2.1.2. Dependence of ozone biomonitoring on meteorological conditions of different sites in Catalonia

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Summary

Evaluation of visual symptoms of ozone damage was conducted in the network of bioindicator rural stations of Catalonia (NE Spain) every 14 days from May to October. Damage rates of ozone (and consequently, ozone biomonitoring capacity of bioindicators) were found to vary highly in time and space depending on the local environmental and meteorological conditions. Lower ozone damage to foliage was produced when meteorological conditions favour stomata resistance. Modulatory effects of meteorological conditions need to be considered in biomonitoring and when modeling plant ozone doses and damage.

Key works: Biomonitoring, meteorology, ozone doses, tobacco plants.

Introduction

Ozone is a highly reactive gas whose increasing tropospheric concentrations may have significant toxic effects on plants and human health (McKee, 1994; Krupa, 1997). The most sensitive plant species are used as bioindicators of ozone concentration (Heggestad, 1991; Ribas et al., 1998). However, damage rates of ozone (and consequently, possible ozone biomonitoring capacity of bioindicators) may vary in time and space depending on the local environmental and meteorological conditions. In fact, higher ozone exposure is not always accompanied by higher symptomatology (Krupa et al., 1993). For example, many previous studies have shown that ozone uptake is controlled by stomata (Thorne and Hauson, 1972; Taylor and Hauson, 1992; Samuelson and Kelly, 1997), and many meteorological conditions such as high vapor pressure deficit (VPDs) favour stomata closing (Willmer and Fricker, 1996). These meteorological conditions are likely to decrease ozone uptake and damage to foliage (Peñuelas et al., 1995; Ribas et al., 1998; ICP Crops, 1997). We aimed to corroborate these possible time and space variations of ozone damage rate and their links with meteorological conditions. We studied plant ozone damage in Catalonia rural network of tobacco bioindicators, covering a large variety of meteorological and environmental conditions, from sea level to 1040 meters high, and from sea-shore to 250 km in-land.

Materials and Methods

We have studied ozone damage in the sensitive tobacco (*Nicotiana tabacum*) cultivar Bel-W3, that is used in most ozone bioindicator programmes conducted all over the world (Heggestad, 1991). Tobacco seeds were germinated in open top chambers, with charcoal filtered air, free of ozone to obtain homogeneous plants and to avoid possible early contamination of ozone. Every fortnight, from May to October, we distributed six to ten plants of this tobacco cultivar in twelve meteorological and air quality monitoring rural stations of Catalonia (N.E. Spain). Seedlings at the 4th leaf stage were transported to the sites where they were transplanted to 8 L pots filled with 25% peat, 25% vermiculite, 25% perlite and 25% sand. Soil pH of those pots was adjusted to 6.0 by adding $CaCO_3$. A slow-release fertilizer (Osmocote plus) was also added. A self-watering system was used in each pot by placing it above individual self-watering reservoirs communicated by two wicks. After two weeks, ozone-induced visible injury was assessed as the percentage of damaged leaf area. Percentage classes of damaged leaf area were estimated in 5% intervals. The average percentage of damaged foliar area was measured in the four first leaves. New plants were replaced in each station every two weeks.

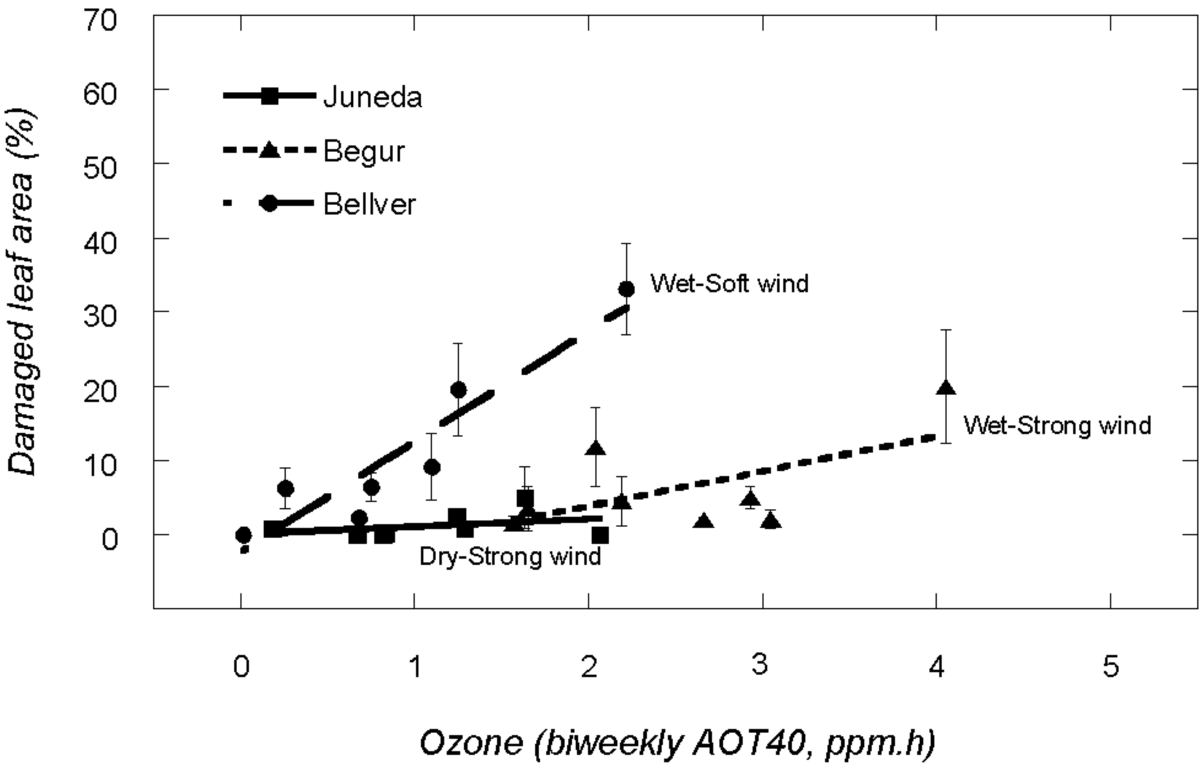
We previously verified the Bel-W3 tobacco plants sensitivity to ozone under controlled conditions in Open Top Chambers where they were exposed to charcoal filtered air, non filtered air and non filtered air plus 40 ppb_v ozone (Ribas et al., 1998).

Ozone concentrations and meteorological data (pressure, solar radiation, rain, wind speed, wind direction, relative humidity and temperature) were recorded continuously in the stations of the Rural Network of the Environmental Department of the Catalan Government. The AOT40 (accumulated ozone over threshold 40 ppb_v) was calculated as the sum of the differences between the hourly concentrations in ppb_v and 40 ppb_v for each hour when the concentration exceeds 40 ppb_v. It is calculated following the definitions stated at the UN/ECE workshops held at Bern in 1993 and Kuopio in 1996.

For statistical analyses (correlation and regression) Statview 4.5 programme package (Abacus Concepts Inc., Berkeley, L.A., USA) was used.

Results and Discussion

Here we present data from 1995 as a representative year. When overall field station data were considered, the tobacco injury symptoms were correlated with ozone concentrations ($r=0.53$, $n=60$, $p<0.01$). Ozone seemed, thus, to explain only a small part of the variance in symptomatology (about 28 %). In fact, the phytotoxicity of ozone strongly differed in different stations and periods. The damage threshold (the appearance of visible foliar injury) was only 0.1 $ppm_v{\cdot}h$ AOT40 in Bellver, a wet-soft wind station, and as high as 1.5 $ppm_v{\cdot}h$ AOT40 in Juneda, a dry-strong wind station (Figure 1). The damage rate was also much higher in wet-soft wind than in dry-strong wind stations (Figure 1). For example, at AOT40 2.0 $ppm_v{\cdot}h$ the leaf damage ranged between 0 % in dry-strong wind sites and 30 % in wet-soft wind stations (Figure 1). Intermediate damage thresholds and damage rates were found in the other stations with intermediate humidity and wind speeds. These results are in accordance with the ICP-Crops (1997) establishment of two different short-term critical levels of ozone injury development: one AOT40 of 0.5 $ppm_v{\cdot}h$ accumulated over 5 days when mean vapour pressure deficit (9.30-16.30) exceeds 1.5 kPa (dry conditions) and another one of only 0.2 $ppm_v{\cdot}h$ when VPD is below 1.5 kPa (wet conditions).



Site	Climate	Average VPD (kPa)	Average Wind speed (m s^{-1})	Damage threshold (ppm_v·h)	Damage rate (%/ ppm_v·h)
Bellver	Wet-Soft wind	0.59	1.18	0.1	15
Begur	Wet-Strong wind	0.70	3.95	1	5
Juneda	Dry-Strong wind	1.13	2.97	1.5	1

Figure 1. Percentage of damaged leaf area in Bel-W3 tobacco plants (±SE) versus AOT40 in three representative stations of Catalonia (Bellver, Begur and Juneda). Each data point corresponds to a fortnight measurement in 1995. Linear regressions are depicted in the figure. Accompanying table shows the average VPD, the average wind speed, the ozone damage threshold (appearance of visible injury) and the ozone damage rate (percentage of damage leaf area/AOT40 in ppm_v·h) for each one of the three stations.

Thus, ozone biomonitoring in different localities and periods will lead to overestimations or underestimations of ozone levels unless factors affecting the expression of ozone foliar symptoms such as temperature, humidity and wind are included in the assessment algorithms. Most of these factors affect the stomata through which ozone enters plant leaves. Low humidity and high temperature (high vapour pressure deficit) or strong wind decrease stomatal conductance, thus decreasing the effective ozone dose. Therefore, local meteorological conditions need to be characterized and included in the algorithms for both the determination of critical ozone levels and the biomonitoring of ozone. They also need to be considered in modelling plant ozone actual doses and damage.

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2.1.3. Effects of Ethylene diurea as a protective antiozonant on beans (*Phaseolus vulgaris* cv Lit) exposed to different tropospheric ozone doses

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Summary

A study to evaluate the effects of ethylene diurea (EDU) as a protective antiozonant for beans (*Phaseolus vulgaris*) submitted to different ambient ozone concentrations was performed in three stations of the rural Network for Air Quality Monitoring (Catalonia, NE Spain). The highest ozone concentrations were accompanied by significant reductions in fruit yield, number of fruits and shoot biomass. These reductions were lower in plants treated with EDU. The results showed toxic ozone effects on sensitive species such as beans and a protective antiozonant effect of EDU. The intensity of the EDU protective effect differed among the different stations characterised by different meteorological conditions and O_3 concentrations.

Key words: AOT40, beans, ethylene diurea (EDU), ozone.

Introduction

The coincidence of high solar radiation with industrialization and the high population density involving vehicular traffic favour ozone formation in some areas of the Mediterranean region (Gimeno et al., 1993b; Peñuelas et al., 1995). Deleterious effects of ozone on Mediterranean crops, fruit trees and natural species have been reported under experimental or field conditions (Reinert et al., 1992; Gimeno et al., 1993a, 1995; Peñuelas et al., 1995 a; Elvira et al., 1995; Paolacci et al., 1995; Velissariou et al., 1996). A large number of native and cultivated perennial and annual plants are known to be sensitive to O_3. Studies in Open-Top Chambers (OTC) and in field conditions have shown that beans (*Phaseolus vulgaris*) is one of these sensitive species (Sanders et al., 1992; Colls et al., 1992; Tonneijck and Van Dijk, 1994). This species presents a clear symptomatology, with older leaves exhibiting extensive bronzing and/or stippling followed by chlorosis and leaf fall (Manning and Feder, 1980). Furthermore, *Phaseolus vulgaris* does not have special cultural requirements, so that its use as bioindicators and biomonitors of O_3 is widely spread (Sanders et. al, 1992; Gimeno et al., 1995; UN/ECE, 1996).

N-(2-(2-oxo-1-imidazolidinyl)ethyl)-N'-phenylurea or ethylene diurea (EDU) has been shown to act as an antiozonant with protective effects against ozone injury (Carnahan, Jenner and Wat, 1978). It is commonly used in the frame of ICP-Crops (International Co-operative Program on effects of air pollution and other stresses on crops and non-wood plants) to assess ozone damage (UN/ECE, 1996).

In the present work the effect of field ozone concentrations on beans and the protective effect of EDU was studied in a typical Mediterranean region (Catalonia, NE Spain). The study was conducted at three different sites with different meteorological conditions. The main objectives (a) to test beans as possible ozone bioindicators and biomonitors, (b) to test the protective effect of antiozonants (c) to assess ozone-induced growth and yield reductions, and finally (d) to study the spatial and temporal variation of ozone-induced effects and the meteorological modulatory effect.

Material and Methods

Measurement sites

The study was performed in 1995 in three rural monitoring stations of the Rural Network of the Environmental Department of the Generalitat de Catalunya (NE Spain) containing ozone monitors and meteorological sensors. The stations were located in Begur (3° 15'E, 41° 55'N), Sort (1° 00'E, 42° 30'N) and Veciana (1° 30'E, 41° 40'N), differing considerably in ozone and meteorological conditions (See chapter 2.1.1, section *Material and Methods*)

Plant bioindicators and cultural techniques

Twenty bean plants (*Phaseolus vulgaris* Lit.) were used as ozone bioindicators in each site. Four seeds per pot were sown at a depth of 2 cm. Once germinated, they were thinned to one plant per pot at the seedling stage. The pots were 8 L containers filled with 25% peat, 25% vermiculite, 25% perlite and 25% sand. Substrate pH was adjusted to 6.0 by adding $CaCO_3$ (0.5 gL^{-1}). A NPK 15:11:13 slow-release fertilizer (Osmocote plus) was also added. A self-watering system was used in each pot by placing it above an individual self-watering reservoir connected to the pot by two wicks.

Ten bean plants were treated with EDU and the other ten remained untreated as a control treatment. The first EDU treatment (100 ppm_v) was made when the first true leaf was unfolded. Increasing doses of EDU (100, 150, 200 and 250 ppm_v) were applied biweekly following the plant development. The EDU solution was applied by carefully pouring 200 ml onto the soil around the base of the plants and avoiding wetting the foliage. The same volume of water was applied on the same dates to the Non-EDU treated plants.

The experiment was performed twice. The first period (from the 17th of May to the 1st of August) was conducted in Begur and Veciana. The second period (from the 2nd of August to the 14th of September) was performed in Veciana and Sort. The experiment was carried out according to the UN/ICP-Crops protocol for the 1995-growing season.

Injury parameters

Number of fruits longer than 4 cm and fruit and shoot dry weights were measured for each individual plant. Leaf percentages of damaged leaf area were assessed every two weeks.

Ozone and meteorological measurements

Ozone concentrations and meteorological data (atmospheric pressure, solar radiation, relative humidity and temperature) were continuously recorded at the stations of Rural Network of the Environmental Department of the Catalan Government. Ozone concentrations were monitored with a MCV analyser, model 48 AUV, based on the UV radiation absorption by ozone.

Whenever the ozone concentrations exceeded 40 ppb_v, the AOT40 (Accumulated ozone exposure Over a Threshold of 40 ppb_v) was calculated by summing up the differences between the hourly concentration (expressed in ppb_v) and a threshold value of 40 ppb_v. It was calculated for those diurnal hours with solar radiation above 50 Wm^{-2} (Fuhrer and Acherman, 1994; Kärenlamp and Skärby, 1996). AOT40 values were calculated for each assessment period and station. VPD (Vapour Pressure Deficit) was calculated by using the period average temperature and relative humidity.

Statistical Analyses

The statistical programme package Statistica 4.0 (Statsoft., Inc.) was used for data analyses. Analyses of variance (ANOVA) were conducted considering AOT40 and EDU treatment as independent variables. Data showed evidence of heteroscedasticity and they were logarithmically transformed and reanalysed.

Results and Discussion

High ozone concentrations were found in spring and at the beginning of summer especially at Veciana and Begur (Figure 1). AOT40 was 17972 $ppb_v{\cdot}h$ in Veciana and 15316 $ppb_v{\cdot}h$ in Begur in the first period (May-July). These values are elevated when comparing to values measured in other European countries (UN/ECE, 1995) but they are in agreement with previous measurements in the same region (Ribas et al., 1998). Later, at the end of summer and beginning of autumn, ozone levels decreased (Figure 1). The AOT40 values of the second period were lower (10184 $ppb_v{\cdot}h$ in Veciana, and 448 $ppb_v{\cdot}h$ in Sort). Such AOT40 values clearly exceeded (except in Sort) the proposed long-term critical level for crop yield protection, a cut-off that has been established at 3000 $ppb_v \cdot h$ over the growing season (approximately 3 months) (Kärenlamp and Skärby, 1996).

No clear visible symptomatoly was observed on the leaf surfaces. Fruit dry weight, fruit number and shoot dry weight decreased with increasing AOT40s ($p<0.01$, ANOVA) (Figure 2) indicating a toxic effect of ozone, but such variables were greater under EDU treatments ($p<0.01$, ANOVA) indicating a protective effect of the antiozonant. For these studied stations the independent terms of regression equations between fruit yield and AOT40, with and without EDU, were significantly different (Figure 2). There were also slightly significant interactions for AOT40 and EDU in fruit yield ($p=0.06$, ANOVA) but not in fruit number or shoot biomass. Ozone-induced yield was reduced approximately to 60 % under an AOT40 value of 10000 $ppb_v \cdot h$. The linear relationships between fruit yield and AOT40 (Figure 2) might be used for biomonitoring ozone in case these results could be confirmed by more data points.

These results are in agreement with previous reports showing beneficial effects of EDU on yield of bean plants under ozone exposure (Gimeno et al., 1996; Fagnano and Zoina, 1995; Schenone et al., 1995; Postiglione and Fagnano, 1995; Fumagalli, Mignanego and Violini, 1997), and other species such as tomatoes (Gimeno et al., 1996; Postiglione and Fagnano, 1995), poplars (Ainsworth et al., 1997) and others (UN/ECE, 1995; Postiglione and Fagnano, 1995). In our study the significance of the EDU protective effects was higher than in many of these previous studies. Although most of the literature indicates that EDU provides protection against O_3-induced visible injury in many species, there is a wide range of responses concerning crop yield which in part could be due to the fact that EDU *per se* might affect plant physiology and growth and finally plant yield. This might be explained by a fertilising effect of EDU. The breakdown of EDU may increase the nitrogen available to plant (Ainsworth et al., 1996). However, Frison et al. (1990) suggest that this effect is negligible, and studies conducted with two different EDU concentrations have not found any increase of plant production (Schenone et al., 1995). In our study, the EDU protective effect was higher at AOT40 10000 $ppb_v \cdot h$ than at AOT40 500 $ppb_v \cdot h$ (Figure 2) indicating that apart from possible fertilizing effect suggested by higher biomass in EDU treated plants at low AOT40, there was also an antiozonant effect.

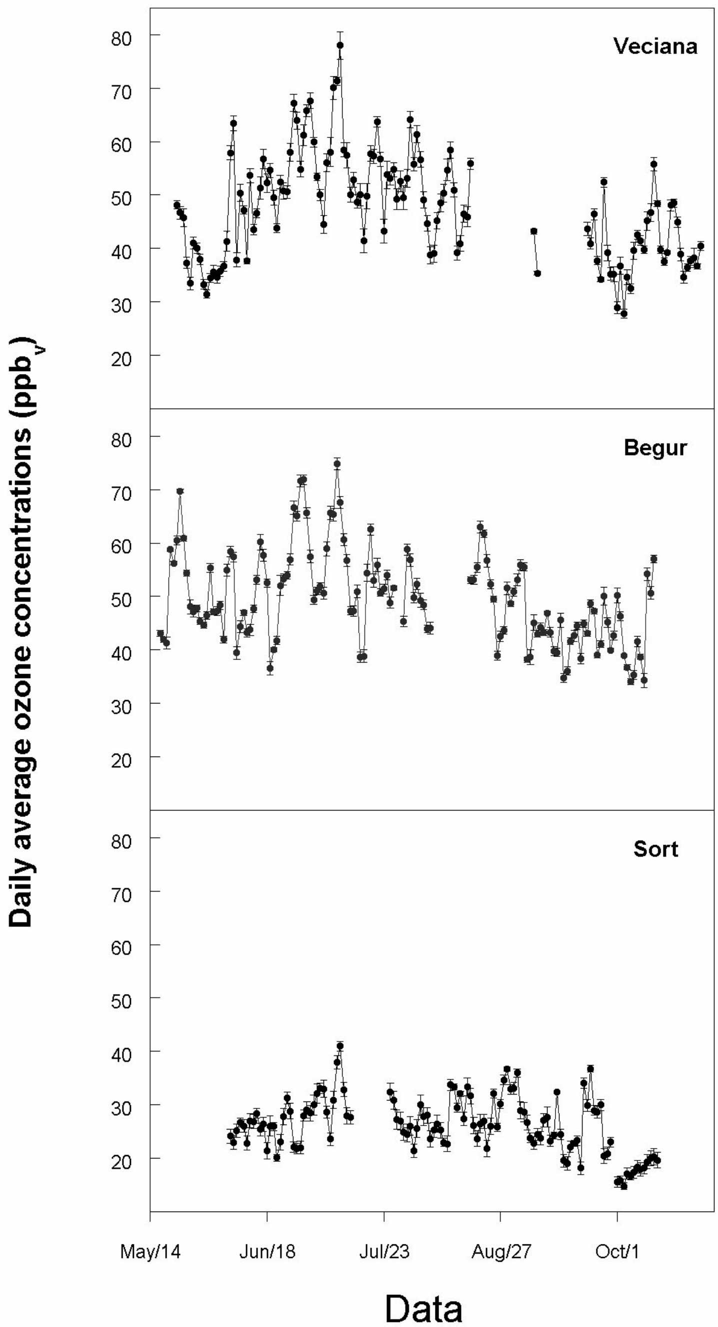

Figure 1. Daily average ozone concentrations during the studied period in the three studied sites.

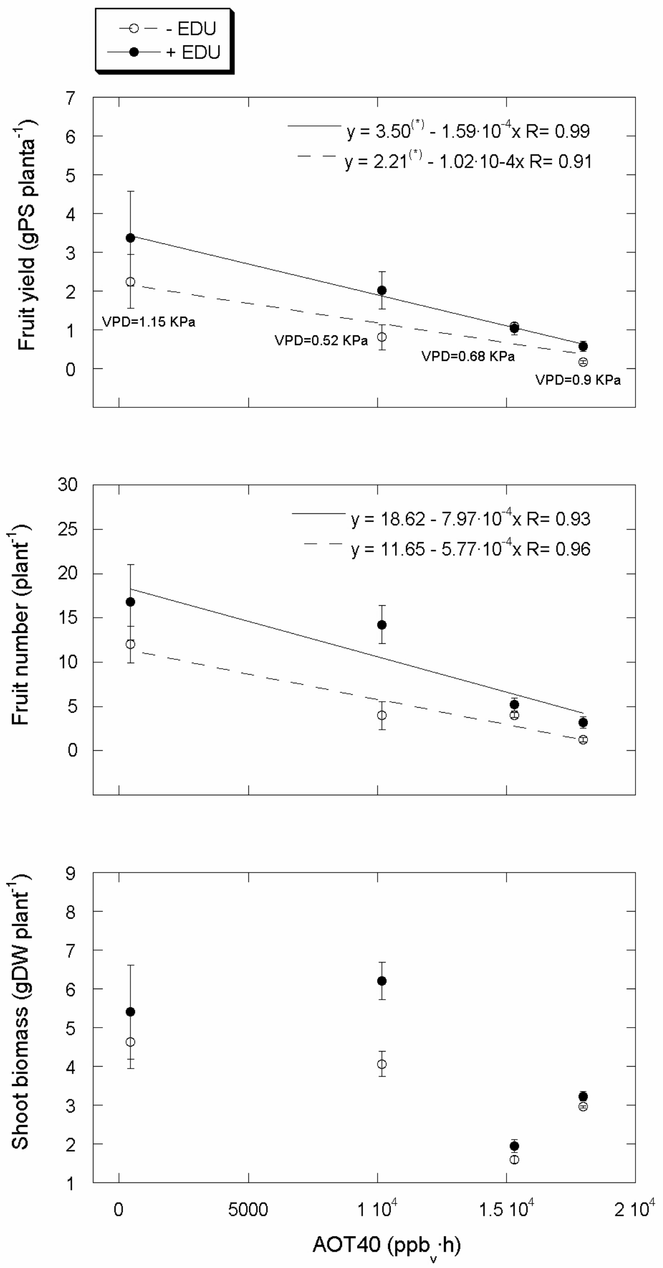

Figure 2. Fruit yield, fruit number and shoot biomass of bean plants grown in different Catalan field sites at different AOT40 levels. Error bars are SEM (n=10). Average VPDs during the growth cycle are also depicted. (*) in the independent terms of the regression equations stands for significant differences with $p < 0.05$.

The protective effect of EDU mitigating ozone effects differed between the sites and periods depending on environmental conditions as has also been found by Toivonen et al. (1982) and Tonneijck and van Dijk (1997a). The EDU protective effect was also more evident when AOT40s were intermediate (10000 $ppb_v{\cdot}h$) and average VPD minimum (0.5 Kpa) than when pollutant concentrations were the highest (AOT40 > 15000 $ppb_v{\cdot}h$) and VPD values were in the range of 0.7-0.9 KPa (Figure2). This is in accordance with results of Regner-Joosten et al. (1994) showing that the amount of EDU absorbed by the roots was proportional to the rate of transpiration, which is higher under lower VPD, providing a possible explanation of the corresponding maximum effect of EDU treatment. Tonneijck and Van Dijk (1997a, b) have also suggested a climate effect on the rate of antiozonant uptake and therefore on protective effect. Increases in ozone sensitivity when mean VPD is low have been reported (Benton et al., 1996; Tonneijck and Van Dijk, 1997b). But, when VPD is low plant stomata will be wide opened in well-watered plants, the absorbed amount of ozone by the plant will be higher than when VPD is high, and therefore it is more likely to find higher EDU protection. However, in this study VPD differences were small and do not seem to be the only explanation of different EDU effect in the different stations and periods, suggesting that other factor/s should be involved. The very high AOT40 value recorded when EDU effect was minimum (Figure 2) may have overcome EDU protection. Miller et al. (1994) have also found a lower effectivity of EDU in sites with elevated concentrations of ozone than in stations with lower concentrations of ozone.

Conclusions

The high ozone concentrations of most of the studied sites in Catalonia produced a significant decrease in fruit yield, fruit number and shoot biomass of bean plants. They could thus be used as ozone bioindicators and possibly as biomonitors if further studies involving a wider range of ozone levels confirm these results (Figure 2). Results also indicate again that EDU may be an antiozonant protector apart from possibly having fertilizing or other physiological effects. They also indicate possible meteorological and ozone concentration modulatory effects on EDU protective capacity. However, no clear relationships between O_3 exposure and EDU protection effectiveness could be established.

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2.2. Biomonitoring of tropospheric ozone phytotoxicity in rural Catalonia

Àngela Ribas and Josep Peñuelas

Summary

The ozone (O_3) phytotoxicity in rural areas of Catalonia (N.E. Spain) and the biomonitoring capacity of Bel-W3 tobacco (*Nicotiana tabacum)* cultivars were assessed by determining the percentage of leaf area injured by ozone in plants of this cultivar exposed from spring to autumn since 1995 to 1999. The study was conducted simultaneously on nine field sites where ground level ozone concentrations and meteorological parameters were continuously monitored. Geographical, seasonal and annual variations of ozone damage rate and their links with meteorological conditions were studied. Ozone concentrations and leaf damage increased at the end of spring and the beginning of summer. Coastal sites generally presented higher O_3 concentrations than inland and mountain sites. These mountain sites were the most sensitive ones to ozone toxicity. The ozone concentrations correlated well with ozone injury. However, at this local scale the ozone levels did not fully account for all the observed injury (only 11%). The response of tobacco plants to ozone concentrations and therefore its biomonitoring capacity depended also on different environmental conditions, mainly those linked to stomatal behaviour such as VPD. The categorisation of leaf damage in 10% intervals and its averaging throughout the whole study period and the whole region, strongly improved (99% of variance accounted) the relationship with ozone concentrations expressed as AOT20 (accumulated over a cut off of 20 ppb_v). *Nicotiana tabacum* cv. Bel-W3 is thus a very good biomonitor of ozone concentrations in the long term at the regional scale. Taking into account the phytotoxical response of this sensitive tobacco cultivar, we propose the 1.28 $ppm_v \cdot h$ biweekly AOT40 (with a solar radiation threshold of 50 W m^{-2}) as a damage threshold level for sensitive species.

Key words: Damage levels, Mediterranean conditions, *Nicotiana tabacum* Bel-W3, Ozone, VPD.

Introduction

Ground level ozone is one of the gaseous air pollutants that is significantly exceeding the permissible concentrations in several regions (Ballaman, 1993; Proyou et al., 1991). Its background concentrations have been reported to increase during the last decades and it is expected that they follow rising next years (Chameides et al., 1994; Jonson et al., 2001). High ozone concentrations have long been known to affect many physiological and biochemical characteristics, as well as growth and yield, of agricultural crops (Krupa et al., 1997), and natural vegetation (Hogsett et al., 1997).

Extensive research has been summarized in Level I Ozone Critical Levels for European Vegetation (Kärenlampi and Skärby, 1996). Level I values are based on plant response to ambient ozone concentrations. However, it is widely recognized that plant response is actually more closely related to the internal ozone dose (ozone taken into the plant through the stomata) (Pleijel et al., 2000). Other parameters such as temperature and VPD (Vapour Pressure Deficit) are widely recognized as factors influencing the flux of O_3 into the leaf, and therefore are expected to be important components of a Level II model for O_3 (e.g. Grünhage et al., 1999). Thus, damage rates of ozone may vary in time and space depending on the local environmental and meteorological conditions (Benton et al., 2000; Peñuelas et al., 1999).

The observation of visual plant responses is a simple and cheap technique that can be used as early toxicity warning systems (Manning, 1998; Mulgrew and Williams, 2000). Some plant species exhibit typical foliar injury symptoms when exposed to ambient ozone, making them useful as bioindicators. Their use as bioindicators has been extensively reviewed elsewhere (Guderian, 1985; Manning, 1998). Most bioindicator programmes conducted all over the world have involved the use of only tobacco cv. Bel-W3 to assess ozone phytotoxicity (Bytnerowicz et al., 1993; Lorenzini, 1994; Ribas et al., 1998).

A distinction is increasingly being made between bioindicators and biomonitors in air pollution studies. The former reveals the presence or absence of an air pollutant while the biomonitors provide additional information about the amount and intensity of the exposure (Market et al., 1997). However, the response of plants to elevated concentrations of air pollutants is modified by other environmental factors and by the physiological status of the plant. The monitoring of these plants assesses the integrated effects of these factors together with those of ozone.

Many biomonitoring studies using tobacco plants have already been conducted (Saitanis and Karandinos, 2001). However, it is important to provide data on long-term temporal and spatial trends of air pollutants and their effects to establish better biomonitoring relationships and their temporal evolution in spatially heterogeneous regions such as the Mediterranean one.

In this Mediterranean region, previous works have already reported harmful morphological and physiological ozone effects on plants (Gimeno et al., 1999; Paolacci et al., 1995; Reinert et al., 1992). Several authors (Fumagalli et al., 2001; Alonso et al., 1999) have shown smaller yield losses in this Mediterranean region than expected from the exposure -response equation presented by Fuhrer et al. (1997) for wheat grown in northern and central Europe. Therefore, the actual O_3 impact cannot be derived from this equation.

High solar radiation and high density of population are characteristics that favour ozone formation in the Spanish Mediterranean coast (Peñuelas et al., 1995a). Besides, in areas like Catalonia (N.E. Spain), the complex orography determines the existence of differences in local ozone concentrations (Martin et al., 1991; Millán et al., 1996) and meteorological conditions. Preliminary studies to assess ozone phytotoxicity involving the use of tobacco cultivars Bel-W3, Bel-C and Bel-B have been carried out in this area (Gimeno et al., 1995; Ribas et al., 1998). However, because of the scarcity of dose-response information in field conditions, we aimed (a) to study possible time and space variations of ozone damage rate and their links with meteorological conditions. We also aimed (b) to study ozone biomonitoring capacity of the widely used tobacco (*Nicotiana tabacum*) Bel-W3 bioindicator (Heggestad, 1991) in a whole region such Catalonia along four years of study. With the analysis of data we finally aimed (c) to define ozone damage thresholds for the sensitive species in Catalonia, and in the Mediterranean region in general.

Material and methods

Measurement sites

From 1995 to 1999 (except in 1997) and since spring to autumn, different bioindication campaigns were performed at Catalonia (N.E. Spain). The bioindicator plants were distributed in different rural sites of Catalonia covering a variety of environmental conditions ranging from sea level to 1040 meters of altitude and from sea-shore to 200 km in-land (Fig. 1 and Table 1). The plants were placed in the monitoring stations of the Rural Network of the Environmental Department of the Generalitat de Catalunya containing ozone monitors and meteorological stations. The number of sites involved in the study varied through the years, eight in 1995 and 1998 and four-five in 1996 and 1999 (see Fig. 3).

Site	Latitude	Longitude	Altitude(m)	T(ºC)		Precipitation		EVTP (mm)	β	
				July	anual	July	anual	anual		
Manlleu	42º 0'	2º 17'	459	22-23	13-14	50-60	800-850	572-712	0,93	±0.13
Bellver de Cerdanya	42º 20'	1º 45'	1043	18-19	10-11	60-70	700-750	572-712	0,86	(± 0.09)
La Sénia	40º 35'	0º 15'	356	22-23	15-16	20-30	800-850	712-855	0,79	(± 0.10)
Sort	42º 30'	1º 00'	682	20-21	10-11	80-90	800-850	572-712	0,75	(± 0.19)
Sta. Maria de Palautordera	41º42'	2º 30'	215	23-24	14-15	30-40	750-800	712-855	0,73	(± 0.20)
Santa Pau	42º10'	3º 00'	569	18-19	12-13	80-90	950-1000	572-712	0,70	(± 0.12)
Begur	41º 55'	3º 15'	198	23-24	15-16	10-20	600-650	712-855	0,69	(± 0.21)
Veciana	41º 40'	1º 26'	726	21-22	12-13	30-40	500-550	712-855	0,66	(± 0.15)
Juneda	41º 35'	0º 50'	275	24-25	14-15	10-20	400-450	712-855	0,60	(± 0.17)

Table 1. Latitude, longitude, altitude, mean July and annual temperature, mean July and annual rainfall, and potential evapotranspiration of the nine studied rural field stations of Catalonia. Standardised simple regression coefficients (β) for intercept 0 models between percentage of damaged leaf area in Bel-W3 and AOT20 in the nine stations with SE (standard error of β between brackets) are also depicted in the last column. For all sites, $p<0.01$.

Plant material and assessment of ozone-induced damage

Six tobacco (*Nicotiana tabacum*) cultivar Bel-W3 plants were used as ozone bioindicators every fortnight in each site. Tobacco seeds were germinated in open top chambers with charcoal filtered air, free of ozone to obtain homogeneous plants and to avoid possible early contamination of ozone. When seedlings were at the 4th leaf stage, they were transplanted to 8 L pots filled with 25 % peat, 25 % vermiculite, 25 % perlite and 25 % sand. Soil pH of these pots was adjusted to 6.0 by adding $CaCO_3$. A NPK 15:11:13 slow-release fertilizer (Osmocote plus) was also added. A self-watering system was used in each pot by placing them above individual self-watering reservoirs communicated by two wicks. New plants were replaced every two weeks from May to October. Phytotoxic levels were defined according to the percentage of damaged leaf area in the Bel-W3 tobacco cultivars (Bytnerowicz et al., 1993; Lorenzini, 1994). After each exposure, percentage of ozone-induced lesions on the oldest four leaves was visually recorded. These percentages were estimated in 5 % intervals.

Ozone and meteorological measurements

Ozone concentrations and meteorological data (atmospheric pressure, solar radiation, wind, relative humidity and temperature) were continuously recorded at the stations of Rural Network of the Environmental Department of the Catalan Government. Ozone concentrations were monitored with a MCV analyser, model 48 AUV, based on the UV radiation absorption by ozone. The air was sampled at 2.5 m height.

The AOT20 (the sum of the differences between the hourly concentrations in ppb_v and 20 ppb_v for each hour when the concentration exceeds 20 ppb_v) was calculated for those diurnal hours with solar radiation above 50 W m^{-2} following the definitions stated at the UN/ECE workshops held at Bern in 1993 and Kuopio in 1996. AOT20 values were calculated for every fortnight assessment period. We also calculated AOT20 without considering a cut off of diurnal radiation, and AOT20 considering different cut offs of vapour pressure deficit from 0 to 1.5 kPa. Parallel AOT40 and AOT60 calculations were conducted also taking different cut offs (40 and 60 ppb_v respectively). Moreover, average and maximal ozone values were also calculated. We thus considered most of the reported ozone variables in the literature.

To characterize weather conditions, meteorological data (pressure (HPa), solar radiation (W m^{-2}), rain (mm), wind speed (m s^{-1}), wind direction (º), air vapour pressure deficit (VPD) (kPa), relative humidity (%) and temperature (ºC) were also recorded. We also calculated accumulated humidities over cut offs of 50 % and 70 % (AHOT50 and AHOT70 respectively) in a similar way to the above mentioned AOTs for ozone. Additionally to average values, wind speeds were also calculated for values above 3, 5 and 9 m s^{-1}. All these meteorological variables were used to distinguish possible interactive effects with O_3. VPD was calculated hourly by using the air temperature and the relative humidity (Jones, 1992).

For statistical analyses (simple and multiple, linear and non-linear regression analyses), SSPS for Windows 10.0.6 (SPSS Inc., 1989-1999) and Statistica 5.5 (StatSoft Inc., Tulsa, USA) were used. For circular statistical analyses (wind direction) we used Oriana for Windows 1.01 (Kovach Computing Services, Wales, UK). A logarithmic transformation was also conducted to normalise AOT20 variable for some of the linear regression models used in this study.

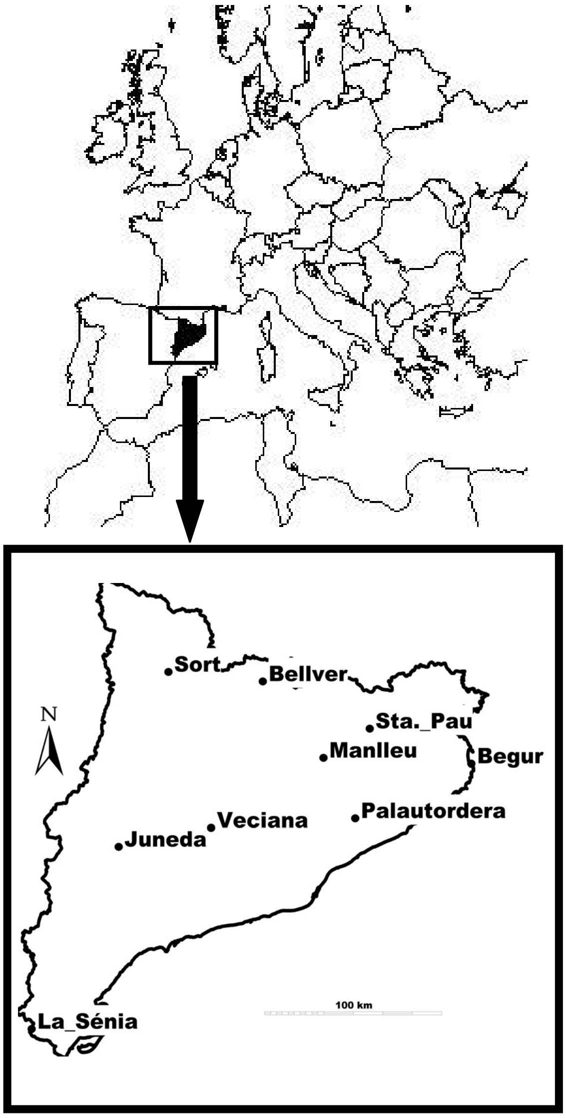

Figure 1. Map of the studied rural sites in Catalonia.

Results

Temporal and spatial variation of ozone concentrations

Throughout 1996-1999 period (except 1997), the AOT20 mean values ranged between 3.5 and 10 $ppm_v \cdot h$ and the AOT40 mean values between 0.5 and 4 $ppm_v \cdot h$ (Fig. 2). Maximum ground level O_3 concentrations were found both in spring and summer (Fig. 3). At the end of summer and beginning of autumn, ozone levels decreased. There were not high differences among the four studied years (Fig. 3).

There was also a clear geographical variation. Coastal sites (Palautordera, Begur, La Sénia) generally presented higher values (AOT20 for two weeks of 8 - 11 $ppm_v \cdot h$, or AOT40 for two weeks of 2- 4 $ppm_v \cdot h$) (Fig. 2), while more inland sites such as Juneda presented lower values (usually AOT20 biweekly values between 4 - 5 $ppm_v \cdot h$, and AOT40 biweekly values always below 2 $ppm_v \cdot h$). The lowest values were recorded in the Pyrenees valleys (Sort presented AOT20 values between 2.5 - 4 $ppm_v \cdot h$ and AOT40 values always below 1 $ppm_v \cdot h$) (Fig. 2).

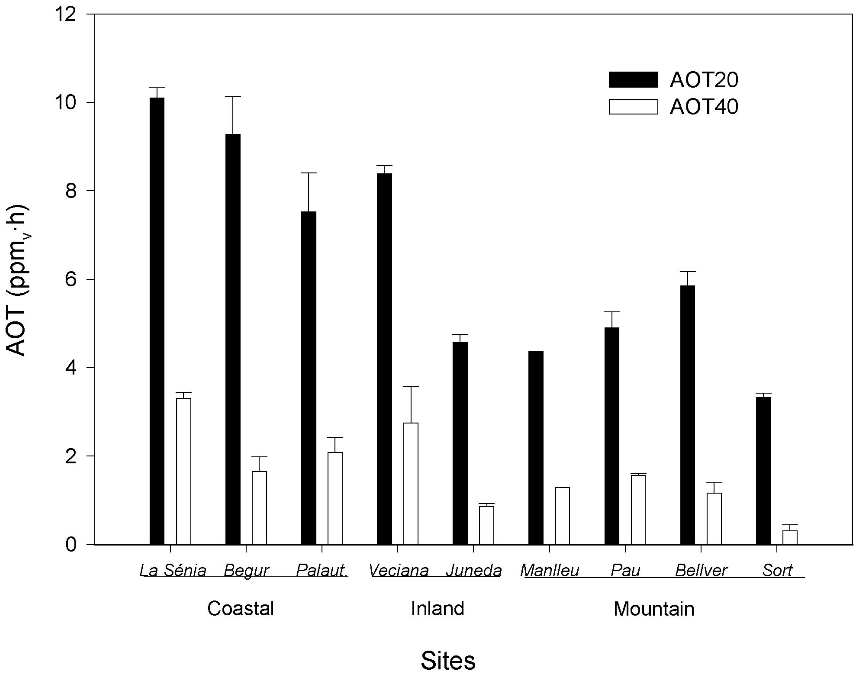

Figure 2. Mean biweekly AOT20 ($ppm_v \cdot h$) and AOT40 (calculated for diurnal hours, with a Solar Radiation Threshold of 50 W m^{-2}) values for each sampling station during the spring-autumn period. Values are to annual average ±SE. (n= 4 annual means of 8-11 biweekly periods per year).

Temporal and spatial variation of leaf damage

Substantial ozone injury symptoms developed on the Bel-W3 tobacco plants exposed to ambient air at every site (Fig. 3). The percentage of ozone-induced damaged leaf area in all these sampling stations followed a similar general temporal and geographical pattern than ozone concentrations. However, there was not always a perfect match between visual ozone damage (or phytotoxicity) and ozone levels (Fig. 3).

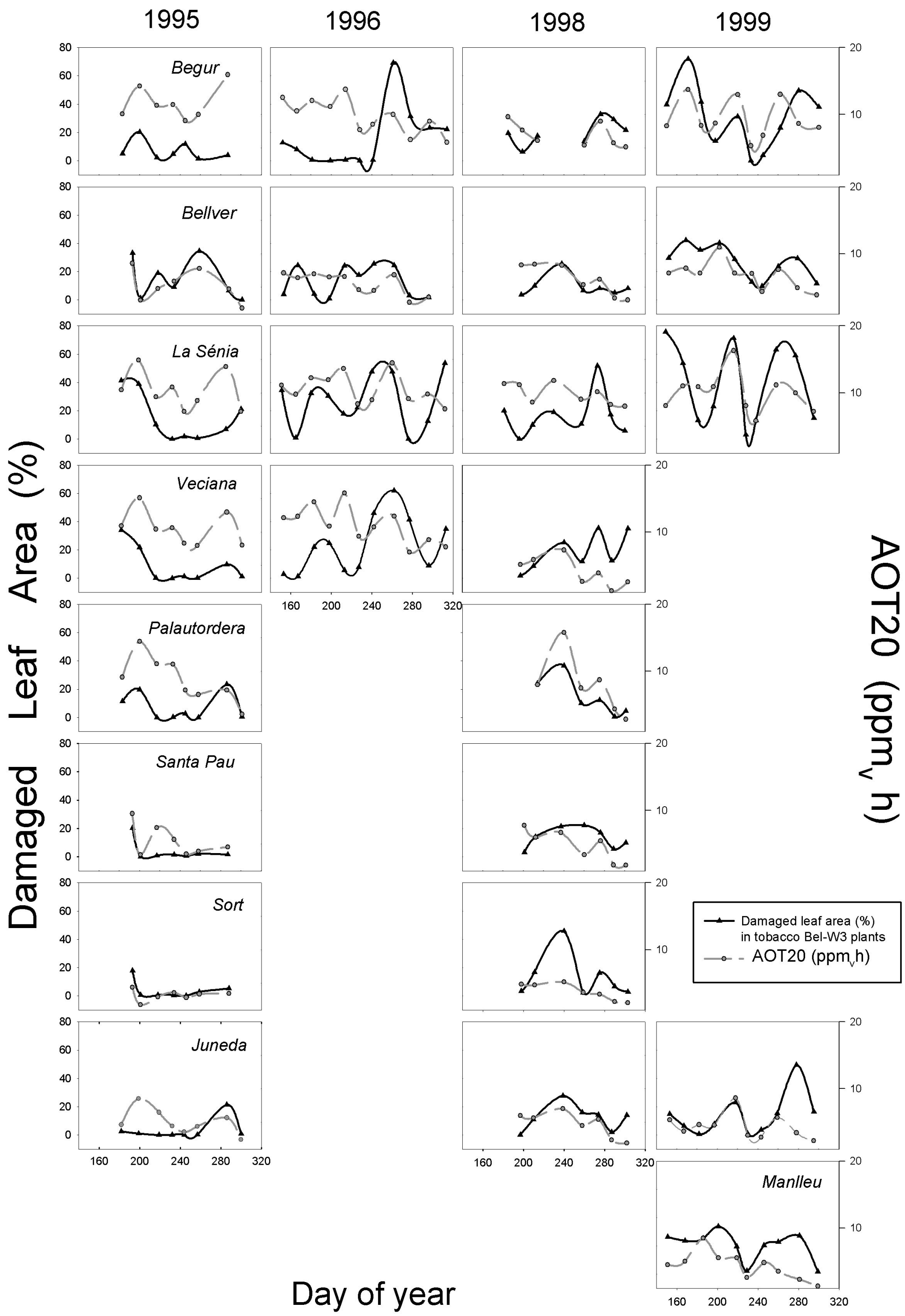

Figure 3. Damaged leaf area (%) in *Nicotiana tabacum* Bel-W3 cultivar and AOT20 ($ppm_v \cdot h$) evolution, for each station and year, throughout the four year monitoring period.

Geographically, coastal areas (La Sénia, Begur) reached the highest damage values (Fig. 3). However, the most sensitive sites were those of the Pyrenees mountains and wet Pre-Pyrenees plains (Manlleu station) where smaller increases in the ozone concentrations produced stronger responses of tobacco plants (Table 1). Furthermore, these sites showed intensive damage response, even though ozone concentrations were below the sensitivity threshold described for this cultivar Bel-W3 (40 ppb_v) (Heggestad, 1991). A gradient of sensitivity of different sites can thus be established based on the slope of the regression between visual damage and AOT20. Mountain-influenced zones were the most sensitive ones, followed by coastal and inland zones (Manlleu > Bellver > La Sénia > Sort > Palautordera > Begur > Santa Pau > Veciana > Juneda) (Table 1).

Leaf damage relationships with ozone and meteorological variables

In order to assess the relationships between leaf damage and environmental parameters, we conducted correlation analyses. Although the relationships between damage and ozone were not strong, they were still significant for AOT20 and AOT40. The percentage of Bel-W3 damaged leaf area was the damage variable that had the strongest correlations with ozone. The ozone parameter best correlated with Bel-W3 damaged leaf area was AOT20 ($r=0.34$, $p<0.000$, $n=201$). The use of radiation thresholds in the calculations of these environmental variables did not introduce any significant improvement in the relationships.

Ozone seemed, thus, to account for only a small part of the variance in symptomatology (about 11%). Therefore, other meteorological variables such as humidity must be considered to quantitatively link ozone to plant response. A multiple regression analysis was conducted to find a better explanation of the visual symptomatology. A non-linear multiple quadratic regression model including VPD significantly increased the fitness of the relationship:

$$DLA(\%)=143.97*\exp(-0.5*(((AOT20-34.97)^2/15.13)+(VPD+0.08)/0.84)^2))$$

$r=0.47$, F-value$=13.72$, $p<0.0001$.

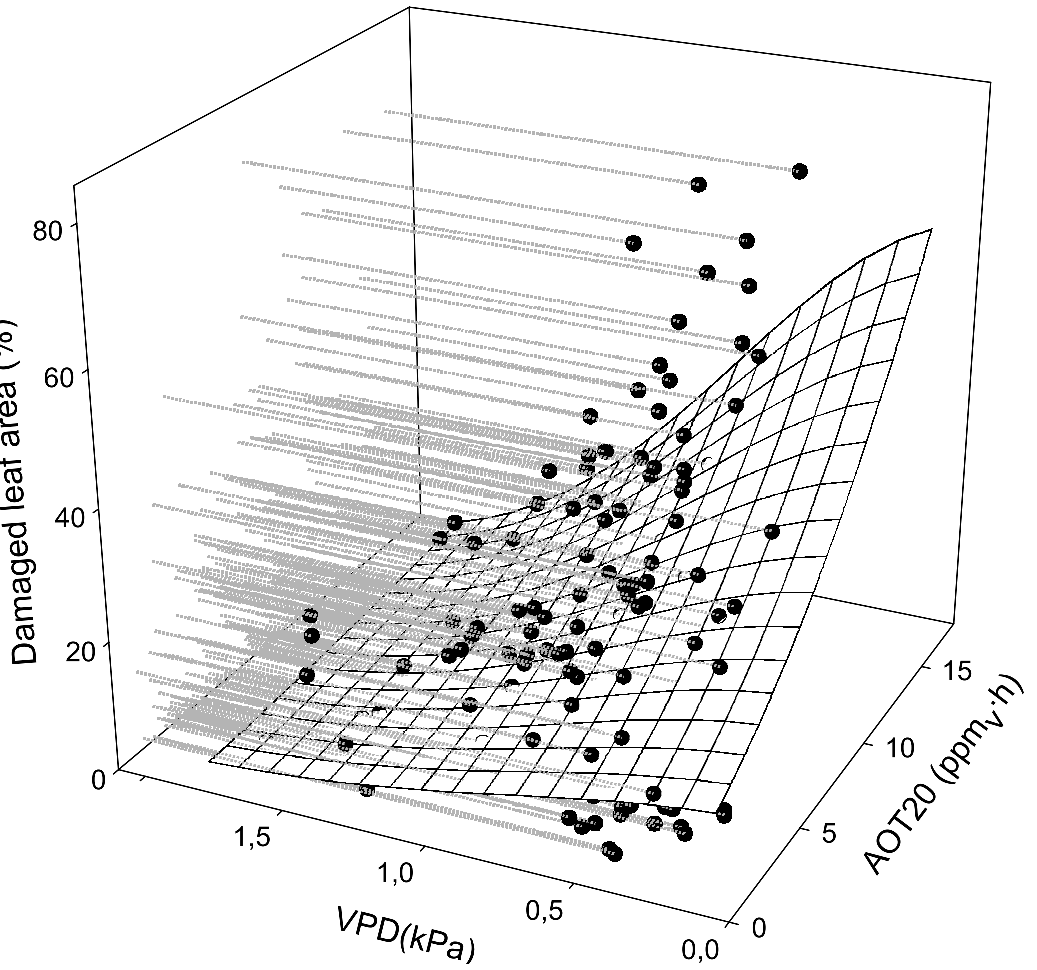

Figure 4. 3-D plot of actual data and fitted non-linear multiple regression model of Damaged leaf area (%) as a function of average biweekly VPD (kPa), Vapour Pressure Deficit) and biweekly AOT20 (ppm$_v$·h). See text for more details.

Fig. 4 shows these modulation effects of VPD on ozone damage. High O_3 concentrations (10-15 ppm$_v$·h) had stronger effects (up to 75% damaged leaf area) at low VPD (0.5-1 kPa) while at the highest VPD values, these high O_3 concentrations produced much lower damage (almost no damage at all). However, all these models for local data still presented low correlation coefficients. We established a categorised damage response (defined as damaged leaf area grouped by intervals of 10%) that averaged the biweekly values obtained for the whole period of assessment and the whole region of Catalonia and eliminated much of the noise linked to the lack of precision of the damage assessment. We found a very strong relationship (r=0.997, n=8) between the log of Exposure (calculated as $\log_{10}$ AOT20) and this new categorised damage variable. Similar relationships were obtained between ozone exposure calculated as AOT20 and categorised damage (linear r=0.982, n=8, and exponential r=0.992, n=8) or AOT40 (linear r=0.936, n=8, and exponential r=0.975, n=8). With this categorisation of leaf damage, Bel-W3 tobacco plants behaved not only as ozone bioindicators but as very good regional ozone biomonitors in the long term, even for this climatically diverse region.

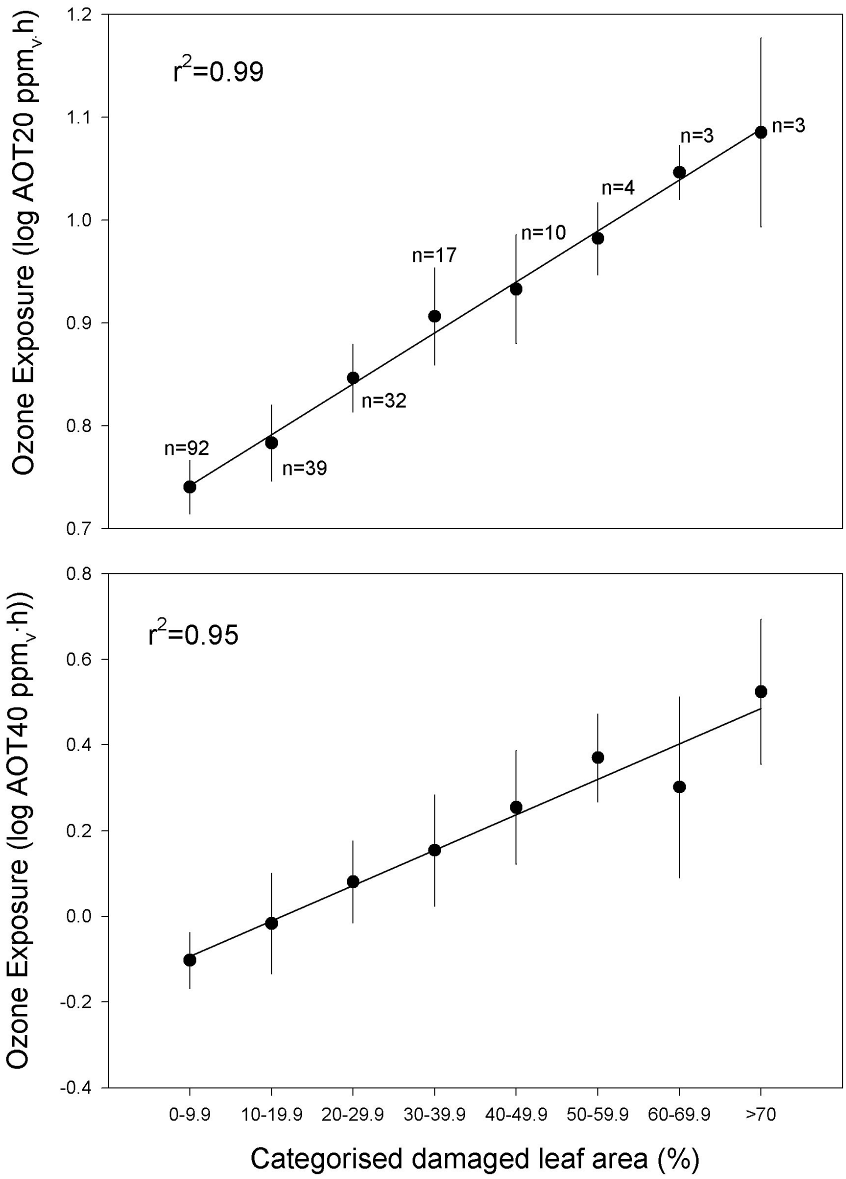

Figure 5. Plots of Ozone concentrations expressed as $\log_{10}$ of biweekly AOT20 and $\log_{10}$ of biweekly AOT40 (calculated for diurnal hours, with a solar radiation cut off of 50 W m^{-2}) *versus* Categorised Damaged leaf area (%). Data include all data obtained throughout four years and nine sampling sites. The number (n) of biweekly values measured for each category of damage are also depicted.

These results led us, moreover, to establish a possible classification of ozone damage levels for sensitive species in Catalonia, which could be extrapolated to most Mediterranean regions. In Table 2 we consider that AOT20 < 6.07 $ppm_v \cdot h$ (or AOT40 < 0.96 $ppm_v \cdot h$) in 15 days define low phytotoxical conditions, AOT20 between 6.07 and 8.84 $ppm_v \cdot h$ define medium phytotoxical conditions, and AOT20 greater than 11.63 $ppm_v \cdot h$ (or AOT40 > 2.59 $ppm_v \cdot h$) very high phytotoxical conditions.

Damage	Categorised response Damage (%)	AOT20 ($ppm_v \cdot h$)	AOT40 ($ppm_v \cdot h$)
very weak	0-9.9	5,50	0,79
weak	10-19.9	6,07	0,96
medium	20-39.9	7,36	1,28
high	40-59.9	8,84	1,94
very high	>60	11,63	2,59

Table 2. Critical ozone levels expressed as biweekly AOT20 and AOT40 (for diurnal hours, solar radiation > 50 W m^{-2}) for the different categories of Bel-W3 tobacco leaf damage in Catalonia.

Discussion

There was a general seasonal pattern of O_3 concentrations in all the studied field sites, with maximum values during spring and summer. This general pattern matches the general net ozone production in middle northern latitudes, resulting from the strong emission and transport of anthropogenic O_3 precursors coincidence with high solar radiations (Mauzerall et al., 2000). There was a large variation in the ozone concentrations recorded at the different sampling sites, but in general these Catalonian sites presented similar or higher values throughout these years than most European sites, and were similar to those described in other sites of the Mediterranean region (Mills et al., 2001).

Even under relatively low ozone concentrations there was visible injury on sensitive tobacco cultivars in all sites and years. Seasonal phytotoxical pattern corresponded to the described O_3 concentrations patterns as also found in our preliminary studies (Ribas et al., 1998) and by other authors (Blum et al., 1997). Previous works in the region (Gimeno et al. 1995; Peñuelas et al., 1995b) showed an injury pattern with high phytotoxical levels associated to the sites close to the coast, and low phytotoxicity at the most inland sites. Here lower phytotoxical levels were also found at inland sites than at coastal sites (Fig. 3). The seasonal pattern was similar in all sites but a different sensitivity (defined as the rate of damage response to O_3 concentrations) was found for the different sites with different climate. Table 1 depicts the greater sensitivity of mountain-influenced sites followed by coastal sites and finally inland sites. Some other authors have also found increasing sensitivity in forest systems with higher altitudes (Wieser et al., 1999). This could be attributed to an increase in stomatal conductance with increasing altitude. And the higher conductance values might be attributed to more favourable water relations (Wieser et al., 1999).

Cultural conditions of the bioindicator plants were standardized as much as possible, but microclimatic conditions induced variations depending on site-specific environmental characteristics. Ozone phytotoxicity was the result not only of ozone exposure but also of the interaction with other environmental factors. One of the strongest influences was that of VPD. At VPD values below 0.5 kPa there was a strong response of the biological variable (leaf damage) to increasing ozone concentrations. On the contrary, at higher VPD values than 1 kPa the uptake or effective dose and the consequent leaf damage decreased (Fig. 4). Many authors have found influences of other environmental variables on ozone toxicity. They are mainly variables related with RH and T. Their incorporation to the models improves the fittings of the regression between ozone exposure and biological response (Krupa et al., 1995; Sanders et al., 1995). Many previous studies have shown that ozone uptake is controlled by stomata (Thorne and Hanson, 1972; Samuelson and Kelly, 1997), and many meteorological conditions such as high VPD favour stomata closing (Benton et al., 1995; 2000). These meteorological conditions are thus likely to decrease ozone uptake and damage to foliage (Peñuelas et al., 1999; Ribas et al., 1998; Fumagalli et al., 2001).

Last years' research efforts in this field have focussed on characterization of ozone exposures in relation to plant damage and on the establishment of critical thresholds for different types of vegetation based on these previous observations. But the exceedance of these critical levels (Level I) only provides an indication that some risk exists of damage to vegetation from ozone. The degree of exceedance cannot be used to provide a measure of the relative risk of damage to vegetation in different areas of Europe (Fuhrer et al., 1997). It is demonstrated that plant damage is related to the internal ozone dose, not always equivalent to ambient concentrations. Consequently, a flux approach based on cumulative ozone uptake and therefore on its meteorological modulation rather than ambient ozone concentrations provides a more consistent relationship between ozone exposure and effects (Baumgarten et al., 2000; Pleijel et al., 2000).

Our results show, however, that an even simpler approach to establish damage levels could be based on "biomonitoring describers", probably a better approach to derive "biologically meaningful" exposure indices. As Fig. 5 shows, the use of annually and regionally averaged categorised responses of the *N. tobacco* Bel-W3 resulted in a very useful tool to describe the regional exposure ozone conditions. This result shows that it is possible to estimate the ozone levels through the visible injury in the Bel-W3 tobacco cultivar under the variable environmental conditions of a complex heterogeneous region such as Catalonia. Although there are some studies and networks of bioindication, they are just considered a supplementary information for the inventories of pollutants because it was not possible to use this response to quantitatively assess air quality (Manning, 1998). Our results show that it is possible to make quantitative inference about air quality for ozone on the basis of plant symptom expression alone, at least at the annual and regional scales.

These results led us to define damage levels for most sensitive species in rural Catalonia by using the data obtained for *N. tabacum* cv. Bel W3 during this 1995-1999 period (Table 2). We consider that situations of significant damage for the most sensitivity species are reached when biweekly AOT20 for growing season are greater than 7.36 $ppm_v \cdot h$ or biweekly AOT40 are greater than 1.28 $ppm_v \cdot h$ and produce more than 40% damaged leaf area.

In summary, these results have shown the great seasonal and geographical variability of ozone concentrations and ozone damage linked to the temporal and spatial variability of environmental conditions. These results have also shown that tobacco cultivars behave as very good biomonitors of ozone phytotoxicity when the temporal and spatial variability is integrated at the regional scale. Finally, these results have allowed the establishment of ozone damage levels for sensitive species in Catalonia (N.E. Spain). These damage levels could be generalized to most Mediterranean regions. Further studies of relations between ozone internal effective dose, species-specificity and environmental conditions are warranted to redefine with greater precision biomonitoring and ozone damage at the local scale. Modulatory variables such as the VPD must be especially considered since they are especially important in the Mediterranean region.

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2.3. Using biomonitors and modelling to develop O_3 dose-response curves

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Abstract

We aimed to test the use of tobacco Bel-W3 biomonitoring data together with ozone flux modelling, by using the WINDEP model, for the development of the absorbed dose-response relationship, and also to compare this approach with the most commonly used AOT40 (accumulated hourly ozone concentration above 40 ppb) in the estimation of exposure-damage curves. Leaf damage values were more related to OAD (ozone absorbed dose) than to AOT40 in all the three stations. An OAD of 180000 $\mu g\ m^{-2}$ accumulated over 15 consecutive days has been found to be the threshold for significant damage to the most sensitive species in this region under well watered conditions. The results show the applicability of the flux approach for risk assessment at the local scale; the improvement of the ozone damage estimation when the absorbed dose is modelled, in this case by means of WINDEP, instead of the ozone exposure approach; and finally the possibilities opened by the use of biomonitoring networks.

Keywords: Bel-W3 tobacco plants, biomonitor, Catalonia, ozone uptake modelling, ozone absorbed dose, WINDEP model.

Introduction

Ground level ozone concentrations have been reported to increase during the last decades and it is expected that they follow rising next years (Chameides et al., 1994; Jonson et al., 2001). High ozone concentrations have long been known to affect many physiological and biochemical characteristics, as well as growth and yield, of agricultural crops (Krupa, 1997; Fumagalli et al., 2001; Gimeno et al., 1995, 1999) and natural vegetation (Hogsett et al., 1997; Inclán et al., 1999; Peñuelas et al., 1995).

Critical levels for ozone damage to plants have been developed both by the United Nations Economic Comission for Europe (UN-ECE) and the European Union (EU). Current critical levels values, the so-called Level I standard (critical levels) (Kärenlampi and Skärby, 1996), are based on exposure-response relationship mainly derived from open-top chamber experiments and are expressed as cumulative ozone exposures using the index AOT40 (the sum of hourly ozone concentrations above a cut off of 40 ppb or nl l^{-1} during daylight hours, when global radiation exceeds 50 W m^{-2}). They correspond to the ozone exposure which is associated with significant negative effects on the yield of crops, the biomass increment of forest trees, or the species performance of semi-natural plant communities (Fuhrer et al., 1997; Karlsson et al. 2003). However, these critical levels are subject to several uncertainties, such as that they are based on a relatively small number of experiments and species considered (Grünhage and Jäger, 2003). Moreover, plant response to ozone is more closely related to the instantaneous flux of ozone through the stomata than to the ambient ozone exposure (Amiro et al., 1984; Fuhrer et al., 1992), and level I approach ignores any factor which may influence plant's response to ozone, such as soil moisture content, vapour pressure deficit, wind speed, or temperature. Thus, an adequate tool to establish a threshold level that ensures actual effective protection against adverse effects of O_3 on vegetation is the derivation of critical cumulative fluxes/stomatal uptake (critical absorbed dose). Level II approach considers the influence of environmental factors (phenology, soil moisture and nutrients, radiation, vapour pressure deficit, etc.) on stomatal conductance and ozone uptake, and also the effects of these environmental factors on plant response.

Several micrometeorological models of energy and trace gas exchange between phytosphere and atmosphere developed the last decades can be helpful in the quantification of O_3 exchange at the field size scale (Grünhage et al., 2000; Emberson et al., 2000a,b), the first step in the derivation of these critical absorbed dose-response relationships. However, the current database for the derivation of the flux-response relationship is more questionable. Only some works have attempted the development of ozone-flux response relationships for wheat (Fuhrer et al., 1992; Pleijel et al., 2000; Grünhage and Haenel, 2000) or potato and the grass *Phleum pratense* (Pleijel et al., 2002), and, there is an uncertainty of these relationships associated to the fact that they were derived from open-top chamber experiments (Nussbaum and Fuhrer, 2000).

There are some plant species that exhibit typical foliar injury symptoms when exposed to ambient ozone, which makes them useful bioindicators. Their use as bioindicators has been extensively reviewed elsewhere (Guderian, 1985; Manning, 1998). Most bioindicator programmes conducted all over the world have involved the use of tobacco cultivar Bel-W3 to assess ozone phytotoxicity (Bytnerowicz et al., 1993; Lorenzini, 1994; Ribas et al., 1998; Ribas and Peñuelas, 2003). *N. tabacum* cultivar Bel-W3 is a very good biomonitor of ozone concentrations in the long term at the regional scale (Ribas and Peñuelas, 2003). The ozone phytotoxicity in rural areas of Catalonia (NE Spain) have been assessed by determining the percentage of leaf area injured by ozone in plants of this cultivar exposed from spring to autumn (Ribas and Peñuelas, 2003). These authors concluded that the response of tobacco plants to ozone concentrations and therefore its biomonitoring capacity strongly depended on environmental conditions.

In the monitoring sites ground level ozone concentrations, meteorological parameters and percentage leaf area injured by ozone were continuously monitored. There are models, such as the big leaf model WINDEP (Worksheet-Integrated Deposition Estimation Program; Grünhage and Haenel, 2000), that allow the calculation of the ozone flux to leaves of the upper canopy as a function of ozone exposure, meteorology and stomatal function. An alternative approach to test exposure-effect relationship could be, thus, the calculation of O_3 stomatal uptake of bioindicator plants from monitoring sites, where the input parameters for WINDEP are usually available, and where the leaf ozone damage values can therefore be compared with simultaneous estimated ozone absorbed dose (OAD) values. Because Bel-W3 is a sensitive variety and the same species and cultivar is compared, we could obtain a good relationship between damage and absorbed dose without the disturbing effects of the species-specific vulnerability to absorbed dose. Moreover, with this approach we also would avoid the problems associated to undesirable chamber effects.

We used tobacco Bel-W3 biomonitoring data of Catalonia together with ozone flux modelling (WINDEP model) for the development of the absorbed dose-response relationship, with the aim to compare this approach with the most commonly used AOT40 in the estimation of exposure-damage curves. We compared the modelled integral of ozone absorbed dose (OAD), with the AOT40 index, and the relationship of both indices with the final ozone leaf damage observations in the monitored tobacco plants.

Materials and methods

Measurement sites

Several bioindication campaigns were conducted in Catalonia (NE Spain) in 1999, from May to October. The bioindicator plants were distributed in three rural sites of Catalonia covering a variety of environmental conditions ranging from sea level (Begur) to 1040m of altitude (Bellver) and from sea-shore (Begur) to 200 km inland (Bellver, Manlleu). The plants were placed in the monitoring stations of the Rural Network of the Environmental Department of the Generalitat de Catalunya containing ozone monitors and meteorological stations.

Plant material and assessment of ozone-induced damage

Six tobacco (*Nicotiana tabacum*) cultivar Bel-W3 plants were used as ozone bioindicators every fortnight in each site. Tobacco seeds were germinated in open top chambers with charcoal filtered air, free of ozone to obtain homogeneous plants and to avoid possible early contamination of ozone. When seedlings were at the 4th leaf stage, they were transplanted to 8 L pots filled with 25% peat, 25% vermiculite, 25% perlite and 25% sand. Soil pH of these pots was adjusted to 6.0 by adding $CaCO_3$. A NPK 15:11:13 slow-release fertilizer (Osmocote plus) was also added. A self-watering system was used in each pot by placing them above individual self-watering reservoirs communicated by two wicks. New plants were replaced every 2 weeks from May to October. Phytotoxic levels were defined according to the percentage of damaged leaf area in the Bel-W3 tobacco cultivars (Bytnerowicz et al., 1993; Lorenzini, 1994). After two weks, percentage of ozone-induced lesions on the oldest four leaves was visually recorded. These percentages were estimated in 5% intervals.

Ozone and meteorological measurements

Ozone concentrations and meteorological data (atmospheric pressure, solar radiation, wind, relative humidity and temperature) were continuously recorded at the stations of Rural Network of the Environmental Department of the Catalan Government. Ozone concentrations were monitored with an MCV analyser, model 48 AUV, based on the UV radiation absorption by ozone. The air was sampled at 2.5 m height. The AOT40 (accumulated hourly ozone concentration above 40 ppb) was calculated as the sum of the differences between the hourly concentrations in ppb and 40 ppb for each hour when the concentration exceeds 40 ppb and for each fifteen days period. It was thus calculated following the definitions stated at the UN/ECE workshops held at Bern in 1993 and Kuopio in 1996.

Ozone uptake modelling

For the estimation of O_3 stomatal uptake, the big leaf model WINDEP (Worksheet-Integrated Deposition Estimation Program; Grünhage and Haenel, 2000) was used. WINDEP is a resistance model based on the soil-vegetation-atmosphere-transfer (SVAT) model PLATIN (Plant-Atmosphere Interaction; Grünhage and Haenel, 1997) and can be downloaded from: http://www.uni-giessen.de/~gfl034/ENGLISH/WINDEP.htm. We applied the version parameterized for agricultural crops and wild plant species. According to our experimental conditions, irrigation of soil up to full field capacity, optimal water supply was assumed.

WINDEP partitions the total atmosphere-canopy O_3 flux into the fluxes reaching the stomata caves, the external plant surfaces and the soil beneath the canopy. The integral of molecular diffusion of O_3 into the leaf ($F_{absorbed}$) over time is the pollutant absorbed dose (PAD) and, in our case, ozone absorbed dose (OAD).

$$OAD = \int_{t1}^{t2} F_{absorbed}(O_3) \,.\, dt$$

In this study OAD was calculated over the same fifteen consecutive days considered for calculating the AOT40s.

Statistical analyses

For statistical analyses (correlation and regression), the Statview 4.5 programme package (Abacus Concepts Inc., Berkeley, L.A., USA) was used.

Results and Discussion

Fig. 1 shows the evolution of the AOT40, the OAD and the leaf damage during the studied period in Begur, Manlleu and Bellver stations. The Begur station presented higher ozone and leaf damage values than the other two stations. Leaf damage values were more related to OAD than to AOT40 in all the three stations. Stronger correlations were found for OAD (r=0.84 in Begur, r=0.76 in Manlleu, and r=0.94 in Bellver) than for AOT40 (r=0.34 in Begur, r=0.63 in Manlleu, and r=0.44 in Bellver). The ozone exposure- and absorbed dose-response relationships are shown in Figs. 2 and 3. The modelled O_3 absorbed dose (OAD) was better related to leaf ozone damage (r=0.83, $p<0.001$) than the accumulated exposure index AOT40 (r=0.62, $p<0.001$) (Fig. 2) when all field stations data were considered all together. When the factors affecting the expression of ozone foliar symptoms such as temperature, humidity and wind were considered, as it is the case in the calculation of OAD, the prediction of ozone damage improved (Peñuelas et al., 1999; Ribas and Peñuelas, 2003), as is clearly illustrated in Fig. 3, where the two stations with the extreme values in VPD and wind speed are depicted. Fig. 3 also shows that similar AOT40 ranges were splitted in different OAD ranges as a result of the local meteorological conditions. Therefore, local meteorological conditions need to be characterized and considered in the algorithms for both the determination of critical ozone levels and the biomonitoring of ozone.

For plant protection, the accepted accumulated exposure threshold AOT40, commonly used for agricultural and semi-natural vegetation is 3000 ppb_v h (for O_3 1 ppb_v =1.96 μg m^{-3} at 20ºC and 101.325 kPa) during daily hours for 3 consecutive months in growing season (Kärenlampi and Skärby, 1996), what would correspond to a biweekly AOT40 of 500 ppb_v if assuming mostly uniform concentrations. In the studied area ozone levels are usually higher (Figs. 1, 2 and 3). Ribas and Peñuelas (2003) defined damage levels for most sensitive species in rural Catalonia by using the data obtained for *N. tabacum* cultivar Bel W3 during the 1995–1999 period. They considered that situations of significant damage for the most sensitivity species, more than 20% damaged leaf area in the tobacco plant, are reached when biweekly AOT40 are greater than ca. 1.300 ppbv h. In the present study a similar relationship between leaf ozone damage and AOT40 has been obtained. Similarly, a critical OAD of approximately 180000 μg m^{-2} accumulated over fifteen consecutive days can be deduced as the damage threshold level for sensitive species in this area under well watered conditions (Fig. 2).

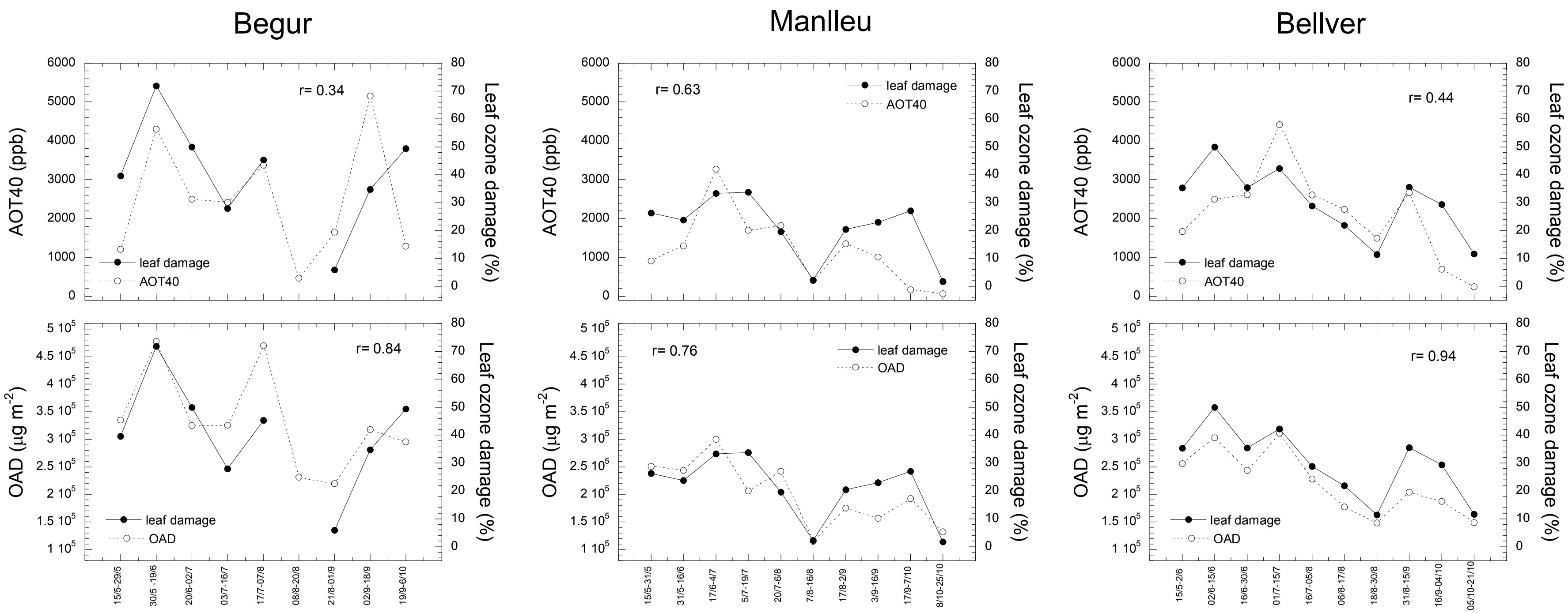

Fig. 1. Time course values of the AOT40, OAD, and tobacco Bel-W3 leaf damage during the studied period in Begur, Manlleu and Bellver stations.

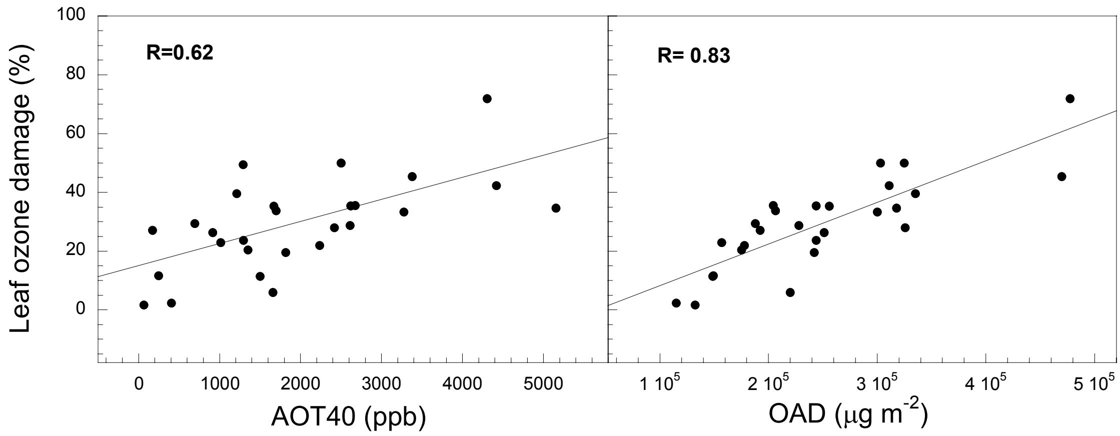

Fig. 2. Tobacco Bel-W3 leaf ozone damage versus AOT40 and OAD for all field stations data.

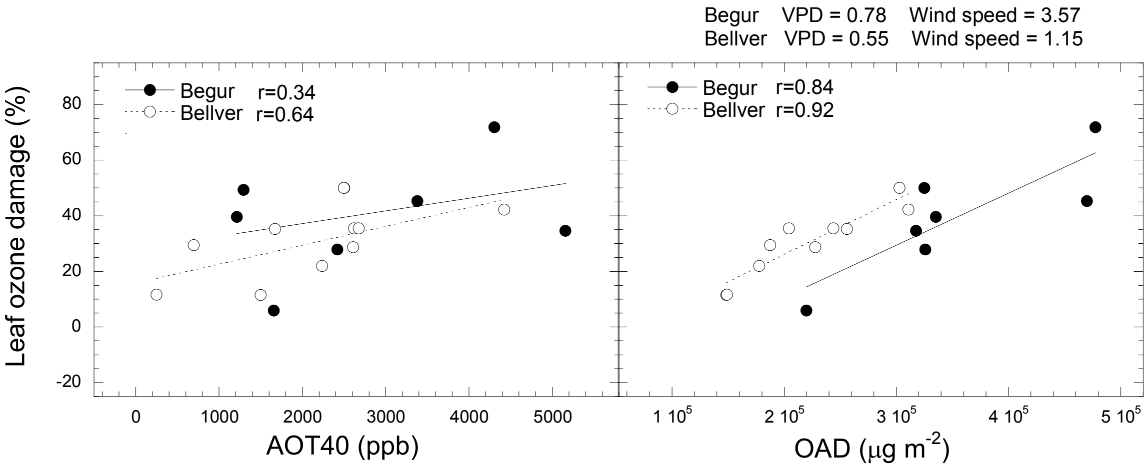

Fig. 3. Tobacco Bel-W3 leaf ozone damage vs. AOT40 and OAD for Begur and Bellver stations, two stations of contrasting VPD and wind conditions as depicted on the panel.

These results demonstrate the usefulness of biomonitoring networks as a new approximation in the development of the flux response relationship. Similarly, other biomonitoring networks could be used to test the derivation of flux-response relationship and to validate the model in different conditions, although in many cases sensitivity of the biomonitors is higher than that of most autochthonous species of the studied geographic area.

For the development of accurate (widely applicable) critical loads, some points need to be previously solved. First of all, a specific parameterization of the model for the species used is needed. Moreover, it must be considered that moderate water stress reduces the impact of O_3 significantly due to reduced stomatal aperture (Grünhage and Jäger, 2003). Since biomonitoring plants, such as the ones used in this study, are well watered, an optimal water supply (soil moisture at field capacity) has to be assumed in the modelling, and therefore, the obtained flux-response relationship is not representative of areas with lower water availability. Additionally, the precise calculation of toxicologically effective stomatal uptake depends on the accuracy of non-stomatal deposition estimates (Grünhage et al., 2003). Equal doses of ozone uptake do not generate equal effects, as the same accumulated stomatal ozone uptake (OAD) can cause more damage the shorter the time in which the dose is absorbed. Consequently, ozone fluxes should be weighted and the frequency of the occurrences of sequentially high fluxes should be taken into account (Grünhage and Jäger, 1996). Furthermore, it must also be considered that in plant communities responses to ozone will be different than in an isolated plant because of competition for resources (Andersen et al., 2001). However, these results show the applicability of the flux approach for local scale risk assessment, the improvement in the ozone damage estimation when modelled absorbed dose, in this case by means of WINDEP, is used instead of the ozone exposure approach, and also the possibilities opened by the use of biomonitoring networks.

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