

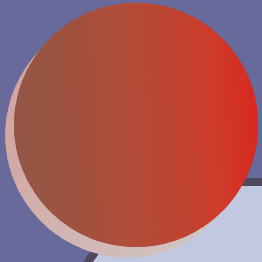


# **Overview of ozone bio-monitoring and chemical protectant study in South Asia**

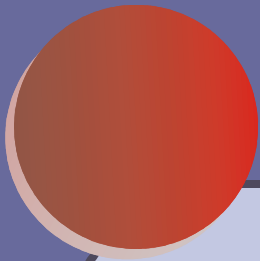
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**15 August 2007**  
**Dhaka, Bangladesh**

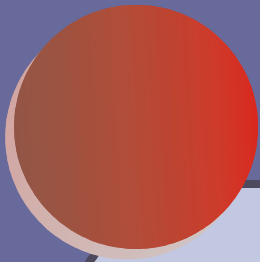


- **Introduction to bio-monitoring**
- **Aims of RAPIDC clover experiment**
- **Introduction of RAPIDC EDU study**
- **Support**



**Bio-monitoring** uses the responses of plants at several organisational levels to detect or predict changes in the environment and to follow their evolution as a function of time (De Temmerman et al., 2004)

- well established, cheap procedure used world-wide
- gives integrated response of plants to air pollution, i.e. biological effects indicate risk potential over time
- different organisational levels of plants can be used for bio-monitoring, ranging from leaves to ecosystems
- multi- and single-pollutant bioindicators available



## Bio-monitoring

**accumulative indicators  
(e.g. for detection of  
heavy metal burden)**

**reactive indicators  
(e.g. for detection of  
ozone effects)**

**active monitoring**

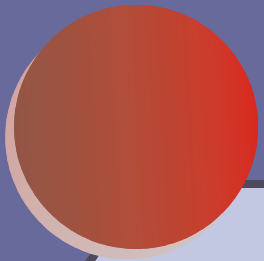
**passive monitoring**



Photo: ICP Vegetation



Photo: ALM



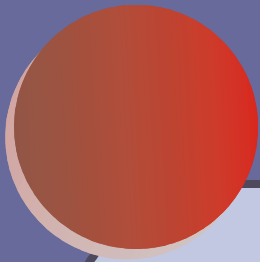
## **Detectable air pollutants**

### using accumulative indicators:

- heavy metals (Pb, Cd, Cu, Zn, Hg, Sb etc.)
- F, S, Cl, N
- (Persistent) organic pollutants (PCB, dioxins, DDT etc.)
- PAH's

### using reactive indicators:

- O<sub>3</sub>
- SO<sub>2</sub>, NO<sub>2</sub>, HCl, HF



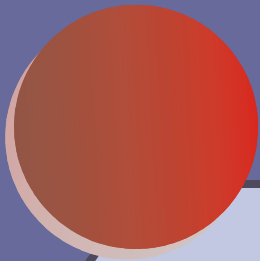
## Effect parameters used in ozone bio-monitoring

- **visible leaf injury** (stipples, chlorosis, necrosis etc.)
- reduced (relative) growth rate and **yield**
- premature senescence

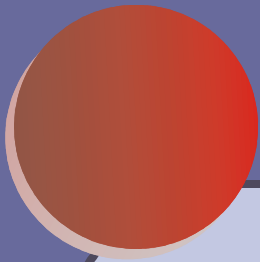


Photo: A. Wahid





- **White clover (*Trifolium repens* L.)**
  - common plant of productive grasslands (forage plant) in temperate and sub-tropical climates
- Cultivar used here: Regal; ozone-sensitive (NC-S) and ozone-resistant (NC-R) genotypes
- Developed in fairly hot climate of North Carolina
- Extensively used in standardised bio-monitoring activities within ICP Vegetation (Europe)

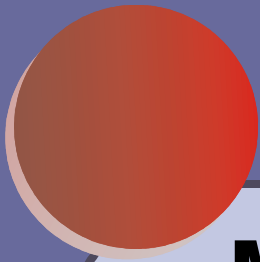


Ozone-sensitive genotype (NC-S)



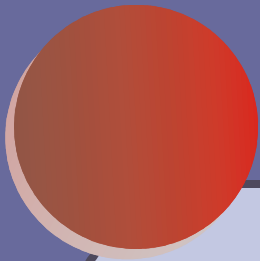
Ozone-resistant genotype (NC-R)





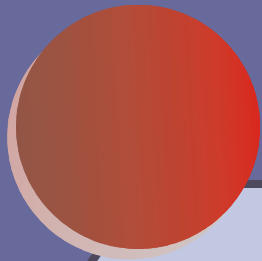
## **Main aims of clover bio-monitoring**

- To assess the biological impacts of increased ozone concentrations on plants in South Asia
- To identify areas at risk from ozone impacts in South Asia
- To verify the results of the model-based provisional risk assessment predictions
- To establish a scientific crop-effect community within Malé Declaration countries



## **Experimental requirements/site set-up**

- Greenhouse for plant cultivation
- Fenced area of approx. 6 x 7 m in good distance from major pollutant point sources (e.g. smelters); 200 m from main roads and 50 m from high buildings
- site with water (and electricity) access
- oven for drying harvested biomass and precision scale

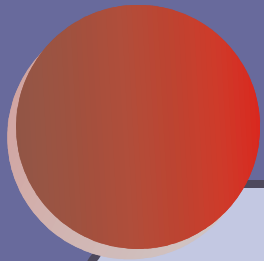


## **Bio-monitoring experimental protocol**

- based on protocol used within ICP Vegetation
  - provides **standardised** methodology to be adopted by all participating Malé countries
  - should function as a field manual
- Details will be discussed in technical session 2 of workshop

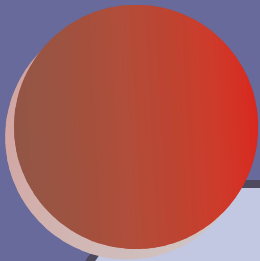
## **Experim. requirements/site set-up (cont.)**

- 1 litre pots for growing of cuttings (at least 40)
- 15 litre pots for exposed plants, plus buckets for water supply (each at least 40)
- fibreglass wicks for water supply → will be provided
- soil mixture of ideally local soil, sand and peat in ratio 1:1:1
- slow-release fertilizer (13N:13P:13K) → will be provided
- scissors, paper bags, wooden sticks



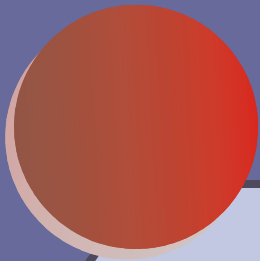
## **Experimental requirements/ site set-up (cont.)**

- Passive samplers for record of ozone concentration (will be exchanged every 4 weeks)
- Tinytag micro-meteorological data loggers for record of air temperature and relative humidity (should be read out every 4 weeks)  
→ will be provided



## Schedule of experiment

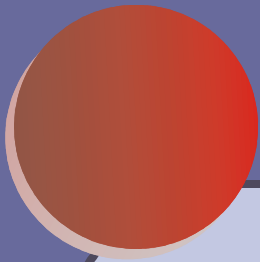
- Day 1: Clover cuttings arrive and are transplanted to 1 litre pots filled with soil mixture (1:1:1 local soil:sand:peat) in greenhouse where they will be kept well watered
- Day 21: Fertilizing of plants with nutrient solution of approx. 5:11:26 (N:P:K)
- Day 26/27: Filling of 15 litre pots with soil mixture
- Day 28: Transplanting of plants from 1 to 15 litre pots and exposure to ambient air at field site
- Day 35, 42, 49 etc.: weekly visible injury assessment
- Day 42, 56, 70 etc.: monthly exchange of passive samplers and read out of Tinytags



## **Schedule of experiment (cont.)**

- Day 56: First harvest after fourth visible injury assessment; first exchange of ozone passive sampler and read-out of Tinytag data logger
- Day 84: second harvest after eighth visible injury assessment; exchange of ozone passive sampler, read-out of Tinytag
- Day 112: Third harvest after twelfth visible injury assessment; exchange of ozone passive sampler, read-out Tinytag
- Day 140: Fourth and final harvest after 16<sup>th</sup> visible injury assessment; last passive sampler will be sent to IVL; final read out of Tinytag data loggers

→ data to be sent to Patrick

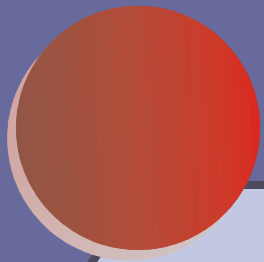


Healthy plant



Injured plant





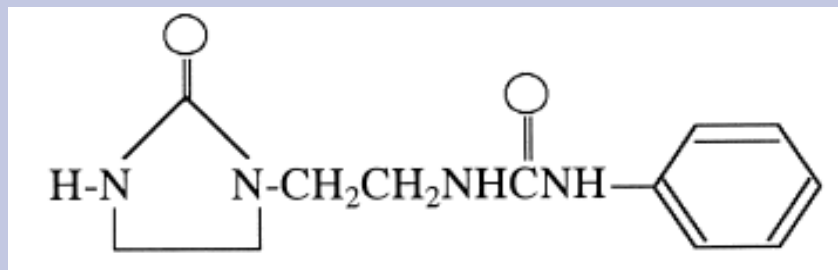
## **Chemical protectant study - aims**

**To use chemicals that protect crops from ozone damage under ambient ozone conditions and to compare yield losses of chemically protected and unprotected staple crops**

**Proposed protective chemical:**

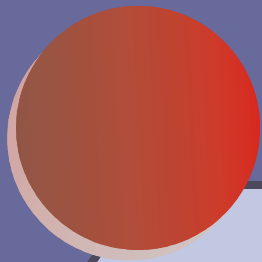
**Ethylenediurea (EDU)**

## EDU (ethylenediurea)



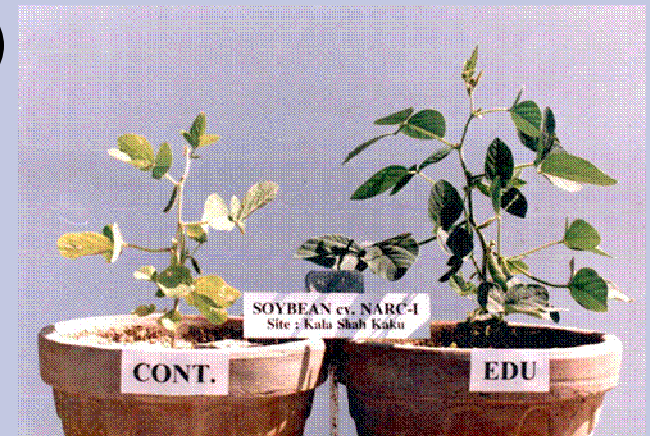
*Structural formula for N-(2-(2-oxo-1-imidazolidinyl)ethyl)-N'-phenylurea*

- antioxidant widely used in Europe and Asia to suppress acute and chronic ozone injury under ambient O<sub>3</sub> conc.
- used for detection of ozone-induced plant injury in bioindicator program in Europe
- easy to use, but expensive

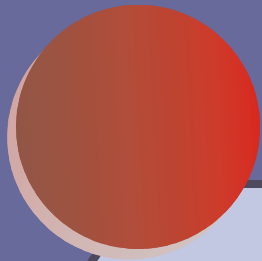


## Reported successful use of EDU

- mung bean, India (Agrawal et al., 2003)
- soybean, Pakistan (Wahid et al., 2001)
- tobacco (chamber study), USA (Godzik & Manning, 1998)
- common bean, Germany, Netherlands etc. (Büker 1997; Kostka-Rick & Manning, 1993; Tonneijck et al., 1997)
- grapevine, USA (Musselman, 1985)

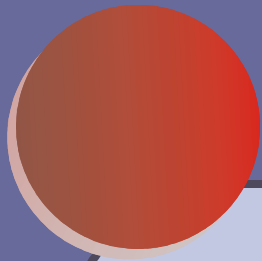


*Pakistan soybean cv. NARC-1 showing protective effect of EDU at a roadside rural site in Lahore, Pakistan (photo courtesy of A. Wahid)*



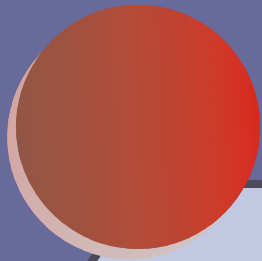
## **chemical protectant study - experimental protocol**

- pilot study in India (Varanasi) and South Africa (Potchefstroom)
- used bioindicators: locally grown/important crops, such as mung bean, potato, tobacco, spinach, maize etc.
- EDU will be supplied by SEI (with health and safety notice)
- effect parameters: visible injury, biomass



## **experimental protocol - methods**

- two batches of plants (EDU-treated and control) will be grown in glasshouse prior to 3-month ambient air exposure
- 100 ml of EDU (150 ppm w/v) or 100 ml of water (control) will be applied to exposed potted plants in 10-day intervals
- visible injury will be assessed twice a week
- final harvest will determine biomass ratio between control and EDU-treated plants



**Experimental protocols will function as manual/guide and for first aid support**

**However, please do not hesitate to contact any of the following persons if you have any specific inquiries or problems:**

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