‘Seeing is Believing’

Ozone injury on *Phaseolus vulgaris* (common bean)

Bean Biomonitoring Protocol

ICP Vegetation, 2020

Essential Requirements

- Pot size must be 10-15 litre volume
- **Soil must be well drained, and without big lumps so that roots can grow properly. Add potting compost, mulch or sand if necessary**
- Use slow release fertiliser
- Exposure site away from main roads and buildings if possible
- Record date of first flowering
- Record date when 50% of pods have dried and gone brown
- Visible injury assessments
- Take photos of visible leaf injury suspected to be from ozone
- Harvest of pods when they have dried and gone brown on the plant
- Ozone and meteorological data from site or nearby if possible
- Send data to Felicity Hayes (fhay@ceh.ac.uk)

Experimental aim

- To demonstrate whether ambient ozone concentrations cause visible damage to sensitive crops.
- To identify the extent of occurrence of visible injury on ozone-sensitive and ozone-resistant *Phaseolus vulgaris* in the differing ozone and physical climates.
- To quantify the reduction in pod yield of the ozone-sensitive *Phaseolus vulgaris* (but this might not be possible in very hot conditions, as pods will not develop).
- To establish a flux-effect relationship for the ozone-sensitive and ozone-resistant *Phaseolus vulgaris* – **we now have sufficient stomatal conductance data for developing a flux model, but if possible we would like to validate the model in differing climatic conditions.**

Background

This study will use the ozone-sensitive (S156) and ozone-resistant (R123) genotypes of *Phaseolus vulgaris* (Bush bean, French Dwarf bean) that have been selected at the USDA-ARS Plant Science Unit field site near Raleigh, North Carolina, USA. The bean lines were developed from a genetic cross reported by Dick Reinert (described in Reinert and Eason (2000)). Individual sensitive (S) and tolerant (R) lines were identified, the S156 and R123 lines were selected, and then tested in a bioindicator experiment reported in Burkey et al. (2005). This system has been tested in central and southern parts of Europe since 2008. Kent Burkey of USDA-ARS kindly provided seeds for the trials. However, it is recommended to also grow a local variety of bean at the same time.

Experimental Requirements

Experimental Plot: The site should be situated at least **200m from major roads and 20m from large buildings** if possible. The plot may need to be fenced to prevent birds, rabbits and small mammals from eating the bean plants and should ideally be surrounded by grass or an artificial weed reducing material to prevent excessive dust formation, mud-splashes or overshadowing by weeds. Slugs like bean plants, so slug pellets or biological control should be used if necessary.

Replication: Use a minimum of 8 pots for each genotype of bean plants.

Pots: **10 - 15 litre volume** with a surface diameter of approximately 25cm. Each pot should be kept well-watered using a method suitable for the climate. For hot climates, some modifications may be necessary to try to minimise heat uptake by the pots, evaporative losses from the soil surface and wilting when the beans have a lot of foliage. Suggestions are:

- Regular, possibly daily watering
- White pots and/or reflective material covering pots
- Stones or other material placed on the soil surface

However, it is important that the pots are well drained, as **bean plants rot if the soil is too wet**.

It is also important that the soil does not contain large clay lumps, as roots cannot grow through this. It might be necessary to add commercial potting compost, mulch or sand to give a good soil texture as **roots will not grow through solid clay soil**.

Soil mixture and fertilizer: Use whichever soil mixture/compost is appropriate for the area. Please add **slow-release fertilizer** according to the manufacturer's instructions.

Seeds: Seeds of ozone-sensitive (strain S156) and ozone-resistant (strain R123) lines of *Phaseolus vulgaris* will be supplied by the ICP Vegetation Coordination Centre. These seeds have been provided by Kent Burkey of USDA-ARS. If possible, please also use a local variety of bean.

Monitoring equipment: If there is any data on ozone and meteorological data at or close to the experimental site, please send this to us to cover the duration of the experiment. Hourly mean data for ozone, temperature, humidity and solar radiation are the most useful.

Setting up the experiment

The experiment will last until the pods have dried and gone brown on the plant. As a guideline, this could be for approximately 80-120 days, but depends on local climate. The seeds should be sown at an appropriate time, depending on climate and local bean growing practice.

At the experimental site, fill the pots with compost and water well to ensure that the soil is fully wetted. For eight pots per genotype either:

a) Sow two seeds 5 cm apart near to the centre of each pot at a depth of 3 cm. Ensure that the pots are protected from birds, animals, slugs and snails. When the first true leaves have emerged, thin to one plant per pot. **Day 0** of the experiment is the day that the plants are thinned to one per pot.

Or

b) Sow one seed per pot into degradable fibre pots (approx 6 cm diameter) at a depth of 3 cm. When the first true leaves have emerged, place one fibre pot containing a bean plant into each large experimental pot. Do not remove the plant from the fibre pot, the roots can grow through. **Day 0** of the experiment is the day that the plants are transplanted into the large pots.

Note: bean seedlings can be susceptible to root pathogens (root rot). There are commercial chemical treatments (e.g. Ridomil Gold, a fungicide containing the active ingredients, mefenoxam and chlorothalonil) that can be added as a soil drench if this becomes a problem. However, it helps if the soil is well drained and not too wet.

Note: the first true leaves are very susceptible to a variety of environmental and biotic stresses. The plants will usually grow through this. Any injury symptoms on these leaves that look like ozone injury are usually non-specific and should not to be included in assessments.

Core activities

1: Assessment of ozone injury

Ozone injury on *Phaseolus vulgaris* consists of bronze-coloured lesions that gradually join together to cover large parts of the leaf surface (Figure 1). Weekly assessments of injury are encouraged wherever practical. If this is not possible, as a minimum, there will be two assessment times:

1. The onset of flowering (when 50% or more of the plants are flowering)
2. Two weeks after the onset of flowering

Please take photographs of any injury that develops.

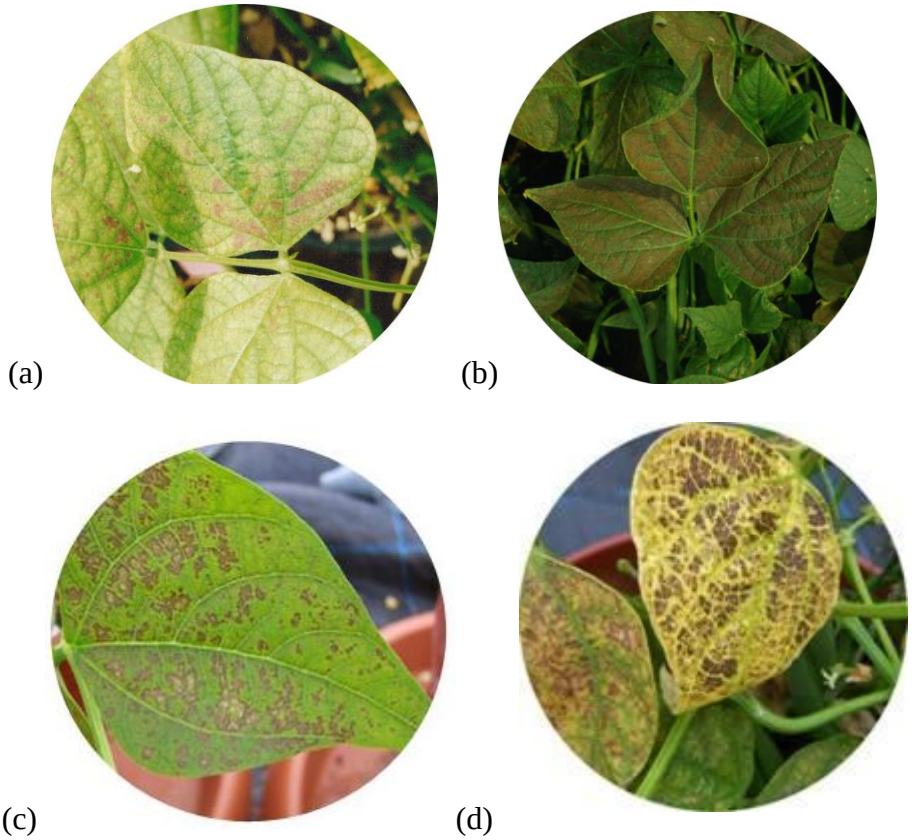

Figure 1: Ozone injury on a trifoliate leaf of *Phaseolus vulgaris* (a) scored as 5-25% of leaf injured, see below, (b) and (c) severely damaged leaf (scored as >25% injury) and (d) senesced leaf (these are yellow, rather than green, but may also show signs of previous ozone injury)

At each visual assessment, please ignore any symptoms on the primary leaves as this is often non-specific, and record the following for each plant:

- Plant genotype
- Plant number
- Health of plant (key described below)
- **Total number of trifoliate leaves[1]** (excluding primary leaves and any cotyledons still present) – please include this so that the % injured leaves can subsequently be calculated
- No of trifoliate leaves[1] with 1 – 5% injury
- No. of trifoliate leaves[1] with 5 – 25% injury
- No. of trifoliate leaves[1] with >25% injury
- No. of dead/senesced trifoliate leaves that remain on the plant
- Number of pods per plant (only count pods > 2cm long)

[1] One trifoliate leaf comprises one central and two side leaflets

<u>Key for the health of each plant</u>

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:

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

Please record the date of flowering (when 50% or more plants have flowers) – we use this when we calculate ozone flux to the plants.

It is important to check for 'red spider mite' which can cause visible damage to the leaves that looks similar to some types of ozone injury as it causes yellow mottles on the leaves (Figure 2). Red spider mite are very small and are usually found on the under-surface of leaves. When infestation is severe, small 'webs' are visible too.

Figure 2: Red spider mite injury on bean. It is yellow mottles, rather than reddish-brown. On the underside of the leaf there are very small 'mites' visible, <1mm and often yellow in colour.

2: Final Destructive harvest

Pods should be harvested when at least 50% of the pods on all plants have reached the brown pods stage. The date on which each genotype reaches the brown pods stage (50% of pods are dry and brown) should be recorded for each genotype because with high levels of ozone stress, all of the S156 leaves may have abscised and all pods will have become brown before the R123 genotype reaches the 50% brown pods stage. We would like a minimum of 50% brown pods for each genotype. We will use the date of the 50% brown pods to accumulate the ozone exposure.

By harvest, a large proportion of the leaves will have already fallen from the plant and thus we only require information about the biomass of the pods. Do not harvest or count pods < 2cm. **Separate pods into two categories, dried brown pods and all other pods (green, light green, yellow), and then separate each category into two size classes**, <4cm and >4cm. Dry the pods to constant weight. For each category/class size, count and record the dry weight of (1) pods that have seeds in and (2) pods that have failed (i.e. have no seeds in).

Optional additional studies

3. Other effects

a) Pod quality. This could include counts of the number of beans per pod, mean weight per bean and chemical analysis of pod quality.

b) Photosynthesis measurements and A/Ci curves.

4. Stomatal conductance: Collection of stomatal conductance data for flux-effect modelling

We have sufficient data to parameterise a DO_3SE[1] stomatal flux model for *Phaseolus vulgaris.* However, if you would like to compare your own stomatal conductance measurements to those collated as part of the flux-modelling work, please follow the following procedure for stomatal conductance measurements.

The final dataset indicates the dependence of stomatal conductance to meteorological parameters such as Photosynthetically Active Radiation (PAR, μmol m^{-2} s^{-1}), air temperature (°C) and Vapour Pressure Deficit (VPD, kPa). Single-sided porometers as well as steady-state gas exchange systems (IRGA) can be used for stomatal conductance measurements, however, when using the former make sure to take measurements on both sides of the leaf since *Phaseolus vulgaris* has amphistomatous leaves (i.e. stomata on both sides of the leaf). Once you have taken approximately 50 alternate measurements with the single-sided porometer (i.e. lower-upper-lower-etc.) on each genotype, subsequent measurements can then be made just for one leaf surface (that with the highest conductance). The ratio of the two surfaces calculated from the 50 alternate measurements can then be used to convert single-sided conductance to conductance for the entire leaf (total g_s).

If the measuring device measures conductance in cm s^{-1} (velocity units), air pressure and temperature data are required to convert the values into mol units.

Stomatal conductance should be measured according to the following criteria:

- Use a range of climatic conditions, including sunny and overcast days. Please make sure that for the derivation of g_{max} enough measurements (200 +) are made under "ideal" meteorological conditions for the gas exchange of the plants (i.e. sunny, warm and humid days);
- Measure at various developmental stages of the plants (e.g. before flowering, at flowering, during pod formation);
- Measure at various times over the course of the day, ideally at frequent intervals between sunrise and sunset;
- Use the **youngest fully developed** and **healthy** leaf, e.g. the second or third from the top of the plant;

The measurements should be recorded in an Excel table (see Appendix 1), indicating:

Leaf conductance
Meteorological data (with measuring height)
Genotype
Leaf number (count from the top of the plant)
% injury of the measured leaf

[1] DO_3SE: Deposition of Ozone and Stomatal Exchange model: https://www.sei.org/projects-and-tools/tools/do3se-deposition-ozone-stomatal-exchange/

Phenological stage (before flowering, flowering, during pod formation)
Leaf surface used (when using a single-sided porometer, i.e. upper or lower surface)
Measuring device
Gas measured (e.g. H_2O, CO_2)
Measuring unit (e.g. mmol H_2O m^{-2} s^{-1}, cm s^{-1})
Time and date of the measurement.

Collated stomatal conductance data can be sent to Felicity Hayes at the ICP Vegetation Coordination Centre (fhay@ceh.ac.uk), where it can be compared to the existing database.

Data collection

We request that all data is returned to Felicity Hayes (fhay@ceh.ac.uk) at the ICP Vegetation Coordination Centre.
Pollutant and climate data: should be sent by email as a spreadsheet preferably in Excel format. The file should have a separate row for each hour of data i.e. the spreadsheet should have parameters such as ozone concentration and temperature along the top, and day and hour in separate columns down the left-hand side. It is important that gap-filled data is readily distinguishable from 'real' data. Template spreadsheets and instructions for 'gap filling' for missing data are available from Felicity Hayes and are also available on the ICP Vegetation website at http://icpvegetation.ceh.ac.uk/manuals/experimental_protocol.html The template spreadsheet will automatically calculate parameters such as AOT40, 12h mean (7am – 7pm, local time) and mean daily maximum ozone concentration.
Plant data: should also be sent to Felicity Hayes by email. Example spreadsheets are available on request.

References cited

Burkey, K.O., J.E. Miller and E.L. Fiscus (2005). Assessment of ambient ozone effects on vegetation using snap bean as a bioindicator species. J. Environmental Quality 34:1081-1086.

Reinert, A. and G. Eason (2000). Genetic control of O_3 sensitivity in a cross between two cultivars of snap bean. J. American Society of Horticultural Science 125(2):222-227.

Appendix 1: Example table for recording stomatal conductance

Name:												
Site:												
Measuring device:												
Surface measured (upper, lower or both):												
Gas and unit of measurements:												
Height of meteorological sensor(s):												
Additional information:												
Date	Time	Leaf number (from top)	Geno-type	% injury of measured leaf	Phenological stage	Leaf surface (upper/lower)	PAR/PPFD (e.g. µmol m^{-2} s^{-1})	Air temperature (°C)	VPD (kPa)	RH (%)	Air pressure (e.g. hPa)	gs (e.g. mmol H_2O m^{-2} s^{-1})

Appendix 2: Self-watering pots

Here are some ideas (we have not examined or tested these products):

Adjustable Reservoirs Create Self-Watering Planters

These Adjustable Water Reservoirs convert your favourite pots into self-watering planters, keeping your plants from drying out and reducing the time you spend watering. Just place this self-contained reservoir in the bottom of your pot, adjust the refill tube and cover with soil. The Large Reservoir holds 4 quarts and fits pots between 16" and 20" in diameter. The Small Reservoir holds 1 quart and fits pots up to 10-14" diameter. Simply fill the reservoir with water through the convenient Fill Tube. The soil will be moistened through evaporative action from the reservoir below.

- A vacation watering solution!
- Includes a water level indicator
- May be used with a liquid or a water-soluble fertilizer.

Website:
http://www.gardeners.com/Adjustable%20Reservoir%2c%20Large/OutdoorPlanters_SelfWatering,34-507,default,cp.html?SC=

Riviera Vision Planter - 13" Grey

The hidden reservoir catches and stores water inside the planter, while a unique fabric wicking system delivers steady, measured hydration to thirsty roots. The handy water gauge completely eliminates guesswork - you know exactly when its time to refill. Caring for plants has never been this easy! Attractive, contemporary design in durable polypropylene never cracks, chips, or peels. Available in Speckled Beige and Speckled Gray. Outer Diameter: 13.7" Inner Diameter: 12"

Appendix 3: Fibre pots

Here are some ideas (we have not examined or tested these products):

6CM ROUND FIBRE POTS (VALUE PACK OF 96)
08325-GN

FIBRE POTS
Value Pack of 96
Colour: Natural

Ideal pots for seeds, seedlings, transplanting and cuttings
Easy to plant as you plant the whole pot the roots will grow uninterrupted through the pot and they are not damaged
No pot to discard

Now with reduced peat (maximum 25%)
Comprise mostly of wood pulp from renewable sources
Better for the environment
Better for plants as wood pulp is easier to re-wet

Website:
http://www.shrubs.co.uk/6-cm-round-fibre-pots-value-pack-of-96-08325-gn-14582-p.asp