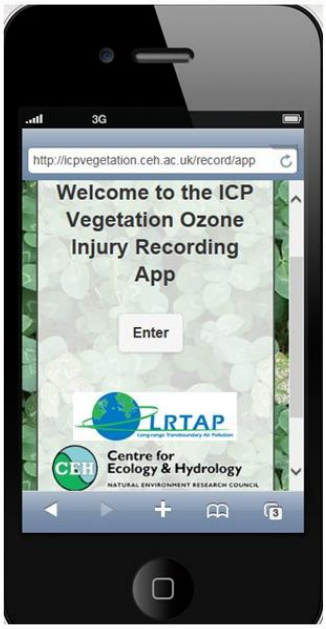
Recording the presence/absence of ozone injury using the smart-phone App or online recording form

Experimental Protocol

ICP Vegetation*, 2015



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* International Cooperative Programme on Effects of Air Pollution on Natural Vegetation and Crops. Reporting to the United Nations Convention on Long-Range Transboundary Air Pollution (LRTAP).

ICP Vegetation Protocols: Ozone gardens and smart phone App

Aims

- To provide evidence of the geographical extent of damaging effects of ozone pollution on vegetation by developing a photographic record of occurrences of ozone injury on sensitive species.
- To use this evidence to support the development of risk assessment methodologies used by the LRTAP Convention to predict areas where vegetation is at risk of damage.
- To determine whether ozone injury is now occurring in cities as well as in rural areas.

Background

In 2007, a synthesis report was published documenting over 500 incidences of visible ozone injury on crops, grassland species and shrubs growing in the field under ambient air conditions in 17 countries across Europe (Figure 1; Hayes et al., 2007). We plan to build on this study and create a worldwide database of new records of visible ozone injury.

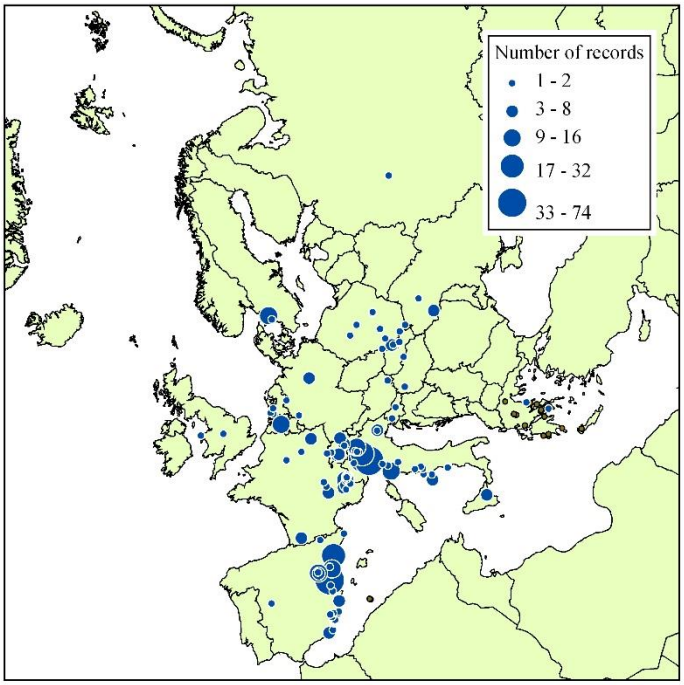


Figure 1: Locations of records of visible injury (1990-2006) attributed to ozone on crops, shrubs and semi-natural vegetation species, under ambient air conditions (Hayes et al., 2007).

Approach

To gain full understanding of the extent of the ozone problem, we are as much interested in the lack of occurrence of ozone injury symptoms as the occurrence of ozone injury symptoms. We would like to record injury presence or absence in a systematic manner. Should ozone symptoms be found on other species or the same species growing in fields or parks, records made on an *ad hoc* basis are also encouraged. Thus, there are three ways of recording presence/absence of ozone symptoms:

1. On known species planted in an ozone garden
2. On plants growing in a park, garden or field visited regularly
3. On an *ad-hoc* basis if you happen to see symptoms (or potential symptoms)

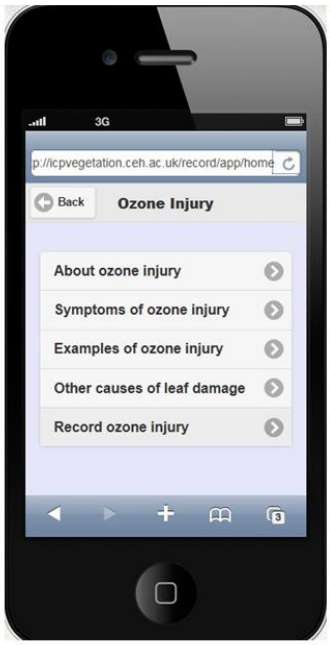
Smart-phone App and online form

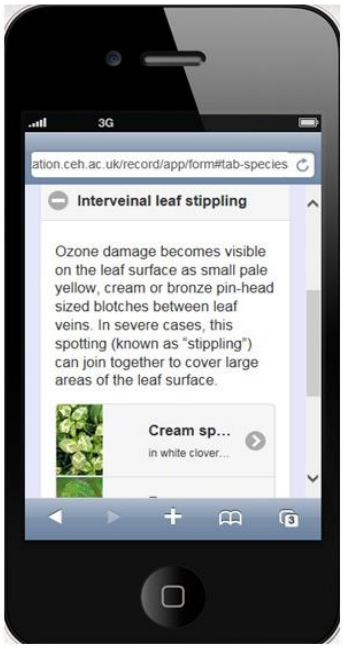
The ICP Vegetation smart-phone App can be used on iPhones and android phones. It allows instantaneous access in a phone-compatible format to the web-based recording form present on the ICP Vegetation web-page. As an alternative, records can be completed online at the ICP Vegetation web-page under "Record Injury." Both the online form and the App can be found at the following:

http://icpvegetation.ceh.ac.uk/record/index

Participants are asked to register before they download the App or use the online form for the first time. This will allow us to contact you for any further details if necessary. When using the App/online form, first, we collect information on where you have seen the ozone injury. If using the App, coordinates for the location where the injury was detected are recorded automatically using the phone's GPS. On the web form, you can choose your location on a map. The broad vegetation type of the damaged plant and the species name can then be selected from a list (or added by hand). We next ask the user to upload photographs of the ozone injured leaves (or uninjured leaves if participating in weekly assessments)and answer a few additional questions about their experience of identifying ozone damage, the injury symptoms, the weather and current ozone levels (if known).

For guidance, the App (and website) contain an 'Ozone information' section, which includes details of the key symptoms of ozone injury, and other causes of leaf damage that may be mistaken for ozone injury. There is also an 'Examples of ozone injury' page, containing photos of ozone injury on a variety of species.





(1) Detecting ozone injury on sensitive plants within an "ICP Vegetation Ozone Garden"

This activity builds on the "Ozone Gardens" concept developed by NASA for which a detailed protocol, including information about the development of ozone symptoms, can be found at:

http://science-edu.larc.nasa.gov/ozonegarden/pdf/Bio-guide-final-3_15_11.pdf

There is a network of 14 ozone gardens in the USA, based at Universities (e.g. St Louis University, see Fishman et al. 2014), museums and National Parks.

We have adapted the concept, allowing the use of the ICP Vegetation smart-phone App/online form for recording incidences of injury and building on previous experience from our biomonitoring experiments.

One of the St Louis ozone gardens, summer 2014

Overview of experiment

To participate in the ozone gardens biomonitoring experiment, you will need an area of bare soil of minimum size 2 x 1 m, in full sunlight, away from trees and buildings. If needed, the area should be protected from rabbits and other herbivores. Hand watering may be needed in dry periods. An appropriate density of plants should be used depending on the species, with 1 m^2 allocated to each species. The plants should be checked for ozone injury symptoms at weekly intervals. The presence or absence of symptoms should be recorded using the smart phone App or by completing the online form via the ICP Vegetation web-page. For those interested in providing more detailed information, please record the severity of ozone symptoms when they occur using the scale in the "Severity of symptoms" Excel spreadsheet, and if available from a nearby monitoring station, send the hourly mean ozone concentration data (and optionally climate data if also available).

Potential locations of ozone gardens

- Botanical gardens or parks if permission given
- Gardens at your place of work
- Your own home garden
- Farmer's field, providing permission has been sought

Choice of species

You may grow as many species as you wish. As a minimum, we ask you to grow ozone-sensitive bean (*Phaselous vulgaris*, cv. S156) at four plants per m^2 using seed supplied by the Coordination Centre. We can also provide seed for an ozone-tolerant variety (cv. R123), which is useful for confirmation of symptoms. Further information about these varieties can be found in Burkey et al. (2005) and in the ICP Vegetation bean biomonitoring protocol, available at the ICP Vegetation web-page. It is recommended that bean plants are raised first in pots and then transplanted into the garden at a density of four plants per m^2.

We can also provide seed for two further species, white clover (*Trifolium repens* cv. Crusader, see paper by Hewitt et al., 2014) and wheat (*Triticum aestivum* cv. Soissons (winter wheat) and Mulika (spring wheat)).

You may wish to explore the sensitivity of local varieties of crops, grassland species or shrubs known to be ozone sensitive and purchase these yourself. We suggest that you choose from the following list as photographs of ozone symptoms for these species can be found on the App as well as on the ICP Vegetation web-page (under "Record Injury").

<u>Species List</u>

Crop

Agricultural – cereal: Wheat (*Triticum aestivum*), Maize (*Zea mays*), Rice (*Oryzia sativa*).

Agricultural - non-cereal: Soybean (*Glycine max*), Potato (*Solanum tuberosum*); French bean (*Phaseolus vulgaris*); Onion (*Allium cepa*).

Fruit crops: Tomato (*Lycopersicon esculentum*); Grape (*Vitis vinifera*); Watermelon (*Citrullus lanatus*); Muskmelon (*Cucumis melo*); Courgette (*Cucurbita pepo*).

Leaf salad crops: Lettuce (*Lactuca sativa*); Spinach (*Spinacia oleracea*); Chard (*Beta vulgaris*); Chicory (*Cichorium intybus*).

Cooking herbs: Parsley (*Petroselinum crispum*).

Tree

Deciduous: Birch (*Betula pendula*); Beech (*Fagus sylvatica*); Ash (*Fraxinus excelsior*); Narrow-leaved ash (*Fraxinus angustifolia*); Flowering ash (*Fraxinus ornus*); Sessile oak (*Quercus petraea*); Poplar (*Populus ssp*); Field maple (*Acer campestre*); Italian maple (*Acer opalus);* White mulberry (*Morus alba*).

Evergreen: Aleppo pine (*Pinus halepensis*).

Shrubs: Hawthorn (*Crataegus monogyna*); Honeysuckle (*Lonicera implexa*); Wayfaring tree (*Viburnum lantana*).

Grassland

Clovers: White clover (*Trifolium repens*); Red clover (*Trifolium pratense)*.

Other grassland herbs: Ribwort plantain (*Plantago lanceolata*); Brown knapweed (*Centaurea jacea*); Black knapweed (*Centaurea nigra*).

Heathland

Mediterranean macchia: Strawberry tree (*Arbutus unedo*); Myrtle (*Myrtus communis*); Mastic tree (*Pistacia lentiscus*); Turpentine tree (*Pistacia terebinthus*).

Preparation and maintenance of the garden

- Select a suitable location (described under "Overview") and prepare the soil for planting once the last frost has occurred. It may be necessary to dig in some garden compost to improve the fertility thereby avoiding any nutrient deficiencies.
- Provide barrier protection against rabbits etc. as needed.
- Seeds can be sown directly into the soil or started off in pots in a greenhouse. It will be necessary to protect the plants from slugs using an appropriate method.
- Keep the area weed free.
- Provide water by hand if needed to maintain adequate moisture in the soil - this will enhance ozone uptake by the plants. Note: an optional further activity in drier areas would be to have an additional garden that is not watered to see if drought affects the level of ozone injury.
- If needed, use pest/disease control methods appropriate to the species and make a note of what/when used.

Weekly recording of injury/non-injury

We recommend that you check each species every week and using the smart-phone App or web-page send in a photograph of a typical mature, older leaf. Please answer the questions on the App/online form to help us with our quality control and to give some indication of recent weather and ozone (if known) conditions. Please start recording once the bean plants have two trifoliate leaves that are fully expanded, and make records for at least 6 weeks, continuing for longer if feasible. If you are unable to take a record one week, please make the next one as soon as you can thereafter and return to your weekly schedule as soon as feasible.

Assessment of ozone injury

While symptoms can vary between plant species, there are several diagnostic features that tend to be commonly found: 1) Spotting on the leaves occurs between the leaf veins; 2) Damage appears on the upper surface of the leaves, spreading to the underside in severe cases; 3) Older leaves (towards the base of the stem and branches) tend to be more affected than younger leaves as damage is determined by the accumulated uptake of ozone over time.

French bean (*Phaseolus vulgaris*)

Ozone injury on *Phaseolus vulgaris* consists of bronze-coloured lesions that gradually join together to cover large parts of the leaf surface (Figure 2).

At each visual assessment, please ignore any symptoms on the primary leaves as this is often nonspecific.

a)

b)

c)

d)

Figure 2: Ozone injury on a trifoliate leaf of *Phaseolus vulgaris* (a) scored as 5-25% of leaf injured, (b) and (c) severely damaged leaf (scored as >25% injury) and (d) senesced leaf.

For further details and photographs of visible ozone injury in *Phaseolus vulgaris*, see the NASA ozone gardens protocol:

http://science-edu.larc.nasa.gov/ozonegarden/pdf/Bio-guide-final-3_15_11.pdf

White clover (*Trifolium repens*)

Visible ozone injury appears between the leaf veins, on the upper surface, as fine pale yellow specks. As symptoms worsen, the spotting on leaves merges to form necrotic patches.

Figure 3: Visible ozone injury on the leaves of white clover (*Trifolium repens*).

Wheat (*Triticum aestivum*)

Injury appears on older leaves as yellow mottling and necrotic patches.

Figure 4: Visible ozone damage on wheat leaves (*Triticum aestivum*).

For further information and photographs of ozone injury symptoms in other species, see:

http://icpvegetation.ceh.ac.uk/publications/documents/CEHOzoneInjury_webmidres.pdf

http://www.ozone.wsl.ch/

http://www.ozoneinjury.org/

If you would like to provide further details on the severity of leaf injury, please request an Excel spreadsheet from Felicity Hayes (fhay@ceh.ac.uk) or download from our website. There are also online training exercises to help you estimate the proportion of injury on leaves, for example:

http://www.nature.nps.gov/air/edu/O3Training/index.cfm

Ozone and climate data (optional).

If participating in this part of the study, please request a template Excel spreadsheet from Felicity Hayes (fhay@ceh.ac.uk) or download one from our website.

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 spreadsheets are complete and that gap-filled data is readily distinguishable from 'real' data. Instructions for 'gap filling' for missing data are included in the spreadsheet. Preferably, please send data for the period from May (or earlier if your experiment starts earlier) through to October. The template spreadsheet will automatically calculate parameters such as AOT40, 12h mean (7am – 7pm, local time) and mean daily maximum ozone concentration.

There are three separate recording sheets, depending on if daylight data is in W m^{-2}, PAR or if there is no daylight data available.

(2) Detecting ozone injury on sensitive plants within parks, gardens or fields

Identify a location where at least one known ozone-sensitive species is growing (see Species List in section 1). Starting in April/May, we recommend that you check each selected species every week and using the smart-phone App or web-page send in a photograph of a typical mature leaf. Please answer the questions on the App/online form to help us with our quality control and to give some indication of recent weather and ozone (if known) conditions. Please make records for at least 6 weeks, continuing for longer if feasible. If you are unable to take a record one week, please make the next one as soon as you can thereafter and return to your weekly schedule as soon as feasible.

If you would like to provide further details on the severity of ozone injury on leaves, please request an Excel spreadsheet from Felicity Hayes (fhay@ceh.ac.uk) or download one from our website.

Ozone and climate data (optional).

If participating in this part of the study, please request an Excel spreadsheet from Felicity Hayes (fhay@ceh.ac.uk) or download one from our website.

(3). Reporting ozone injury symptoms on an *ad-hoc* basis if you happen to see symptoms (or potential symptoms)

Using the photographs on the App/web-page as a guide, please record symptoms as you see them. If you would be willing for us to send you a text when injury symptoms are likely (for example, during "ozone episodes"), please email Katrina Sharps (katshar@ceh.ac.uk) or Felicity Hayes (fhay@ceh.ac.uk). We speak a few languages between us, but we may need to guide you to a location in your country where ozone data can regularly be found if web-pages are not in English.

These records will have the least certainty, but will provide added information on the range of species injured and the geographical extent of the injury.

References cited

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

Fishman, J., Belina, K.M. & Encarnación, C.H. (2014) The St. Louis ozone gardens: visualizing the impact of a changing atmosphere. *Bulletin of the American Meteorological Society*, **95**, 1171–1176.

Hayes, F., Mills, G., Harmens, H. & Norris, D. (2007) Evidence of widespread ozone damage to vegetation in Europe (1990-2006). ICP Vegetation Programme Coordination Centre, CEH Bangor, UK.

Hewitt, D.K.L., Mills, G., Hayes, F., Wilkinson, S. & Davies, W. (2014) Highlighting the threat from current and near-future ozone pollution to clover in pasture. *Environmental Pollution*, **189**,111-117.