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Evaluation of Ozone Phytotoxicity in the Greater Area of a Typical Mediterranean Small City (Volos) and in the Nearby Forest (Pelion Mt.), Central Greece

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Tropospheric (ground level) ozone is a widespread and highly toxic photochemical air pollutant affecting - directly and/or indirectly - not only the citizen's health but also the ornamental plants in the open spaces of the cities as well as the urban and suburban trees and forests in the outskirts (Fumagali et. al., 2001; Inclan et al., 1999; Pehnec et al., 2003). It is produced by the photolysis of nitrogen dioxide and the photochemical degradation of hydrocarbons under sunlight. Ozone concentrations in remote rural areas of Europe range from 20 to 40 ppb and are expected to rise in the near future. It has been estimated that ozone levels in the northern hemisphere have doubled during the 20th century and are increasing by an annual rate of 0.5-2.0% (Guicherit and Roemer, 2000). Over recent decades ambient ozone has been recognized as a real threat for cultivated species and forest trees (Benton et al., 2000).

In Greece, except of the metropolitan area of Athens city, that has been extensively studied, there is a lack of information about the levels of air pollution in mid-sized and small cities. Volos is a municipality inhabited by 120,000 residents, which face atmospheric pollution problems due to traffic, port activities and emissions of the local industries. There is not any research on the possible effects of urban air pollution of Volos city on the nearby forest of Pelion Mt., which is recognized world wide for its magnificent natural beauty, although it is known that air pollution, and particularly tropospheric ozone, affects certain species of pine and it is considered one of the factors, contributing as a secondary cause, to "forest decline" phenomenon (Matyssek and Innes, 1999). Recent investigations have shown that ozone occurs at potentially phytotoxic levels not only in Athens city but also in the nearby Mesogia plain (Saitanis et al., 2003) as well as far away from Athens, in the greater region of Corinth (Saitanis, 2003), suggesting that ozone constitutes a real threat to agricultural plants and forest trees. The present study is one of a series undertaken in order to obtain data of ozone phytotoxicity in Greece (Saitanis and Karandinos, 2001). Its purpose was to find out a) whether or not the ozone concentrations should be considered phytotoxic for the ornamental plants grown in the city of Volos, a city with urban and industrial pollution and b) whether the ambient ozone constitutes a real threat to the plants grown in the greater peripheral areas which include cultivated land and forests of particular natural beauty (Pelion Mt.).

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MATERIALS AND METHODS

Two approaches are usually used in the assessment of ozone phytotoxicity. The first concerns the *instrumental recording* of ozone concentrations and the assessment of the AOT40 (accumulated exposure over a threshold of 40 ppb) index. The AOT40 is calculated as the sum of the differences between the hourly concentration and the 40 ppb for each hour when the concentration exceeds 40 ppb and the global radiation exceeds 50 Wm^{-2}. The long-term critical level above which adverse effects on crops, trees and natural vegetation may occur has been defined as an AOT40 of 3,000 ppb*hours over three months for crops and 10,000 ppb*hours over six months for forest trees. Moreover, based on the calculation of the AOT40 over five days, two relevant short-term critical levels for visible injury on crops have been defined: the 200 ppb*hours for "humid" and the 500 ppb*hours for "dry" (related to mean day time: 09:30-16:30 and to vapour pressure deficit: below 1.5kPa means "humid", otherwise "dry") air conditions (Fuhrer et al., 1997; Benton et al., 2000). The second approach is *phytodetection* which refers to the use of higher plants (usually Bel-W3 tobacco variety) in detecting ozone occurrence at potentially phytotoxic levels, by scoring the extent of ozone-induced injury on their leaves (Nali et al., 2002; Saitanis, 2003).

Both approaches were used in this investigation. The records of the hourly concentrations of O_3 (ppb), NO and NO_2 ($\mu g/m^3$), as well as of the wind direction, monitored in the city of Volos during the period from Dec. 1, 2000 to Jan. 31, 2003 were provided by local agencies of the Prefecture of Magnesia. The data were analyzed to reveal the diurnal and seasonal pattern of NO, NO_2 and O_3 concentrations, as well as to calculate the long term AOT40s and the short term AOT40s. For the calculation of the long term AOT40 index we followed the definition given above. For the short term AOT40 index we proceed to the analysis of the five-days moving average to depict the time course of this index.

The phytodetection study was conducted during August 2001, using as bioindicators plants two tobacco (*Nicotiana tabacum* L.) varieties: i) Bel-W3 which has an ozone sensitivity threshold of 40-50 ppb and ii) Zichnomirodata, a Greek tobacco variety which has a threshold of about 60-80 ppb. Because ozone usually causes characteristic - different for each variety - visible macroscopic symptoms, these two varieties can be used as a pair of bioindicators for the tropospheric ozone in phytodetection studies (Saitanis and Karandinos, 2002; Saitanis, 2003). Seedlings of the two varieties were initially planted in pots with commercial soil and grown under laboratory conditions, in a walk-in controlled-environment chamber where the ambient air was purified by passing it through activated charcoal-purafil filters. When plants reached the stage of four to six small leaves, the pots were transferred to the city of Volos (three sites) as well as to 25 sites around the Pagasitikos gulf and on Pelion Mt. (Figure 1). The locations were carefully chosen to provide plant protection against strong wind and direct sunlight that are known to affect the response of plants to ozone.

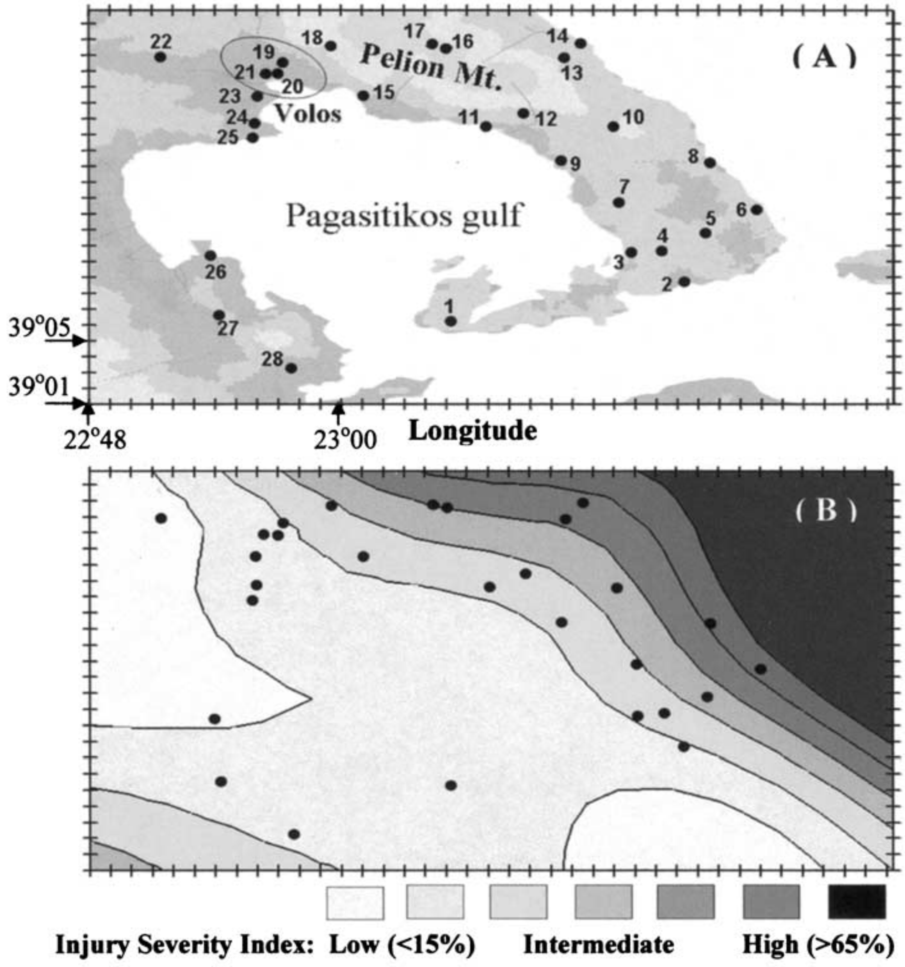


Figure 1a,b. (a) Map of the greater region of Volos and Pelion. The biomonitored sites were: 1-Trikeri, 2-Platanias, 3-Milina, 4-Lafkos, 5-Promiri, 6-Mourtias, 7-Argalasti, 8-Xinovrisi beach, 9-Afissos, 10-Neoxori, 11-Kala Nera, 12-Milies, 13-Tsagarada, 14-Damouhari, 15-Agria, 16-Agriolefkes, 17-Chania, 18-Makrinitsa, 19-20-21-Volos, 22-Velestino, 23-Alikes, 24-Soros, 25-Kritharia, 26-Amaliapoli, 27-Sourpi, 28-Pteleos. **(b)** The isolines of Injury Severity Index resulting from the analysis of the leaf injury developed on the leaves of Bel-W3 plants exposed at each site. The black points correspond to the sites indicated in the figure 1a.

After a week of exposure the extent of macroscopic symptoms on each leaf was evaluated. To this purpose, the percentage of each leaf area showing the characteristic ozone induced symptoms was estimated and then the mean of the percentages of injury from all the leaves of each plant was calculated and it constituted the per plant averaged visible injury. The mean of the per plant averaged visible injury, of all the plants of each experimental site was used as an index of ozone phytotoxicity of that site.

In order to evaluate any possible depression of photosynthesis, we measured the quantum yield of photosynthesis (dimensionless) and the gross photosynthesis ($\mu mol*m^{-2}sec^{-1}$) in a number of leaves of the exposed Bel-W3 plants showing symptoms of a different extent (from healthy to severely injured). In order to explore the effects of different light conditions, both parameters were recorded continually from dawn to sunset. It was also of interest to compare quantum yield and photosynthesis of the leaves of the exposed plants with those of healthy leaves of non-exposed plants. Thus, both parameters were measured in the leaves of a group of plants that had been grown, till then, in growth chambers. Two days before the day of measurement, those plants (called chamber-control plants) were transferred to the city of Volos and placed at a shaded place, to be protected from the midday intense direct sunlight in order to be gradually adapted to the ambient environment and so to perform better during the measurement of photosynthesis under sunlight. The measurement of each plant under sunlight lasted only 3-4 minutes, so that the plants were not depressed by long exposure to direct sunlight, and after that they were returned to the shaded place. Both parameters were measured using a portable Photosynthesis Meter instrument (Model LEAF; EARS Corp.).

RESULTS AND DISCUSSION

The ozone concentrations exhibited a diurnal and a seasonal fluctuation. High ozone levels occurred from middle spring to middle autumn, when sunlight intensity was high enough to induce photochemical formations of ozone. The diurnal pattern was the typical one for urban areas; ozone maximized during early afternoon hours, when sunlight also maximizes, and minimized during the night (Figure 2a). The minimum ozone levels occurred during the dawn and morning hours when the ozone-destroying pollutants (mainly dust and NOx), of anthropogenic origin (mainly traffic), began accumulated in the atmosphere while the sunlight was not sufficient enough to induce photochemical formation of ozone. The AOT40 calculated over the periods from May to July and from April to September were 11391 and 10351 ppb*hours for the first year (2001) and 19234 and 14276 ppb*hours for the second year (2002) respectively. The short-term AOT40, illustrated as 5-days moving average (Figure 3), showed that ozone occurred at phytotoxic levels from early spring to late autumn period. These values of AOT40s were much higher in comparison to the corresponding threshold for visible injury in vegetation. Since it is well documented that ozone levels in suburban areas are usually higher compared to those in downtown (Velissariou et al., 1992; Saitanis et al., 2003), the ozone levels are expected to be even higher in the outskirts areas including the west side of Pelion Mt. These suggest that ornamental plants and trees grown in the parks and along the streets of Volos city but also in the outskirts areas are expected to undergo potentially phytotoxic levels of ozone.

NO and NO_2 exhibited a very different diurnal pattern (Figure 2b,c). The comparison of the diurnal patterns of all three gases indicated negative correlation coefficients between O_3 and NO ($r = -0.517$; $p<0.001$) and between O_3 and NO_2

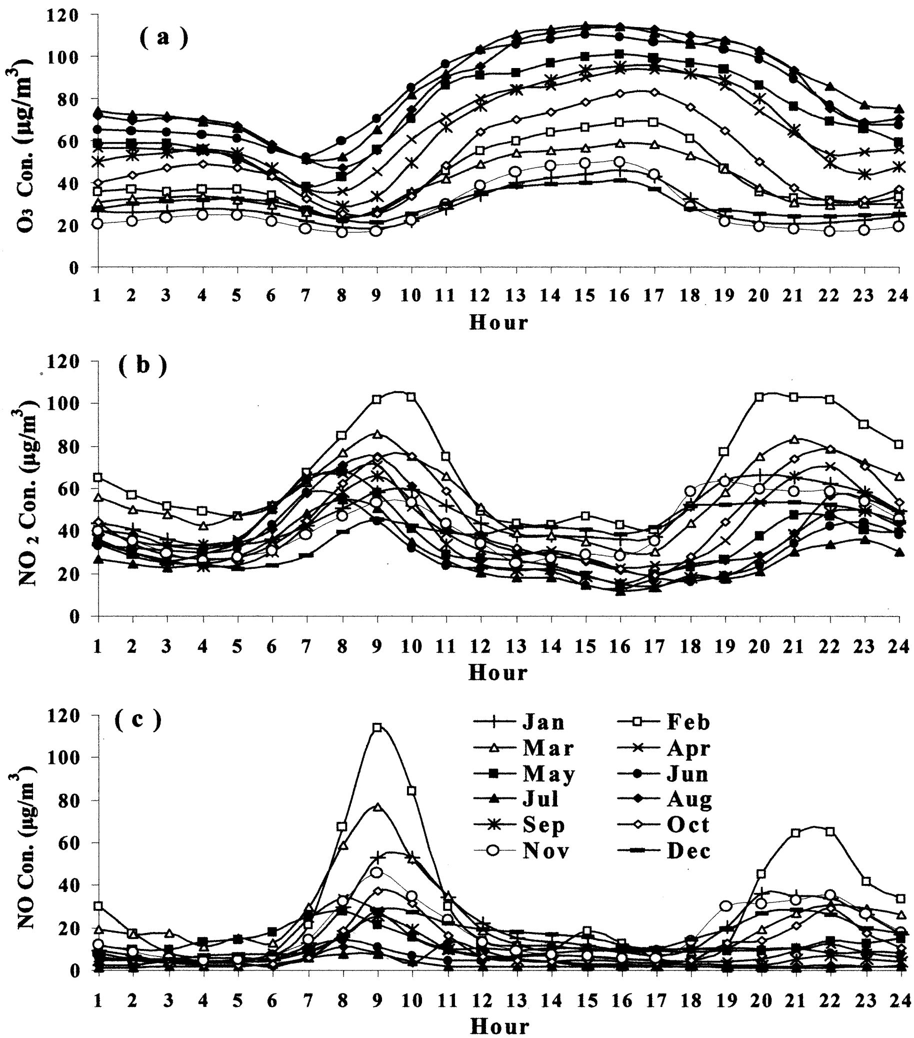


Figure 2a,b,c. The diurnal pattern of O_3 (a), NO_2 (b) and NO (c) concentrations per month and per hour of day.

($r = -0.621$; $p<0.001$) and a positive correlation coefficient between NO and NO_2 ($r = 0.759$; $p<0.001$).

It is known that at certain concentration levels, ozone and NOx may cause damage to humans, animals and vegetation. The ozone threshold, adopted by the EU (Directive 92/72/EEC), for human health protection is an 8 hour average of 110 $\mu g\, m^{-3}$ (56 ppb), while for plants the threshold has been set to a 1 hour average concentration of 200 $\mu g\, m^{-3}$ (100 ppb) (acute exposure threshold) or a 24 hour average concentration of 65 $\mu g\, m^{-3}$ (33 ppb) (chronic exposure threshold).. The percentage of days for which ozone exceeded the above three thresholds, in the city of Volos, were 12% for the threshold for human health protection, 5%

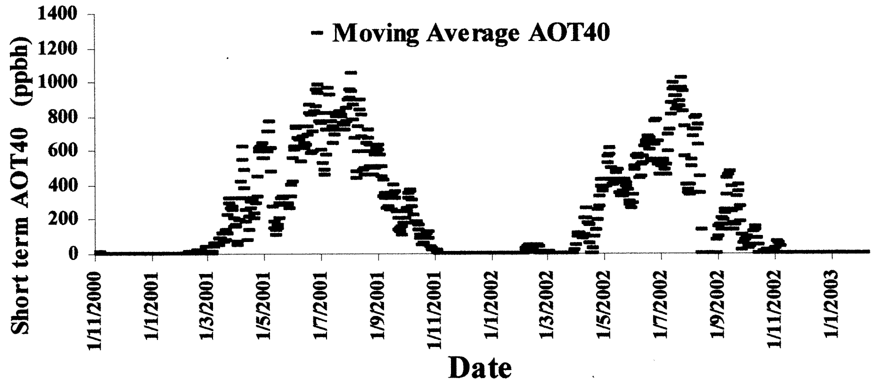


Figure 3. The time course (moving average) of the short-term (5 days) AOT40 index.

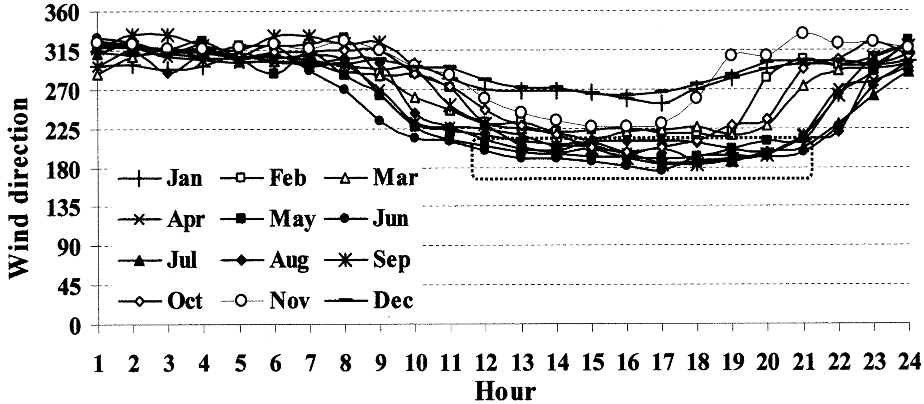
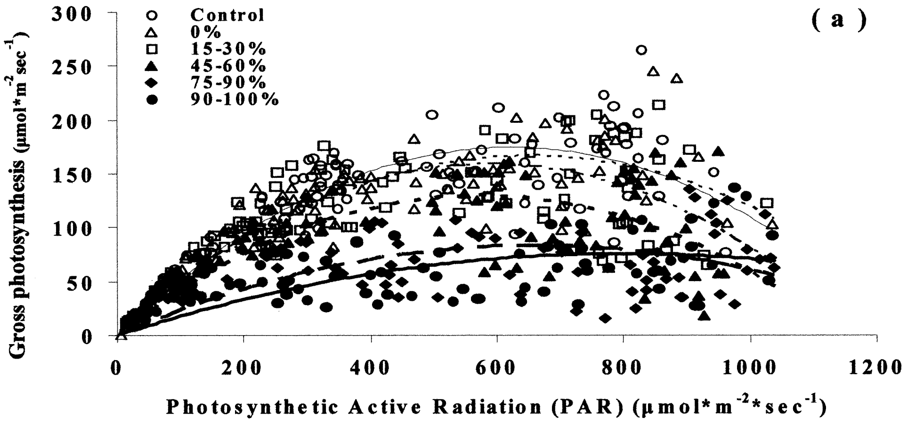


Figure 4. The prevailing wind directions per month and per hour of the day. Zero corresponds to north (clockwise) direction. The frame inside indicates that the prevailing wind during the high ozone hours of the high ozone months are those blowing from south and south-west directions.

for the threshold of acute exposure and 37% for the threshold of chronic exposure of plants. Moreover, the EU thresholds related to the protection of human health from NO_2 and of vegetation from NOx have been set to an annual average of 40 $\mu g/m^3$ and 30 $\mu g/m^3$ respectively (Directive 1999/30/EC). These limits were exceeded in the city of Volos (annual averages 42 and 56 $\mu g/m^3$ for NO_2 and NOx respectively) indicating the occurrence of these gases at levels that should be considered marginally toxic for citizens and unambiguously phytotoxic for the urban plants of the Volos city.

Analysis of the wind direction frequencies (Figure 4) showed that the prevailing wind directions changed during the 24 hours. During the afternoon hours of the warm months – when ozone, ozone precursors and sunlight occur at high levels –



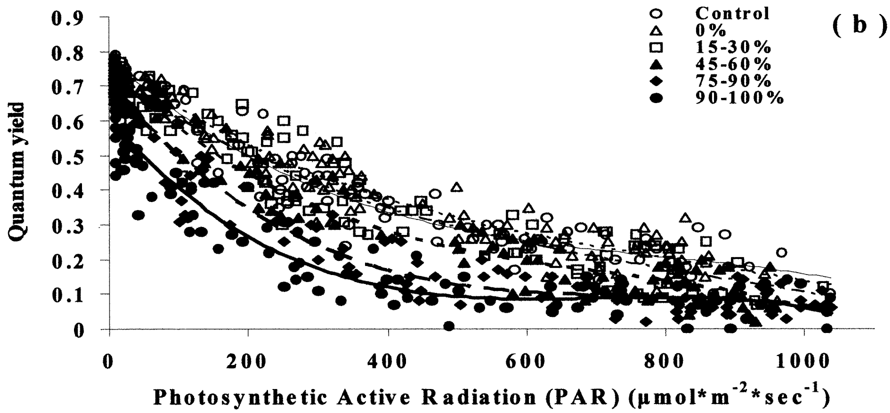


Figure 5a,b. The gross photosynthesis (μmol/m^{2}sec) **(a)** and the quantum yield of photosynthesis **(b)** in leaves of ambient exposed Bel-W3 plants, showing different degree of injury (from 0 to 90-100% injury), under different Photosyntheticaly Active Radiation (PAR) (μmol/m^{2}sec), in comparison with that of the leaves of chamber control plants (more details in the text).

the winds were of south and south-west directions. In addition to the ozone coming from the city, some amounts of ozone are probably formed *in situ* over the forest canopy. It is known that, under high intensity sunlight conditions, ozone precursors react with volatile organic compounds (VOCs) to form ozone, in a feedback process (Peñuelas et al., 1999). The VOCs necessary for local formation of ozone may be provided from the forest itself since trees release terpens, especially during the warm season.

Severe symptoms were observed on the Bel-W3 plants at the majority of the sites studied. Symptoms were also observed on plants of the Zichnomirodata variety at a sufficient number of sites. More severe symptoms were observed at the eastern-site of Pelion Mt. (Mortias, Xinovrisi, Tsagarada, etc.) and at the sites of higher altitude (Makrinitsa, Chania). This is clearly depicted in the Figure 1b which shows increased injury at the region outside the gulf. Obviously the prevailing wind directions facilitate the transportation of ozone and ozone precursor from the source – the city of Volos – to Pelion Mt. leading to an increased injury on the plants exposed over there. The macroscopic appearances of the symptoms were those already known for Bel-W3 and Zichnomirodata varieties. When some injured plants were transferred back to the laboratory and left to continue growing for two weeks, no new injury was observed on the newly expanded leaves. Moreover, no viruses were found when injured leaves were tested. The severity of symptoms observed on the leaves of Bel-W3 plants at the majority of the sites, suggests that the levels of ozone concentrations exceeded the 50 ppb, which is the threshold for symptoms appearance on this variety. Similarly, in some sites the ozone concentrations exceeded the 60-80 ppb for sufficient time to induce severe symptoms on the leaves of Zichnomirodata plants. The severity of injury observed on the leaves of Zichnomirodata plants (X) was always lower to that on the leaves of Bel-W3 plants (Y) exposed in the same site ($Y=0.8546+19.122$; $R^2=0.5616$).

In all leaves, photosynthesis showed the classic quadratic curve (Figure 5b). In low light intensity photosynthesis increased by increasing light intensity, reaching a pick during early midday hours. The further increase of the light intensity causes photo-inhibition of photosystem II (PSII) leading to a decrease of gross photosynthesis. The corresponding quantum yield, showed a gradual- initially linear – decrease (Figure 5b). This parameter reveals the performance of the photosynthetic machine; its measurement is based on the changes in chlorophyll fluorescence and it is an, easy to use, non-destructive method for *in vivo* detection of plants stress (Saitanis et al., 2001).

The maximum quantum yield is achieved under very low light conditions and is usually about 0.80-0.82. Both parameters, quantum yield and gross photosynthesis, were found to be lower in the more severe injured leaves and higher in the non-injured ones. This difference was clear when leaves were measured under high intensity light conditions, when plant can not overcome photoinhibition. Quantum yield and photosynthesis were higher in the leaves of chamber-control plants. Although this difference is difficult to be explained, since many other environmental factors may interfere, it would be, at least partially, attributed to ambient ozone. It has been demonstrated in the relevant literature that, even without visible injury, ozone depresses plants' growth and yield.

In addition, injury symptoms probably due to ozone were observed on the leaves of nearby cultivated grape-vines (leaf bronzing, yellowing and premature senescence). These symptoms were identical to those that appeared on grape-vine plants fumigated by ozone in controlled environment chambers (our unpublished data). They were also similar to those described in the literature and shown in relevant pictorial atlases (Flagler, 1998). The extent of the symptoms differed between vineyards, from very low (0-5%) to severe (about 30%). However, since in different vineyards, different varieties of vitis were cultivated and different agronomic care was provided, we are planning another research project focusing on the effects of ozone on cultivated vines.

Our bioindicators clearly showed that ambient ozone, during the period of our study, in the region around Pagasitikos gulf and at Pelion Mt., occurred at levels that were phytotoxic at least for sensitive species. The phytotoxicity of ozone levels for the plants grown in the urban and suburban region of Volos city, including those on the west side of Pelion Mt. were also marked out by analyzing the data of ozone levels occurring in the city.

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