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Ozone air pollution and foliar injury development on native plants of Switzerland

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"Capsule": *Visible ozone-induced foliar injury on native forest species of Switzerland was identified and confirmed under ambient OTC-conditions and related to the current European AOT40 standard.*

Abstract

The objectives of this study were to examine the foliar sensitivity to ozone exposure of 12 tree, shrub, and herbaceous species native to southern Switzerland and determine the seasonal cumulative ozone exposures required to induce visible foliar injury. The study was conducted from the beginning of May through the end of August during 2000 and 2001 using an open-top chamber research facility located within the Lattecaldo Cantonal Forest Nursery in Canton Ticino, southern Switzerland (600 m asl). Plants were examined daily and dates of initial foliar injury were recorded in order to determine the cumulative AOT40 ppb h ozone exposure required to cause visible foliar injury. Plant responses to ozone varied significantly among species; 11 species exhibited visible symptoms typical of exposures to ambient ozone. The symptomatic species (from most to least sensitive) were *Populus nigra, Viburnum lantana, Salix alba, Crataegus monogyna, Viburnum opulus, Tilia platyphyllos, Cornus alba, Prunus avium, Fraxinus excelsior, Ribes alpinum,* and *Tilia cordata*; *Clematis* spp. did not show foliar symptoms. Of the 11 symptomatic species, five showed initial injury below the critical level AOT40 10 ppmh O_3 in the 2001 season.

Keywords: Ozone; Visible foliar injury; Critical levels; AOT40; Switzerland

1. Introduction

The long-range transport of ozone and its precursor chemicals is known to occur throughout Europe, resulting in elevated ozone concentrations many kilometers from the source region (Derwent and Jenkin, 1991; Vecchi and Valli, 1999; Wotawa et al., 2000). Canton Ticino is located in the sub-alpine region of southern Switzerland and is bordered by the Swiss Alps to the north and the heavily industrialized and populated Po Plain and the city of Milan, Italy to the south. The Swiss and Italian Alps act as a barrier to northern and central European air mass transport and as a result, much of Canton Ticino is influenced by a Mediterranean climate; weather patterns consist of hot, sunny days during the summer season extending from early May through to late October. Ozone concentrations are influenced by the "Milan urban plume" which contains elevated levels of primary pollutants, including nitrogen oxides, non-methane hydrocarbons (NMHCs), and volatile organic compounds (VOCs). A northwesterly wind can transport this plume into northern Italy and southern Switzerland where it enters the complex topography of the sub-alpine region (Bacci et al., 1990; Staffelbach et al., 1997; Staffelbach and Neftel, 1997; Wotawa et al., 2000). These air masses then circulate on a diurnal cycle and create a cumulative daily build-up of ozone (Bacci et al., 1990; Wunderli and Gehrig, 1990). Staffelbach et al. (1997) reported ozone concentrations of 166 ppbv near Chiasso, located in the southern tip of Ticino only a few kilometers from the site of the current study. The increased elevation of the Alps also influences ozone concentrations; concentrations increase as elevation increases (Wunderli and Gehrig, 1990; Bacci et al., 1990;

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Brönnimann et al., 2000). More recent studies at the Lattecaldo research site have shown ozone concentrations continuing to reach hourly averages of ca. 140 ppb (VanderHeyden et al., 2001).

The current European critical level of ozone as set to protect forest vegetation is expressed as an AOT40 of 10 ppm.h (the amount of ozone accumulated over a threshold of 40 ppb) during daylight hours when solar radiation exceeds 50 Wm^{-2}, over a six-month growing season (Ashmore and Davison, 1996; Fuhrer et al., 1997). The critical level has been exceeded at numerous monitoring stations throughout Switzerland and southern Europe (NABEL, 1995; de Leeuw and de Paus, 2001; Gerosa et al., 1999; VanderHeyden, 1999; VanderHeyden et al., 2001). Various plant species throughout Europe have proven to be symptomatic of ozone exposures, with most incidences of visible foliar symptoms occurring in southern Europe and the Mediterranean region (Skelly et al., 1999). The occurrence of ozone-induced injury to crops and forest species in Italy and other Mediterranean areas has also been reported (Lorenzini et al., 1995; Schenone and Lorenzini, 1992; Schenone, 1993; Bussotti and Ferretti, 1998; Inclan et al., 1999).

In 1995, an open-top chamber facility was established at the Lattecaldo Cantonal Forest Nursery in Ticino. Innes and Skelly (1996) exposed seedlings of black cherry, *P. serotina,* European beech, *Fagus sylvatica* L., and European ash, *Fraxinus excelsior* L. to ambient air, non-filtered air (~90% ambient O_3), and charcoal filtered air (~50% ambient O_3) at the Lattecaldo Nursery site. All species grown in ambient conditions displayed visible ozone symptoms; the type and severity of symptoms varied within and among species. Black cherry was found to be the most sensitive of the three species investigated. In addition, these symptoms occurred at exposures below the AOT40 critical level for ozone (Innes and Skelly, 1996; Ghosh et al., 1998). Additional field surveys conducted from 1995 through 1998 within and around the Lattecaldo Nursery and in areas of southern Spain (Skelly et al., 1999) resulted in a list of approximately 80 herbaceous and woody species native to Switzerland and 42 species native to southern Spain showing typical ozone-induced foliar symptoms (Skelly et al., 1999). In the spring of 1997, six additional plots were added to the open-top facility at Lattecaldo and 16 species (also selected from previous surveys, Skelly et al., 1999) were investigated during the 1997 and 1998 summer seasons. VanderHeyden et al. (2001) confirmed ozone as the cause of foliar injury on 14 of the 16 species investigated with several species showing injury below the AOT40 critical level for ozone.

As a part of continuing research within the Lattecaldo open-top chamber research facility, additional species were selected for investigation during 2000 and 2001. The objectives of the current research were to assess the foliar sensitivity of 12 native tree, shrub, and herbaceous species to ozone in southern Switzerland and to examine the seasonal cumulative ozone exposures required to induce visible foliar injury on those species.

2. Materials and methods

2.1. Study site and plot design

The research site was located in the sub-alpine region of southern Switzerland at the Lattecaldo Cantonal Forest Nursery in the Valle di Muggio, Canton Ticino (9°3′ E, 45°51′ N, 600 m asl) and was also used previously by VanderHeyden et al. (2001). In spring of 2000, the site was cleared of previously used plants and open-top chambers, and the soil was tilled during the time period of 17–19 April. The current study consisted of three treatments with four replications (12 plots): four filtered air chambers, four non-filtered air chambers, and four open plots (Fig. 1). Activated charcoal filters were expected to reduce ambient ozone concentrations by approximately 50% (Heagle et al., 1973).

Twelve species of native trees and shrubs (Table 1) were examined in the 2000 and 2001 growing seasons; species were selected from a list of apparent ozone-sensitive species developed during surveys in the Lattecaldo nursery and surrounding areas (Skelly et al., 1999). During 26–29 April 2000, seedlings were planted in two concentric circles at a spacing of ca. 50 cm. Each plot contained 36 plants consisting of three representatives of each species; species were arranged identically in each of the 12 plots (Fig. 1). Following the initial planting, several dead or dying individuals of *Clematis* spp., *P. nigra*, *P. avium, T. platyphyllos*, and *V. lantana* were replaced with healthy plants from nursery stock.

2.2. Visible foliar ozone injury evaluation

Visible foliar injury evaluations were made from 16 May to 22 August in 2000 and from 2 May to 20 August

Table 1
Scientific and common names of species studied in the 2000 and 2001 growing seasons (Lattecaldo Nursery, Canton Ticino, Switzerland)

Scientific Name	Common name
Clematis spp. L.	Clematis
Cornus alba L.	Tatarian Dogwood
Crataegus monogyna Jacq.	Hawthorn
Fraxinus excelsior L.	European Ash
Populus nigra L.	Black Poplar
Prunus avium L.	Sweet Cherry
Ribes alpinum L.	Alpine Currant
Salix alba L.	White Willow
Tilia cordata Miller	Small Leaf Linden
Tilia platyphyllos Scop.	Linden
Viburnum lantana L.	Wayfaring Tree
Viburnum opulus L.	European Cranberry

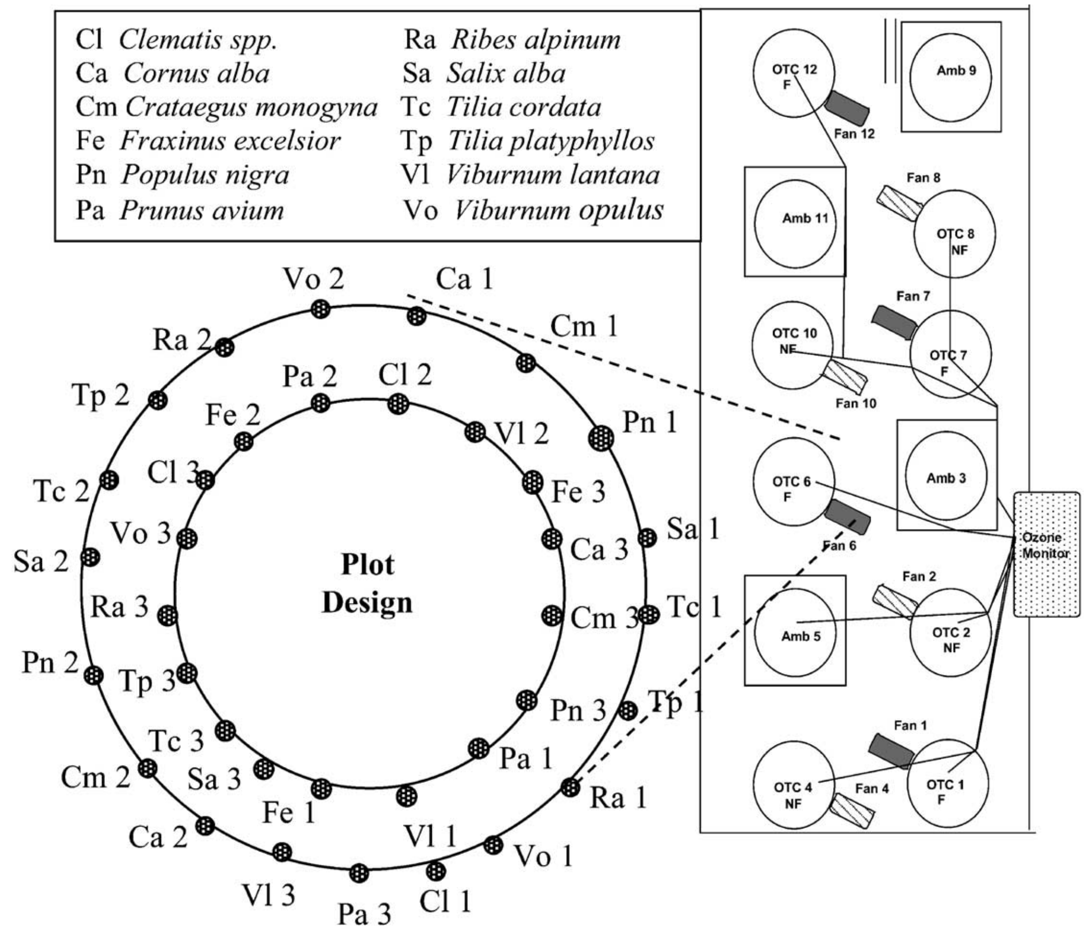

Fig. 1. Layout of open-top research facility and plot design at the Lattecaldo Nursery, Canton Ticino, Switzerland. OTC = Open-top Chamber; NF = Non-filtered air; F = Filtered air; Amb = Open Plots (ambient air).

in 2001. In 2000, fully expanded leaf maturity dates following bud break were recorded for each species with the exception of *Clematis* spp. and *Ribes alpinum*, which had mature leaves prior to the beginning of the observation period. Since leaf maturity had occurred prior to the start of observations in 2001, fully expanded leaf maturity for all species was estimated to be 15 April based on observations made during site set-up in April. Evaluations took place daily to determine dates of initial visible foliar injury. All plants were observed macroscopically; potential ozone-induced symptoms were further examined using a 10× hand lens. The following general criteria were used and combined as shown in Fig. 2 (Innes et al., 2001), when diagnosing ozone-induced foliar injury: (1) symptoms usually appeared as dark-colored stipple or discoloration (reddening or bronzing) on the upper leaf surface only; (2) symptoms did not occur on leaf veins or veinlets; and (3) symptoms initially appeared with greater severity on older leaves toward the base of the plant. Timing of initial injury was also considered and a defined progression of symptoms following initial injury was also recorded. Chlorosis was noted but not recorded as ozone-induced injury. Symptoms on plants growing in non-filtered and open plots were compared to plants growing in the filtered air chambers to further confirm ozone as the cause of foliar symptoms.

Following the onset of injury, all plants were examined every 3–5 days. A 5% scale (0, 5, 10, 15…100%) was used to evaluate the percentage of symptomatic leaves per plant and a modified Horsfall–Barratt scale (0, 1, 3, 6, 12, 25, 50, 75, 88, 94, 97, 99, 100%) was used to evaluate percent area injured on symptomatic leaves (Horsfall and Barratt, 1945). The number of abscised leaves was not recorded but was noted and scored with the highest rating class for symptomatic leaves still remaining on the plant. A Forest Health Expert Advisory System (Nash et al., 1992a,b) was used for eye calibration to insure accuracy and consistency when evaluating. All evaluations were made by the same observer throughout the 2000 and 2001 growing season. Relative amounts of yellowing and leaf abscission were noted via photographic records for all plants within each of the respective plots at the conclusion of 2000 and 2001 seasons; photographic records of injury progression on symptomatic species were also kept

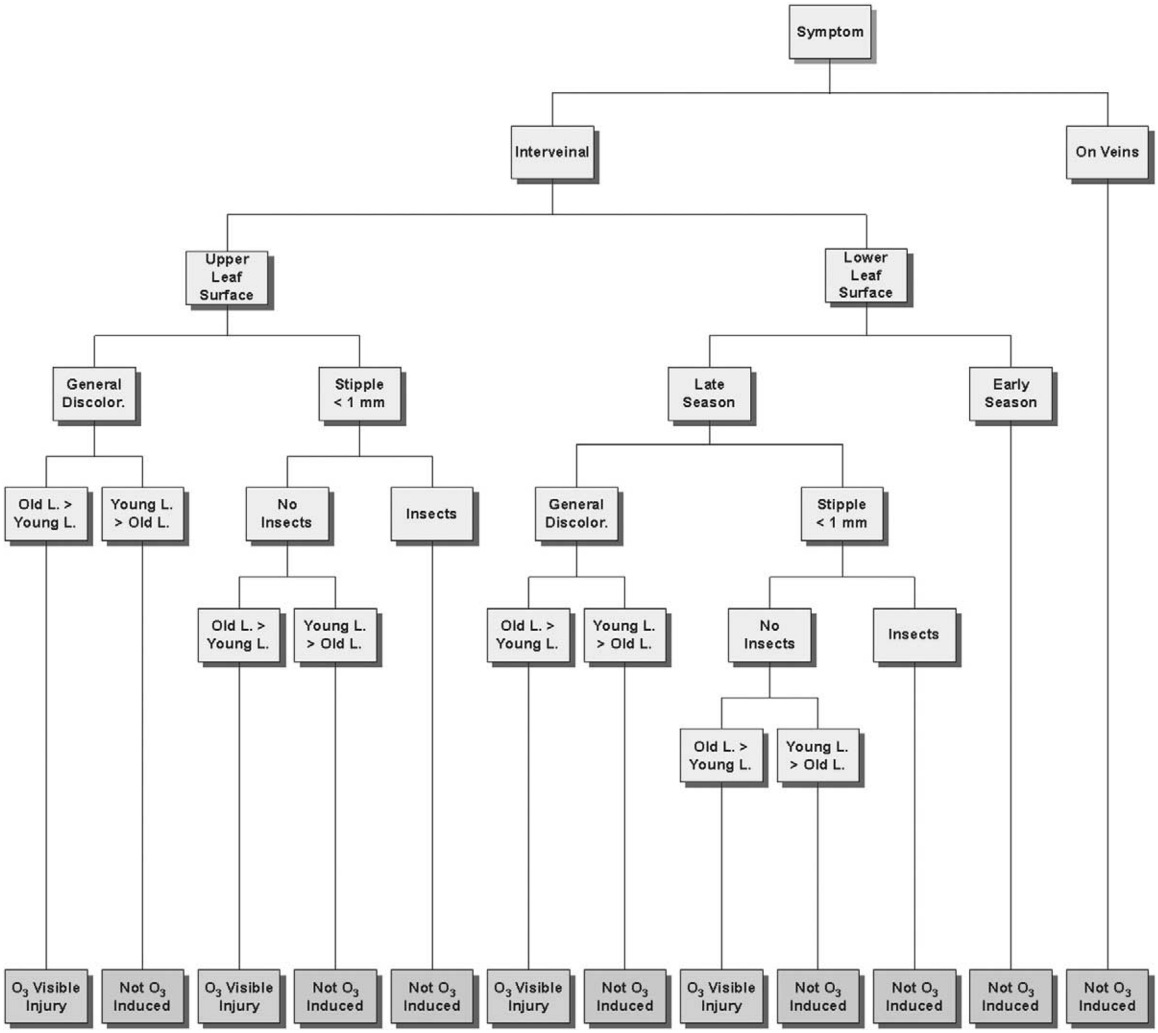

Fig. 2. Flowchart for the diagnosis of ozone-induced injury on broadleaved species (L. = leaves) (from Innes et al., 2001).

throughout the 2001 season. In addition to foliar rating, notes on insect damage, and the presence of chlorotic or necrotic spots, discoloration of the leaves, obvious changes in plant vigor, and any noticeable abnormalities were recorded for each plant during each evaluation period.

2.3. Ozone monitoring

Ozone concentrations were monitored throughout each growing season using a Monitor Labs model ML 8810 ozone monitor that was calibrated monthly. Hourly average ozone data were downloaded several times daily and displayed graphically on a website maintained by the Swiss Federal Institute for Forest, Snow, and Landscape Research (WSL) (http://www.wsl.ch/forest/wus/ozone/tio3mess/tio3mess1e.htm). Ozone concentrations were recorded from 9 May to 25 October 2000 and from 1 May to 2 October 2001. Two-minute air samples drawn at 1 m height were taken from one ambient plot No. 5 (100% ambient air) and each of the open-top chambers (Plot No.s 1, 2, 4, 6, 7, 8, 10, and 12) (Fig. 3); a repeating 20-min sampling interval was used 24 h/day.

2.4. Data analysis

The cumulative AOT40 ppbh ozone threshold required to cause visible foliar injury was calculated by subtracting the AOT40 values for dates of leaf maturity from the AOT40 values for dates of initial foliar injury appearance. This last value was considered as the total parts-per-billion hours (ppbh) of ozone exposure greater than 40 ppb that induced visible foliar injury. Injury thresholds were expressed as averages per species, per treatment, using daylight hours (07:00–18:59). A split plot design with ozone treatment as the main plot and species as the subplot was used for comparing species and treatment differences for mean AOT40 injury thresholds using analysis of variance in the General Linear Model procedure of the Statistical Analysis System (SAS) (SAS Institute Inc., 1999).

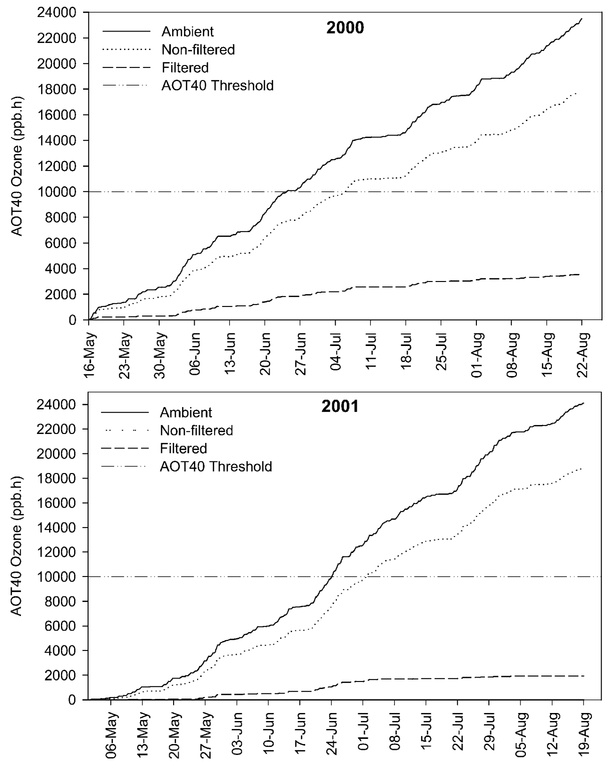

Fig. 3. Cumulative AOT40 ozone (ppbh) exposures for ambient, non-filtered, and filtered air treatments from 16 May to 22 August 2000 and 2 May to 19 August 2001 for daylight hours (07:00–18:59). Lattecaldo Nursery, Canton Ticino, Switzerland.

3. Results

3.1. Ozone exposures

Peak hourly ozone concentrations as well as monthly average ozone concentrations per treatment are shown in Table 2. The highest hourly ozone concentration (128.5 ppb) was recorded on 3 June 2000 during the 15:30–16:29 h and on 23 July 2001 (139.1 ppb) during the 16:30–17:29 hr period. Monthly peak-hour ozone concentrations were generally higher in 2001 as compared with 2000, with the exception of August 2000. Monthly 24 h and 12 h averages were higher in May, June, and August of 2000; July averages were higher during 2001.

Elevated ozone exposure episodes were more pronounced in 2001 with a series of major episodes occurring in late June and July as well as early August. Lower ozone exposures observed in July of 2000 corresponded to a high frequency of rain events and unfavorable meteorological conditions for ozone formation. Filtered air chambers reduced ambient ozone concentrations by as much as 44.2% in 2000 and 38.5% in 2001; non-filtered air chambers received 89.4% and 91.2% ambient ozone concentrations in 2000 and 2001, respectively. Cumulative AOT40 ozone exposures were based on daylight hours (07:00–18:59) and the AOT40 critical level of 10,000 ppbh was exceeded on 24 June 2000 and 25 June 2001 (Fig. 3).

3.2. Foliar injury onset

The overall plant response to ozone exposure varied significantly among species in both 2000 and 2001 growing seasons. Most species had an increased number of symptomatic plants in 2001 as compared to 2000 with exception of *C. monogyna, R. alpinum*, and *T. cordata*. Visible foliar injury onset varied among seasons, among species, and, to a lesser extent, within species (Table 3). Most plants showed injury by late July or early August, with the exception of *P. nigra*. Several of the more sensitive species including *P. nigra, S. alba, V. lantana,* and *V. opulus* exhibited symptoms beginning in late May 2001 (Table 3) and were among those species with the greatest number of symptomatic individuals. In 2000, initial symptoms were observed earlier in the non-filtered air chambers on *P. avium* and *C. alba*.

3.3. Foliar injury development and sensitivity of species

Based on foliar injury onset in response to ambient ozone exposures in 2001, symptomatic species (from most to least sensitive) were *P. nigra, V. lantana, S. alba, C. monogyna, V. opulus, T. platyphyllos, C. alba, P. avium, F. excelsior, R. alpinum,* and *T. cordata* (Fig. 4) with *Clematis* spp. not showing any foliar injury induced by ozone exposure. There were significant differences ($P<0.0001$) in AOT40 injury thresholds among species for both observation periods (Table 4). There was no significant block effect, but significant differences ($P<0.0001$) were observed among treatments in 2000 as a result of a greater number of symptomatic plants in the filtered air treatment as compared to 2001. The type, onset, and progression of symptoms varied among species, illustrating the species-specific nature of

Table 2
Monthly and seasonal peaks and 24 and 12 h (07:00–18:59) average ozone concentrations for ambient (Amb), non-filtered (NF), and filtered (F) air treatments for 2000 and 2001 (Lattecaldo Nursery, Canton Ticino, Switzerland)

	Peak h	Amb		NF		F	
		24 h	12 h	24 h	12 h	24 h	12 h
2000							
May	110.4	44.3	50.7	39.3	45.3	19.2	25.5
June	128.5	54.1	62.9	48.3	56.7	25.5	35.0
July	120.8	48.9	54.4	44.0	49.0	22.1	28.6
August	116.3	52.1	58.1	46.6	52.2	21.3	28.0
Seasonal	128.5	50.2	56.9	44.9	51.2	22.2	29.5
2001							
May	136.0	43.9	48.0	39.4	43.2	17.9	21.8
June	138.8	54.3	60.2	49.3	54.7	23.3	30.0
July	139.1	53.4	59.9	49.3	54.7	19.2	24.0
August	97.2	48.9	52.9	45.0	48.4	16.8	20.0
Seasonal	139.1	50.2	55.3	45.8	50.3	19.3	24.0

Table 3
Dates of first observed foliar injury, average injury onset dates, and standard error (days) for ambient, non-filtered, and filtered air treatments within the Lattecaldo Nursery, Canton Ticino, Switzerland for 2000 and 2001

Species	Year	Ambient			Non-filtered			Filtered		
		Date of first symptom	Avg Injury Onset	S.E.	Date of first symptom	Avg Injury Onset	S.E.	Date of first symptom	Avg Injury Onset	S.E.
Cornus alba	2000	18 July	4 August	4	27 June	27 July	8		no injury	
	2001	12 June	29 June	5	19 June	11 July	6		no injury	
Crataegus monogyna	2000	18 July	1 August	3	26 July	5 August	4	15 August	15 August	0
	2001	7 June	15 June	6	15 June	16 July	0	31 July	31 July	1
Fraxinus excelsior	2000	18 July	8 August	6	1 August	19 August	4		no injury	
	2001	22 June	12 July	6	17 June	14 July	6		no injury	
Populus nigra	2000	13 July	21 July	1	18 July	31 July	4		no injury	
	2001	25 May	25 May	5	25 May	28 May	6	17 June	18 July	7
Prunus avium	2000	21 July	25 July	5	27 June	23 July	7		no injury	
	2001	7 June	2 July	4	5 June	3 July	6		no injury	
Ribes alpinum	2000	27 June	9 July	4	27 June	23 July	5	13 July	26 July	5
	2001	10 July	15 July	3	10 July	15 July	5		no injury	
Salix alba	2000	21 July	28 July	2	26 July	2 August	1	3 August	7 August	3
	2001	25 May	10 June	6	25 May	16 June	8	31 July	5 August	1
Tilia cordata	2000	26 July	10 August	4	15 August	16 August	3	8 August	15 August	2
	2001	7 August	7 August	0	7 August	7 August	0		no injury	
Tilia platyphyllos	2000	5 July	29 July	11	18 July	2 August	6	11 August	15 August	2
	2001	1 June	23 June	7	19 June	19 June	0		no injury	
Viburnum lantana	2000	27 June	17 July	6	18 July	29 July	7		no injury	
	2001	25 May	31 May	2	25 May	6 June	4		no injury	
Viburnum opulus	2000	13 July	24 July	5	11 August	11 August	0		no injury	
	2001	27 May	21 June	4	26 May	14 June	6	23 July	23 July	0

Average injury onset and the corresponding standard error in days for each species per treatment was calculated from the dates of injury onset for symptomatic individuals.

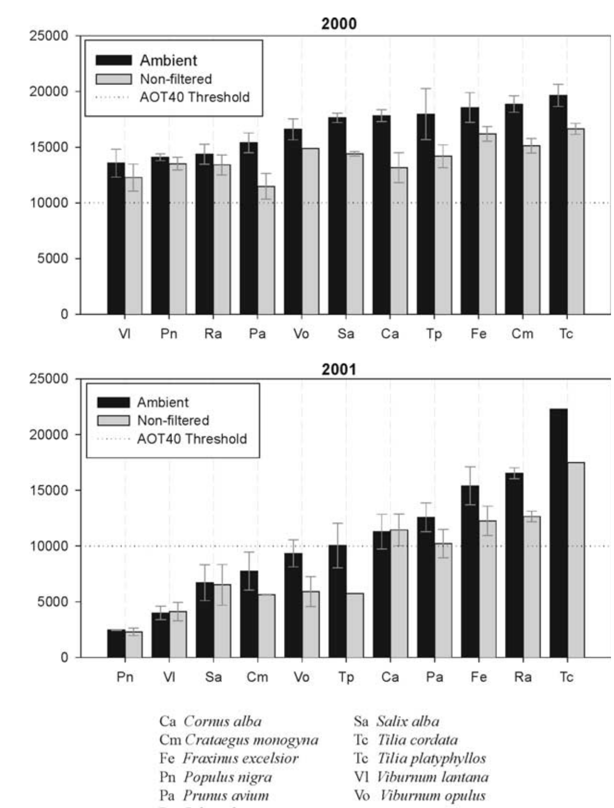

Fig. 4. Species-specific average AOT40 ozone thresholds (ppb.h) required to induce foliar injury for ambient and non-filtered air treatments, from 16 May to 22 August 2000 and 2 May to 19 August 2001. Lattecaldo Nursery, Canton Ticino, Switzerland.

visible symptom responses to ozone. In 2000, none of the species investigated were symptomatic at exposures below the standard (Fig. 4). In comparison, 5 of 12 species exhibited visible injury below the current standard in 2001, based on average injury onset dates. When comparing the date of the first symptom observed to the date of exceedance for the AOT40 critical level, we found that 9 of 12 species showed initial injury below an AOT40 of 10,000 ppbh. Of the symptomatic species, *P. nigra, V. lantana,* and *S. alba* appear to be the most sensitive as determined by the 2001 rankings (Fig. 4).

Plants of *P. nigra, V. lantana, F. excelsior*, and *P. avium* were among the most severely injured at the end of the 2001 season with total leaf area affected per plant (LAA) injury ratings approaching 25, 15, 15, and 7%, respectively (Figs. 5–8). *Tilia cordata* and *R. alpinum* exhibited initial symptoms late in the season and were among the least injured species at the conclusion of the experiment. Several species such *P. nigra, P. avium,* and *S. alba* fluctuated in their injury development as a result of new leaf and shoot growth. In 2001, foliar injury steadily increased throughout the season on *V. lantana, V. opulus,* and *T. platyphyllos* as cumulative ozone exposures increased. Other species such as *C. alba, F. excelsior, P. avium, and S. alba* showed distinct increases in injury toward the latter part of the research season. Nevertheless, all symptomatic species showed increased injury in response to seasonal cumulative AOT40 ozone exposures in 2000 and 2001.

Table 4
Degrees of freedom (d.f.), mean squares (MS), and *F* values (*F*) for the split-plot design of analysis of variance for AOT40 O_3 ppbh thresholds for the onset of visible foliar injury among 11 species grown in ambient, non-filtered, and filtered air treatments during 2000 and 2001 (Only symptomatic plants were included in the analysis.)

Source of variation		AOT40 O_3 (ppb.h)	
	d.f.	MS	F
2000			
Block (A)	3	2548981.0	0.35
O_3 Treatment (B)	2	855840620.0	129.05***
AB	6	6631900.0	0.91
Species (C)	10	26505785.9	11.50***
BC	14	9246356.7	4.01***
ABC	24	2304247.0	0.32
Error	77	7295858.0	
2001			
Block (A)	3	1378239.0	0.10
O_3 Treatment (B)	2	72620482.1	2.67
AB	6	27182180.0	1.89
Species (C)	10	249573978.0	12.78***
BC	13	9147532.0	0.47
ABC	21	19528024.0	1.35
Error	104	14415063.0	

***$P<0.001$.

4. Discussion and conclusions

Visible foliar symptoms observed during the current investigation were characteristic of ozone-induced injury and nearly identical to those previously identified during field surveys and OTC studies conducted in and around the Lattecaldo Nursery by Skelly et al. (1999) and VanderHeyden et al. (2001). Differences in timing of symptom expression, number of symptomatic plants per species, and progression of symptoms throughout the observation period were observed between the two seasons. Although exact leaf maturity dates for 2001 were not recorded, these dates were considerably earlier than leaf maturity dates in 2000, as observed during site set up in April 2001. Thus, there was most likely an earlier start of ozone uptake by the plants that coincided with the considerably earlier injury start dates in 2001 ranging from 25 May to 7 August as compared with 27 June to 15 August in 2000 (Table 3). This may also be a result of the plants having better-established root systems as well as more vigorous foliage in the 2001 season. Having well established and more vigorously growing plants supports the observation by VanderHeyden et al. (2001) that plant responses among seasons

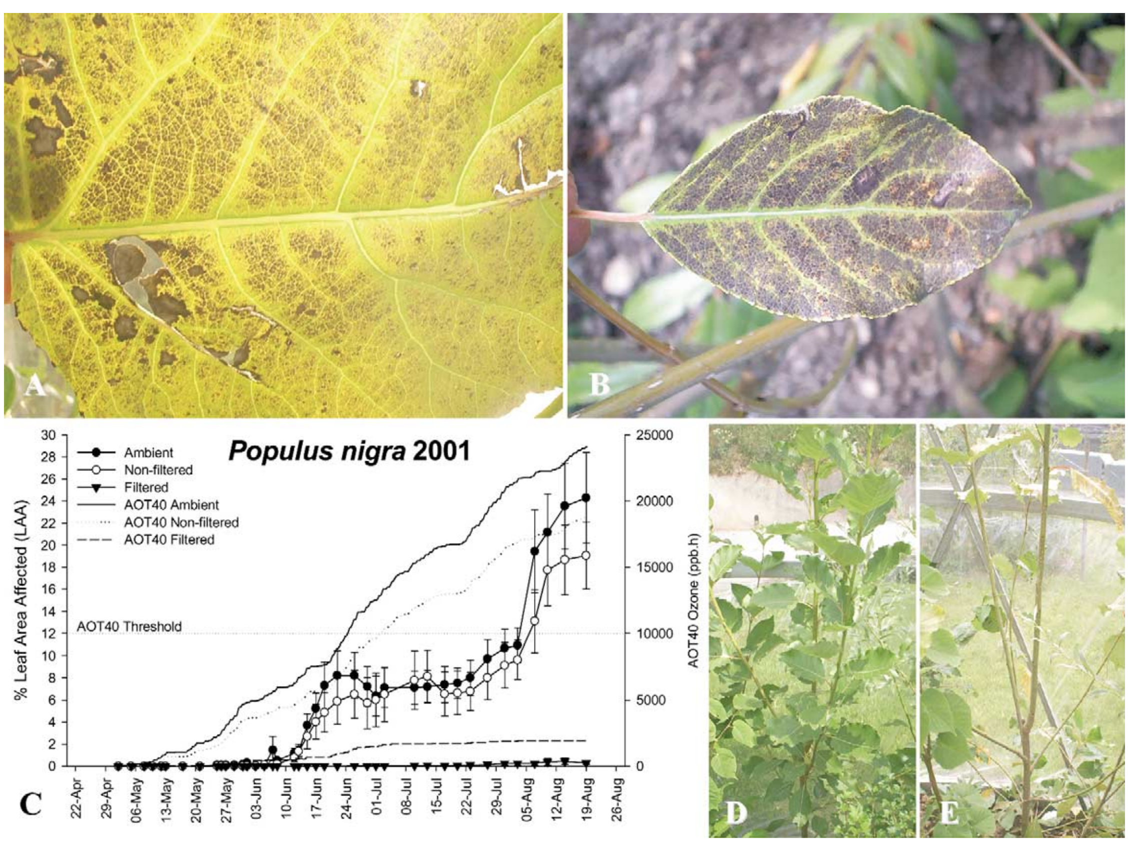

Fig. 5. *Populus nigra*: ozone-induced injury on upper leaf surface (A, B); the AOT40 ozone exposures and the % total leaf area symptomatic per plant (LAA) for plants grown in ambient, non-filtered and filtered air, 2 May to 19 August 2001 (C) and; full green leaf retention (D) and symptomatic older leaves and defoliation (E) for seedlings in the filtered air and non-filtered air chambers, respectively. Lattecaldo Nursery, Canton Ticino, Switzerland.

vary considerably due to transplant-shock involved with initial planting.

Variability in plant responses between 2000 and 2001 may also be explained by differences in ozone exposures. Fewer symptomatic plants were observed in the filtered air treatment in 2001 most likely due to an increased filtering efficiency following the replacement of the charcoal filters in the beginning of the 2001 observation period. Ozone peaks were considerably higher and the episodes were more pronounced during 2001 as compared with 2000 (Table 2). The distribution of peak ozone episodes appeared to be associated with meteorological conditions and driven mainly by sunlight and higher temperature intermixed with periods of precipitation. The 2000 summer season had relatively frequent episodes of elevated ozone exposure; however, cloudy and cool weather conditions with extended periods of heavy rain persisted throughout the latter portion of the summer season. As a result, symptom expression was likely suppressed for many species toward the end of the observation period. Several species such as *P. nigra, P. avium*, and *S. alba* showed a leveling or decreasing trend in injury rating during the last several rating periods as new growth occasionally surpassed the advance of symptoms on the older leaves. Similar trends in injury progression were observed in 2001 as a result of slightly lower ozone exposures in mid to late August. These observations support the findings of Reich (1987), who suggested that some species might produce additional leaves to compensate for effects of ozone exposure. In general, all symptomatic species showed increasing injury in response to increasing cumulative ozone exposures and therefore, exceeded the compensating growth of new leaves. In addition, several species such as *C. alba, F. excelsior, P. avium,* and *S. alba* showed distinct increases in injury following a high ozone episode in late July, during which peak ozone concentrations exceeded 130 ppb for several days, thus illustrating the influence of peak ozone episodes on symptom expression.

The type of symptoms observed also varied among and within species. All symptomatic plants observed in this study exhibited some form of upper leaf surface stippling; leaf discoloration, such as reddening, yellowing, or bronzing often occurred simultaneously with stippling. These symptoms were clearly shown on *P. nigra,* which simultaneously exhibited typical dark colored stippling, yellowing, and necrotic spotting throughout both summer seasons. The relative amount of each symptom increased in response to peak ozone

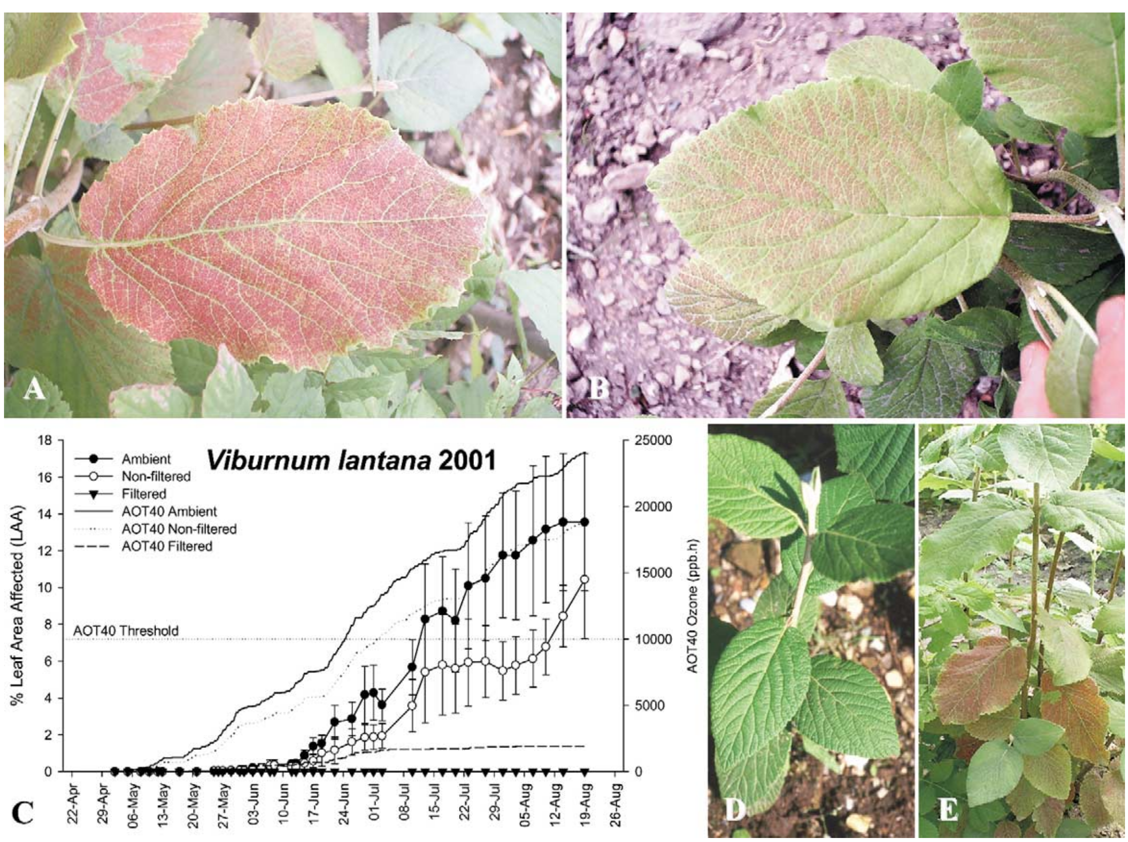

Fig. 6. *Viburnum lantana*: ozone-induced injury on upper leaf surface (A, B); the AOT40 ozone exposures and the % total leaf area symptomatic per plant (LAA) for plants grown in ambient, non-filtered and filtered air, 2 May to 19 August 2001 (C) and; full green leaf retention (D) and symptomatic older leaves and defoliation (E) for seedlings in the filtered air and non-filtered air chambers, respectively. Lattecaldo Nursery, Canton Ticino, Switzerland.

episodes and eventually led to early leaf abscission. Bortier et al. (2000), exposing *P. nigra* and *F. sylvatica* to ozone, described nearly identical symptoms on *P. nigra* with yellow discoloration occurring throughout the season from the time of first symptom expression. *Fagus sylvatica* also showed ozone symptoms comparable to those observed during previous studies at the Lattecaldo Nursery (VanderHeyden et al., 2001). Bortier et al. (2000) concluded that faster growing species, such as *P. nigra*, exhibit a greater sensitivity and response to ozone as compared with slower growing species such as European beech. Most of the faster growing species observed in the current study followed this trend, with *P. nigra* being the most notable example.

Significant levels of senescence and leaf abscission were observed on *P. nigra* and *S. alba*, with as much as 50% ozone-induced defoliation occurring during the 2001 growing season prior to the onset of natural leaf senescence. Plants growing in the filtered air treatment retained most of their leaves throughout the summer season, with only slight senescence and abscission occurring in late August.

Although symptoms typical of ozone exposures have been noted at the Lattecaldo nursery in previous years on wild type *Clematis* spp., no injury was observed within any of the open plots or non-filtered plots during the 2000 or 2001 seasons on the rooted cuttings selected for the study. It is important to note that a commercial variety was selected (rather than a field or wild type selection) and hence the lack of symptoms is most likely a result of a tolerant genotype.

Several genera and species investigated in the current study appear to be sensitive to ozone across a variety of regions in Switzerland, Italy, Spain, and southern Europe in general (Skelly et al., 1999); these genera include *Prunus, Viburnum, Salix, Populus, Fraxinus,* and *Cornus*. In addition, the more sensitive species within these genera could serve as bioindicators of ozone in the field. A variety of studies have investigated the response of *Populus* species to ozone. Ballach (1997) suggested that poplar clones exhibiting a range of ozone sensitivities might serve as bioindicators of ozone air pollution. Similarly, Bergmann et al. (2000) investigated the relative ozone sensitivities of 25 German native plants and also suggested the possibility of using ozone sensitive herbaceous species as biomonitoring systems in the field.

The relative species sensitivity rankings (Fig. 4) were based on average dates of initial injury per species; 5 out

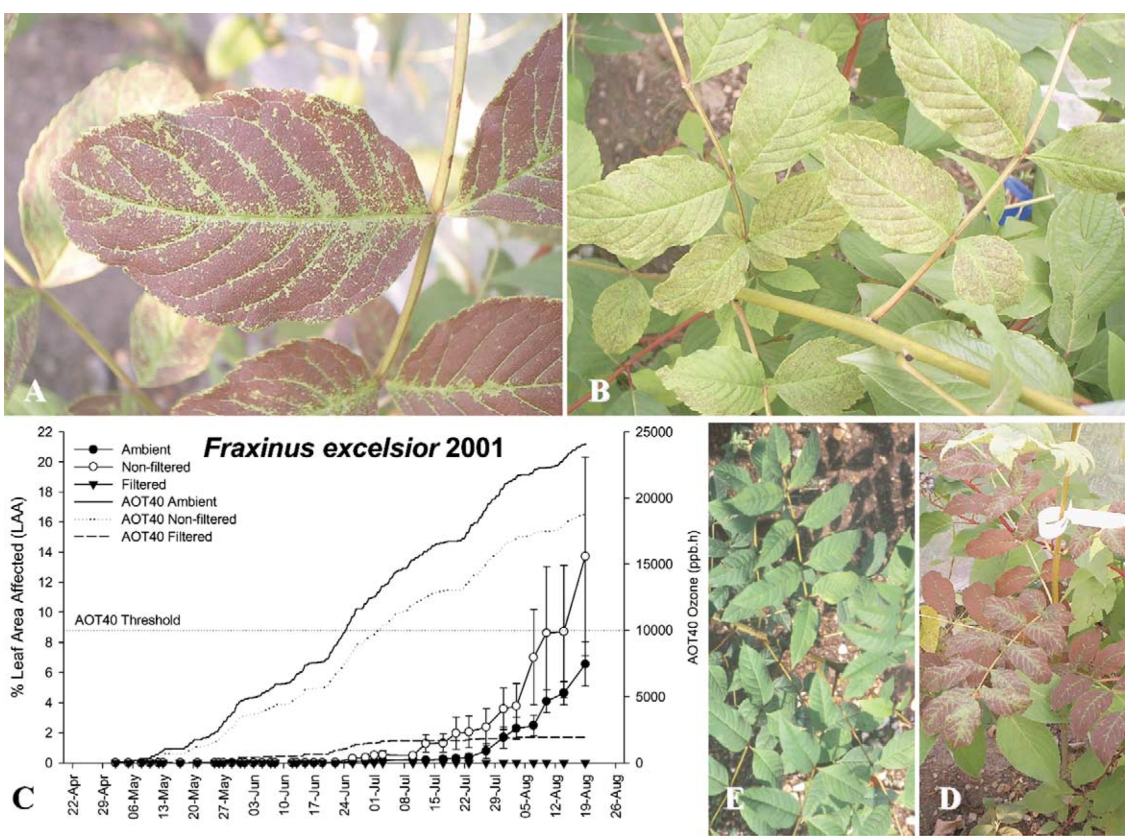

Fig. 7. *Fraxinus excelsior*: ozone-induced injury on upper leaf surface (A, B); the AOT40 ozone exposures and the % total leaf area symptomatic per plant (LAA) for plants grown in ambient, non-filtered and filtered air, 2 May to 19 August 2001 (C) and; full green leaf retention (D) and symptomatic older leaves and defoliation (E) for seedlings in the filtered air and non-filtered air chambers, respectively. Lattecaldo Nursery, Canton Ticino, Switzerland.

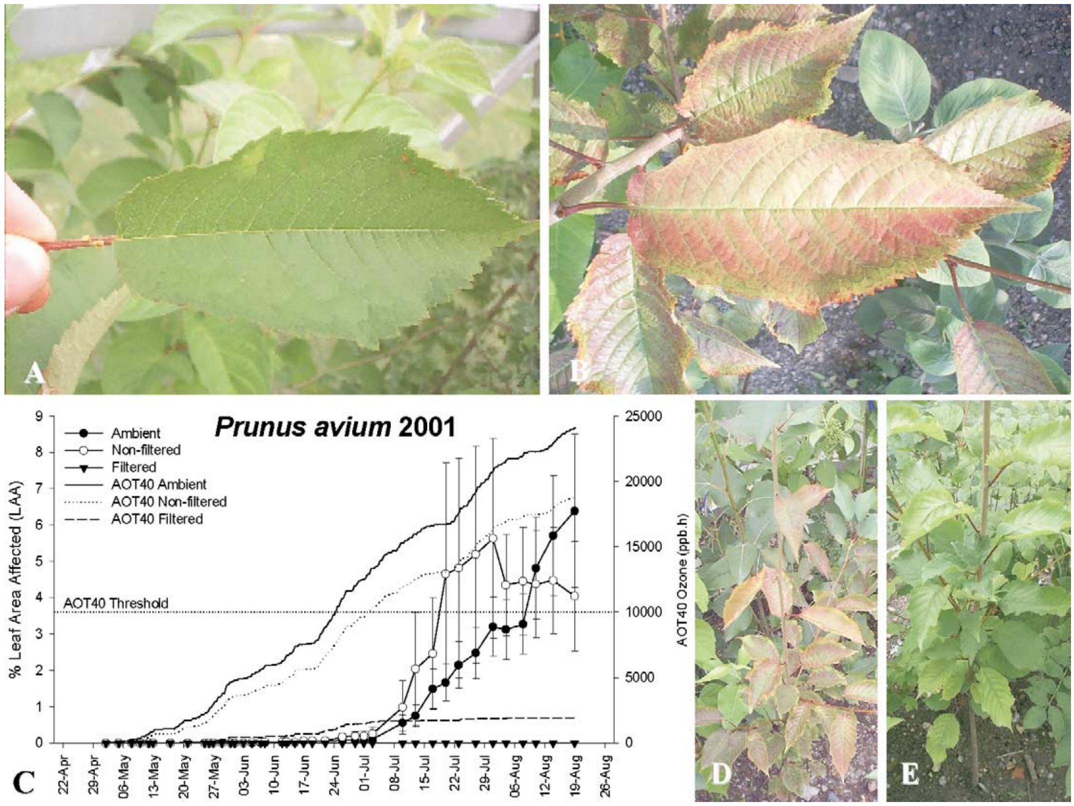

Fig. 8. *Prunus avium*: ozone-induced injury on upper leaf surface (A, B); the AOT40 ozone exposures and the % total leaf area symptomatic per plant (LAA) for plants grown in ambient, non-filtered and filtered air, 2 May to 19 August 2001 (C) and; full green leaf retention (D) and symptomatic older leaves and defoliation (E) for seedlings in the filtered air and non-filtered air chambers, respectively. Lattecaldo Nursery, Canton Ticino, Switzerland.

of 12 species were shown to be symptomatic below the current AOT40 critical level. In comparison, 9 out of 12 species were symptomatic below this critical level, based on the plant-specific date of initial injury (Table 3). To make an accurate assessment of the relative sensitivity of a species, it becomes necessary to determine whether injury onset as averaged for each individual species or injury onset as observed on a plant-by-plant basis (within species) is more relevant. Most species also showed lower AOT40 injury thresholds for non-filtered air plots across both seasons. Although no measurements were made that could explain this observation, we believe it may be a result of differing microclimatic conditions between the non-filtered chambers and open plots (Nussbaum and Fuhrer, 2000). VanderHeyden et al. (2001) observed similar trends at the Lattecaldo site in 1997 and 1998.

The difficulty in assessing the sensitivity of species further emphasizes the species-specific nature of plant responses to ozone exposure, which is primarily determined by uptake of ozone through the stomata (Reich, 1987; Wieser et al., 2000; Zhang et al., 2001; Schaub, 2001). Many environmental factors such as relative humidity, temperature, light, and soil moisture affect stomatal conductance and therefore affect the overall sensitivity of plants to ozone. The current critical level to protect forest vegetation is expressed as AOT40 10 ppmh (the amount of ozone accumulated over a threshold of 40 ppb) during daylight hours when global radiation exceeds 50 Wm^{-2}, over a 6-month growing season. The current critical level is based on the Level I approach, which largely ignores the environmental factors that determine the impacts of ozone on a plant (Fuhrer et al., 1997; Fuhrer, 2000). Therefore, to accurately determine the critical level required to protect vegetation from harmful effects of ozone, a transition needs to be made from a simplified Level I approach, on which the current AOT40 critical level is based, to a more inclusive and ecologically meaningful flux-based Level II approach (Fuhrer et al., 1997, Fuhrer, 2000; Grünhage et al., 2001).

The current study was successful in validating ozone-induced foliar injury on 11 of 12 species investigated in 2000 and 2001. These results add to the ongoing list of plant species showing confirmed visible foliar injury as a result of ambient ozone exposures in southern Switzerland (Skelly et al., 1998, 1999; VanderHeyden, 1999; VanderHeyden et al., 2001; Innes et al., 2001). Furthermore, these results can serve as an important tool for further identification and evaluation of ozone symptomatic species for an assessment of ozone risk across European forest ecosystems.

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