coordination team (see above) at the beginning of the experiment about the exact composition of the chosen fertilizer.**

Plant Material: The following species (in descending order of importance) have been identified as being suitable for this experiment due to their ozone-sensitivity and local economic and nutritional importance:

Mung bean	*Vigna radiata*
Tobacco	*Nicotiana tabacum*
Potato	*Solanum tuberosum*
Spinach	*Spinacia oleracea*

EDU supply: Ethylenediurea (EDU) will be supplied in sufficient quantities by SEI, York.

Monitoring equipment: Ideally, participants should have access to pollution and climate data monitored at or close to the experimental site. Half-hourly mean data for ozone, temperature, humidity and solar radiation are the most useful. However, where such data are not available, passive samplers will be supplied by our project partners from IVL, Gothenburg (Sweden) (cf. chapter 2).

5.3. Setting up and maintaining the experiment

5.3.1. Receiving and establishing the plants

Acquisition of seeds

Seeds of locally available cultivars of mung bean, tobacco, potato and spinach will be acquired by the participants. If known, ozone-sensitive cultivars of these bioindicators will be chosen.

Planting procedure

For **mung bean**, three seeds will be sown directly into 15 litre pots already exposed to ambient air. One week after the emergence of seedlings, they will be thinned to one plant per pot (keep the best developed and healthiest seedling).
For **spinach**, five seeds will be sown directly into 15 litre pots already exposed to ambient air. One week after the emergence of seedlings, they will be thinned to one plant per pot (keep the best developed and healthiest seedling).
For **potato**, 50 tubers of the same size (45 – 60 mm diameter) will be planted into 15 litre pots (n = 50) already exposed to ambient air. After 4 weeks, the 40 best ones, i.e. healthy plants of approx. similar size and shape, will be selected for the further experiment.

For **tobacco**, sufficient seeds for approx. 50 plants will be sown into plant trays and protected from direct sun-light, for instance by placing the plant tray in a greenhouse. After 5 weeks, seedlings will be transplanted into 15 litre pots, with five seedlings per pot. After a further week (week six after sowing), the seedlings will be thinned to one plant. This is also the day, when the plants will be exposed to ambient air.

Use the above mentioned soil mixture (chapter 5.2.) for all plants. Thoroughly wet the soil after the sowing procedure but prevent over-watering.
The pots have to be prepared according to section 5.3.2 and arranged according to Figure 6. All plants should be fed with approx. 30g of slow release 'Plantacote pluss 8M' (14N:8P:15K (+ 2 MgO)) or a product with a similar nutrient composition.

The minimum number of replicate plants to be placed at the experimental site is 20 per treatment, i.e. 40 altogether. However, it is advisable to grow more plants in case some have to be discounted because of e.g. insect damage. Label the pot to identify the treatment (EDU/non-EDU).

Maintenance of the young plants
The young plants will suffer if the growth medium is too dry or too moist (e.g. over-watering may increase the susceptibility of plants to fungi). They will probably need protection from dry, hot conditions (high sunlight levels), especially during the first two weeks. We expect that local knowledge of growing plants in the specific climates will help minimizing any damaging effects caused by extreme weather situations.
Keep aphids and other insects off the plants to avoid the spread of viruses and diseases.

5.3.2. Experimental set-up

Safety Information: Strong gloves, a laboratory coat, eye protection and a dust mask should be worn at all times when handling the fibreglass wicks.

Two days before planting
Make three evenly distributed holes in the bottom of each pot. Pre-cut the fibreglass wicks supplied into three of 60 cm lengths per pot, and soak overnight in a bucket of water.

One day before planting
Fill the pots with the soil mix, inserting the wicks and applying the fertiliser using the following procedure:

1. Place the wicks inside the pot as indicated in Figure 4 with 20 cm extending through each drainage hole and outside the bottom of the pot.

2. Place approximately 6-7 cm depth of soil mix into the pot and pat down firmly. Add 10 g of slow release fertilizer uniformly to the surface using a scoop calibrated to 10 g. Lay one of the 60 cm wicks in a partial circle approximately 2.5 cm in from the pot perimeter (Figure 5).

3. Repeat step 2 using the adjacent wick (clockwise) and including a further 10 g of fertilizer as before.

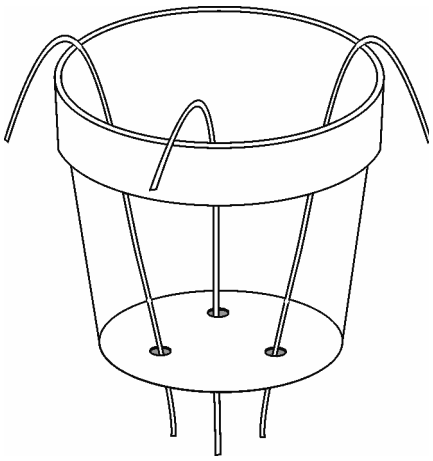


Figure 4 Placing the wicks in the pots.

4. Repeat step 2 for the last remaining wick, and fill with further soil mix until close to the top of the pot, taking care to ensure that the last wick cannot dry out by being too close to the surface.

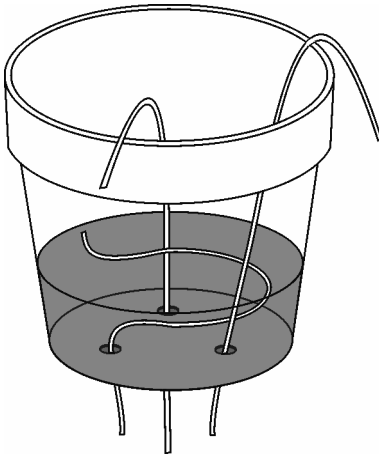


Figure 5 Winding of first wick in a partial circle approximately 2.5 cm in from the pot perimeter.

5. Place the pots into the filled water reservoir (a bucket underneath the pot as can be seen in Figure 6) and water the pots thoroughly from above, top up with soil mix as necessary to make the soil surface 3 cm below the rim of the pot. The

distance between the pot base and the water level should be approximately 3 cm; ensure that the pot base is not immersed in water. Drainage holes in the reservoir at this height would prevent this happening (Figure 5). In the field, the pots are arranged according to Figure 7, i.e. alternate EDU-treatment and non-EDU-treatment pots in 4 rows of 10 pots with 0.5m distances within rows and 1m distances between rows.

6. It is important to reduce over-heating by using white-coloured pots.

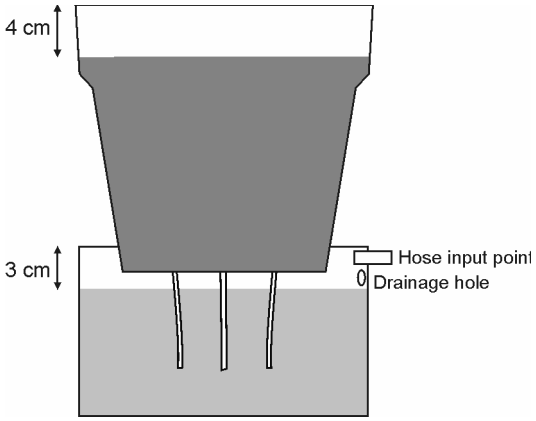


Figure 6 The pot and water reservoir system

+	-	+	-	+	-	+	-	+	-
-	+	-	+	-	+	-	+	-	+
+	-	+	-	+	-	+	-	+	-
-	+	-	+	-	+	-	+	-	+

Figure 7 Suggested experimental design of chemical protectant study with 20 EDU-treated (+) and 20 non-EDU-treated (-) plants.

5.3.3. EDU application/treatment

EDU will be supplied in sufficient quantities by either

Stockholm Environment Institute at York (SEI-York)
University of York
Heslington
York YO10 5DD
U.K.
Tel: ++44 1904 432890 (Patrick Büker)
Fax: ++44 1904 -432890
E-mail: pb25@york.ac.uk

For mung bean and spinach, day 1 of the experiment is when the seedlings emerge (germination). For potato and tobacco, day 1 of the experiment is when the 40 most healthy plants are selected for the experiment (4 weeks after planting the tuber) or when the plants are exposed to ambient air, respectively.
At day 7 (one week after seedling emergence/germination), 50% of the plants (approx. 20) are treated with 100 ml EDU solution freshly prepared in deionized water. **An EDU-concentration of 300 ppm has to be applied to spinach, whereas mung bean, potato and tobacco require a concentration of 400 ppm EDU.** Please note that it takes a long time to dissolve EDU in water, so allow enough time for this working step.
The EDU application is repeated every 10 days up to the maturity of reproductive parts with an increasing amount of solution after every second treatment: day 7 – 100ml, day 17 – 100ml, day 27 – 150ml, day 37 – 150ml, day 47 – 200 ml, day 57 – 200ml. The EDU solution is applied as a soil drench in the early morning (7 or 8 a.m.). Control plants (approx. 20) are treated with the similar amount of deionized water only.
Avoid wetting the foliage with the EDU solution and wear gloves during preparation and application of the solution since EDU has some toxic properties.

5.3.4. Care of plants

Watering:
The plants are watered via the wicks that project into the water reservoirs and natural rainfall. The reservoirs should be checked daily, especially during the last week prior to cutting back to ensure the plants are watered sufficiently. Also ensure that the media is not holding too much water so that it is waterlogged.

Weeding:
Remove weeds from the pots and around the pots as necessary.

Damaged plants:
Remove any pots from the experimental plot if their clover plants become severely damaged by anything other than ozone pollution. Do not continue to harvest these.

Insect Pests
Careful observation for insect pests is required, for example, aphids sometimes hide below stolons coiled around the inside of the pot rim. A suggested insecticide is Avid (Manufactured by Syngenta) for control of aphids, thrips, leaf miners and red spider mites. If the use of insecticides is necessary, please take notes of the product's name, chemical composition and dosage as well as the time when it was applied.

Viruses
Virus infection normally appears as large, whitish-yellowish round spots on the upper leaf side. Remove any affected plants from the site.

Fungal infections
If the plants are infected by a fungal pathogen, fungicides can be used but please ensure that the chosen chemical will not have an interaction with ozone. For example, it is believed that benomyl and triadimefon offer some protection against ozone, and thus these and chemically related pesticides should not be used. Leaf Spot fungi can be a problem when there is a lot of rain during the growing season and Chlorothalonil (Bravo) is suggested for control. Systhane can be used to control mildew. Neither of these fungicides are thought to alter the response of plants to ozone. Use of any pesticides should be made with consideration of prescribed safety rules.

Herbivores
Birds, rabbits and other rodents as well as possibly larger mammals feed on clover. A rabbit exclusion fence and bird netting is a requirement where these risks exist. For slug control use slug pellets (metaldehyde) or commercial biological control methods, e.g. 'nemaslug' (nematodes).

Advice:
Advice on any aspect of the experimental work can be sought by emailing Patrick Büker (pb25@york.ac.uk), Prof. Håkan Pleijel (hakan.pleijel@miljo.gu.se) or Prof. Madhoolika Agrawal (madhoo58@yahoo.com). If you are requesting help with identifying symptoms of any type, please send photos with your email.

5.4. Assessments

5.4.1. Identifying visible ozone injury on crops

The photographs presented in Figure 8 to 11 provide examples of ozone injury on mung bean, potato, tobacco and spinach.

For mung bean, these injury symptoms appear as white/silver spots or bronzing on the upper leaf surface, as can be seen in Figure 8.

Figure 8 Example of ozone injury on mung bean

In potato, ozone-induced foliar injury appears mainly as reddish-brown lesions and/or bronzing on both sides of the leaves, as can be seen in Figure 9.
Many cultivars of tobacco are known to be ozone-sensitive; especially the cultivar BelW3 has extensively been used to demonstrate the impact of ozone on plant tissues. Figure 10 shows typical ozone-induced injury symptoms on leaves of the BelW3 cultivar. These symptoms – also called weather flecks - appear as necrotic and/or chlorotic spots of increasing size with increasing ozone uptake.

Figure 9 Typical ozone injury on potato leaves (courtesy of A.S. Heagle)

Figure 10 Typical ozone-induced foliar injury on the ozone-sensitive tobacco cultivar BelW3 (courtesy of A.S. Heagle).

Figure 11 Typical ozone-induced foliar injury on spinach (courtesy of Håkan Pleijel).

In spinach, ozone-induced foliar injury is usually expressed as chlorosis and sometimes necrosis. First symptoms appear as brown-yellowish flecks on the upper leaf surface, which quickly expand with ongoing ozone-exposure.
For further pictures of typical ozone-induced foliar injury on several crops cultivated in Europe and North America, please check Flagler et al. (1998) and the following websites:

http://icpvegetation.ceh.ac.uk/
http://www.ncl.ac.uk/airweb/

5.4.2. Weekly injury assessments

The following assessments based on the fraction of fully developed/expanded leaves exhibiting injury should be made on a weekly basis, ideally on the same day per week and by the same (two) person(s) to minimise variation of the assessments. If possible, more than one observation of the plants per week should be made so that the date of first occurrence of ozone injury can be noted.

Assess each plant for ozone injury every week using the following scale:

0: no injury
1: slight injury, < 5 % of fully expanded leaves with slight injury
2: moderate injury, 5-25 % of fully expanded leaves with injury
3: heavy injury, 25-50 % of fully expanded leaves injured
4: very heavy injury, > 50 % of fully expanded leaves injured

Please also count the number of damaged fully expanded leaves and the total number of fully expanded leaves.

Grade each plant as either healthy (H) or abnormal, with the cause of the abnormality graded as 1 (slight), 2 (moderate), or 3 (severe) using the following key:

O	Ozone
S	Stunted
D	Diseased
I	Insect damage
Sl	Slug damage
A	Animal (rabbits, deer, birds etc.)
V	Virus

If the plants are damaged by anything other than ozone pollution, please indicate on the recording form whether you think the data obtained at this harvest should be used.

5.4.3. Harvest

Bean (mung bean, soybean):
Conduct the harvest when pods are turning brown (start of maturity). Record the day when the harvest is conducted!
At the harvest, morphological characteristics, such as number of leaves and pods per plant, are assessed. Furthermore, the total above-ground biomass of each plant is determined by summing the dry weight of all separately harvested plant components.
Each plant is separated into seeds and all remaining above-ground biomass. Each component is put into a labelled paper bag (i.e. one for seeds and one for remaining above-ground biomass per plant), dried to constant weight (ideally at approx. 80°C for approx. 48 hours in an electric oven) and subsequently weighed using a precision scale. Before the weighing procedure, please cool down the dried samples to air temperature in an exsiccator or any other drying apparatus equipped with a desiccant such as silica-gel (samples not cooled down will show a too low weight due to thermal buoyancy).

Potato:
The potato plants are harvested six weeks after planting day one of experiment (i.e. when the 40 healthiest plants are selected for the further experiment).
The entire above-ground biomass of each plant is put into a labelled paper bag (i.e. one bag per plant). The biomass is dried at approx. 80°C for approx. 48 hours in an electric oven before being weighed using a high precision scale. Before the weighing procedure, please cool down the dried samples to air temperature in an exsiccator or any other drying apparatus equipped with a desiccant such as silica-gel (samples not cooled down will show a too low weight due to thermal buoyancy).

Tobacco:
The tobacco plants are harvested after six weeks of exposure to ambient air.
The entire above-ground biomass of each plant is put into a labelled paper bag (i.e. one bag per plant). The biomass is dried at approx. 80°C for approx. 48 hours in an electric oven before being weighed using a high precision scale. Before the weighing procedure, please cool down the dried samples to air temperature in an exsiccator or any other drying apparatus equipped with a desiccant such as silica-gel (samples not cooled down will show a too low weight due to thermal buoyancy).

Spinach:
The spinach plants are harvested six weeks after germination.
The entire above-ground biomass of each plant is put into a labelled paper bag (i.e. one bag per plant). The biomass is dried at approx. 80°C for approx. 48 hours in an electric oven before being weighed using a high precision scale. Before the weighing procedure, please cool down the dried samples to air temperature in an exsiccator or any other drying apparatus equipped with a desiccant such as silica-gel (samples not cooled down will show a too low weight due to thermal buoyancy).

Please note: We would strongly like to advise people to keep the dried plant samples stored (for example in their paper bags, with each harvest enclosed in a separate plastic bin-liner) for further analyses (e.g. leaf-nitrogen content, heavy metal content etc.) in the future.

Data collection

Pollutant and climate data:
At the end of the experiment, we would kindly like to ask you to read out all meteorological data and create an Excel-file in the following format:

Country:			
Site:			
Geographical coordinates of site:			
Person in charge:			
Email address:			
Phone number:			
Fax number:			
Contact address:			
Comments (e.g. on missing data):			
Date	**Time**	**Temperature (°C)**	**Relative humidity (%)**
01/11/06	11:00		
01/11/06	11:30		

The file should have a separate row for each hour of data, i.e. the spreadsheet should have the parameters temperature and relative humidity (plus any additional parameters recorded on sites) along the top, and day and hour in separate columns down the left-hand side. Missing data values should be represented by " * ".

Please also list the four-weekly mean ozone concentration data as recorded by the passive samplers and analyzed by IVL (see Section 2) in a separate worksheet of the same file.

This file should then be sent by email to Patrick Büker (pb25@york.ac.uk), where the data will be pooled and analyzed according to agreed standards. A receipt will be sent for any data received.

Plant data:
The injury assessment and biomass data should also Patrick Büker (pb25@york.ac.uk) on spreadsheets provided at the start of the growing season.

6. References

Agrawal, M., Rajput, M., Singh, R.K., 2003. Use of ethyelendiurea to assess the effects of ambient ozone on *Vigna radiata*. International Journal of Biotronics 32, 35-48.

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

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

Ainsworth, N., Fumagalli, I., Giorcelli, A., Mignanego, L., Schenone, G., Vietto, L., 1996. Assessment of EDU stem injections as a technique to investigate the response of trees to ambient ozone in field conditions. Agriculture, Ecosystems and Environment 59, 33-42.

Astorino, G., Margani, I., Tripodo, P., Manes, F., 1995. The response of *Phaseolus vulgaris* L. cv. Lit. to different dosages of the anti-ozonant ethylenediurea (EDU) in relation to chronic treatment with ozone. Plant Science 111, 237-248.

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

Bennett, J.H., Lee, E.H., Heggestad, H.E., 1978. Apparent photosynthesis and leaf stomatal diffusion in EDU bean plants. In: Abdel-Rayman (Ed.). Proceedings Plant Growth Regulator Working Group, 5th Annual Meeting. Virginia Polytechnic Institute and State University, Blacksburg, Virginia, Longmont, CO, USA. pp 242-246.

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

Brunschön-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.

Brunschön-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.

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.

Elagöz, 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.

Flagler, R. (Ed.), 1998. Recognition of Air Pollution Injury to Vegetation: a Pictorial Atlas. Air & Waste Management Association, Pittsburg, U.S.A.

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

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.

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.

Kuehler, E.A., Flagler, R.B., 1999. The effects of sodium erythorbate and ethylenediurea on photosynthetic function of ozone-exposed loblolly pine seedlings. Environmental Pollution 105, 25-35.

Lee, E.H., Upadhyaya, A., Agrawal, M., 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., 2005. Establishing a cause and effect relationship for ambient ozone exposure and tree growth in the forest: Progress and an experimental approach. Environmental Pollution 137, 443-454.

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

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.

Mills, G., Benton, J., Skärby, L., Fuhrer, J., Gimeno, B. (1997). ICP Vegetation experimental protocol for the ICP-CROPS. ICP-CROPS coordination centre, The Nottingham Trent University, U.K., 47 pp.

Paoletti, E., Manning, W.J., Spaziani, F., Tagliaferro, F., in press. Gravitational infusion of ethylenediurea (EDU) into trunks protected adult European ash trees (*Fraxinus excelsior* L.) from foliar ozone injury. Environmental Pollution.

Pleijel, H., Almbring Norberg, P., Selldén, G., Skärby, L., 1999. Tropospheric ozone decreases biomass production in radish plants (*Raphanus sativus*) grown in rural south-west Sweden. Environmental Pollution 106, 143-147.

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.

Tingey, D.T., Olysk, D.M., Herstrom, A.A., Lee, E.H., 1993. Effects of ozone on crops. In: McKee, D.J. (Ed.), Tropospheric ozone: Human health and agricultural impacts. Lewis Publishers, Boca Raton, U.S.A., pp175-206.

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.

Tonneijck, A.E.G., Van Dijck, C.J., 1997a. Effects of ambient ozone on injury and yield of *Phaseolus vulgaris* L. at four rural sites in the Netherlands as assessed by using ethylenediurea (EDU). New Phytologist 135, 93-100.

Tonneijck, A.E.G., Van Dijck, C.J., 1997b. Assessing effects of ambient ozone on injury and growth of *Trifolium subterraneum* at four rural sites in the Netherlands with ethylenediurea (EDU). Agriculture, Ecosystems and Environment 65, 79-88.

Tonneijck, A.E.G., Van Dijck, C.J., 2002. Injury and growth response of subterranean clover to ambient ozone as assessed by using ehtyleneduirea (EDU): Three years of plant monitoring at four sites in The Netherlands. Environmental and Experimental Botany 48, 33-41.

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., 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.

Appendix 1

The use of passive diffusive samplers and Tinytags

Jenny Sundberg & Håkan Pleijel

PASSIVE SAMPLERS

Why use passive diffusive samplers?

The main advantages include:

- It is a relatively cheap, easy to handle and reliable way of measuring
- The samplers are small, have a light weight and are silent
- No electricity is needed
- Can be used in many environments (outdoor-indoor, rural-urban, arctic-tropical)
- No field calibration is needed
- Due to this the location of the measurement site can be very geographically flexible. The samplers are good to use for regional mapping or long-term monitoring.

The key disadvantage is:

- Low time resolution (usually at least 1 week)

How do the samplers work?

During measurement the gas molecules diffuse into the sampler through the grey net. The net acts as a mechanical protection against particles. Inside the sampler the gas molecules are collected on an impregnated filter or an adsorbent material. Good ventilation of air around the sampler is required for the technique to work. After analysis an average concentration of the air pollutant during the measurement period is received.

How to use them?

The samplers are kept in plastic containers to which the cap fits tight. The containers with the samplers are placed in sealed plastic bags before and after exposure. If possible it is good if the samplers are stored in a fridge if not used immediately.

When measuring outdoors some kind of protection from rain is needed. A metal disc with tool holders mounted underneath will be provided by IVL. The disc can be placed either on a pole, out from a wall or similar. The ozone samplers should be placed about three to four meters above ground. The measurement height must be specified. It is good to describe the measurement site (or take some photos). The location in the landscape can influence the results, since for example a hilltop site is more exposed than a valley site and therefore experience higher ozone concentrations.

Do not take out the sampler from the container until it is time to start measuring at the site. The exposure starts as soon as the cap is taken off the container. Carefully take the sampler out of the container without touching the grey net, which easily can be damaged. Grip the edges. Attach the sampler underneath the metal disc with the help of the tool

holders with the grey net downwards. Enter date, starting time and name of measurement site in the field protocol enclosed in the plastic bag.

How long the measurement period should be depends on the concentration of the air pollutant at the site and the time resolution wanted. The sampler needs to be exposed long enough to pass the lower detection limit but not so long it passes the upper detection limit. A measurement period of approximately 1-4 weeks is usually good. The measurement period will be four weeks for the RAPIDC biomonitoring campaign. At least two replicates are strongly recommended.

When the measurement period is over, carefully take down the sampler and put it back into the container and close the cap tightly. Enter the date and end time in the field protocol. Any special remarks or observations should also be noted. Put the container and field protocol back into the plastic bag and seal it. Send the samplers to the laboratory so they can be analyzed. The average concentration of the air pollutant during the period of interest will be received.

What can go wrong?

- ✓ Do not open the container with the sampler until it is time to put it up. The exposure starts as soon as the cap is taken off. If the container is opened and immediately closed again the sampler will not be destroyed.
- ✓ Do not touch the grey net. It can easily be damaged. Grip the edges of the sampler.
- ✓ Place the sampler with the grey net downwards when attaching it underneath the metal disc; otherwise there will be no exposure and no result.
- ✓ Make sure the sampler is well attached to the metal disc with the tool holders so it won't fall down.
- ✓ Remember to note the start and end times of exposure as well as name of measurement location in the field protocol; otherwise the sampler will not be analysed at IVL.
- ✓ Put up some information and a "please do not touch"-note close to the sampler on the measurement site and a phone number where more information can be obtained, to avoid damage caused by curious people.
- ✓ To avoid vandalism, try to choose a measurement site that is not too exposed to people not informed about the measurements.
- ✓ Make sure the sampler is protected from rain – the metal disc should be horisontal.
- ✓ Make sure the cap is tightly closed after the measurement period is over and the sampler is put back in the container.

TINYTAG

Why use Tinytags?

The amount of an air pollutant at a site is determined by the nature of the relevant emissions and the state of the atmosphere. Meteorological characteristics such as the humidity, air temperature, intensity of solar radiation, wind, etc control the dispersion, transformation and eventual removal of the air pollutant after release.

The most important effects of air pollutants on plants are related to the uptake of the pollutant rather than to the concentration in the ambient air. The pollutant must enter the leaf through the stomatal pores, where the gas exchange between the plant and the surrounding air takes place. The degree of stomatal opening is regulated by the micro-scale climate, for example temperature, air humidity and light. It is therefore important to know how the micro- and local-scale climate varies together with air pollution concentration. If for example the air humidity is very high the stomata will be open to a larger extent and a lower ozone concentration could give rise to the same effects as a higher concentration in a drier micro environment. The stomata respond to the vapour pressure deficit (VPD) in the air. VPD is the difference between the amount of moisture in the air and how much moisture the air can hold when it is saturated. With increased shortage of moisture in the air, the plants close the stomata in order not to lose water. How much moisture the air can hold depends on temperature. Warm air can hold more moisture than cold. Therefore VPD depends on both temperature and relative humidity.

Since exposure and dose of air pollution depends on local climatological characteristics, it is necessary to combine the information about both climatology and the concentration of the air pollutant to assess the risk for effects on vegetation from air pollution. An understanding of representative measurement sites in relation to local climate is important

A relatively cheap and simple way to measure air temperature and relative humidity is to use small, robust Tinytag loggers/sensors. They do not require electricity (except for batteries which last long) and therefore the measurement site location is just as flexible as for the passive diffusive samplers.

How do the Tinytags work?

The temperature is measured by a thermistor while the relative humidity is measured with an externally mounted capacitive sensor. To be able to use the Tinytag you also need a software installed on your computer and a download cable.

How to use them?

It is very important to use a radiation shield when measuring temperature and relative humidity such as with the Tinytags. If the sunlight falls directly on the Tinytag it will warm up and of course measure the temperature of itself which is higher than the surrounding air temperature. If using a Tinytag, it is most likely that it is not possible to use a radiation shield with forced ventilation which requires electricity. One way to solve this is to use self ventilating radiation shields. The Tinytag hangs in the lower part of a tube, the upper part of the tube is black and the lower half has reflective cover. The air

will warm in the upper black part of the tube and rise and new air comes in from below. Alternatively, use a simple arrangement with aluminium foil.

New Tinytag sensors are well calibrated. It could anyway be recommended to put up the Tinytag in connection to a meteorology station with more advanced instruments some days before and/or after the measurement period of the experiment. This gives an indication of the correctness of the Tinytag sensors, the influence of the radiation shield and also if the Tintyag measures as correct in the end of the measurement period as in the beginning.

With a laptop it is easy to collect the Tinytag data on the experimental site. In case something would happen (Tinytag stolen, out of battery, etc) it is good to have collected the Tinytag data a few times during the measurement period even though it is possible to store a large amount of data in it.

Once a year it is recommended to change the battery, seal and desiccant packs of the Tinytag. To make sure that the Tinytag measures correctly it is also recommended to calibrate it once a year. After a year it will also improve the performance of the Tinytag to gently wash the relative humidity sensor with some deionised water to make sure that salt deposits will not influence the relative humidity measurements.

It is problematic to measure relative humidity close to 100%. At very high humidity out of range values may be generated by the Tinytag which have to be removed in the data processing before further calculations.

Calculation of the saturation water vapour pressure (e_s, kPa) at a certain temperature (T, °C) according to Campbell and Norman (1998):

$$e_s = 0.611 \times \exp\left(\frac{17.502 \times T}{T + 240.97}\right) \quad \text{(eq. 1)}$$

Calculation of the vapour pressure (e_a, kPa) at a certain relative humidity (RH, %):

$$e_a = \frac{e_s \times RH}{100} \quad \text{(eq. 2)}$$

Calculation of the vapour pressure deficit (VPD, kPa):

$$VPD = e_s - e_a \quad \text{(eq. 3)}$$

What can go wrong?

- ✓ Make sure the Tinytag really started; download to see if data is stored as you intended.
- ✓ Download data several times during the measurement period to make sure as little as possible is lost in case something happens to the Tinytag.
- ✓ Make sure the battery is not too old.
- ✓ Try to minimize the risk for curious people or vandals to disturb your measurements.
- ✓ Make sure the Tinytag will not be directly exposed to rainfall; it takes a while for the relative humidity sensor to dry up even though water will not damage it.

- ✓ Remove out of range relative humidity values before forming averages over longer times.

READ MORE

http://www.ivl.se/en/
http://www.tinytag.info/

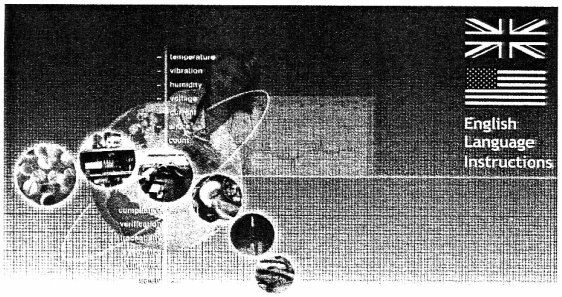
Campbell G. S. and Norman J. M. (1998) *An introduction to environmental biophysics.* Springer Science Inc, USA, page 41.

Carmichael G. R., Ferm M., Thongboonchoo N., Woo J. H., Chan L. Y., Murano K., Viet P. H., Mossberg C., Bala R., Boonjawat J., Upatum P., Mohan M., Adhikary S. P., Shrestha A. B., Pienaar J. J., Brunke E. B., Chen T., Jie T., Guoan D., Peng L. C., Dhiharto S., Harjanto H., Jose A. M., Kimani W., Kirouane A., Lacaux J. P., Richard S., Barturen O., Cerda J. C., Athayde A., Tavares T., Cotrina J. S. and Bilici E. (2003) *Measurements of sulphur dioxide, ozone and ammonia concentrations in Asia, Africa and South America using passive samplers*. Atmospheric Environment 37: 1293-1308

Ferm M. (2001) *Validation of a diffusive sampler for ozone in workplace atmospheres according to EN838*. Proceedings from the International Conference on measuring Air Pollutants by Diffusive Sampling, Montpellier, France, 26-28 September, pp 298-203

Sundberg J., Karlsson P. E., Schenk L. and Pleijel H. (2006) *Variation in ozone concentration in realtion to local climate in south-west Sweden*. Water, Air and Soil Pollution 173: 339-354

Appendix 2



Tinytag Explorer

Quick Start Guide

Tinytag

WHAT YOU NEED

- **Tinytag Explorer software installation CD-ROM**
- **Software Activation Code**
- **One or more data loggers**
- **Download connection cable or inductive pad from data logger(s) to PC**
- **External probe (for loggers requiring an external probe only)**

INSTALLING THE SOFTWARE

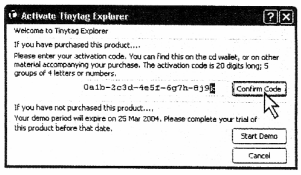
- Start your computer, and insert the Tinytag Explorer CDROM into the drive.
- In a few seconds, the installation process should start automatically
- At the installer welcome screen, click **Next** and follow the instructions.
- Read the **Licence Agreement**. For installation, accept the conditions. Click **Next**.
- **Select Installation Folder**: the system asks you where you want to install the program. We recommend that you accept the default and click **Next**.
- **Ready to Install**: click **Install** to proceed. Installation will take a few moments.
- Once complete, click the **Finish** button to exit. The software is now installed and once you have re-started your computer you can begin to use it.

"Nothing happened after I put the CD in"

- Open **Windows Explorer** and navigate to your CD-ROM drive. It may be labelled Compact Disk or CD-RW drive.
- Look at the files on the CD and double-click on **setup.exe** (if you cannot see it, look for **tinytag.msi**). The installation should now begin.

ACTIVATING THE SOFTWARE

- When you come to the **Activate** screen, type the code into the box provided.



Example Activation screen showing a 'dummy' code...

- Click **Confirm Code** to activate the software.

Activation code not recognised

If the code is not recognised, the system will tell you.

- Click **OK** to clear the warning screen.
- Try entering the code again, typing the letters and numbers carefully. Click **Confirm** to activate. The main screen should now appear.

Note: If you cannot successfully activate the software, please call Gemini Technical Support.

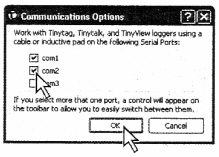
Time-limited demo trial software only

If you are trying the software before you buy it, click **Start Demo**. The system will work until the expiry date shown and then will cease to function.

SERIAL PORT SETUP

Once you have installed the software, it can detect **how many serial ports** there are on your computer.

Start the software and go to **menu > Options > Communications Options.**



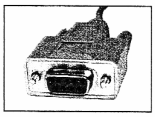
Example where two serial ports are used for loggers...

- Check ☑ those port(s) to which you will connect loggers, and leave the others ☐ unchecked. The default is com1.

CONNECT LOGGER TO PC

There are two types of connection at the logger end of the cable, a conventional **cable plug** or a Tinytag **data transfer pad**. At the computer end, there is either a 9-pin D-connector or a USB connector, depending on what was specified.

Plug the D-Connector into the Computer



- If the cable has a 9-pin D-connector, plug it into a serial port on the back of your PC.
- Make sure the plug is the right way up, push it in firmly.
- Tighten the fixing screws so that the plug is secure. Do not over-tighten.

Plug USB Cable into the Computer

- Another version of the cable uses a USB connector rather than a 9-pin D-type connector.
- Plug this into a USB port on your computer.

Logger Connection by Plug

- If you have loggers that use a plug connection, note that the socket on some loggers has a 'key' for aligning the plug.
- Rotate the plug until it clicks into place, then push firmly home.

- Other loggers use a 3.5mm jack plug (resembling the kind used on headphones).
- Align the plug carefully and push it into the small socket until it clicks into place.

Logger Connection by Inductive Pad

- Just place the logger onto the inductive pad and you are ready to transfer data to and from the computer.

CONNECTION FUNCTIONS

- Use **Toolbar > drop-down** to select Com port to which the logger is connected.

Functions available

- Launch: See **Configure and Launch.**
- Stop: To stop the logger **from recording any more data.**
- Get data: See **Get Data from Logger.**
- Logger's current readings.

Failed to connect to data logger on com...

- If the system cannot 'see' the logger, check the cable connections at both ends.
- Check that the correct Com port is selected.

CONFIGURE & LAUNCH LOGGER

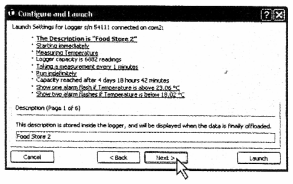
WARNING: Make sure you download and save data **before** you configure and launch a logger, otherwise you will **lose all previous recorded data**.

- To start the setup process, click **toolbar > Go** .

In a few seconds the **Configure and Launch** screen appears. This shows the options available for the logger. The upper part of the screen shows the list of options, configure each option in the lower part. Use **Next** / **Back** to move from step to step.

Page 1 of 6: Enter a Description

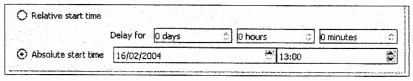
- Enter your own description for the next measurement log. This will be stored with the captured data and appear on charts etc.



- Click **Next.**

SET START TIME

Page 2 of 6: Start Options

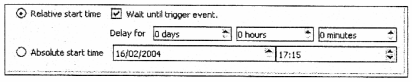


- Tell the logger when to begin logging data.
- Click ⊙ to set a Relative Start Time. Delay = 0 means start now. OR
- Click ⊙ to set a Relative Start Time + Delay = X days, Y hrs and Z mins after you click **Launch**. OR
- Click ⊙ to set an Absolute start time and date.
- Click **Next**.

As well as the common settings shown above, some loggers have extra settings for specific purposes.

SET TRIGGERED START

Page 2 of 6: Select triggered start. (Not all models)

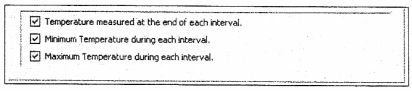


Some loggers have a **magnetic switch** built in. You activate the logger by holding a magnet close to the front of it.

- Check ☑ Wait until trigger event. You can also add a delay if you wish.
- Click **Next** to continue.

SELECT MEASUREMENTS

Page 3 of 6: Measurements



Some models only have the Spot Measurement option; but if there is a choice, check ☑ whether you want Spot Reading, Minimum and/or Maximum values:

- **Spot value =** Instantaneous value **measured at the end of each logging interval.**
- **Minimum =** lowest value **during each logging interval.**
- **Maximum =** highest value **during each logging interval.**

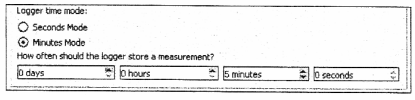
Note: If you want to **record Minimum and Maximum** readings, you must select the **Minutes Mode** in the next screen.

- Click **Next**.

SET LOGGING INTERVAL

Page 4 of 6: Logging Interval

How often do you want to take a reading? Set the logging interval.



There are **two logging modes**:

- Click ⊙ **Seconds Mode** for short intervals where readings may fluctuate quickly. In this mode, you cannot take Maximum and Minimum readings, and you cannot download data while the logger is recording. OR
- Click ⊙ **Minutes Mode** for longer intervals over longer periods of time. This mode also allows you to check the logger's current reading at any time, and to take maximum and minimum readings, and to download while still running.
- Then click **Next**.

Note: You must set a **valid time interval** or you cannot launch the logger.

SET STOP OPTIONS

Page 5 of 6: Stop Options

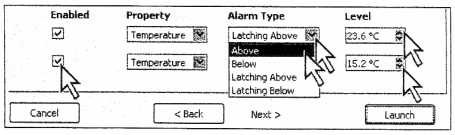
- Run indefinitely **- this fills the memory to the end and then starts writing again at the beginning. This will** overwrite old readings **as it goes.**
- Stop after N readings **- takes N measurements only before stopping. Select the number required.**
- Stop when full **- takes readings until the** memory is full **and then stops.**

- Click **Next**.

SET ALARM INDICATORS

Page 6 of 6: Alarm Options. (Not all models)

This sets a **visible alarm indicator** if a logger detects a reading **above or below preset thresholds**.



- Enabled: Check ☑ to enable each alarm as required.

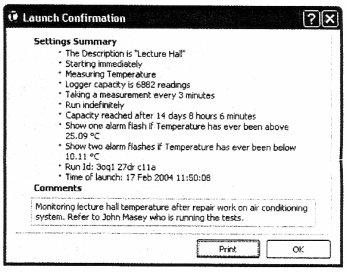
LAUNCH AND PRINT SUMMARY

⚠ **WARNING:** Once you **launch the logger**, you will **lose all previous recorded data**. To download the data first, see **Get Data from Logger**.

- Click **Launch** [Launch] to save the settings to the logger and start it operating with the new settings.

The system confirms the launch and displays the **Settings Summary**.

- You can add Comments if you wish.



Example environment temperature logging in a University Lecture Hall...

Note: The Comments only appear on the printout - they are not stored in the logger.

- Click **Print** to print this information to your computer's printer, then **OK** to finish.

Note: the **Run Id** is created by the software as a unique number for that logging run, except when you use a triggered start. It will appear in the download information.

- You can now unplug the logger and set it in place for recording data.

If the logger has LED indicators, one of them will flash every few seconds when it is recording. (The flash interval depends on the logger settings.)

GET DATA FROM LOGGER

Note: This does **not** clear the stored data, provided you have not launched another recording session - you can download it again if required.

- Connect the logger to the computer, and select the correct Com port (if you have more than one).
- Click **Toolbar > Get Data from logger**.

If a logger is set to **Seconds mode**, it **cannot download** while it is still running. The system will ask you to confirm that it should **stop the recording process** first.

- Click **Yes** to proceed.

Wait a few seconds while the computer downloads the data.

If there is a lot of data, this could take a minute or two. When it has finished, you will see the **Graph** (or chart) on the screen.

SAVE DATA

Note: If the data looks good quality, we recommend that you **Save Data** to a file before you go any further.

- Click on the Save icon.
- Navigate to where you save your data files.
- Enter a suitable file name and click Save.

FURTHER INFORMATION

For further information, refer to the Online Help Guide installed with Tinytag Explorer.

- Start Tinytag Explorer, and then go to **menu > Help > Contents**.

Alternate language versions of manuals and guides are available the Gemini website at: **www.geminidataloggers.com/manuals**

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