JOURNAL OF FOREST SCIENCE, *56*, 2010 (4): 177–182

Visible foliar injury as a tool for the assessment of surface ozone impact on native vegetation: a case study from the Jizerské hory Mts.

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ABSTRACT: Surface ozone is considered to be a very phytotoxic gaseous air pollutant. Its negative impacts at both the cell and the organ level have been shown, mainly as a result of experiments. However, the demonstration of ozone negative impacts on native plants is not explicit. An assessment of ozone impact on vegetation and ecosystems using indicators based on ambient ozone concentrations is insufficient and assessment techniques based on internal ozone dose and on real plant damage are more appropriate. Such a possible technique is the mapping of ozone visible symptoms due to ozone influence. The ICP-Forest method concerning ozone visible symptoms and the assessment of ozone influence were applied for the purposes of this case study. The visible symptoms are characterized by a few typical signs. Nevertheless, the identification of visible symptoms in native conditions can be problematic and misleading conclusions could be drawn. Therefore it is necessary to complete the identification of visible symptoms with a validation in order to confirm ozone as the cause of plant injury.

Keywords: Jizerské hory Mts.; surface ozone; validation; visible symptoms

Ozone is considered to be a significant threat to vegetation as it is a major phytotoxic pollutant that may reduce crop yields and damage plant communities (Ashmore 2005). Increasing ozone concentrations may play an important role in the observed suite of symptoms of forest decline in Central and Eastern Europe (Derwent, Jehlin 1991).

Direct foliar injury in many tree species, biochemical cell changes and a reduction in photosynthesis and growth have been shown under controlled conditions (reviewed by Schraudner et al. 1997; Chappelka, Samuelson 1998, and others). To define a relationship between the ozone influence and the forest tree growth response is more problematic (Manning 2005). Broadmeadow (1998) estimated a 10% loss in forest productivity in Europe due to the ozone impact. Karlsson et al. (2005) estimated the concrete forest growth reduction and, consequently, the economic return of the forest production due to the negative influence of surface ozone. On the other hand, e.g. Stribley and Ashmore (2002) or Dittmar et al. (2004) showed that surface ozone negatively influences only a canopy with a more favourable soil water regime.

The complications and complexity of the ozone problem consist both in ozone production in the ground layer of the atmosphere and in the dependence of plant ozone uptake on environmental con-

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Supported by the Grant Agency of Charles University, Project No. PRF/12507/2007, and by the Ministry of Agriculture of the Czech Republic, Project No. 0002070203

ditions (Davison, Barnes 1998; Coulston et al. 2002). At the same time, it is important to state that the factors ideal for ozone production (i.e. intensive solar radiation, high temperature, low relative air humidity) induce stomatal closure. Consequently, it is not possible to assume that elevated ozone concentrations necessarily cause injury to native plants and lead to growth reduction. Only the internal ozone dose obtained by plant stomata is harmful (Manning 2003). Therefore, it is not sufficient to assess the ozone influence on vegetation using indicators which are based on ozone concentrations, e.g. AOT40 or SUM60 exposition indexes (Vander-Heyden et al. 2001; Manning 2003; Karlsson et al. 2004). An approach with biological significance is needed (Manning 2003).

The ozone visible injury assessment on bioindicators – plants exhibiting typical ozone injury – is one of possible methods (Manning 2003). Ozone pollution, unlike fluoride or sulphur dioxide pollution, leaves no elemental residue that can be detected by analytical techniques. Therefore, visible injury on needles and leaves is the only easily detectable evidence in the field and it is considered to be a result of oxidative stress (UN/ECE 2004). Ozone visible injury is usually the first clear and unequivocal sign of the presence of phytotoxic levels of ozone and is a reliable method of monitoring the effects of ozone air pollution over short periods of time (Bergmann et al. 1999).

Even though visible injury does not include all the possible forms of injury to trees and natural vegetation (for example pre-visible physiological changes, reduction in growth), the observation of typical foliar symptoms in the field appears to be a valuable tool for the assessment of the impact of ambient ozone exposure on plants (UN/ECE 2004). The monitoring of ozone visible injury serves as the first step in identifying possible forest ecosystem health issues that may demand more detailed research (Coulston et al. 2002).

The identification of ozone visible symptoms in Western Europe started in the 1990s (Skelly et al. 1999). The first report of probable ozone injury to native plants in Central and Eastern Europe with an emphasis on the Carpathian Mountain Range was produced by Manning et al. (2002). Nowadays, there is a detailed database of ca 84 ozone bioindicators for Europe and USA operating under the ICP-Forests programme (Schaub et al. 2002).

The aim of this case study is to assess the usefulness of ozone-like visible foliar injury in areas exposed to high ozone levels and to find convenient ozone plant bioindicators for Czech mountain forests. The conclusions of this study may be useful in the planning of a much demanded research project concerning the forest growth reduction due to ozone impact.

METHODS

Ozone-like symptom assessment

The method of symptom assessment is described in detail in the sub-manual for the assessment of ozone injury to European forest ecosystems of the ICP-Forests programme, revised in 2004 (UN/ECE 2004). ICP-Forests was launched in 1985 under the CLRTAP/UNECE. A few signs are typical of ozone visible injury. Visible injury develops on leaves that are well-exposed to sunlight, and middle-aged and older leaves suffer more damage than younger leaves, whilst shaded portions (i.e. if two leaves overlap) do not usually show any injury. Visible injury does not normally extend throughout the leaf tissue, visible symptoms being usually confined to the upper leaf surface, typically expressed as tiny purple-red, yellow or black spots or sometimes as discolouration, reddening or bronzing. Both stippling and discolouration occur only between the veins. Severely injured leaves appear to age faster and drop sooner (UN/ECE 2004).

The assessment proceeded in the framework of the LESS plots (Light Exposed Sampling Site, 2 m × 1 m) which were established in the vicinity of the ozone measurement device. Ozone foliar injury was assessed at the light-exposed edge of the forest

Table 1. Research sites ranked according to increasing altitude

Site	Altitude (m)	Exposition	Characteristics
Jizerka	900	south-east	Forested, clear-cut, containing gaps without trees
Bukovec	920	south	Large meadow, below the beech forest
Jizera Mt.	962	south	Grass slope with scattered young spruces
Smědava Mt.	978	north	Steep slope of the Smědá river valley, scattered spruces
Knajpa	989	south	Wet meadow, scattered with low spruces and dwarf pine

Table 2. Results of the assessment of ozone-like visible symptoms at five research sites at the end of the 2006 and 2007 growing seasons

Species	Site	Ozone-like visible symptoms	
		Scoring 2006	Scoring 2007
Alchemilla sp.	Jizerka	1	1
	Bukovec	0	0
Betula pendula	Jizerka	1	0
	Knajpa	0	0
Betula pubescens	Jizerka	1	0
Cirsium heterophyllum	Jizerka	2	0
	Bukovec	2	2
Epilobium angustifolium	Jizerka	1	0
Fagus sylvatica	Jizera Mt.	0	0[1]
Geranium sylvaticum	Bukovec	2	2
Heracleum sphondylium	Jizerka	1	0
Hypericum maculatum	Jizerka	2	1
Prunus padus	Jizerka	1	0
Picea abies	Jizerka	1	1
Pinus mugo	Jizerka	1	1
Plantago major	Jizerka	1	0
Polygonum bistorta	Jizerka	2	0
Populus sp.	Jizerka	2	0
Potentilla erecta	Jizerka	2	0
Rubus idaeus	Jizerka	1	0
	Smědava Mt.	1	1
Rumex acetosa	Jizerka	1	0
Salix capraea	Jizerka	1	1
Salix cinerea	Jizerka	1	1
Sorbus aucuparia	Jizerka	2	0
	Knajpa	0	0
	Smědava Mt.	0	0
Vaccinium myrtillus	Jizerka	1	0

Scoring and definitions for the percentage of symptomatic leaves: 0 – none of the leaves injured, 1 – 1–5% of the leaves show ozone symptoms, 2 – 6–50% of the leaves show ozone symptoms, 3 – 51–100% of the leaves show ozone symptoms (UN/ECE 2004), [1]ozone-like symptoms found, but in less than 1% of leaves

closest to the ozone measurement device, within a maximum radius of 500 m (78.5 ha). The edge was divided into a number of LESS plots corresponding to the length of the edge of the forest, the southern exposed edge of the forest being preferred (UN/ECE 2004).

Study design

This study was carried out in the Jizerské hory Mts. at five sites, where both ozone concentrations and ozone visible symptoms were monitored. The study was carried out during the 2006 and 2007 growing

Table 3. Results consequent upon the validation made in the Ozone Validation Centre for Central Europe of ozone-like symptoms on the leaves of four species. Symptoms where the ozone influence was validated (Günthardt-Goerg, Menard 2008) are specified as ozone-induced

Species	Site	Ozone-like	Ozone-induced	AOT40F^2 (ppm.h)	Ozone-like	Ozone-induced	AOT40F^2 (ppm.h)
		2006			2007		
Cirsium heterophyllum	Bukovec	2	0	31.7	2	0	20.4
Geranium sylvaticum	Bukovec	2	0		2	0	
Fagus sylvatica	Jizera Mt.	0	0	45.0	0	0^1	26.8
Rubus idaeus	Smědava Mt.	1	1	52.1	1	1	35.5

Scoring and definitions for the percentage of symptomatic leaves: 0 – none of the leaves injured, 1 – 1–5% of the leaves show ozone symptoms, 2 – 6–50% of the leaves show ozone symptoms, 3 – 51–100% of the leaves show ozone symptoms (UN/ECE 2004), ^{1}ozone-like symptoms found, but in less than 1% of leaves, ^{2}Hůnová et al. (2008), Hůnová et al. (unpublished)

seasons. These 5 sites were located at altitudes between 900 and 989 m a.s.l. (Table 1).

Ozone visible symptoms were assessed on the leaves of 22 species. LESS plots were established in the field at the beginning of the 2006 growing season. Potential ozone-induced symptoms were examined using a 15× hand lens. The score or level of damage (the percentage of leaves with ozone symptoms relative to the total number of leaves on the plants, Table 2) was estimated for species with ozone-like symptoms.

Literature and photo-documentation were used to identify ozone-like visible symptoms (Innes et al. 2001; Schaub et al. 2002). The leaves of four symptomatic species (Table 3) supplied as voucher samples were sent to the Ozone Validation Centre for Central Europe for their ozone-like symptoms to be validated.

RESULTS AND DISCUSSION

In assessing ozone-like symptoms, we found 22 symptomatic species in 2006. Level 1 (i.e. 1 to 5% symptomatic leaves) was recorded in 15 species and level 2 was recorded in 7 species. Some species were assessed at more than one site. In the case of *Alchemilla* sp., *Betula pendula* and *Sorbus aucuparia*, recorded levels from different sites were not always the same. Out of these 22 species, only 9 species were symptomatic in 2007. Level 1 was recorded in 7 species, level 2 in only 2 species. In the case of *Alchemilla* sp., *Cirsium heterophyllum*, and *Rubus idaeus* the levels recorded from different sites were not equivalent. The remainder of the species was assessed as asymptomatic in 2007. In consideration of the ozone levels, we assumed a more severe injury. Nevertheless, this confirmed for us that the AOT40 concept is rather inconsistent with the observed ozone injury. Details are shown in Table 2.

Visible ozone injury on coniferous trees as well as on deciduous trees is known, but is difficult to distinguish from other abiotic and biotic symptoms (UN/ECE 2004). Similar symptoms can result from drought, increased light irradiation, presence of pathogens or combination of these factors. The mapping of ozone symptoms using only photographic guides is suitable for orientation purposes only and demands additional verification, especially if the identification of ozone symptoms in the field also depends on research experience (Bussotti et al. 2006a).

For the above-mentioned reasons, we decided to verify the ozone-like symptoms of four species. We chose the most injured species (level 2 recorded at the end of both growing seasons), i.e. *Cirsium heterophyllum* and *Geranium sylvaticum*. We then chose *Fagus sylvatica* as an important woody plant that can substantially increase the stability of forest ecosystems in the Czech Republic (Balcar, Kacálek 2003; Jurásek 2007). The fourth species to be verified was *Rubus idaeus*, chosen as a pioneer species that could be very useful for comparison of ozone impact at many heterogeneous sites because it is widespread.

Visible injury on the leaves of *Fagus sylvatica* was characterized as uniform brown stippling. A purple stippling that is especially complicated to interpret was observed on the leaves of *Rubus idaeus*. Severe leaf reddening was found on *Cirsium heterophyllum* and *Geranium sylvaticum* leaves.

For our purposes, we used the possibility of verifying ozone-like symptoms at the Ozone Validation

Centre for Central Europe. Ozone-like symptoms found by our team in the Jizerské hory Mts. were validated there using voucher samplers. Micrographs were made under a stereomicroscope. Ozone-like symptoms were validated as being ozone-induced on the leaves of two species (*Fagus sylvatica* and *Rubus idaeus*) out of the four (Günthardt-Goerg, Menard 2008). The influence of ozone was not validated on *Cirsium heterophyllum* and *Geranium sylvaticum* leaves (Table 2).

The results of the validation changed our preliminary conclusion and showed that it is important to validate ozone-like symptoms in order to correctly interpret ozone impacts. Even if ozone-like symptoms show as typical signs (see Methods) we have to be careful, especially with the interpretation of reddening. Reddening, typical not only of these two species, can be a natural phenomenon on young leaves, a result of oxidative stress due to intensive irradiation or a result of general senescence. Ozone can contribute to the development of oxidative stress or accelerate senescence but reddening without validation is a misleading sign and should not be interpreted as an ozone-induced symptom (Vollenweider et al. 2003; Bussotti et al. 2006b). According to Bussotti et al. (2006b) it is better to validate other ozone-like symptoms.

The appearance of ozone-like symptoms under controlled conditions (ozone fumigation, open top chambers) has already been verified by many authors (e.g. VanderHeyden et al. 2001; Orendovici et al. 2003) but it is not always possible to use these pieces of equipment in the field (due to cost, technical support etc.). We can instead perform a microscopic analysis using light microscopy for ozone symptom validation (Vollenweider et al. 2003), which is of higher quality than voucher analysis (Günthardt-Goerg, personal communication). The use of EDU, a successful protective chemical that looks very promising, is another possible method of validation. EDU can be applied as a stem injection or foliar spray on native plants (Manning 2005). Paoletti et al. (2009) recommended it as a simple tool for ambient ozone visible injury diagnosis on native plants. Moreover, EDU can be used to assess ozone impact on the growth of tree seedlings and saplings in the field (Manning 2005). For adult trees, application to the soil seems to be an effective method (Paoletti et al. 2009).

CONCLUSION

Visible ozone symptoms may provide us with information about the harmful effects of ozone, but they have to be validated. Only under these conditions can we interpret differences between species, between growing seasons, or investigate relationships between ozone visible symptoms and growth reduction or ozone internal dose.

The preliminary results based on the ozone-induced symptoms of this case study also show that ambient ozone may have a much lower impact on the mountain ecosystem than has been assumed regarding the ambient ozone concentrations. There is a need for a more detailed study with the focus on the probable limitation of ozone uptake due to environmental conditions or a shorter growing season.

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Received for publication May 29, 2009
Accepted after corrections September 29, 2009

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