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An international cooperative programme indicates the widespread occurrence of ozone injury on crops

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Abstract

The UN/ECE ICP-Vegetation[1] routinely investigates the effects of ambient ozone pollution on crops throughout Europe. Each year, a series of co-ordinated ambient air experiments are conducted over a large area of Europe and a range of crop species are observed for the occurrence of injury following ozone episodes. In 1995 and 1996, ozone injury was observed at sites throughout Europe from United Kingdom (Nottingham) to the Russian Federation (Moscow) and from Sweden (Östad) to Italy (Naples). The only site participating in the ICP-Vegetation where it was not observed was that at Finland (Jokioinen). Injury was identified on subterranean and white clover, French bean, soybean, tomato, and watermelon at one or more sites. Injury was also detected in gardens and on crops growing in commercial fields. Two short-term critical levels which incorporate ozone dose and air saturation vapour pressure deficit (VPD) were derived from the 1995 data. These were (i) an AOT40[2] of 200 ppb.h over 5 days when mean VPD (0930–1630 h) is below 1.5 kPa and (ii) an AOT40 of 500 ppb.h over 5 days when mean VPD (0930–1630 h) is above 1.5 kPa. In general, the 1996 data supported these critical levels although injury did occur on two occasions when the AOT40 was less than 50 ppb.h, and the VPD was less than 0.6 kPa. Thus, ICP-Vegetation experiments have shown that ozone injury can occur over much of Europe and that plants are most at risk in conditions of high atmospheric humidity.

Keywords: Critical levels; Crops; Injury; Ozone; Europe

1. Introduction

Over recent decades, photochemical ozone pollution has been identified as a potential threat to crops in rural areas (Photochemical Oxidants Review Group, PORG, 1997). This secondary pollutant forms from a chemical reaction involving oxides of nitrogen and volatile organic compounds (mainly from

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[1] United Nations Economic Commission for Europe International Cooperative Programme on effects of air pollution and other stresses on crops and non-wood plants (formerly ICP-Crops).

[2] The sum of the difference between the hourly concentration (ppb) and 40 ppb when the concentration exceeds 40 ppb for the hours when global radiation (GR) exceeds 50 $\mathrm{Wm^{-2}}$.

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vehicle exhaust fumes and industrial processes) in the presence of sunlight. Annual average concentrations of ozone have doubled during the last 100 years (Voltz and Kley, 1988). Ozone episodes, where the concentration exceeds 60 ppb (parts per billion) by volume commonly occur during the spring and summer when crops are actively growing (PORG, 1997). The main entry route into plants is via the stomata; ozone subsequently reacts with cell wall and membrane components (Kangasjärvi et al., 1994) resulting in the formation of reduced oxygen species such as hydroxyl and superoxide radicals and hydrogen peroxide which are highly reactive with biological molecules. Consequently, membrane integrity is disturbed, thus modifying cell permeability (Heath, 1987) and osmotic pressure, membrane potentials and the activity of membrane-bound enzymes such as ATPases are affected (Dominy and Heath, 1985). Membrane disruption, leading to cell death, causes chlorotic flecking, necrosis and bronzing on foliage. This has important implications for crops grown for their foliage as symptoms of visible injury may affect market value. In addition to causing visible injury, ozone exposure can modify physiological processes such as carbon dioxide assimilation which influences plant productivity. In 1983, a European Open-Top Chamber programme was initiated to establish if ambient concentrations of atmospheric pollutants in Europe were sufficient to cause loss of crop yield or induce visible injury (CEC, 1993). The majority of studies using *Triticum aestivum* (spring wheat) showed that yield was reduced with increasing levels of ozone indicating that ambient ozone concentrations in Europe were sufficient to reduce the grain yield of this crop (Skärby et al., 1993).

As with other major air pollutants, such as sulphur dioxide, the precursors of ozone can be transported over long distances and thus present a transboundary air pollution problem. In 1983, the United Nations/Economic Commission for Europe Convention on Long-Range Transboundary Air Pollution (UN/ECE LRTAP Convention) came into force to address this problem and an effects-orientated approach was adopted for establishing successful pollutant abatement policy (UN/ECE, 1996a). This included establishing critical levels for sulphur, reduced/oxidised nitrogen and ozone i.e. the concentrations of pollutants in the atmosphere above which direct adverse effects on receptors, such as plants, ecosystems or materials may occur (Bull, 1991). Five International Cooperative Programmes (ICPs) and a Mapping Programme operate under the Convention’s Working Group on Effects to assess and monitor air pollution effects.

The UN/ECE ICP-Vegetation (International Cooperative Programme on effects of air pollution and other stresses on crops and non-wood plants) came into force in 1988 and involves research in 15 European countries. The programme aims to establish the crop and non-wood plant species at risk from ozone pollution by determining those species which show symptoms of visible injury and/or a reduction in biomass or yield following exposure to ambient ozone. This information, in conjunction with known ambient air ozone concentrations and climatic conditions, can be used to establish critical levels for ozone and to identify areas of Europe at risk from ozone pollution.

Two short-term critical levels of ozone for visible injury were set at the UN/ECE Workshop on Critical Levels for Ozone in Europe-Testing and Finalising the Concepts (Kuopio, Finland, 1996) following analysis of the 1995 ICP-Vegetation experimental data (Benton et al., 1996). These were an AOT40 of 200 ppb.h accumulated over 5 days when mean VPD (0930–1630 h) was less than 1.5 kPa, and an AOT40 of 500 ppb.h when mean VPD exceeded 1.5 kPa. The ozone dose parameter, AOT40, is calculated as the sum of the difference between the hourly concentration (ppb) and 40 ppb (when the concentration exceeds 40 ppb) for the hours when global radiation (GR) exceeds 50 $\mathrm{W m^{-2}}$. AOT40 is used because it enables data from different experiments to be combined, and it allows for a linear relationship between ozone exposure and yield and it considers the effects of cumulative exposures to concentrations above a cut-off of 40 ppb (Fuhrer and Achermann, 1994). Concentrations below this cut-off value are strongly influenced by natural background processes in the northern hemisphere (UN/ECE, 1996a).

Vapour pressure deficit was included in the definition for the short-term critical levels because it is one of the main factors influencing stomatal conductance and thus the flux of ozone into the plant. For example, when VPD is high, conductance is reduced (Grantz and Meinzer, 1990) and the flux of ozone into the leaf is restricted. In these conditions, higher ambient ozone concentrations (or a higher AOT40) are required before an adequate dose is absorbed to cause injury. However, when VPD is lower, stomatal conductance increases which boosts the flux of ozone into the leaf. Thus, it is possible that the absorbed dose is sufficient to cause injury even though ambient ozone concentrations are low. Indeed, it has been suggested that higher yield reductions or injury levels occur when ozone concentrations are moderate (Grünhage and Jäger, 1994; Krupa et al., 1995) because high ozone concentrations often coincide with conditions which are not conducive to ozone uptake, for example, high VPD. Gimeno et al. (1995) described how ozone injury on tobacco cultivars in Spain was more prevalent in coastal areas but decreased at sites further inland. It was suggested that the high relative humidity at the coastal sites could favour ozone phytotoxicity. Similarly, Balls et al. (1996) reported how the level of injury observed on subterranean clover increased as VPD decreased and attributed this to an influence on stomatal conductance.

This paper describes the ozone concentrations in 1995 and 1996 across the ICP-vegetation experimental sites and the geographical extent of visible injury on selected species grown throughout Europe. The crops were chosen because of their commercial importance in Europe, and because previous studies with white clover (Horsman et al., 1981; Becker et al., 1989), subterranean clover (Horsman et al., 1981), bean (Tonneijck, 1983; Guzy and Heath, 1993), watermelon (Fernandez-Bayon et al., 1993), tomato (Lorenzini et al., 1984) and soybean (Salleras et al., 1989) had shown that these species were ozone-sensitive. The data from the 1996 experiments have been used to validate the short-term critical levels for visible injury that were set at the Kuopio Workshop. ICP-Vegetation experiments that monitored the effects of ozone on biomass are reported by Ball et al. (1998).

2. Materials and methods

2.1. Experimental sites and crop species

In 1995 and 1996, experiments designed to investigate the onset of ozone-induced visible injury were conducted at sites in 15 countries across Europe (UN/ECE, 1995; UN/ECE, 1996b). Table 1 gives details of the sites involved and species grown are shown in. The most northerly and southerly sites were Finland-Jokioinen (60°47′N) and Italy-Naples (40°48′N), respectively. Participation in the programme is on a voluntary basis. Hence there has been no effort to influence the location of sites, nor their representation of ozone conditions in Europe. Nevertheless, the spread of the sites across Europe has allowed for plant responses to a wide range of ozone and physical climatic conditions to be determined.

At each experimental site, one or more of the following crop species were grown: French bean (*Phaseolus vulgaris* L. cv. ‘Lit’ and ‘Groffy’); subterranean and white clover (*Trifolium subterraneum* L. cv. ‘Geraldton’ and *T. repens* L. cv. ‘Menna’); tomato (*Lycopersicon esculentum* Miller. cv. ‘Tiny Tim’); soybean *(Glycine max* (L.) Merr. cv. ‘Ceresia’) and watermelon (*Citrullus lanatus* (Thunb.) Matsum. and Nakai cv. ‘Sugar baby’). The seeds were purchased from commercial suppliers in the UK (French bean, subterranean and white clover), Spain (tomato and watermelon), and Austria (soybean), and then distributed to all participants in the programme.

2.2. Cultivation

2.2.1. Subterranean and white clover

Twenty 15 cm diameter plastic plant pots were filled with an appropriate soil mixture for the site (typically a mixture of peat, sand and soil). Two 20 cm glass-fibre wicks (Vitrulan Textilglas GmBH, Bayern, Germany) were inserted vertically into the growing medium of each pot. One end of each wick protruded through a hole in the pot base and the other end was 2 cm below the soil surface. Nine clover seeds were sown in each pot to a depth of 0.5 cm with the soil depth being 1 cm below the pot rim. Seedlings were raised in a glasshouse, thinned to three per pot when the first leaf had emerged (approximately 14 day after sowing) and placed outside in ambient conditions once the first trifoliate leaf had unfolded. Pots were placed 25 cm apart in trenches (to reduce the influence of temperature) with the pot rim being 5 cm above ground level. The pots were positioned on a second pot containing water in which the protruding wicks were placed. This formed a self-watering system. The experiment was repeated twice at each site in each year. Typically,

Table 1
The location of ICP-vegetation experimental sites and crop species grown at each site in 1995 and/or 1996

Country-site	Altitude (m.a.s.l)[a]	Coordinates	Crop species grown
Austria (Seibersdorf)	190	47°58′N, 16°30′E	Subterranean clover, white clover, bean, soybean
Belgium (Tervuren)	80	50°49′N, 04°31′E	Subterranean clover, white clover, bean soybean
Finland (Jokioinen)	100	60°47′N, 23°28′E	White clover
France (Pau)	120	43°18′N, 00°22′W	White clover, bean
Germany (Braunschweig)	85	52°15′N, 10°30′E	White clover, bean
Germany (Essen)	60	51°22′N, 06°55′E	White clover
Germany (Trier)	129	49°46′N, 06°39′E	White clover
Germany (Giessen)	190	50°32′N, 08°41′E	White clover
Hungary (Keszthely)	n.a.[b]	46°47′N, 17°16′E	Bean
Italy (Milan)	120	45°28′N, 09°12′E	White clover, soybean
Italy (Rome)	n.a.	41°53′N, 12°03′E	White clover, bean
Italy (Naples)	20	40°48′N, 14°20′E	Bean
Netherlands (Westmaas)	−0.5	51°47′N, 04°27′E	Subterranean clover and bean
Netherlands (Schipluiden)	−2.0	51°59′N, 04°16′E	Sbterranean clover and bean
Netherlands (Zegveld)	−1.5	52°08′N, 04°50′E	Subterranean clover and bean
Netherlands (Wageningen)	7.0	51°58′N, 05°38′E	Subterranean clover and bean
Poland (Kornik)	n.a.	52°15′N, 17°06′E	White clover, bean
Russian Federation Moscow	n.a.	55°45′N, 37°42′E	Subterranean and white clover, bean
Slovenia (Ljubljana)	250	46°03′N, 14°30′E	White clover, bean
Slovenia (Kovk)	780	46°08′N, 15°06′E	White clover, bean
Slovenia (Zavodnje)	800	46°25′N, 15°01′E	White clover
Spain (Ebro Delta)	0	40°75′N, 00°45′E	Tomato, bean, watermelon
Spain (Pamplona)	n.a.	42°49′N, 01°39′W	White clover, bean
Spain (Begur)	190	41°55′N, 03°15′E	Bean
Spain (Veciana)	725	41°40′N, 01°30′E	Bean
Sweden (Östad)	60	57°54′N, 12°24′E	Subterranean and white clover
Switzerland (Cadenazzo)	200	46°10′N, 08°56′E	Subterranean, white clover, bean, soybean
UK (Nottingham)	47	52°53′N, 01°11′W	Subterranean and white clover, bean

[a] m.a.s.l.: Metres above sea level.
[b] n.a.: Not available.

exposure of the first set of plants started in mid-June and the second set of plants a month later. At 28 and 56 days after exposure to ambient air, the dry weight of leaves/petioles and flowers from each plant was determined and at 84 days, the entire above-ground biomass (including stolons) for each pot was determined (data not presented).

2.2.2. *French bean cv. Lit and Groffy, and soybean*

Twenty (for bean experiment) and 10 (for soybean) 20 cm diameter plastic plant pots were filled with soil mixture and three 30 cm wicks inserted as described above. Four bean or soybean seeds were sown in each pot to a depth of 2 cm and raised in the glasshouse. The seedlings were thinned to one per pot and placed in ambient conditions when the first true leaf had unfolded. The pots were placed in holes and the plants watered by the self-watering system already described.

2.2.3. *Experiments at the Ebro Delta (North East Spain)*

French bean cv. Lit, tomato and watermelon were grown using a modified experimental protocol described by Gimeno et al. (1996).

2.3. *Assessment of ozone injury*

Photographs of injury on all species were distributed to participants (UN/ECE, 1994; Sanders and Benton, 1995) in order to confirm that the symptoms recorded were those of ozone injury.

2.3.1. Subterranean and white clover

Plants were observed daily and the date on which injury was observed for the first time before each harvest was recorded. Injury was always seen on the upper surface and appeared as chlorotic flecking on white clover but as necrotic lesions on subterranean clover. The plants were also assessed at 28, 56 and 84 days after exposure to ambient conditions when the total number of leaves and the number of ozone injured leaves per pot were determined.

2.3.2. French bean cv. Lit and Groffy, and soybean

Both species were observed daily for the appearance of ozone injury. Typical symptoms included necrosis and bronzing on the upper leaf surface. Bean plants were also assessed when 50% of plants had flowers (flowering) and when the skin of the pods was flat (green harvest) for the number of ozone injured leaves and the total number of leaves per plant. Assessments made at the Ebro Delta site in Spain of French bean, tomato and watermelon are described by Gimeno et al. (1996).

2.3.3. Surveys of gardens/commercial fields

Nearby gardens and commercial fields of crops were surveyed for visible injury after injury had been detected on the experimental plants at the field sites at Belgium (Tervuren), France (Pau), Spain (Ebro Delta), and Switzerland (Cadenazzo).

2.3.4. Measurement of ambient ozone concentrations and climatic conditions

Ambient ozone concentrations and climatic conditions were measured at the sites throughout the experimental season. Ozone concentrations were measured at a height of 3 m by UV photometric methods; standard calibration procedures were used at each site. The concentrations presented in this paper are expressed as a mean daily maximum (ppb) and as AOT40. The AOT40 for the 5 days preceding the onset of visible injury on white clover was calculated for data obtained in both 1995 and 1996. The climatic parameters measured during the experimental season included mean daily temperature (°C), percent relative humidity (RH) and global radiation (GR; $\mathrm{Wm^{-2}}$). The 7 h mean (0930–1630 h, GMT) air saturation vapour pressure deficit (VPD) for the 5 day period preceding injury was calculated from these data.

3. Results

3.1. Pollution climate and the occurrence of visible injury

The range of ambient ozone concentrations experienced across Europe was demonstrated by the data recorded at experimental sites in 1995 (Table 2). For example, at Finland (Jokioinen) and Sweden (Östad) the mean daily maximum ozone concentrations were 35 and 41 ppb, respectively whereas at sites in central Europe, the mean daily maximum ozone concentrations were typically between 50 and 60 ppb for the same period. Concentrations were even higher in the southern European sites (Austria, Italy and Switzerland) where the mean daily maximum ozone concentrations were 64, 68 and 72 ppb, respectively. Variation in the mean daily maximum ozone concentration was reflected in the AOT40 values for each site with the total AOT40 for the period of June 21st to Sept 2nd was 290 ppb.h at Finland (Jokioinen), whereas at Switzerland (Cadenazzo) it was 15665 ppb.h. The cut-off value of 40 ppb was exceeded on only 15 days at the site in Finland but at sites in France and Switzerland, 40 ppb was exceeded on most days although the extent of exceedance was much greater in Switzerland than in France (Fig. 1). These data clearly indicate the variation in ozone pollution climate throughout Europe in 1995.

Table 2
Mean daily maximum ozone concentrations and AOT40 (for hours with GR $\geq 50\,\mathrm{Wm^{-2}}$) at ICP-vegetation experimental sites in Europe from 21st June to 2nd September 1995 inclusive. This represented the longest time period of ozone measurement common to all sites listed in this table

Country-site	Mean daily maximum (ppb)	Total AOT40 (ppb. h)
Austria (Seibersdorf)	64	12900
Belgium (Tervuren)	55	7988
Finland (Jokioinen)	35	290
France (Pau)	52	3893
Germany (Braunschweig)	57	8241
Italy (Milan)	68	11781
Netherlands (Westmaas)	57	7465
Slovenia (Zavodnje)	60	16800
Sweden (Östad)	41	1734
Switzerland (Cadenazzo)	72	15665
UK (Nottingham)	67	8842

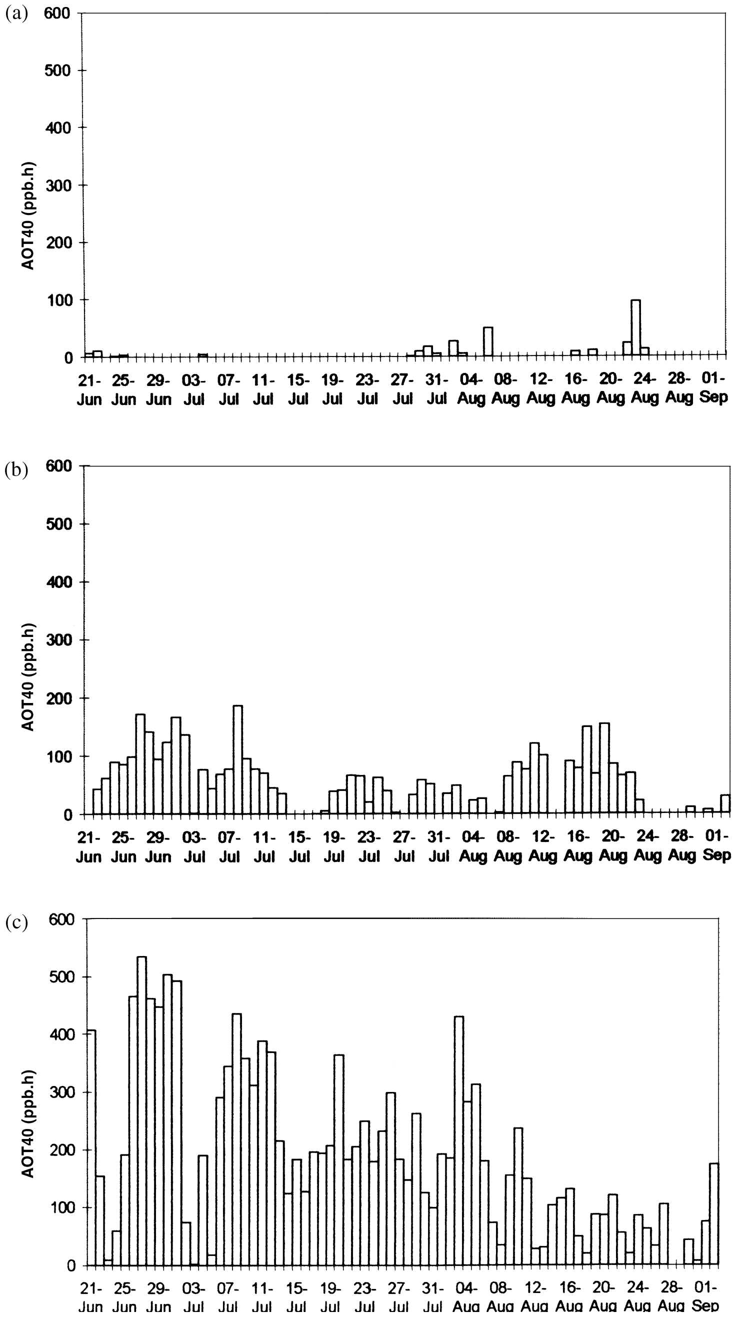

Fig. 1. Daily AOT40 values (ppb.h) for ozone (for hours with $GR \geq 50\,Wm^{-2}$) at ICP-vegetation experimental sites from 21st June to 2nd September 1995 (inclusive) in (a) Finland (b) France and (c) Switzerland. This represented the longest time period of ozone measurement common to all sites listed in Table 2.

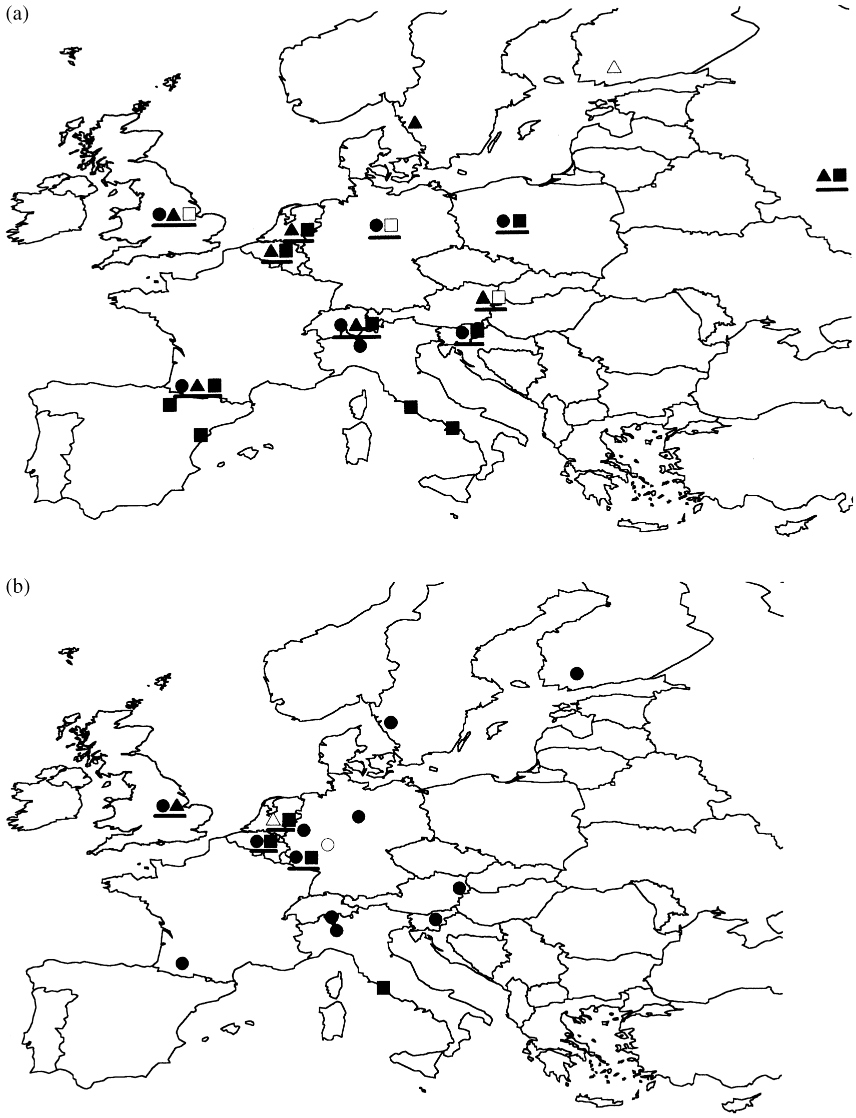

Fig. 2. Ozone injury at ICP-vegetation experimental sites in (a) 1995 and (b) 1996. Key to symbols: (●) injury on white clover, (○) white clover grown, but injury not detected, (▲) injury on subterranean clover, (△) subterranean clover grown, but injury not detected, (■) injury on French bean cv. Lit, (□) French bean grown, but injury not detected. Where more than one symbol is present at a site, the symbols are underlined to avoid confusion with symbols for adjacent sites. The national boundaries shown on the maps do not reflect the official view of the United Nations or any subsidiary body.

Injury occurred on clover species at all sites throughout Europe in 1995 and 1996 with the exception of the site at Finland (Jokioinen) in 1995 (Fig. 2). Table 3 shows the extent of ozone injury to white and subterranean plants recorded at the first and second harvest in 1995. The highest levels of injury (>10% of injured leaves per pot) were seen at sites in Austria, Belgium, Germany, the Netherlands, Poland, Sweden and Switzerland, whereas lower injury levels (<10% of injured leaves per pot) were recorded at sites in France, Italy, The Russian Federation, Slovenia and the United Kingdom. The mean total leaf number per

Table 3
The percentage of ozone injured leaves at harvest 1 and 2 on white and subterranean clover grown throughout Europe in 1995 (values are a mean per pot ± SD where $n = 20$ and are from experiment 1). AOT40 calculated for hours with GR ≥ 50 $\mathrm{W m^{-2}}$

Country-site	Date harvest 1[a] Date harvest 2	Total number of leaves	Number of ozone injured leaves	% injured leaves	AOT40[b] (ppb.h)
White clover					
France (Pau)	3 July	61.6 ± 15	1.6 ± 1.8	2.6	n.a.[c]
	31 July	96.4 ± 30.5	9.0 ± 6.2	9.3	n.a.
Germany (Braunschweig)	21 July	106.1 ± 12.6	15.5 ± 10.2	15	2889
	16 August	226.7 ± 60.9	6.5 ± 9.2	2.9	3307
Italy (Milan)	5 July	52 ± 19.6	0.05 ± 0.2	0.1	4943
	7 August	47.5 ± 22.8	3.8 ± 5.1	7.9	6848
Poland (Kornik)	11 July	52.5 ± 12	5.5 ± 4.6	10.5	n.a.
Slovenia (Zavodnje)	10 July	100.8 ± 4.2	4.2 ± 3.8	4.2	n.a.
	2 August	384.9 ± 97.1	18.9 ± 11.2	4.9	n.a.
UK (Nottingham)	24 July	38.1 ± 8.1	0	0	1645
	21 August	45.4 ± 8.4	0.06	0.1	5764
Subterranean clover					
Austria (Seibersdorf)	5 July	90.3 ± 15.1	0.16 ± 0.5	0.18	3568
	31 July	287.3 ± 105.3	66 ± 32.9	23	6325
Belgium (Tervuren)	13 July	86.3 ± 12.1	12.4 ± 9.6	14.4	1997
	10 August	217.2 ± 43	63.5 ± 21.9	29.2	4062
Netherlands (Westmaas)	18 July	173.7 ± 34.1	40.4 ± 15.3	23.3	2662
	15 August	177.6 ± 35	27.6 ± 7.9	15.5	5690
Netherlands (Schipluiden)	15 August	152.5 ± 23.2	47.4 ± 14.1	31.1	4067
	12 September	302 ± 40.4	46.3 ± 6.7	15.3	1391
Netherlands (Wageningen)	18 July	168 ± 19.7	34.9 ± 12.8	20.8	3558
	15 August	185.1 ± 31.1	22.4 ± 10.8	12.1	4770
Netherlands (Zegveld)	18 July	190.3 ± 16.2	28.2 ± 14.3	14.8	2270
	15 August	211.2 ± 31.7	40.5 ± 12.3	19.2	3649
Russian Federation (Moscow)	11 August	13.9 ± 1	0.4 ± 0.7	2.5	n.a.
	8 September	37 ± 4	0.5 ± 0.7	1	n.a.
Sweden (Östad)	19 July	67.3 ± 14.2	1.3 ± 3.1	1.9	378
	16 August	146.9 ± 36.6	27.7 ± 13	18.8	954
Switzerland (Cadenazzo)	6 July	139 ± 21	62 ± 12	44.6	6564
	10 August	209 ± 83	66 ± 37	31.6	8335

[a] Harvest 1–28 day after exposure; harvest 2–56 day after exposure to ambient conditions.
[b] AOT40 for 28 day period.
[c] n.a.: Data not available.

pot differed considerably among sites and could be attributed to climatic variation. For example, at the second harvest at Germany (Braunschweig) and Italy (Milan), the mean number of leaves per pot of white clover was approximately 227 and 48, respectively. Injury was also observed on the leaves of French bean cv. Lit and Groffy (Table 4) at both the flowering and green harvest growth stages. However, injury to this species was not as widespread as injury to clover species (Fig. 2). For example, injury was not observed on bean at either Germany (Braunschweig) or the United Kingdom (Nottingham) in 1995. Highest levels of injury (>10% of leaves injured) were recorded at sites at Austria (Seibersdorf), Belgium (Tervuren), France (Pau), The Netherlands (Schipluiden), Poland (Kornik) and Slovenia (Kovk). Injury was also seen on soybean at Switzerland (Cadenazzo) and Austria (Seibersdorf), and on bean, tomato and watermelon at the Ebro Delta site in Spain (Gimeno et al., 1995). Ozone injury was observed during and after flowering on beans and when the flowers were already formed on watermelon. Injury was also recorded on plants

Table 4
The number of ozone injured leaves on bean cv. Lit and cv. Groffy plants grown throughout Europe in 1995 (values are a mean per plant ± SD where $n = 20$)

Country-site	Growth stage	Total number of leaves	Number of ozone injured leaves	%
Austria (Seibersdorf)	Fl[b]	16.9 ± 2.1	1.7 ± 0.7	10.1
	GH[c]	18.5 ± 4	3.7 ± 1.4	19.7
Belgium (Tervuren)	FL	22.8 ± 3.3	2.8 ± 0.7	12.1
	GH	33.8 ± 5	14 ± 4.1	41.5
France (Pau)	GH	19 ± 3	2.9 ± 1.3	15
Hungary (Keszthely[a])	FL	13.7	4.2	30.6
Italy (Naples[a])	FL	17.1 ± 3.6	1.8 ± 1.5	10.51
Italy (Rome)	FL	8.9 ± 1.6	0.2 ± 0.4	2.3
Netherlands (Westmass)	GH	28.2 ± 4.8	1.4 ± 2.3	4.6
Netherlands (Wageningen)	GH	30.6 ± 3.7	1.4 ± 1.8	4.6
Netherlands (Schipluiden)	GH	28.3 ± 2.3	5.2 ± 4.2	18.3
Netherlands (Zegveld)	GH	30.1 ± 2.4	2.1 ± 1.7	6.6
Poland (Kornik)	FL	13 ± 2	2.6 ± 0.9	20
Russian Federation (Moscow)	FL	4.4 ± 0.6	0.3 ± 0.4	5.8
Slovenia (Kovk)	FL	14.8 ± 4.1	4.8 ± 1.8	32.5
United Kingdom	FL	24.5 ± 5.1	0	0

[a] Groffy.
[b] FL: Flowering (when 50% of plants have flowers).
[c] GH: Green harvest (when pods are still flat).

grown in gardens and commercial fields in addition to those grown for experimental purposes (Table 5). For example in 1995 and 1996, injury was recorded on watermelon, soybean, French bean, potato, maize, wheat and butter bean.

The differences in ozone concentrations at each site were reflected to a certain extent in the occurrence and amount of visible injury. For example, injury did not occur on the subterranean clover grown for ICP-Vegetation experiments at Finland (Jokioinen) in 1995 where ozone concentrations were lower than in the rest of Europe (Fig. 2, Tables 2 and 3). The high ozone concentrations at Switzerland (Cadenazzo) injured 45 and 32% of subterranean clover leaves per pot (Table 3). The AOT40 values for the 28 day period before each harvest were 6564 and 8335 ppb.h, respectively. However, at Sweden (Östad), where the ozone concentrations were lower, 19% of subterranean clover leaves were injured at the second harvest following a 28 day period of 954 ppb.h and in Italy (Milan), where the AOT40 for 28 day was 6848 ppb.h, injury at the second harvest was approximately 7.9%. This indicated that there was not a definite relationship between long-term exposure and the extent of injury development.

Table 5
Crops which showed ozone injury in gardens/commercial fields in 1995 and 1996

Crop	1995	1996	Country
Bean (*Phaseolus vulgaris*)	√		Belgium (Tervuren)
Soybean (*Glycine max*)	√		France (Pau)
Watermelon (*Citrullus lanatus*)	√	√	Spain (Ebro Delta)
Potato (*Solanum tuberosum*)	√	√	Belgium (Tervuren)
	√	√	Switzerland
Maize (*Zea mays*)	√		Belgium (Tervuren)
Wheat (*Triticum aestivum*)		√	Belgium (Tervuren)
Butter bean (*Phaseolus lunatus*)		√	Belgium (Tervuren)

3.2. Validation of the short-term critical level of ozone for visible injury

The short-term critical levels defined in the introduction to this paper were set from the 1995 data shown in Fig. 3. Because of the episodic nature of ozone pollution, AOT40 and mean VPD for the 5 days before injury expression proved to be the best fitting parameters (Benton et al., 1996). Plotting the 1996 data on the same axes showed that, with the exception of three outliers, the original critical levels held up to validation with data from a year in which VPD at the ICP-Vegetation sites was generally lower.

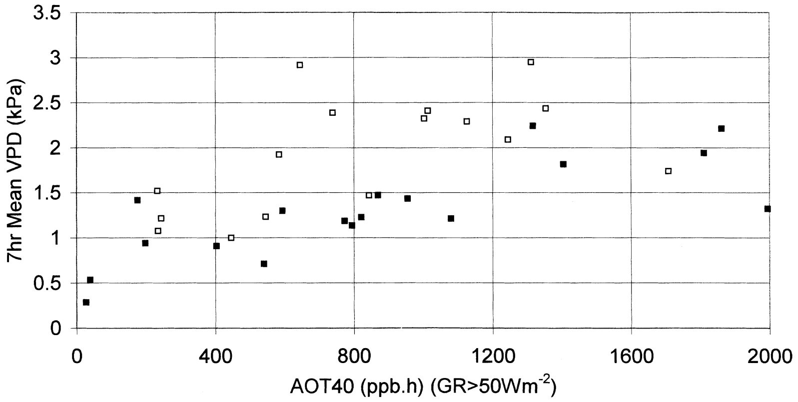

Fig. 3. The AOT40 (for hours with GR ≥ 50 Wm^{-2}) and the mean vapour pressure deficit (0930–1630 h) during the 5 days preceding the presence of injury on *Trifolium* species in 1995 (☐) and 1996 (■).

4. Discussion

Ozone concentrations differed at sites across Europe and were generally lower in the Scandinavian countries and higher in central and southern Europe in 1995 and 1996. ICP-Vegetation experiments showed that the ozone concentrations were sufficient to induce visible injury on two clover species and bean at the sites in southern and central Europe, and most of those in northern Europe. Injury was also detected on eight other species growing at or near some of the experimental sites. The only site where injury was not observed on any of the species monitored in either year, was that at Finland (Jokioinen) where ozone concentrations were lower than at any other site in the study. Thus, the ICP-Vegetation programme has documented the widespread occurrence of damaging ozone episodes in Europe, and has indicated that a range of crops are potentially at risk from ozone pollution.

The extent of injury to clover and French bean varied among sites and no clear relationship existed between ozone dose in the 28 day period before harvest and the extent of injury on clover at harvest. This may be because injury usually develops following short-term ozone exposure rather than after exposure to a long-term average concentration (Sanders et al., 1994, 1995). Pihl-Karlsson et al. (1995) showed that injury to subterranean clover was greater following a short period with high ozone than following a longer period with lower ozone concentration. However, Amiro et al. (1984) found that ozone concentration and length of ozone exposure were not sufficient to explain the onset of injury on *P. vulgaris*, and Tonneijck (1994) suggested that ozone-climate interactions were important.

The two critical levels for visible injury (defined in the introduction) were set from the 1995 data and included terms for VPD and ozone (Benton et al., 1996). In general, data from the 1996 ICP-vegetation experiments supported these critical levels although injury did occur on three occasions before the 5 day AOT40 exceeded 200 ppb.h, when VPD was below 1.5 kPa. Thus, it appears that when VPD is very low, injury can occur following a 5 day AOT40 as low as 27 ppb.h. Similar findings have been reported by Tonneijck and Van Dijk (1997) following studies with subterranean clover. Therefore, it may be necessary to refine these critical levels to address the occurrence of injury when VPDs are small. Other climatic factors that modify the outcome of ozone exposure may become important in these conditions. For example, temperature and solar radiation influence stomatal conductance, and windspeed, through an influence on the laminar boundary layer and atmospheric resistance determines the actual ozone dose available for absorption by the plant (Grünhage and Jäger, 1994, 1996).

Data from the ICP-Vegetation experiments have shown that injury occurs on a range of crop species throughout Europe following exposure to ambient ozone episodes. Injury is especially likely when ozone episodes coincide with periods of high atmospheric humidity, and can occur at relatively low ozone concentrations. Surveys of commercial fields have shown that injury also occurs on crops growing under normal agronomic practices. Thus, several crops are at risk from ozone pollution. The critical levels for visible injury based on 1995 ICP-Vegetation data held for 83% of incidences of injury in 1996. Thus, a combination of VPD and accumulated ozone dose provides a good indicator of the likelihood of injury. However, the presence of outliers suggests that the critical levels need further refinement, possibly by the inclusion of other climatic factors that modify ozone flux into the plant.

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