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Study of ambient ozone phytotoxicity in Ukraine and ozone protective effect of some antioxidants

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Abstract

The aim of the study was to assess ambient ozone phytotoxicity in Kyiv (Ukraine) using bioindicator clover plants (*Trifolium subterraneum* cv. Geraldton) and to test some natural and synthetic antioxidants as ozone protectants. The results obtained showed that ambient ozone concentrations were high enough to cause visible leaf injury in clover. All used substances showed partial ozone protective effect on clover. Water extracts from the leaves of plants, known to contain flavonoids–antioxidants showed weaker ozone protective effect and were less stable in the field conditions than synthetic antioxidants. Among the studied extracts, those from *Ocimum basilicum* and *Tagetes patula* were more effective as ozone protectants than the one from *Salvia sclarea*.

Keywords: Ambient ozone; Phytotoxicity; Biomonitoring; Ozone protectants; Synthetic antioxidants; Natural antioxidants

1. Introduction

Ambient (tropospheric) ozone (O_3) is one of the most widespread pollutants of ambient air of the highest category of toxicity for human and vegetation. Its concentration in the air is legally regulated in all developed countries of the world. Together with carbon dioxide (CO_2) and methane (CH_4), ozone is one of the most important gases implicated in greenhouse effect and causing undesirable climatic changes [1–3].

The problems of increase in ozone pollution of the atmospheric air and overcoming of its negative consequences are arising sharply in a number of regions of the world due to considerable losses, which could be caused by phytotoxic effect of ozone on agricultural crops and forests. Ambient ozone reduces plants yield by impeding photosynthesis and weakening them [1,2,4]. Average yield losses for cereals in Ukraine due to antropogenic pollution of atmospheric air by ozone during the last decade are estimated as 12%, while biomass growth reduction for deciduous trees is estimated as 9%, respectively [5].

Investigations of the atmospheric air pollution by ozone and the study of its toxic effect on vegetation are very urgent today. As a result of conducted researches scientists succeeded to select from natural vegetation and to breed special cultivars of domestic plants sensitive and tolerant to ozone. Alternative method for ambient ozone biomonitoring, using bioindicator plants, has also been developed [6].

In spite of great attention paid by scientists of the leading countries of the world towards study of the ambient ozone and its phytotoxic effect, such investigations in Ukraine are not developed enough. Though in the economy of this country, agriculture and forestry are of special importance, extremely little is known about the levels of ozone concentrations on its territory up to now. While according to some preliminary data, ambient ozone could occur in Ukraine in potentially phytotoxic concentrations [7,8].

The problem of reduction of yield loses due to ambient ozone could be solved by two ways: selection of tolerant to ozone crops and application of substances protecting plants from ozone damage. Today a number of such substances are already known. Out of them ethylene diurea (EDU)—*N*-[2-2-oxo-1-imidazolidinyl)ethyl]-*N*-phenylurea is one of the most effective and perspective [9,3]. EDU is an antioxidant that prevents acute O_3 injury and inhibits senescence in plants. EDU does not affect photosynthesis, but may affect stomata opening [10]. This substance is effective both as a foliar spray and as soil drench [11].

Significant amount of chemicals utilized presently in agriculture, when intensive methods of plant cultivations are implemented, causes serious ecological concern as it poses hazard to human health. Existing substances with ozone protective

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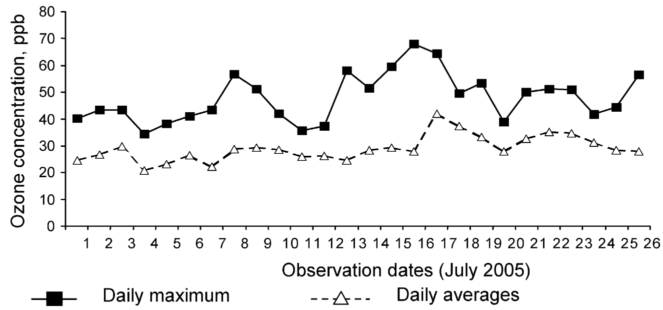

Fig. 1. Dynamics of ozone concentrations in the atmosphere during the period of the bioindication experiments.

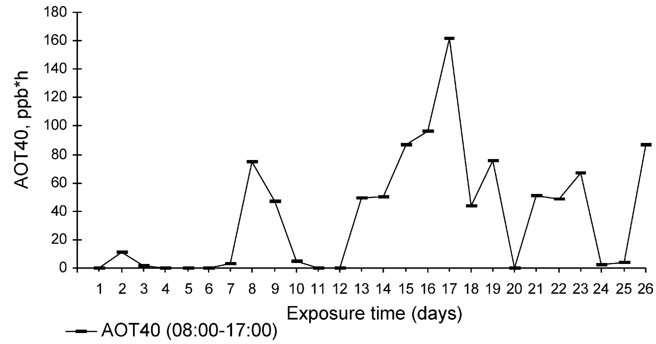

Fig. 3. The moving averages of the short-term (5-day) AOT40 recorded from 1 to 26 July 2005.

properties (EDU, penconazol, benomyl, etc.) are also a source of chemical pollution of environment and agricultural production. Therefore it is urgent to search for effective, environment friendly natural antioxidants.

Many species of plants are known to contain substances with high antioxidant effect (flavonoids, carotinoids and some other phenolics). High antioxidant effect of the mentioned above substances is due to their ability to bind ions of heavy metals catalyzing oxidation reactions and to stop chain reactions with free radicals as they are able to give electron to almost every kind of free radicals [12]. As obtainment of individual biologically active substances originated from plants is very expensive it is more promising from practical point of view to study crude plant extracts with analogues properties rather than separate antioxidant chemicals. However, as far as we know from literature, no study in this direction has been conducted so far. Our preliminary experiments with extracts from marigold plants (*Tagetes patula* L.) applied for protection of plants from ozone damages showed considerable promise of this approach [13].

The aim of the study was to assess ambient ozone phytotoxicity using bioindicator test-plants of subterranean clover (*Trifolium subterraneum* cv. Geraldton) as well as to test some natural and synthetic antioxidants as ozone phytoprotectants.

2. Materials and methods

Ozone biomonitoring experiment using test-plants of subterranean clover (*T. subterraneum* cv. Geraldton) was conducted at semi-urban monitoring station located at the National Botanic Garden of the National Academy of Sciences of Ukraine in Kyiv city from 1 to 26 July of 2005. The test-plants were cultivated in ozone-free air until they reach four-leaves stage. Afterwards clover plants were sprayed with one of the following: (1) control distill water, (2) solution of EDU (300 mg/l), a well known synthetic antioxidant, (3) Topaz, a fungicide agrochemical containing 10% of penconazol (0.3 ml/l), and 5% water extracts from fresh leaves of plants rich in flavonoids with antioxidant properties such as (4) clary (*Salvia sclarea* L.), (5) marigold (*Tagetes patula* L.), (6) basil (*Ocimum basilicum* L.). The leaves were collected at the flowering stage of plants-donors. On the following day after application of antioxidants the test plants were exposed in the field. In 2 weeks after exposition the application of antioxidants was repeated.

The visible symptoms of ozone foliar injuries (%) were evaluated once a week for the whole period of exposition. On the last day of exposition apart from foliar injury, photosynthetic pigments (chlorophylls *a* and *b*, carotenoids) in leaves and biomass of above-mentioned test-plants were evaluated. Each treatment had seven replicates.

Statistical analysis of the results obtained was conducted with the usage of descriptive statistics: average values and standard errors for the all measured physiological and morphological parameters of the test plants were calculated [14].

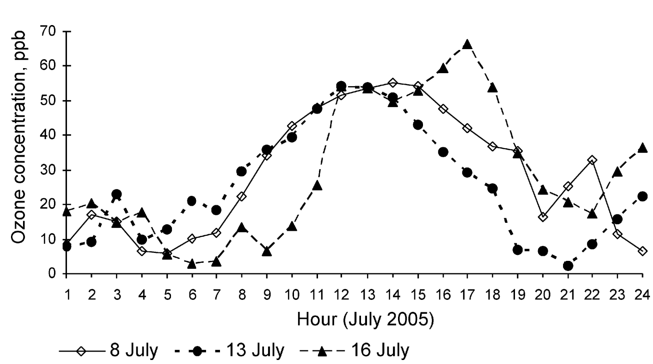

Fig. 2. The diurnal profile of ozone concentrations recorded in Kyiv during ozone episode on 8, 13 and 16 July 2005.

Fig. 4. Typical ambient ozone injuries on leaves of clover (*Trifolium subterraneum*) test plants.

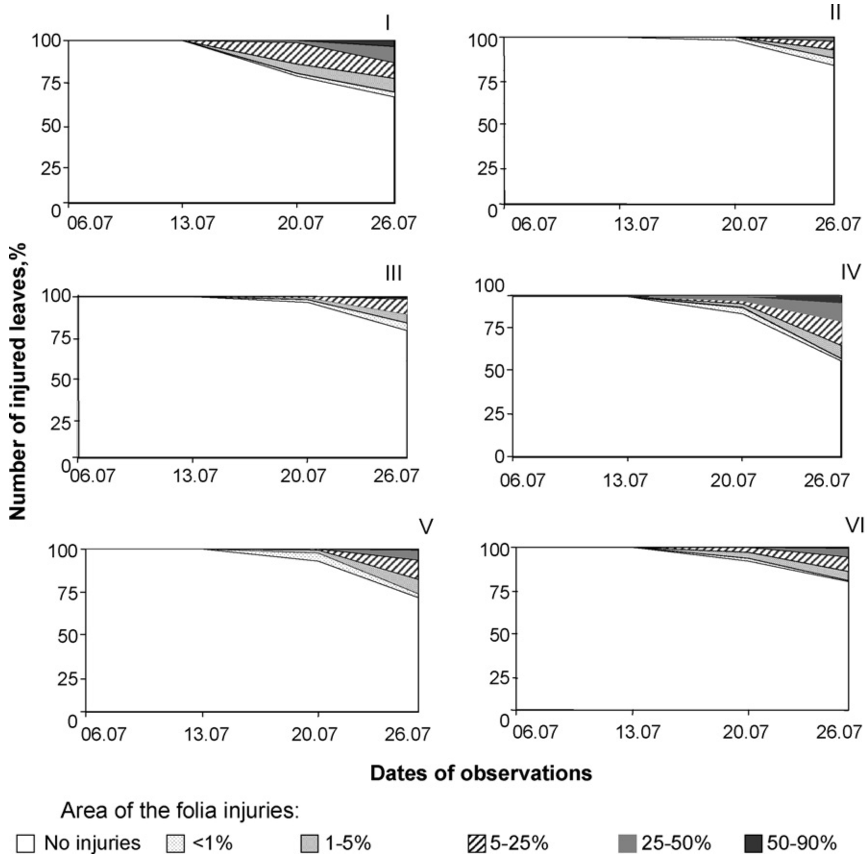

Fig. 5. Development of ozone-induced foliar injuries in clover test-plants in control (I), and sprayed with the solutions of EDU (II), Topaz (III), extracts of leaves of *Salvia sclarea* (IV), *Tagetes patula* (V), and *Ocimum basilicum* (VI).

During the period of the experiments ambient ozone concentrations were continuously monitored by Thermo Environmental UV Photometric Ozone Analyzer Model 49 and the moving averages of the short term AOT40 were calculated. Temperature and relative humidity of the atmospheric air were also measured and recorded by special memory devices (HOBO H8 Data Logger).

3. Results and discussion

Dynamics of ozone concentrations in the atmosphere during the period of the bioindication experiments is shown in Fig. 1.

The results obtained show that ambient ozone concentrations during the whole period of the bioindication experiments (1–26 of July) exceeded the threshold of subterranean clover sensitivity to ozone of 30 ppb. In Fig. 1 it could be seen that three ozone episodes occurred on 8, 13 and 16 of July. Analysis of the diurnal profile of ozone concentrations during these days showed exceedences of threshold of 30 ppb for 8–11 h (Fig. 2).

These profiles are typical for urban and semi-urban locations with high automobile emissions of NOx, which are precursors of ozone. The lowest ozone concentrations were observed in the early morning (5–6 a.m.) after NOx react with ozone in ambient air during the night before sunrise. Then ozone concentrations begin to rise gradually. Fig. 3 shows the moving averages of the short-term (5-days) AOT40s during the experimental period.

During the frequent rains, period from 1 to 7 and from 10 to 12 of July short-term AOT40s were essentially low. The highest value of this index of 180 ppb h was observed on 16 July, when the weather was hot and dry.

Because of the rainy weather during the first week of July, visible symptoms of ozone induced foliar injuries on clover test plants appeared only on 14th day of exposition (Figs. 4 and 5)

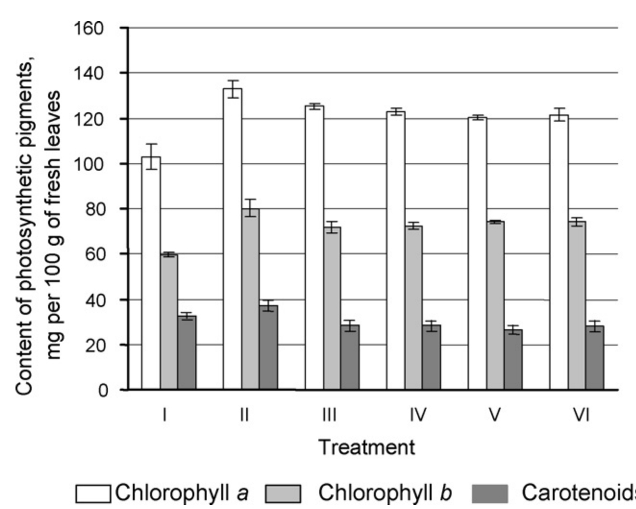

Fig. 6. Content of photosynthetic pigments in leaves of clover test-plants in control (I), and sprayed with the extracts of leaves of *S. sclarea* (II), *O. basilicum* (III), *T. patula* (IV) and the solutions of Topaz (V), and EDU (VI).

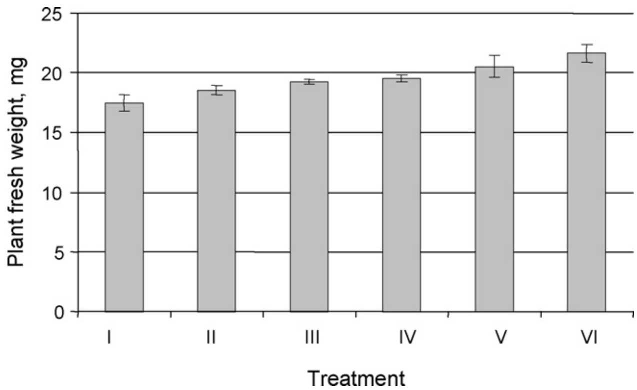

Fig. 7. Fresh weight of clover test-plants in control (I), and sprayed with the extracts of leaves of *S. sclarea* (II), *O. basilicum* (III), *T. patula* (IV) and the solutions of Topaz (V), and EDU (VI).

after the weather became more hot and dry, and maximum daily ozone concentrations increased. From 14th till 26 of July ozone induced foliar injuries were increasing steadily. This trend is in good correspondence with the rise in daily maximum ozone concentrations observed in the atmosphere from 11 till 16 and from 20 till 23 of July as well as with the pick of daily average on 16 of July.

All the applied antioxidants effectively protected clover plants from ozone injury during first week after application. However, as the period of the exposition increased the effectiveness of the ozone-protective effect of the applied antioxidants, especially of natural ones from plant extracts, decreased. This is the evidence of the low stability of natural antioxidants (flavonoids), which could be decomposed by epiphytic microflora. At the end of the experiment (on the 14th day after the second application of antioxidants) clover plants treated with the extract from clary were not different from control. While for the rest of the tested antioxidants some ozone protective effect was observed. Test-plants treated with the 5% extracts from marigold and basil were only to a small extent more injured than those treated with the solutions of the well know synthetic antioxidant EDU (in concentration of 300 mg/l). Though the concentration of the extracts from plants is much higher as compared to EDU, the former as natural products are safer for the environment and had no direct phytotoxic effect on plants. While EDU in higher concentration was shown to be phytotoxic [10].

The content of photosynthetic pigments (chlorophylls *a* and *b*) at the end of the experiment in the leaves of test-plants treated with any of the tested antioxidants were reliably higher than that in control (Fig. 6).

Photosynthesis is one of the major physiological processes affected by ozone. Caused by ozone reduction in photosynthetic capacity affects stomatal conductance, carbohydrate allocation and respiration. The photosynthetic capacity of a plant is known to play a major role in plant response to stresses in the environment and to affect leaf repair as well as overall growth and reproductive capacity to compensate for foliar loss resulting from ozone stress. Thus, observed increase in photosynthetic pigment in the leaves of test-plants sprayed with the antioxidants in comparison with the ones in control evidenced that the former had better chances for further adaptation and regeneration than the latter. The results of the measurements of the fresh phytomass of the clover plants on the last date of the exposition confirmed this suggestion (Fig. 7). The fresh weight of the test plants was reliably higher in the all treatments than in the control.

Thus among the tested antioxidants the most effective was synthetic ozone protectant—EDU. The water extracts from the leaves of plants, known to contain flavonoids–antioxidants showed weaker ozone protective effect than synthetic antioxidants. Among the studied extracts, those from basil and marigold were noticeably more effective as ozone protectants than the one from clary.

4. Conclusion

The results obtained showed that ambient ozone concentrations were high enough to cause visible leaf injury in clover. All used substances showed partial ozone protective effect on clover. Among the tested antioxidants the most effective was synthetic ozone protectant—EDU. The water extracts from the leaves of plants, known to contain flavonoids–antioxidants showed weaker ozone protective effect than synthetic antioxidants. Besides they were less stable in the field conditions. Among the studied extracts, those from basil and marigold were noticeably more effective as ozone protectants than the one from clary. However to reach the effectiveness of a synthetic antioxidant such as EDU, plant extracts should be applied in concentrations several orders higher than the former. Nevertheless plant extracts as natural products are safer for the environment as they are rapidly decomposed by microflora. Further investigation of the ozone protective properties of the natural antioxidants and the search of their more stable forms are needed for the working out of the environment friendly techniques of plant protection from ozone injury.

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References

[1] J. Benton, J. Fuhrer, B.S. Gimeno, L. Skarby, D. Palmer-Brown, G. Ball, C. Roadknight, G. Mills, An international cooperation programme indicates the widespread occurrence of ozone injury on crops, Agric. Ecosyst. Environ. 78 (2000) 19–30.

[2] W.W. Heck, O.C. Taylor, D.T. Tingey (Eds.), Proceedings of an International Conference on Assessment of Crop Loss from Air Pollutants, Raleigh, NC, USA, 1987.

[3] W.J. Manning, S.V. Krupa, Experimental methodology for studying the effects of ozone on crops and trees, in: A.S. Lefohn (Ed.), Surface Level Ozone Exposure and Their Effects on Vegetation, Lewis Publisher, Chelsea, 1992, pp. 93–155.

[4] C.J. Saitanis, M.G. Karandinos, Instrumental recording and biomonitoring of ambient ozone in Greek countryside, Chemistry 44 (2001) 813–821.

[5] S.M. Semenov, I.M. Kunina, B.A. Koukhta, Troposphere ozone and plant growth in Europe, Publishing Center “Meteorology and Hydrology”, Moscow, 1999.

[6] B. Godzik, Surface ozone concentrations in southern Poland: tobacco cultivar exposure study, Fragm. Flor. Geobot. 42 (1997) 161–172.

[7] O. Blum, Biomonitoring of atmospheric heavy metal deposition and tropospheric ozone monitoring in Ukraine: results and perspectives, in: Proceedings of the 4th Training-workshop on the Calculation and Mapping of Critical Loads for Air Pollutants Relevant for the UN/ECE Convention on LRTAP in East and South East European Countries (Sudak, Crimea), Simferopol, 2002, pp. 77–84.

[8] O. Blum, A. Bytnerowicz, W. Manning, L. Popovicheva, Ambient tropospheric ozone in the Ukrainian Carpathian mountains and Kiev region: detection with passive samplers and bioindicator plants, Eviron. Pollut. 98 (1997) 299–304.

[9] G. Hofstra, D.A. Littlejohns, R.T. Wukasch, The efficacy of the antioxidant ethylenediureu (EDU) compared to carboxin and benomyl in reducing yield losses from ozone in navy beans, Plant Dis. Rep. 62 (1978) 350–352.

[10] W.J. Manning, Assessing the effects of ozone on plants: use and misuse of ethylendiurea (EDU), in: Proceedings of the 85th Air and Waste Management Association Meeting. Kansas City, Missouri, 1992, pp. 95–100.

[11] B. Godzik, W.J. Manning, Relative effectiveness of ethylenediurea, and constituent amounts of urea and phenylurea in EDU, in prevention of ozone injury to tobacco, Environ. Pollut. 103 (1998) 1–6.

[12] M.N. Zaprometov, Phenolic substances. Distribution, metabolism and functions in plants, Nauka Publication, Moscow, 1993.

[13] O. Blum, N. Didyk, C. Saitanis, Ambient ozone phytodetection with sensitive clover (*Trifolium subterraneum* L. cv. Geraldton) in Ukraine, in: G. Arapis, N. Goncharova, Ph. Baveye (Eds.), Ecotoxicology, Ecological Risk Assessment and Multiple Stressors, NATO Security though Science Series C: Environment Security, Springer, Netherlands, 2006, pp. 279–289.

[14] G.N. Zaytsev, Mathematical statistics in experimental botany, Nauka Publication, Moscow, 1984.