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The Impact of Ozone Stress on Herbaceous Semi-Natural Ecosystems: Analysis of Physiological and Biochemical Markers Related to Plant Sensitivity to Ozone

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S u m m a r y

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The aim of the present work was to evaluate suitable physiological and biochemical markers to relate plant sensitivity to ozone stress in selected species of herbaceous semi-natural ecosystems of England, investigating the impact of ozone on plant species diversity. Data obtained from the physiological and biochemical analyses were used to perform different statistical analyses. The multivariate analysis of variance proved that the factor ozone, the factor species as well as their interaction have a significant effect on the system. By means of a two-way analysis of variance it was possible to select the parameters, which proved to be suitable and reliable biomarkers of sensitivity to the pollutant. A hierarchical clustering allowed us to prove that among the species analyzed, *Trifolium repens* was the one which showed a response to ozone different from all the other species; from the physiological and biochemical analyses performed this species revealed in fact a higher sensitivity to the pollutant.

I n t r o d u c t i o n

Although there is a great deal of concern about the effect of human activities, in particular to the generation of surface ozone, on biodiversity, until recently there has been very little interest in the effects of this pollutant on herbaceous species of natural and semi-natural communities. However some authors have reported that ambient ozone levels in many regions have dramatically

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increased in the past years (ALTSHULLER 1987, RUNECKLES & CHEVONE 1992, KHAN & al. 1996) reaching levels, which can influence the composition of native plant communities. There is evidence that these ambient ozone levels can be high enough to threaten some sensitive species and drive the selection of resistant genotypes so determining subtle shifts in the composition of natural or semi-natural ecosystems (FUHRER & al. 1994, BUNGENER & al. 1999).

Until now the attention on ozone effects has been focused almost exclusively on crops and forest trees and only recently it has moved to herbaceous species which constitute the natural and semi-natural communities (DAVISON & BARNES 1998). Besides, most of the studies on these species have dealt with ozone-induced damages mainly from the ecological point of view, while the effects in terms of biochemical responses have been poorly analyzed.

As the analysis of the metabolic changes induced by ozone, that can occur even before the appearance of visible symptoms on leaf surfaces, could help to identify and/or quantify plant responses to ozone, the aim of the present work was to evaluate suitable physiological and biochemical markers to relate plant sensitivity to ozone for species of a herbaceous semi-natural ecosystem of England, and to investigate the impact of ozone on plant species diversity.

Material and Methods

Six different species of a herbaceous ecosystem from the north of England (annual weed community with *Epilobium hirsutum*, *Plantago lanceolata*, *Poa pratensis*, *Rumex obtusifolium*, *Trifolium pratense*, *Trifolium repens*) were grown in chambers with controlled conditions (14 h photoperiod, 400 µmol photons m^{-2} s^{-1} PPFD, 25°/18°C day/night temperature and 70% RH). At the stage of the third true leaf, seedlings were exposed to 150 ppb ozone for 3 h.

Chlorophyll *a* fluorescence measurements were performed by means of a pulse amplitude modulated fluorometer (PAM-2000, Heinz Waltz, Germany) after 40-min dark adaptation. Parameters were directly or indirectly calculated (GENTY & al. 1989). Peroxidase activity was determined according to RANIERI & al. 2000 measuring the increase in absorbance at 460 nm due to *O*-dianisidine oxidation. The activity was expressed as units (µmol *O*-dianisidine oxidized min^{-1}) mg $proteins^{-1}$. Pigments were analyzed by means of HPLC, according to CIOMPI & al. 1997. Leaves were homogenized in 100 % acetone and the separation was performed using a Zorbax ODS column (Chrompack SA, 5 µm particle size, 250 × 4.6 mm Ø). Ascorbate and dehydroascorbate content was determined by means of spectrophotometer as reported in RANIERI & al. 2000 measuring the product of the reaction between ascorbate, $FeCl_3$ and α, α'-dipyridyl at 525 nm.

Multivariate Analysis of Variance, two-way Analysis of Variance and Hierarchical Clustering were performed by means of NCSS Statistical Software (Kaysville, Utah).

Results and Discussion

The results of the two-factor multivariate analysis of variance (Table 1) for the effects of ozone and species on the biomarkers considered showed that the treatment with the pollutant significantly influenced the various physiological and biochemical biomarkers analyzed. In addition, besides a clear effect by the specie factor (Wilk's lambda = 63.11), also the interaction between the two factors

resulted statistically significant showing that the ecosystem considered is characterized by plant species with different strategies of adaptation.

Table 1. Two factors (ozone and species) MANOVA for the different species of the ecosystem studied. d.f (H) and d.f. (E) represents the degrees of freedom, for numerator and denominator respectively, of the corresponding "F-ratio".

	d.f. (H)	d.f. (E)	Wilks' Lambda	P Level	Decision (P<0.05)
Ozone	2	5	17.58	$1x10^{-6}$	Reject
Species	0	4	63.11	0.000	Reject
Ozone x Species	0	4	11.17	0.000	Reject

More in detail, the two way analysis of variance for the parameters measured (Table 2) showed that both the actual yield of PSII and the electron transport rate, within the physiological parameters considered, were influenced by ozone treatment, while, among the biochemical parameters total chlorophylls and carotenoids, peroxidase activity and ascorbate content represent the parameters which significantly changed following ozone treatment. All parameters were influenced by ozone although at a different extent for the various plant species, indicating a quite strong biodiversity within the ecosystem considered. On the contrary the interaction between ozone treatment and plant traits resulted in a statistically significant difference among the physiological parameters only for the actual yield of PSII and the electron transport rate, among the biochemical parameters only for POD activity and ascorbate content.

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By means of the parameters analyzed, comparisons between species in relation to the response to ozone treatment were performed by means of hierarchical clustering. The results showed (Fig.1) that differences between species were bigger then differences between control and treated samples within each species, thus confirming the high degree of biodiversity between the species. The

analysis revealed a marked difference between *Trifolium repens* and the other species analyzed. *Trifolium repens* showed in fact, as a consequence of ozone treatment, stronger changes in most of the parameters analyzed, changes which showed a higher sensitivity of this species to the pollutant.

Table 2. Two way ANOVA for the physiological and biochemical parameters analyzed. Numbers in the table are the *F*-ratios.

	d.f.	Fv/Fm	Yield	PQ	TR	HL tot	Car total	β-Car
Ozone	1	.30	4.29*	6.02*	5.94*	21.66*	12.55*	3.99
Species	5	.91*	0.56*	9.16*	4.12*	17.59*	3.57*	3.02*
Ozone x Species	5	.59	6.42*	6.71*	6.68*	1.38	0.48	0.50

	d.f.	β-Car	AZ	EPS	OD	ASA	Redox state
Ozone	1	3.99	3.59	2.56	86.79*	78.28*	0.89
Species	5	3.02*	6.59*	14.77*	3445.1*	3205.7*	93.93*
Ozone x Species	5	0.50	1.53	3.14	102.50*	239.72*	5.61*

*P<0.05.

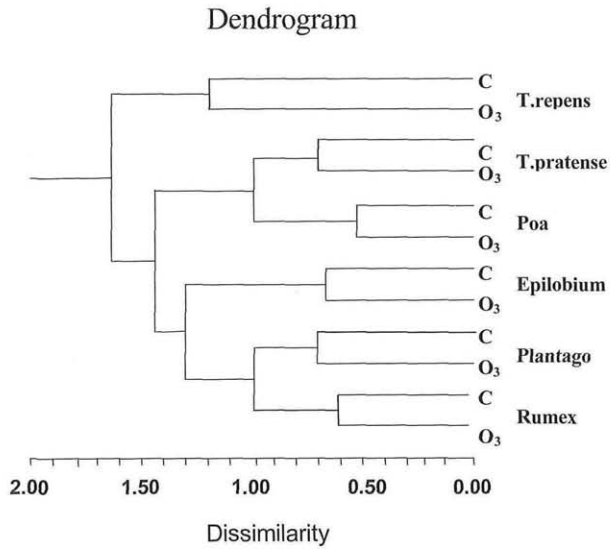

Fig. 1. Hierarchical clustering for the species analyzed. Control and treated samples of each species have been kept separate.

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References

ALTSHULLER A. P. 1987. Atmospheric chemistry and long range transport. - In: HECK W. W., TAYLOR O. C. & TINGEY D. T. (Eds.), Assessment of crop loss from air pollutants, pp. 141 - 179. - Proceedings of the International conference, Raleigh, NC., Elsevier Applied Sciences, London.

BUNGENER P., NUSSBAUM S., GRUB A. & FUHRER J. 1999. Growth response of grassland species to ozone in relation to soil moisture condition and plant strategy. - New Phytol. 142: 283 - 293.

CIOMPI S., CASTAGNA A., RANIERI A., NALI C., LORENZINI G. & SOLDATINI G. F. 1997. CO_2 assimilation, xanthophyll cycle pigments and PSII efficiency in pumpkin plants as affected by ozone fumigation. - Physiol. Plant. 101: 881 - 889.

DAVISON A. W. & BARNES J. D. 1998. Effects of ozone on wild plants. - New Phytol. 139: 135 - 151.

FUHRER J., SHARIAT-MADARI H., PERLER R., TSCHANNEN W. & GRUB A. 1994. Effects of ozone on managed pasture. II. Yield, species composition, canopy structure, and forage quality. - Environ. Pollut. 86: 307 - 314.

GENTY B., BRIANTAIS J. M. & BAKER N. R. 1989. The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. - Biochim. Biophys. Acta 990: 87 - 92.

KHAN M. R., KHAN M. W. & KHAN A. A. 1996. Evaluation of the sensitivity of some vegetable crops to ozone. - Tests Agrochem. Cultiv. 17: 94 - 95.

RANIERI A., PETACCO F., CASTAGNA A. & SOLDATINI G. F. 2000. Redox state and peroxidase system in sunflower plants exposed to ozone. - Plant Sci. 159: 159 - 168.

RUNECKLES V. C. & CHEVONE B. I. 1992. Crop responses to ozone.- In: LEFOHN A. S. (Ed.), Surface level ozone exposures and their effects on vegetation, pp. 189 - 270. - Lewis Publishers Inc., Chelsea, MI.