Environmental Pollution 157 (2009) 865–870

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Deciduous shrubs for ozone bioindication: *Hibiscus syriacus* as an example

Elena Paoletti [a,*], Anna Maria Ferrara [b], Vicent Calatayud [c], Júlia Cerveró [c], Fabio Giannetti [b], María José Sanz [c], William J. Manning [d]

[a] Institut Plant Protection (IPP), National Council Research (CNR), Via Madonna del Piano 10, 50019 Sesto Fiorentino, Florence, Italy
[b] Istituto per le Piante da Legno e l'Ambiente (IPLA), Corso Casale 476, 10132 Turin, Italy
[c] Fundación C.E.A.M., Charles R. Darwin 14, Parc Tecnològic, 46980 Paterna, Valencia, Spain
[d] Department of Plant, Soil and Insect Sciences, University of Massachusetts, Amherst, MA 01003-9320, USA

An Italian population of the deciduous shrub Hibiscus syriacus, a common ornamental species in temperate zones, is recommended as ozone bioindicator

ARTICLE INFO

Article history:
Received 11 July 2008
Received in revised form
3 November 2008
Accepted 5 November 2008

Keywords:
Bioindication
Biomonitoring
Ozone sensitivity
Tropospheric O_3
Visible injury

ABSTRACT

Ozone-like visible injury was detected on *Hibiscus syriacus* plants used as ornamental hedges. Weekly spray of the antiozonant ethylenediurea (EDU, 300 ppm) confirmed that the injury was induced by ambient ozone. EDU induced a 75% reduction in visible injury. Injury was more severe on the western than on the eastern exposure of the hedge. This factor of variability should be considered in ozone biomonitoring programmes. Seeds were collected and seedlings were artificially exposed to ozone in filtered vs. not-filtered (+30 ppb) Open-Top Chambers. The level of exposure inducing visible injury in the OTC seedlings was lower than that in the ambient-grown hedge. The occurrence of visible injury in the OTC confirmed that the ozone sensitivity was heritable and suggested that symptomatic plants of this deciduous shrub population can be successfully used as ozone bioindicators. EDU is recommended as a simple tool for diagnosing ambient ozone visible injury on field vegetation.

1. Introduction

Northern Italy is an area of Europe that experiences high ozone (O_3) concentrations and AOT40 exceedances (Sanz et al., 2007). In the surroundings of Turin, O_3 reaches phytotoxic levels and may induce visible injury in a range of plant species (Paoletti et al., 2007a,b). Among all species, some *Hibiscus syriacus* plants, used as ornamental hedges, have shown O_3-like visible injury every year.

H. syriacus, the common garden hibiscus, is a flowering shrub in the family Malvaceae, native to Asia. In temperate zones, it is among the most commonly grown ornamental species, widely planted in areas with hot summers. It is a deciduous and fast-growing species, reaching 2–4 m in height, with very attractive white, pink, red, lavender, or purple flowers. Ozone-like visible injury in unidentified hibiscus species has been already reported (Sanz Sáanchez et al., 2001; Bergweiler et al., 2008). Other hibiscus species have been artificially exposed to O_3 and have shown high sensitivity (Kasana, 1992; Sung et al., 1998; Chang and Yu, 2001).

Since the detection of grape leaf stipple (Richards et al., 1958), many plant species have been suggested for O_3 bioindication, including forbs, vines, shrubs, and trees (Manning and Godzik, 2004; Hayes et al., 2007), and species from temperate (Novak et al., 2003) and tropical zones (Furlan et al., 2007, 2008; Bergweiler et al., 2008). Sensitive cultivars of tobacco, clover and bean have been often suggested for active-bioindication networks (e.g., Nali et al., 2006; Burkey et al., 2005), because they are easy to be reproduced and managed for limiting the intra-specific variability in O_3 sensitivity (Bassin et al., 2004). Herbaceous species need frequent cares (watering and surveys), being thus not well suited for long-term bioindication studies at remote sites. Tree species have been usually suggested as passive bioindicators (e.g., Yuska et al., 2003). As incidence and severity of O_3 injury on passive bioindicators is dependent upon many environmental variables, interest is increasing in using woody plants for long-term monitoring of O_3 effects (Gravano et al., 2003).

Our objectives were to determine whether ambient O_3 was the agent of the observed visible injury in an Italian population of the shrub *H. syriacus* (hibiscus) and whether the O_3 sensitivity of this population was heritable. Therefore, in-situ applications of the antioxidant ethylenediurea (EDU) were performed, seeds were collected and seedlings were ex-situ exposed to O_3 in Open-Top Chambers (OTC). EDU (N-[2-(2-oxo-1-imidazolidinyl)ethyl]-N′-phenylurea), used as a foliar spray or soil/potting medium drench, has been widely used to prevent foliar O_3 injury and determine O_3 effects on many plant species (Manning, 2000, 2005).

* Corresponding author. Tel.: +39 055 5225 591; fax: +39 055 5225 666.
E-mail address: e.paoletti@ipp.cnr.it (E. Paoletti).

0269-7491/$ – see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envpol.2008.11.009

2. Materials and methods

2.1. Field experiment

2.1.1. Location

Based on past observations of foliar ozone-like injury, one symptomatic hibiscus hedge was selected and regularly treated by spraying EDU over one growing season. The cultivar was *H. syriacus* L. 'Minerva' with lavender–pink flowers. Bud break occurred on April 8. The site was located at the 34-ha "Millerose" park on the hills around Turin (45°4′25″N, 7°43′46″E, 240 m a.s.l.). Ambient O_3 was continuously monitored by means of an O341M–LCD (Ansyco, Karlsruhe, Germany). The accumulated exposure over a threshold of 40 ppb (AOT40) was calculated on the basis of mean hourly values from 8 to 20 CET (EU, 2002). Ozone descriptors are in Table 1 and Fig. 1. The seasonal trend of O_3 was similar to that in previous years (Paoletti et al., 2007b).

2.1.2. EDU regime

At 7-day intervals, both sides (western and eastern exposure) of the hedge were sprayed until dripping. One linear meter of hedge was sprayed with 300 ppm EDU (100% active ingredient in distilled water, provided by University of Massachusetts), followed by half a meter of not-sprayed hedge (isolation zone) and one meter of hedge sprayed with distilled water. The sequence was repeated twice and labelled. At least two individual plants were included in each sprayed meter, for a total of four plants per treatment.

2.1.3. Symptoms

The plants were examined every week to record the date of symptom onset. A microscopical analysis on fresh cross-cuttings (Fig. 2) helped to diagnose O_3 injury. On September 14, visible injury was assessed as stippling on three random shoots per plant. As the hedge was dense and the inner leaves were unequally exposed to ambient air and difficult to evaluate, only the 5 terminal leaves of each shoot were surveyed. The number of symptomatic leaves was counted and expressed as percentage of injured leaves per shoot (LA), and the percent surface injury was visually assessed and expressed as percentage of injured leaf surface per symptomatic leaf (AA). The assessment used a 5%-step scale with the help of a phototable (Fig. 3) elaborated by means of preliminary scanning (Epson twain 5) and a supervised classification method which used a manual delineation of pixels considered as injured and uninjured for defining spectral signatures and related spectral statistics. Once a set of spectral signatures was created, each pixel was assigned according to a Maximum Likelihood Classification Algorithm implemented in a software for image analysis (ENVI 4.4., ITT, Boulder CO). A Plant Injury Index (PII) was calculated combining the two parameters: $PII = (LA \times AA)/100$.

2.1.4. Statistical analysis

Percent values were arcsine transformed. After checking for normal distribution (Kolmogorov–Smirnov *D* test) and homogeneity of variance (Levene test), a one-way analysis of variance (ANOVA) was applied to the percent surface injury of any leaf to test for the effects of treatment (EDU vs. water application), individual plant, leaf position (from 1 = top to 5) and lighting exposure of the hedge (west vs. east). A two-way ANOVA was applied to PII to test for the effects of EDU application and lighting exposure.

2.2. OTC experiment

2.2.1. Plant culture

Seeds were collected from symptomatic plants of the hedge, sown on April 22, grown in a charcoal-filtered, partially shaded open-top chamber (39°16′14.8″N, 00°26′59.6″W, 30 m a.s.l.), and transplanted to containers on June 12. The 7-l containers were filled with 40% coconut-peat, 40% peat, 10% sand, and 10% vermiculite, soil pH being close to 7.0. A slow release fertilizer was incorporated (Osmocote plus, NPK 20:10:20). Plants were irrigated using a droplet irrigation system, twice a day. Twelve plants were kept in charcoal-filtered air, and 12 were fumigated.

Table 1
Mean ozone concentrations for different daily time windows, peak hourly value and AOT40 over the experiments: from leaf sprouting (April 8) to injury onset (July 15) and injury survey (September 14) in the field experiment; and from start of exposure (June 29) to injury onset (July 12) and end of exposure (September 22) in the OTC experiment. The 8-h window covered the time in which plants of the NF + 30 treatment were exposed to Non-Filtered air + 30 ppb ozone. CF = Charcoal-Filtered OTC. AA (ambient air) is not a treatment but refers to the ozone levels measured at the experimental site outside the Open-Top Chambers.

	24-h mean	12-h mean	8-h mean	Peak	AOT40
		[8–20 CET]	[10–18 CET]		[8–20 CET]
	(ppb)	(ppb)	(ppb)	(ppb)	(ppb × h)
Field exp.					
Injury onset	52.5	52.4	54.3	89.5	16,632
Injury survey	53.8	54.3	56.1	102.5	30,009
OTC exp.					
NF + 30 Inj.onset	37.5	60.2	66.7	102.3	3900
NF + 30 final	38.5	62.6	70.3	104.3	24,854
CF – final	10.9	10.7	12.7	29.5	0
AA – final	30.6	43.7	46.7	77.0	6582

2.2.2. OTC exposure

Plants were placed in eight Open-Top Chambers with two O_3 treatments (4 OTCs per treatment): Charcoal-Filtered air (CF) and Non-Filtered air plus 30 ppb O_3 (NF + 30). Air quality inside and outside the OTCs was monitored at regular intervals with an O_3 monitor (Dasibi 1008-AH, Environmental Corp.) and a nitrogen oxide monitor (Dasibi 2108, Environmental Corp.); these monitors were periodically calibrated. Plants were fumigated 8 h a day, from 10:00 to 18:00 CET. Ozone was generated from oxygen using a high-voltage electrical discharge generator (Sir sa, Madrid, Spain). Fumigation started on June 29, and ended on September 22. Ozone descriptors are in Table 1 and Fig. 1.

2.2.3. Symptoms

All plants were examined every two days to record the first date of symptom onset in each individual plant. Once a week, both the percentage of affected leaves per plant (LA), and the 5%-step percentage of area affected in the symptomatic leaves (AA) were scored, and PII was calculated. Growth and physiological responses will be included in a further paper (Calatayud et al., personal communication).

3. Results

3.1. Field experiment

In the field experiment, the injury onset was detected in all the four plants on July 15, i.e. 97 days after leaf sprouting ($AOT40 = 16{,}632$ ppb × h), at the time of a sharp increase of daily AOT40 (up to 482 ppb × h), after a 1.5 month of better air quality (mean daily $AOT40 = 118$ ppb × h, Fig. 1). On September 14 ($AOT40 = 30{,}009$ ppb × h), 46% of the leaves scored on water-sprayed plants were affected, and the injured leaves had on average 16% of their surface covered by stippling. The injured surface of individual leaves (AA) never exceeded 45%. The visible injury was a fine interveinal yellow stippling, quickly becoming brown bronze (Fig. 4). The affected surface might also show a diffuse chlorosis. Stippling was only on the upper leaf surface and was irregularly distributed on it, apparently reflecting shade effects inside the hedge. Areas with stipples showed accumulation of secondary metabolism compounds in the only palisade cells (Fig. 2). Individual plant and leaf position did not significantly affect the percent of injured leaf surface ($p > 0.01$). The western side of the hedge had higher PII than the eastern side (on average, 7.8 vs. 2.3; $p = 0.004$). EDU-sprayed plants had lower PII than water-sprayed plants, on both sides of the hedge (on average, 2.0 vs. 8.1; $p = 0.008$).

3.2. OTC experiment

In the OTC experiment, plants of the CF treatment did not show any symptom during the experiment. The first symptom in the NF + 30 OTCs was detected after 14 days of fumigation in two plants ($AOT40 = 3900$ ppb × h). Injury rapidly affected all the 12 plants so that after 23 days ($AOT40 = 6885$ ppb × h), they all were injured to some extent. Initially, visible injury was a fine interveinal yellow stippling, while finally the stippling became brown and more marked (Fig. 5). In the affected leaves, a chlorosis was associated with the stippling. PII increased progressively (Fig. 5), as a consequence of almost parallel increases of affected leaves and leaf surface. In the last injury score, after 78 days of O_3 exposure, plants of the NF + 30 treatment had 66% of the leaves affected on average, and the injured leaves had an average of 70% of their surface covered by stippling.

4. Discussion

Ozone was shown to cause foliar injury symptoms in *H. syriacus*. Symptoms consisted of interveinal chlorosis and brown stipples on