



## Plant Species Sensitivity Distributions for ozone exposure



T.M.W.J. van Goethem<sup>a,\*</sup>, L.B. Azevedo<sup>a</sup>, R. van Zelm<sup>a</sup>, F. Hayes<sup>b</sup>, M.R. Ashmore<sup>c</sup>,  
M.A.J. Huijbregts<sup>a</sup>

<sup>a</sup> Department of Environmental Science, Institute for Water and Wetland Research, Radboud University Nijmegen, P.O. Box 9010, 6500 GL Nijmegen, The Netherlands

<sup>b</sup> Centre for Ecology and Hydrology, Environment Centre Wales, Deiniol Road, Bangor, Gwynedd LL57 2UW, UK

<sup>c</sup> Stockholm Environment Institute, University of York, York YO10 5DD, UK

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### ABSTRACT

This study derived Species Sensitivity Distributions (SSD), representing a cumulative stressor–response distribution based on single-species sensitivity data, for ozone exposure on natural vegetation. SSDs were constructed for three species groups, i.e. trees, annual grassland and perennial grassland species, using species-specific exposure–response data. The SSDs were applied in two ways. First, critical levels were calculated for each species group and compared to current critical levels for ozone exposure. Second, spatially explicit estimates of the potentially affected fraction of plant species in Northwestern Europe were calculated, based on ambient ozone concentrations. We found that the SSD-based critical levels were lower than for the current critical levels for ozone exposure, with conventional critical levels for ozone relating to 8–20% affected plant species. Our study shows that the SSD concept can be successfully applied to both derive critical ozone levels and estimate the potentially affected species fraction of plant communities along specific ozone gradients.

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### 1. Introduction

Northern Hemisphere tropospheric background ozone concentrations have increased over recent decades, as peak concentrations have fallen in North America and Europe (Derwent et al., 2007; Vingarzan, 2004). Background concentrations are predicted to further increase with 0.5–2% per year over the next 50 years primarily due to elevated emissions of nitrogen oxides and volatile organic compounds (Emberson et al., 2003; Royal Society, 2008). The adverse effects of ozone pollution on plants, including trees and grassland species, are of considerable concern (Emberson et al., 2007; Mills et al., 2007a,b). Some of these effects include growth and seed production reduction (Booker et al., 2009), premature senescence (Tonneijck et al., 2004), reduced ability to withstand stressors (Wilkinson and Davies, 2009), and an increase in leaf injury (Manning et al., 2002).

Critical levels are based on relationships between ozone concentrations and effects such as yield loss and biomass reduction (Hayes et al., 2006; Pleijel et al., 2007; Tuovinen et al., 2007). These levels are expressed as an Accumulated exposure Over a Threshold of 40 ppb (AOT40) and are based on sensitive but ecological relevant

species (LRTAP, 2010; Matyssek et al., 2007). These species, and corresponding critical levels, are used as indicators to determine the risk for species groups or plant communities (Musselman and Lefohn, 2007). For example, critical levels of *Trifolium* sp. are assumed representative for all species of the productive grassland community (Klingberg et al., 2011). For monoculture arable crops and productive trees, such an approach of defining a critical level based on a single species for that community is possible. However, for semi-natural plant communities, with the large range of species present, an approach based on a single indicator such as *Trifolium* ignores the wide range of sensitivity across all the component species (Hayes et al., 2007; Mills et al., 2007b). To date, an approach which gives the affected fraction of a species assemblage due to ozone exposure is lacking in risk assessment for semi-natural vegetation (Ashmore, 2005; Paoletti and Manning, 2007).

In contrast, in most areas of ecotoxicology, Species Sensitivity Distributions (SSDs) are used (1) to derive environmental quality objectives of chemicals set equal to the concentration at which 5% of the species are affected (HC<sub>5</sub>), and (2) to estimate the fraction of species affected at different exposure concentrations of chemicals (Posthuma et al., 2002). An SSD is a cumulative distribution of responses of different biological species to the same stressor (Vanstralen et al., 1989). The SSD concept is a standard approach in ecotoxicology which is applicable to ozone risk assessment. It offers opportunities to both derive critical levels and estimate the affected

\* Corresponding author.

E-mail address: [tgoethem@science.ru.nl](mailto:tgoethem@science.ru.nl) (T.M.W.J. van Goethem).

fraction of species within a plant community along a specific ozone gradient.

The goal of this study was to develop SSDs for ozone exposure on natural vegetation. Our study includes 96 plant species. SSDs were constructed from species-specific ozone–response data provided by a comprehensive review of scientific literature and databases. Species were grouped according to response type (decrease or no decrease of biomass) and taxonomy (trees, annual and perennial grassland species). Critical threshold levels for ozone based on HC<sub>5</sub> were compared with AOT40-based critical levels commonly used in environmental policy assessment for ozone exposure. Finally, we show how the SSDs can be applied in practice by deriving spatially explicit estimates of potentially affected fraction of plant species in Northwestern Europe.

## 2. Methods

In order to derive SSDs, we first gathered species-specific ozone exposure–response functions from the literature. In these functions the measure of ozone exposure was expressed as AOT40, calculated as the sum of the differences between the hourly mean ozone concentration (in ppb) and 40 ppb during daylight hours. The exposure–response functions were used to calculate for each species the AOT40 value related to a 10% effect (EC<sub>10</sub>). These species-specific EC<sub>10</sub> values were subsequently used to derive the average and standard deviation of the SSD for each vegetation type. The steps from gathering species-specific data on ozone effects and acquiring SSDs to deriving HC<sub>5</sub> values are described below.

### 2.1. Data gathering

Data on the effects of ozone concentrations on plants were collected from peer-reviewed studies published up to April 2012. The following keywords were used in the Boolean search (incl. keyword extensions) in Web of Science: (1) ozone; and (2) either vegetation, plant, tree, grassland; and (3) either critical levels, dose–response relationship, exposure, response, biomass; and (4) either open top chamber (OTC), AOT40, Free-Air Concentration Enrichment (FACE), exposure based model. This literature search provided 980 peer-reviewed studies to be considered. In addition to the Boolean search we used the data from the OZOVEG database (Hayes et al., 2007).

### 2.2. Data selection

Following Mills et al. (2007a) and Hayes et al. (2007), ozone exposure–response data from individual species were only included when the following criteria were met:

- (1) It should not be a factorial experiment, testing for the effect of a treatment variable in addition to ozone, e.g. CO<sub>2</sub> + O<sub>3</sub> exposure, except when the specific effect of ozone without the treatment variable could be quantified.
- (2) Experiments should be conducted under ‘close to field’ conditions, either using an open-top chamber (OTC), field release system (e.g. Eastburn, 2006) or solardome (e.g. Rafarel et al., 1995).
- (3) The accumulated exposure above the critical 40 ppb level should be at least be 21 days to ensure chronic exposure.
- (4) The mean ozone concentration for any hour of the day should be maximum 100 ppb to take only realistic field conditions into account.
- (5) Only ozone response data for individual species and not higher taxonomic groups (e.g. family, class, etc.) were considered. An exception was made for genus-level records in case no other species belonging to that particular genus was listed.
- (6) Experiments should report the change in biomass. This endpoint is commonly used for ozone risk assessment in plants (LRTAP, 2010).

Ozone exposure–response relationships were found for a total of 96 species. For grassland species functions available from the OZOVEG database, along with new data for the additional species were used (Hayes et al., 2007), for trees data presented in Calatayud et al. (2011), Karlsson et al. (2003, 2004), Landolt et al. (2000), Skärby et al. (2004) was used.

### 2.3. Data handling

First, species synonyms were excluded using The Plant List (2010) to avoid double counting of species names. The effects of ozone on biomass were calculated relative to the charcoal-filtered air treatment (or occasionally non-filtered air if no charcoal filtered control was used). EC<sub>10</sub> values were then calculated using the standardized dose–response functions. Species exhibited two types of response when exposed to ozone, either biomass reduction (negative slope) or no biomass decrease (positive slope). The linear functions for biomass decrease were converted as follows:

$$EC_{10} = \frac{-0.1 \cdot b}{a} \quad (1)$$

where  $b$  is the intercept and  $a$  is the slope of the linear function.

A list of all species with their dose–response functions and EC<sub>10</sub> values can be found in the [Supplementary information \(S1, S2 and S3\)](#).

### 2.4. Species Sensitivity Distributions

Species Sensitivity Distributions (SSDs) were developed for three separate groups of species, i.e. trees, annual grassland species and perennial grassland species. For each group there were two effect definitions:

- one SSD was derived based on EC<sub>10</sub> values for biomass reduction only;
- one SSD was derived for biomass reduction, corrected for the fraction of species with no biomass reduction ( $f_{nbd}$ ).

SSDs were derived in the following way. First the EC<sub>10</sub> data were log-transformed. Second, the mean ( $\mu$ ) and standard deviation ( $\sigma$ ) of the log EC<sub>10</sub>-data were calculated. Assuming a lognormal SSD for ozone exposure, the parameters  $\mu$  and  $\sigma$  were then used to derive the Potentially Affected Fraction (PAF):

$$PAF = \frac{a}{\sigma \cdot \sqrt{2} \cdot \pi \cdot AOT40 \cdot \ln 10} \cdot \int_0^{AOT40} \exp\left(-\frac{1}{2} \cdot \left(\frac{\log(AOT40) - \mu}{\sigma}\right)^2\right) dAOT40 \quad (2)$$

where  $a$  is 1 for the SSD derived based on EC<sub>10</sub> values for biomass reduction only and  $a$  equals  $1 - f_{nbd}$  for the SSD derived including the fraction of species with no biomass reduction. AOT40 represents the ambient ozone exposure.

Differences in sensitivity between the species groups were investigated by comparing the means ( $\mu$ ) and variances ( $\sigma$ ). The log<sub>10</sub>-transformed EC<sub>10</sub> values were tested for normality with the Kolmogorov–Smirnov test. The means were compared with the Independent  $t$ -test and the variances ( $\sigma$ ) were compared using the Levene's test. All tests were executed with SPSS 17.0 for Windows.

### 2.5. Critical levels

Hazardous exposure concentrations for which 5% of the species assemblage remains unprotected (HC<sub>5</sub>) were derived for each species groups and their respective response types. The HC<sub>5</sub> for the species with biomass reduction only was calculated following the procedure described by Aldenberg and Jaworska (2000):

$$\log(HC_5) = \mu - k \cdot \sigma \quad (3)$$

where  $k$  is the extrapolation constant for 95% species protection. Aldenberg and Jaworska (2000) present extrapolation constants for the estimation of the log(HC<sub>5</sub>) based on the assumption of normal Species Sensitivity Distributions for the log-transformed toxicity data. To assess the uncertainty of the HC<sub>5</sub> the 90% confidence interval was calculated following Aldenberg and Jaworska (2000).

The HC<sub>5</sub> for the species assemblage including the fraction of species with no biomass reduction was derived by calculating the concentration at which  $5/(1 - f_{nbd})\%$  of the sensitive species is affected.

PAF levels corresponding to the critical levels recommended by the LRTAP Convention (2010) were determined using the lognormal SSD function. The 90% confidence interval was calculated following methods adapted from Aldenberg and Jaworska (2000).

### 2.6. Impact assessment

Maps of the potentially affected fraction (PAF) of species were compiled to determine the impact of ozone exposure on annual and perennial grassland species in Northwestern Europe. A spatially explicit grid-based approach on a  $0.5 \times 0.5^\circ$  (i.e. ca. 50 km  $\times$  50 km at 60° N) resolution was applied. Grid-specific AOT40 exposure concentrations for 2010 were obtained using the EMEP model (Jonson et al., 2001). The AOT40 values were based on a growing season of May–July at a height of 1 m above the ground. In each grid the PAF was derived for each species groups using the AOT40 exposure values as input in the SSD (equation (3)).

## 3. Results

### 3.1. Species Sensitivity Distributions

Exposure–response functions were determined for 25 annual grassland species, 62 perennial grassland species, and 9 tree species. The full data set is given in the SI (Tables S1, S2 and S3). The percentage of species in the dataset that exhibited a biomass reduction was 88% for annual grassland species, 63% for perennial

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