Protocol for assessing ozone injury using vegetation surveys

ICP Vegetation, 2022

Citation: Hayes, F. & Sharps, K. 2022. Protocol for assessing ozone injury using vegetation surveys. ICP Vegetation, UK Centre for Ecology & Hydrology. http://icpvegetation.ceh.ac.uk

CONTACT: ICP Vegetation Programme Coordination Centre*, UK Centre for Ecology & Hydrology, Environment Centre Wales, Deiniol Road, Bangor, Gwynedd, LL57 2UW, United Kingdom. Telephone: +44 (0) 1248 374500

Website: http://icpvegetation.ceh.ac.uk

* 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).

Contents

Aims/objectives

- To provide evidence of the geographical extent of damaging effects of ozone pollution on vegetation, based on presence/absence of ozone injury symptoms on leaves.
- To determine the severity of the impact of ozone pollution, based on the extent of ozone injury of specified sensitive species.

Background and scope

Ozone is a pollutant that causes damage to plants when it enters plant leaves via the stomatal pores. Although plants have some ability to detoxify the harmful molecules that are formed after ozone reacts within the leaf, when ozone concentrations are high the detoxification capacity can be exceeded leading to cell death. This can result in visible symptoms on the leaf surface, including spotting (stipples), mottling, yellowing and bronzing, eventually leading to necrosis. Distinctive visible leaf-injury symptoms occur following ozone exposure for some species. However, some species exhibit non-specific symptoms such as reddening or increased rates of senescence. These symptoms also occur with other forms of stress and cannot be conclusively attributed to ozone.

Visible-leaf injury is a visual indication of ozone stress. This may not always result in quantifiable reductions in growth of native plants or yield of crops. In contrast, alterations in plant physiology including reductions in growth, flowering, seed production and crop yield can occur in the absence of any visible leaf-injury symptoms. Therefore, identification of visible ozone symptoms can be a quick and relatively simple way to tell if a plant is potentially being damaged by ozone.

Survey frequency and timing

It is recommended to survey for ozone injury at least once per year, as the magnitude of ozone pollution can vary between years. Ideally a survey of ozone injury should occur after an ozone 'episode' (a few consecutive days of relatively high ozone concentrations). However, if this is not possible then ozone injury surveys should occur April-September (northern Europe) or February-July (southern Europe) as this is when the vegetation is actively growing. Surveys should be completed before the target vegetation species start to senesce at the end of their growing period.

While this may not always be possible/practical, if surveys could be repeated at regular intervals, this would allow effects of ozone to be tracked throughout the growing season as visible injury is determined by the accumulated uptake of ozone over time. This would also allow the first appearance of ozone injury to be recorded and the ozone concentration at that time.

Information on daily ozone levels from monitoring sites across the UK can be found here:

https://uk-air.defra.gov.uk/latest/currentlevels?view=region

Identification of suitable locations/plots

Plots should be >100m from roads.

For non-forest plots, these should ideally be >100m from the nearest forest/woodland to avoid the influence of the forest on the plot. It is acceptable to have scattered trees near to the plots.

Plot characteristics to record

GPS location at the centre of the plot.

Dominant vegetation type.

Recommended plot size

The area to be surveyed at a site depends on the vegetation type.

Vegetation type	Recommended plot size	Recommended number of plots per site
Grassland	2m x 2m	5
Heath	4m x 4m	5
Wetland	2m x 2m	5
Shrub	4m x 4m	5
Woodland	4m x 4m	5
Crop	2m x 2m or 20 plants (whichever is largest)	5

For all vegetation, it is recommended to prioritise efforts on key species with distinctive and known ozone injury symptoms if these are present, rather than to survey all species present. Priority species are listed on page 5. For deciduous trees, it is recommended to check the leaves of 3 branches per tree, rather than the whole tree. All of the mature leaves per branch need to be examined, under good light conditions. It is preferable to check branches that are exposed to sunlight, as these are more likely to have leaves with ozone-injury symptoms than shade-leaves.For conifers, the current and current+1 year needles should be assessed in good light conditions, using approximately 30 needles per branch, and 3 branches per tree. If possible, needles should be placed close to each other, making a "plane," allowing easier observation of injury.

For other vegetation, it is recommended to base assessments on a maximum of 20 plants per plot. If fewer than 5 plants per species are used for the assessment, this should be recorded.

It is recommended to take a photo of ozone symptoms observed for subsequent verification purposes. Pictures should be taken in good light or with a camera equipped with a flash. The leaf sample should cover at least 3/4 of the final picture area to allow symptom identification. It is also recommended to zoom in on typical and species-specific ozone symptom characteristics.

Equipment needed

Some people prefer to use a hand lens (x10), which allows closer examination of the leaf surface, to investigate potential ozone injury. An alternative is to use the camera on a smartphone, and to enlarge the image to aid identification of key features.

Handheld computer or notebook/pre-printed survey sheets and pencil for recording key information.

Reference photos to aid identification of injury symptoms

Information to record

Date of survey

Species name

Number of plants on which assessment was based (1, 2, 3, 4, 5, >5)

Ozone injury (presence/absence) for each species surveyed

Extent of injury: % of leaves showing symptoms (to nearest 10%)

Severity of injury: % injury per leaf (0, 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100%)

Characterising the % injury per leaf

These photos give a guidance to the amount of injury per leaf.

a)

b)

c)

d)

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 a useful guide on evaluating ozone injury in conifer needles, see the chronic mottling chart in Annex III of the ICP Forest "Assessment of ozone injury manual."

https://storage.ning.com/topology/rest/1.0/file/get/9995552252?profile=original

Species List

These species are known to be sensitive to ozone and to have distinctive visible leaf-injury symptoms. Additional species can be used for assessments if distinctive symptoms are observed.

Crop

Agricultural – cereal: Common wheat (*Triticum aestivum*); Durum wheat (*Triticum durum*); Finger millet (*Eleusine coracana*); Pearl millet (*Pennisetum glaucum*); Rice (*Oryzia sativa*) and Maize (*Zea mays*).

Agricultural - non-cereal: Soybean (*Glycine max*); Onion (*Allium cepa*); Alfalfa (*Medicago sativa*); Potato (*Solanum tuberosum*); Peas (*Pisum sativum*) and French bean (*Phaseolus vulgaris*).

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

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

Cooking herbs: Parsley (*Petroselinum crispum*) and Coriander (*Coriandrum sativum*).

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)* and White mulberry (*Morus alba*).

Evergreen: Aleppo pine (*Pinus halepensis*), Eastern White pine (*Pinus strobus*).

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

Grassland

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

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

Heathland

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

How to identify injury

Symptoms can vary between plant species, with leaf spotting expressed as tiny purple-red, yellow, white or black spots or as a general even reddening or bronzing. However, there are several diagnostic features that tend to be commonly found: 1) Spotting on the leaves occurs between the leaf veins; 2) Damage is evenly distributed and appears on the upper surface of the leaves, spreading to the underside in severe cases; 3) Older, fully developed 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.

For conifer species, the most common ozone-induced symptom is chronic mottling which appears as yellow or light green areas of similar size without sharp borders between green and yellow zones, evenly distributed along the entire needle. However, not all needles in a fascicle may be uniformly

affected. Chlorotic mottling frequently appears only in needles older than 1 year (second-year needles and older). Symptoms increase with increasing needle age. Chlorotic mottling is more distinct on light-exposed needle areas in comparison to shaded ones. Information on identifying visible leaf-injury due to ozone and accompanying photos are also available on the ICP Vegetation ozone injury App, which can be found at the following address:

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

Key photos

Example photos from a range of habitats are shown here. For more examples of photos from specific habitats, please see factsheets on the ICP Vegetation website.

<table>
<tr>
<td>White clover (Trifolium repens)
  </td>
<td>Meadow buttercup (Ranunculus acris)
  </td>
</tr>
<tr>
<td>Hemp agrimony (Eupatorium cannabinum)
  </td>
<td>Wild carrot (Daucus carota)
  </td>
</tr>
<tr>
<td>Common mallow (Malva sylvestris) (light injury)
  </td>
<td>Common mallow (Malva sylvestris) (severe injury)
  </td>
</tr>
</table>

Field scabious (*Knautia arvensis*) 	Common dandelion *(Taraxacum officinale)* 
Pea (*Pisum sativum*) 	Common bean (*Phaseolus vulgaris*) 

For further information and photos of ozone injury on the leaves of trees and crops, see the following online guides:

http://www.ozoneininjury.org/index.php?option=com_content&view=category&id=3&Itemid=4

http://www.ozoneininjury.org/crops/index.php?option=com_content&view=category&id=4&Itemid=23

Other causes of leaf damage

There are also many other potential causes of leaf damage, including pests and diseases, nutrient deficiency and drought. In some cases, these other sources of leaf damage may cause symptoms that could be mistaken for ozone injury. However if leaves are examined carefully, there are ways to distinguish between the different types of leaf damage.

Leaf diseases can cause dark brown or black patches or signs of "powder" on the leaf surface, general leaf surface yellowing or large regular-shaped yellow/brown areas. For example, fungal disease can cause necrotic spots surrounded by yellow halo areas in tomato (*Lycopersicon esculentum*) and powdery mildew can produce white areas (hyphae of the fungus) on the underside of the leaf in ash (*Fraxinus excelsior*). Fungal diseases can also cause concentric circles on the leaf surface and small fruiting bodies can be seen inside the spots.

Leaf viruses can cause regular-shaped injury **between** the veins. There may be spots of dead, brown tissue and leaves may be rolled or curled.

Leaf biting insects can create holes on the leaf, which go straight through the surface. Leaf miners (larvae of insects that eat plant tissue) can cause linear patterns of necrotic tissue. Red spider mites are small, crawling pin-head sized insects and can cause white webbing on the leaves. Unlike ozone damage, spider mite symptoms can be found on the underside of leaves and are not restricted to interveinal areas. Leaves can also be tapped while holding a paper underneath and mites will fall off on to the paper.

Characteristics of ozone injury	Characteristics of other causes
Stipples (spots), colour specific to species for ozone damage	Stipples (spots), of unusual colour for that species
Stipples (spots) are uniform in colour	Stipples (spots) have concentric rings of colour
Older leaves more affected than younger leaves	
Damaged area is between the leaf veins	Damaged area includes the leaf veins
Damaged areas are 'smooth'	Damaged areas are raised (fungal) or pitted (insect)
Damaged areas are only on one surface of the leaf	Damaged areas are on both the top and bottom surface (insect)
Injury is randomly positioned over the leaf	Injured pattern is symmetrical, or concentrated in a small area (fungus or virus)
Spotting due to ozone damage can't be rubbed or washed off	Spotting can be rubbed or washed from the leaves (fungal disease or dust)

Quality Assurance

Check the species is found in the habitat surveyed

Check whether symptoms match the colour of injury expected for that species

Surveys should promote consistency when estimating % injury per leaf and % leaves affected, as this is subjective. Surveyors should refer to indicator guides when making estimates.