

Herbicide induced oxidative stress in lettuce, beans, pea seeds and leaves

D. ŠTAJNER*, M. POPOVIĆ** and M. ŠTAJNER***

*Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8,
21000 Novi Sad, Serbia and Montenegro**

*Faculty of Science, University of Novi Sad, Trg Dositeja Obradovića 3,
21000 Novi Sad, Serbia and Montenegro***

*Faculty of Medicine, University of Novi Sad, Hajduk Veljkova 3, 21000 Novi Sad, Serbia and Montenegro****

Abstract

The effects of different paraquat, alachlor and metolachlor concentrations on superoxide dismutase (SOD), catalase (CAT), guaiacol peroxidase (GPX) activities and pigment contents in lettuce, bean and pea seeds and leaves were studied. Under paraquat (1.0 and 2.0 μM) treatment declined SOD and CAT activities were observed in seeds and undetectable activity of GPX. Germination of all investigated seeds was completely inhibited. All used concentrations of alachlor and metolachlor inhibited antioxidant enzyme activities in seeds but did not prevent germination and growth. In leaves, lower concentrations of these herbicides increased activities of antioxidant enzymes but at the highest herbicide concentrations (200 μM) activities of investigated enzymes declined. The pigment contents the leaves decreased due to alachlor and metolachlor treatment in a concentration dependent manner.

Additional key words: alachlor, carotenoids, catalase, chlorophyll, guaiacol peroxidase, metolachlor, paraquat, superoxide dismutase.

Introduction

Formation of toxic active oxygen species such as singlet oxygen, superoxide, and hydroxyl radicals, and hydrogen peroxide is generally considered to be detrimental to cellular functions, but these molecules are also formed in normal cell metabolism and their destruction is regulated (Gey 1994). During environmental or man-made stresses (e.g. caused by herbicide action) production of active oxygen species increase and could cause non-regulated oxidative damage to membranes (Aioub *et al.* 1993), chloroplasts (Halliwell 1986, Vincente *et al.* 2002) and other cell organelles.

Bipyridyl herbicides, such as paraquat (1,1'-dimethyl-4,4'-bipyridinium chloride), increase oxidative stress directly by generating oxygen radicals. Also known as methyl viologen, paraquat is a redoxactive compound that is photo-reduced by photosystem I and subsequently reoxidized by transfer of its electrons to oxygen, forming the superoxide anion (Štajner *et al.* 1994). Highly reactive hydroxyl radicals produced from this superoxide are presumably the agents that cause cellular damage (Frei 1996). Paraquat is also toxic to non-photosynthetic tissues because it can take electrons from many sources,

in particular from a microsomal NADPH cytochrome P450 reductase both in animals and in plants. The chloroacetamides alachlor (2-chloro-2',6'-diethyl-N-(methoxymethyl)-acetanilide and metolachlor (2'-chloro-2'-ethyl-6'-methyl-N-(1-methyl-2-methoxyethyl)-acetanilide) are the selective pre-emergent or early post-emergent herbicides used to control most annual grasses and certain broad leaf weeds. Under field condition, these compounds could be completely degraded and decomposed under the influence of UV radiation and ozone (Somich *et al.* 1988). During its dechlorination, at the first step of photolysis, reactive oxygen species are formed producing other radicals that contribute to the deleterious action of alachlor and metolachlor on plants (Bowjer *et al.* 1987, Gey 1994, Štajner *et al.* 1994a,b, Štajner *et al.* 1995).

The antioxidant enzyme superoxide dismutase (SOD) plays a key role in the defense of all aerobic organisms against toxic oxygen species, which are generated under oxygen stress (Misra *et al.* 1979, Escobar 1996, Goeffroy *et al.* 2002). Therefore, the SOD activity has often been correlated with the mechanism of herbicide survival.

Received 3 April 2002, accepted 5 June 2003.

Abbreviations: Car - carotenoids; CAT - catalase; Chl - chlorophyll; GPX - guaiacol peroxidase; SOD - superoxide dismutase.

* Fax: (+381) 21 450857, e-mail: stajnerd@polj.ns.ac.yu

Other antioxidant enzymes, such as catalase (CAT) and guaiacol peroxidase (GPX), are also involved in antioxidant protection by removing H_2O_2 . In addition, some non-enzymatic substances, such as carotenoids vitamins E and C, have protective antioxidant properties (Escobar *et al.* 1996).

The precise mechanism of paraquat, alachlor and

metolachlor action is so far insufficiently known (Bowler *et al.* 1992). The present study was therefore undertaken to investigate the changes in the activities of active oxygen scavenging enzymes and pigment contents in vegetable seeds and leaves subjected to stress induced by these herbicides.

Materials and methods

Seeds and young plants of lettuce (*Lactuca sativa* L. cv. Vuka), bean (*Phaseolus vulgaris* L. cv. Zlatko) and pea (*Pisum sativum* L. cv. Dunav) were used. For the experiments, anatomically fully developed seeds without mechanical injuries and necrotic areas were chosen. Three concentrations (0.1, 1.0 and 2.0 μM) of paraquat and seven concentrations (0.2, 1.0, 2.0, 20.0, 100.0, 200.0 μM) of metolachlor and alachlor were applied in Hoagland nutrient solution. Enzyme activities determinations were performed in four repetitions with 1 g of seeds (1 g pea = 7 seeds; 1 g bean = 4 seeds; 1 g lettuce = 1345 seeds) after 48 h.

The surface-sterilized seeds were placed in Petri dishes on filter paper moistened with 5 cm^3 of Hoagland nutrient solution [$g\ dm^{-3}$]: 1.18 $Ca(NO_3)_2 \cdot 4\ H_2O$, 0.306 KNO_3 , 0.136 KH_2PO_4 and 0.25 $MgSO_4 \cdot 7\ H_2O$. They were kept at room temperature in the dark for 48 h and then transferred to the greenhouse and treated with herbicides. The plants were grown 24 d after herbicide treatment in the greenhouse. The second leaf was used for experiments.

For enzyme activity measurements 1 g of soaked

seeds or 24-d-old second leaves were ground with quartz sand in a cold mortar. The ground materials were suspended in 5 cm^3 0.1 M K_2HPO_4 at pH 7.0. After a 10 min centrifugation at 4 $^\circ C$ and 15 000 g, the aliquots of the supernatant were used for enzyme activity measurements. For the superoxide dismutase (SOD) activity measurements, the supernatant after precipitation with Tsuchihashi solution ($CHCl_3$:ethanol, 3:5) and centrifugation was used. SOD activity was determined by the modified method of Misra and Fridovics (1972) based on the autocatalytic transformation of epinephrine-adrenochrome at pH 10.2. The aliquots of phosphate buffer supernatant were used for determination of catalase (CAT) and guaiacol peroxidase (GPX) activities using the method of Simon *et al.* (1974). Protein content was determined by the method of Bradford (1976).

Carotenoids (Car) and chlorophylls (Chl) were extracted with acetone and measured by the method of Rabinowitch *et al.* (1975).

The data represent the average of three experiments. Variations between replicates were less than 5 %.

Results and discussion

SOD and CAT activities declined proportionally to the applied paraquat concentrations in lettuce, bean and pea seeds (Fig. 1). GPX activity in the lettuce seeds was inhibited strongly and was not detectable after treatment

with 1 and 2 μM paraquat. At all concentrations of paraquat used, germination was completely inhibited, what is in agreement with our previous results in wheat (Štajner *et al.* 1994), and statements of other authors that

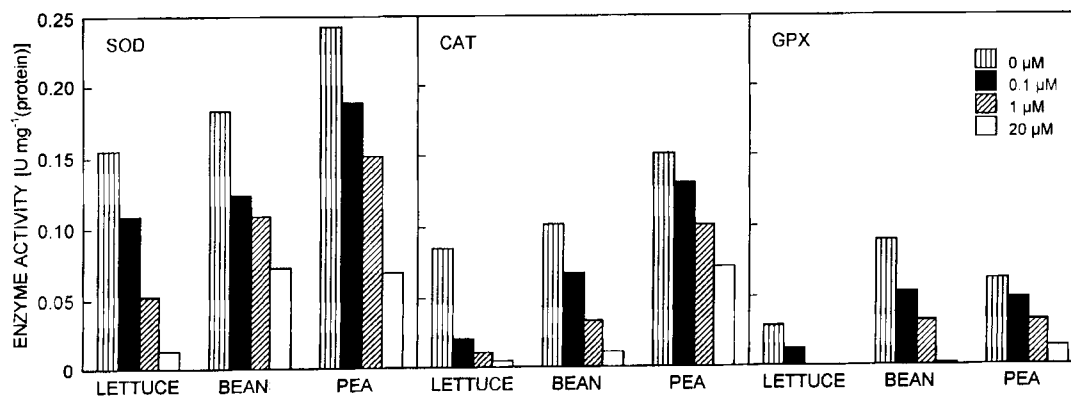


Fig. 1. Effect of different paraquat concentrations on the antioxidant enzyme activities in seeds.

paraquat kill the green plants in the presence of oxygen (Stegman and Schuler 1993).

It was proved that antioxidant enzymes are an important part of the mechanism of paraquat tolerance (Vincente *et al.* 2001). In our previous results osmotic stress in wheat induced by different polyethylene glycol concentrations also induced oxidative stress and increased SOD activity, but germination was not inhibited. Thus the oxidative stress induced by osmotic stress was not so strong as paraquat caused stress (Somich *et al.* 1988). The imposition of salt stress decreased but not prevented germination rate in roots of *Oryza sativa* L. (Khan and Panda 2002).

The antioxidant enzyme activities also declined under alachlor and metolachlor treatments (Fig. 2). The highest inhibition was observed at the highest herbicide

concentration of 200 μM and negligible effect was observed at the lowest herbicide concentration of 0.2 μM , in seeds of all investigated vegetable sorts. In contrast to paraquat which form a bipyridyl radical it was suggested that superoxide can displace chloride from chlorinated compounds such as alachlor and metolachlor followed by forming toxic peroxy radicals. On the other hand, hydroxyl radicals react immediately with chlorinated compounds such as chloroacetamides producing Cl^\cdot and other secondary radicals of variable reactivity and toxicity (Halliwell 1986). Antioxidant enzyme inhibition was stronger in the presence of alachlor than metolachlor due to its faster dechlorination and further degradation when different radicals which contribute to its deleterious action were formed (Gey 1994, Štajner *et al.* 1994a,b).

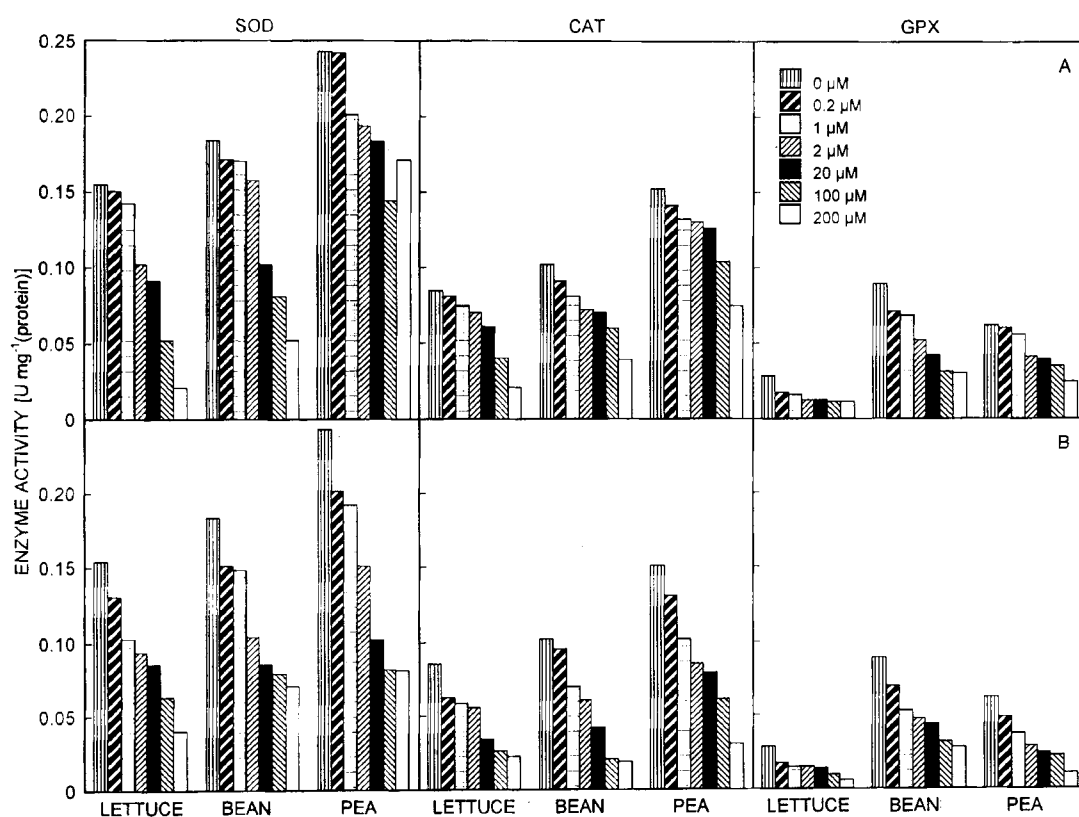


Fig. 2. Effect of different alachlor (A) and metolachlor (B) concentrations on the antioxidant enzyme activities in seeds.

The antioxidant enzyme activities in lettuce, bean and pea young leaves were inhibited after treatments with higher concentrations of alachlor and metolachlor but mostly stimulated at lower concentrations (Fig. 3). Comparing present results with those obtained in young wheat leaves treated by metolachlor we could state that the herbicide induced similar changes in wheat SOD and GPX activities as in lettuce, bean, and pea, however, it had only a slight effect on wheat CAT activity (Štajner *et al.* 1995).

The alachlor and metolachlor induced significant decrease in Chl *a*, Chl *b* and Car contents (Fig. 4). These herbicides damage the plants just by destroying components of the photosynthetic apparatus. Similar destructive effects on photosynthetic pigments were caused by the action of other herbicides (Bowler *et al.* 1992).

Carotenoids quench singlet oxygen and can therefore help to protect chlorophylls. Therefore in photosynthetic tissue of leaves where at low concentrations of alachlor

and metolachlor carotenoids are still present increased SOD, CAT and GPX activities were observed. In contrast, in nonphotosynthetic tissue of seeds where

mitochondria are probably main target of herbicide action, only inhibition of SOD, CAT, and GPX activities was observed.

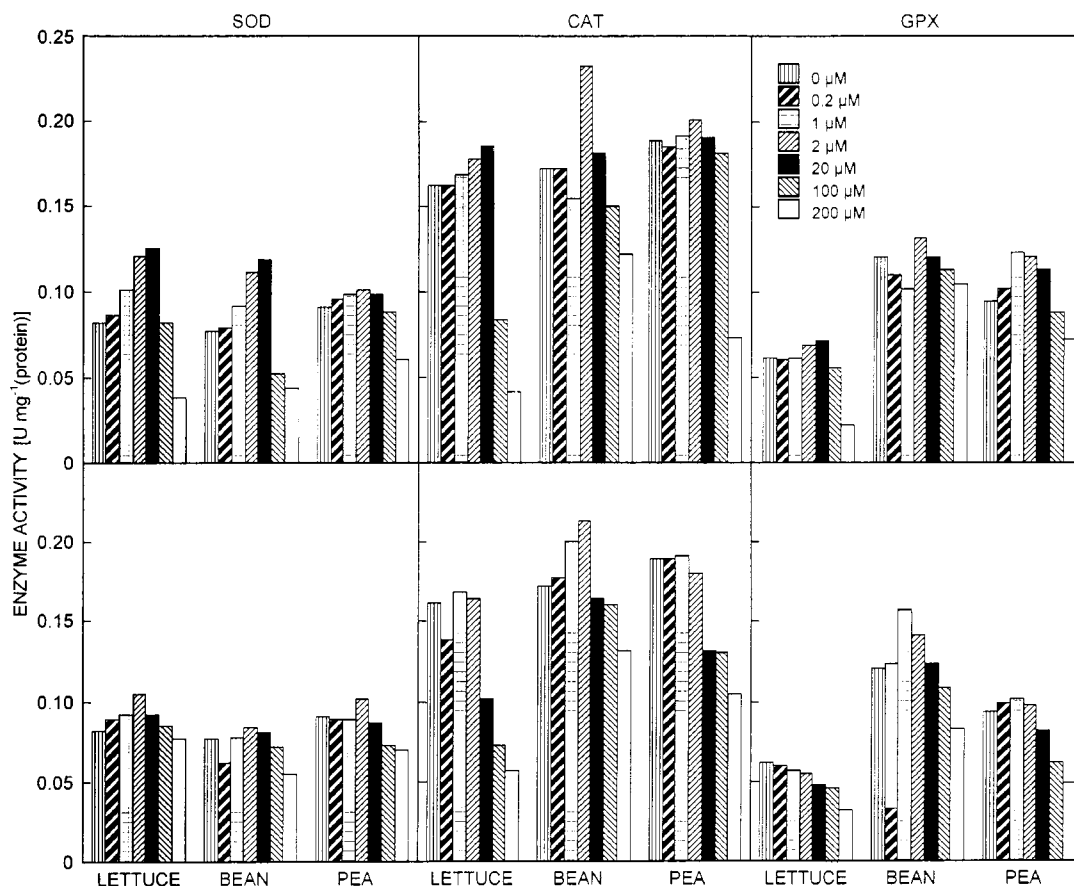


Fig. 3. Effect of different alachlor (A) and metolachlor (B) concentrations on the antioxidant enzyme activities in leaves.

References

- Aioub, A.A., Gullner, G., Komives, T., Brunold, C.: Peroxidation of lipids in corn plants exposed to heavy metal and herbicide stress. - In: Mozsik, G., Emerit, I., Feher, J., Matkovics, B., Vince, A. (ed.): *Oxygen Free Radicals and Scavengers in the Natural Sciences*. Pp. 57-60. Akadémiai Kiadó, Budapest 1993.
- Bowler, C., Van Montagu, M., Inze, D.: Superoxide dismutase and stress tolerance. - *Annu. Rev. Plant Physiol. Plant mol. Biol.* **43**: 83-116, 1992.
- Bowjer, J.R., Camilleri, P.: *Herbicides*. - Wiley and Sons, London 1987.
- Bradford, M.M.: A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. - *Anal. Biochem.* **72**: 248-254, 1976.
- Escobar, J.A., Ruibio, M.A., Lissi, E.A.: SOD and catalase inactivation by singlet oxygen and peroxy radicals. - *Free Rad. Biol. Med.* **20**: 285-290, 1996.
- Frei, B.: Natural antioxidants in human health and disease. - *Free Rad. Biol. Med.* **20**: 257-258, 1996.
- Gey, F.K.: *Free Radicals in the Environment Medicine and Toxicology*. - Richelieu Press, London 1994.
- Geoffroy, L., Teisseire, H., Couderchet, M., Vernet, G.: Effect of oxyfluorfen and diuron alone and in mixture on antioxidative enzymes of *Scenedesmus obliquus*. - *Pesticide Biochem. Physiol.* **72**: 178-185, 2002.
- Halliwell, B., Gutteridge, J.M.C.: *Free Radicals in Biology and Medicine*. - Clarendon Press, Oxford 1986.
- Harper, D.B., Harvey, B.M.R.: Mechanism of paraquat tolerance in perennial ryegrass. II. Role of superoxide-dismutase, catalase and peroxidase. - *Plant Cell Environ.* **1**: 211-215, 1978.
- Khan, M.H., Panda, S.H.: Induction of oxidative stress in roots of *Oryza sativa* L. in response to salt stress. - *Biol. Plant.* **45**: 625-627, 2002.
- Misra, H.P., Fridovics, I.: The role of superoxide anion in the autooxidation of epinephrine and a simple measurement for superoxide dismutase. - *J. Biol. Chem.* **47**: 3170-3175, 1975.

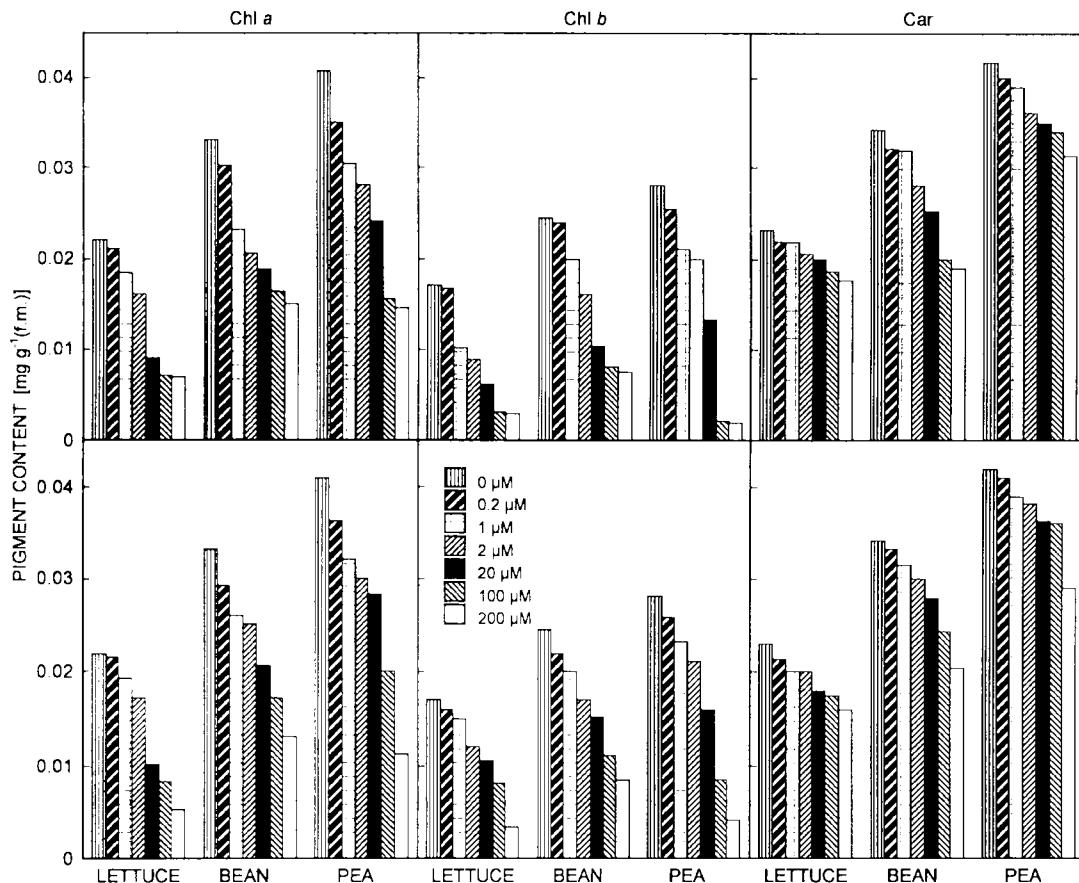


Fig. 4. Effect of different alachlor (A) and metolachlor (B) concentrations on pigment contents in leaves.

Rabinowitch, H.D., Budowski, P., Kedar, N.: Carotenoid and epoxide cycles in mature-green tomatoes. - *Planta* **122**: 91-97, 1975.

Simon, L.M., Fatrai, Z., Jonas, D.E., Matkovics, B.: Study of some metabolism enzymes. - *Comp. Biochem. Physiol.* **166**: 31-34, 1974.

Somich, C.J., Kearney, P.C., Muldoon, M.T., Elsasser, S.J.: Enhanced soil degradation of alachlor by treatment with ultraviolet light and ozone. - *J. agr. Food Chem.* **36**: 1322-1326, 1988.

Štajner, D., Varga, I., Štrbac, D., Matkovics, B., Gašić, O., Kastori, R.: Change in malonyldialdehyde, hydroxyl radical, reduced glutathione and protein content in wheat seeds germinated in polyethylene glycol-6000 solution. - In: Feher, J., Blázovics, A., Matkovics, B., Mézes, M. (ed.): *Role of Free Radicals in Biological Systems*. Pp. 3-9. Akadémiai Kiadó, Budapest 1993.

Štajner, D., Gašić, O., Popović, M.: Effect of herbicides on the antioxidant enzyme activities and soluble protein content in wheat seeds. - *J. serb. chem. Soc.* **59**: 775-779, 1994a.

Štajner, D., Köck, M., Gašić, O., Schlee, D.: Alachlor induced changes of superoxide dismutase activity and pigment content in wheat seeds and leaves. - *J. serb. chem. Soc.* **59**: 7-12, 1994b.

Štajner, D., Gašić, O., Matkovics, B., Varga, S.I.: Metolachlor effect on antioxidant enzyme activities and pigment content in seeds and young leaves of wheat (*Triticum aestivum* L.). - *Agr. mediter.* **125**: 267-273, 1995.

Stegman, H.B., Schuler, P.: Oxidative stress of crops monitored by EPR. - *Z. Naturforsch.* **48c**: 766-772, 1993.

Vincente, J.A.F., Pexoto, F., Lopez, M.L., Madeira, V.M.C.: Differential sensitivities of plant and animal mitochondria to the herbicide paraquat. - *J. biochem. mol. Toxicol.* **15**: 322-330, 2001.