DETECTION OF RESISTANT INDIVIDUALS TO SO_2 POLLUTION BY USING PEROXIDASE ACTIVITY REGULATED BY A GLYCOPROTEIN IN SESSILE OAK SEEDLINGS (*Quercus Petraea* (Matt.) Liebl.)

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The aim of this paper was to demonstrate the importance of the regulation of peroxidase from sessile oak seedlings by a glycoprotein from the same species to induce resistance to SO_2 environmental pollution. In certain concentrations glycoprotein G_2 can promote both high increase and/or decrease in peroxidase activity which might reflect the fighting potential of individuals exposed to SO_2 stress. Moreover the interaction between peroxidase and glycoprotein G_2 was revealed by increasing in UV spectrum absorbance at 217.5 nm. No steady results were found after G_1 glycoprotein interaction with peroxidase. These experiments may open a new chapter in evaluation and understanding of resistant individuals to environmental stress.

Key words: Peroxidase activity; Physiological functions; Glycoproteins; Sessile oak seedlings; Stem infusion; Resistance towards stress.

INTRODUCTION

Pollutants, especially SO_2 compounds have cytotoxic effects in plants at all their levels. Thus, it has been shown that SO_2 compounds affects physiological functions such as: perspiration, respiration and photosynthesis (Malhotra and Khan 1976, Ziegler, 1975, Ianculescu, 2005) These functions are related to trees' leaves apertures, which are blocked due to the air pollutants (Rosen *et al*. 1978). Also, due to SO_2 more of the chloroplasts are destroyed, resulting in a significantly decrease in chlorophylls (Neamtu, 1983). The limitation of photosynthesis in trees affected by SO_2, produced important changes in the transportation of metabolites through phloem (Ten and Swanson, 1982). The phloem's translocation movement becomes very limited, resulted in starch accumulation on the Gymnosperm needles for example (Fink, 1983). In our previous research we also found several modifications related to auxological and physiological aspects of trees species under SO_2 stress (Ianculescu *et al*. 1987, 1989; Ianculescu, 2005).

It was also shown that enzymes such as: acid phosphates, peroxidase and cytocrome oxidase increase their activity in gymnosperm needles (Fink, 1983).

Therefore, one of the important biochemical indicators, related to environmental stress appears to be peroxidase enzyme.

Peroxidase (EC 1.11.1.7) is an enzyme, which catalyzes a large range of aromatic substances under the action of hydrogen peroxide. Peroxidase is distributed everywhere in the plant being implicated in several aspects of growth and development (Trinh *et al*., 1981, Van Huyte and Cairns, 1982) and in hormonal balance (Schneider, 1970).

It has been shown that plant peroxidases are believed to function in diverse physiological processes, including responses to various environmental stresses (Hofferck, 1981, Kim *et al*. 2007, Castillo *et al*., 1989).

It was also indicated that some specific peroxidase isoenzymes, might be specifically involved in the defense mechanism against oxidative stress induced by air pollutants and UV radiation in sweet potato plant (Kim *et al*., 2007). It was also shown that Peroxidase activity increases during drought (about 250% higher than under well watered conditions in mycorrhizal shrub seedlings grown in amended semiarid soil (Roldan *et al*. 2007).

The involvement of Peroxidase has been shown in the defense of yellow Lupin embryo axes against *Fusarium oxysporum* (Morkunas *et al*.. 2005).

All these results make us confident of the importance of peroxidase activity in the physiology of SO_2 stressed trees with respect to sessile oak seedlings.

The usage of SO_2 controlled fumigation chambers, which mimics the SO_2 pollution of industrial areas, may predict the inducible stress of different seedlings, in our case in the sessile oak seedling.

It has been also shown that peroxidase can interact with different proteins in various subcellular compartments with possible functional role (Rouhier *et al*. 2007).

Interaction between peroxidase in glycoproteins has been shown "in vitro" in Fir (*Abies Alba Mill*.) phloem, with significant modifications at the peroxidase activity level (Budu *et al*. 1989).

The aim of this paper was to evaluate the possibility of glycoprotein effectors' interaction with peroxidase and their importance for the physiology of environmental stressed sessile oak seedlings, with special involvement of SO_2.

MATERIAL AND METHODS

Two years sessile oak seedling stems were infused with two glycoproteins G_1 and G_2, from the same species for 24h, using chromatographic paper.

The scheme representing the infusion of the two glycoproteins was shown in Table 1. This procedure was used before SO_2 fumigation.

Table 1

Scheme regarding infusion procedure of sessile oak seedlings by using two glycoprotein effectors

Number used for analyzed seedling	Glycoprotein G_1^* and G_2^{**} concentrations	
	Group A (control)	Group B (used for SO_2 fumigation)
1	control	control
2	0,15 mg/ml G_1^x	0,15 mg/ml G_1
3	0,4 mg/ml G_1	0,4 mg/ml G_1
4	0,1 mg/ml G_2^{xx}	0,1 mg/ml G_2
5	0,2 mg/ml G_2	0,2 mg/ml G_2
6	0,1 mg/ml G_1 + 0,02 mg/ml G_2	0,1 mg/ml G_1 + 0,02 mg/ml G_2

Control = represents seedling without any glycoprotein infused treatment.
G_1* = represents glycoprotein #1; G_2** = represents glycoprotein #2.

Sessile oak seedlings were fumigated with 20 mg/m^3 SO_2 for 8 h before analyses.

Leafs were analyzed before and after the infused glycoproteins and SO_2 fumigation respectively.

- Peroxidase activity was performed using the method of Mateescu (Mateescu *et al*., 1979). Thus, it was measured the enzymatic rate of oxidation from hydroquinone to p benzochinone (pBQ) in the presence of H_2O_2. The enzymatic activity was evaluated in μmoles pBQ/min/ml using the following formulae:

$$\frac{\Delta A/\min \times 2.1}{18.18 \times 0.1},$$

where 2.1 is the volume in ml, 18.18 is the extinction coefficient of pBQ, and 0.1 is the volume of the peroxidase extract in ml.

- The glycoproteins were obtained from a healthy, 40 years old, sessile oak's phloem.

The separation procedure was followed the procedure established by Budu *et al.* (1988), using fir (*Abies alba Mill*.) phloem.

Thus, the sessile oak's glycoproteins were separated in one step procedure using Sepharose 4B, BrCN activated. The unabsorbed material was eluted from the column with 0.04M Tris-0.15M Boric acid, pH = 7.4. The absorbed glycoprotein fractions were eluted using a low salt pH solution 0.1NHCl, 2M. 4 glycoproteins were eluted from the column using this procedure. First two fractions, represented by G_1 and G_2 glycoproteins were used to infuse 2 years old sessile oak seedlings' stem.

RESULTS AND DISCUSSION

The peroxidase activity from foliage material of sessile oak seedlings is presented in Table 2. The enhancement of peroxidase activity was evaluated as a negative difference between the peroxidase activities, before and after glycoprotein stem infusion and SO_2 fumigation respectively.

Table 2

Variation of peroxidase activity, from foliage material acquired from sessile oak seedlings and analyzed in controlled fumigation chambers

No exp.	Group	No. of seedling	Peroxidase activity * µmoli/pBQ/min/ml I	Peroxidase activity** µmoli/pBQ/min/ml II	Difference I – II
1	A	1	38,102	52,170	– 14,07
2	A	2	24,977	19,209	5,770
3	A	3	32,533	29,813	2,720
4	A	4	98,393	48,118	50,270
5	A	5	115,279	128,224	– 12,950
6	A	6	31,969	18,458	13,512
7	B	1	16,643	14,908	1,746
8	B	2	16,642	14,567	2,073
9	B	3	5,636	28,285	– 22,648
10	B	4	14,234	10,079	4,154
11	B	6	20,367	16,643	3,724

* Determination of peroxidase activity was performed on foliage material, before and after infusion with two glycoprotein effectors.

** Determination of peroxidase activity was performed on foliage material analyzed after glycoprotein infusion and/or fumigation with SO_2.

Thus, it was observed that all seedlings respond with an inhibitory effect towards peroxidase activity. Exception was the control, which does not contain any glycoprotein and the infused A_5 seedling with a double concentration of G_2 (0.2 mg/ml). The enhancement of peroxidase activity was also observed in case of glycoprotein G_2 (0.4 mg/ml) in seedling B_3, after SO_2 fumigation. Then the value was 22,648.

In the same time, it was shown a high inhibition of peroxidase activity in the case of low concentration of glycoprotein G_2 (0.1mg/ml) in A_4 seedling, with a value of 50,270 µmoles pBQ/min/ml. G_1 glycoprotein didn't show important changes in peroxidase activity.

Peroxidase activity was changed when the stem was infused with G_2 glycoprotein. Therefore, it may be possible that G_2 glycoprotein interacts with peroxidase. Consequently the peroxidase activity was modified. The interaction between glycoproteins and peroxidase *in vitro* was already shown by us (Budu *et al*., 1989). However the *in vivo* interaction was never established.

In both cases, high inhibition or high increase in peroxidase activity, G_2 played an important role. The extreme value of peroxidase activity may reflect the fighting potential of the stressed seedlings. These results are in agreement with the peroxidase activity values, high or low in different trees, in high polluted areas (Ianculescu and Budu, 2008).

It can be also predict that the infused glycoproteins may enter through the stem's pores and can be involved in peroxidase regulatory metabolic reactions. Moreover, as peroxidase is involved in many stress reactions it becomes possible the theory, in which the glycoproteins can regulate the peroxidase activity resulting in new response of plants to stress.

These analyses are the first analyses of this type, that may open a new era of understanding of the tree response towards environmental stress, such as drought wich is in directly conection with climate change.

The increase in absorbance was calculated as a negative value from the difference between seedlings before and after glycoprotein infusion and SO_2 fumigation respectively (Table 3).

Increase in the absorbance at 217.5 nm was observed in control and A_5 seedlings, the last infused with glycoprotein G_2 (0,2 mg/ml). The calculated values were 0.29 absorbance units for control versus 0,23 absorbance units for infused G_2 glycoprotein. These results are in agreement with the results obtained from the evaluation of peroxidase activity. In contrast, the highest concentration of infused G_2 (0.4 mg/ml), in B_3 seedling, didn't result in any modification at the 217,5 nm wavelength. This result can be explain in part by the interaction of the glycoprotein G_2 in peroxidase enzymatic reaction and its consumption in this reaction.

Table 3

Variation of UV absorbance at 217.5 nm of foliage material analyzed from sessile oak seedlings in SO_2 controlled fumigation chambers

No. Exp	Group	No. seedling	Absorbance* at 217,5 nm I	Absorbance** at 217,5 nm II	Difference I – II
1.	A	1	0,35	0,64	– 0,29
2.	A	2	0,70	0,41	0,29
3.	A	3	0,60	0,58	0,02
4.	A	4	0,43	0,51	– 0,08
5.	A	5	0,41	0,64	– 0,23
6.	A	6	0,46	0,61	– 0,15
7.	B	1	0,30	0,41	– 0,11
8.	B	2	0,28	0,44	– 0,16
9.	B	3	0,44	0,38	0,06
10.	B	4	0,43	0,27	0,16
11.	B	6	0,36	0,54	– 0,18

* Absorbance spectra were performed before and after glycoprotein infusions
** Aabsorbance spectra were performed after glycoprotein infusions and SO_2 fumigation respectively.

No steady results were found after G_1 stem treatment and SO_2 fumigation at the 217.5 nm. This is in agreement with the results found at the peroxidase activity level.

These results are of great importance for the detection of resistant individuals. The extreme values of peroxidase activity, especially the increase in peroxidase activity, can express their response and fighting potential towards stress.

In Figures 1–4 it has been present UV absorbance spectra and variation of UV absorbance at 217,5 nm of foliage materials from sessile oak seedlings for group A (sessile oak seedlings nonfumigates, but infused with 2 glycoprotein effectors), and group B (sessile oak seedlings fumigates in controlled SO_2 chambers, but stimulated by 2 glycoprotein effectors).

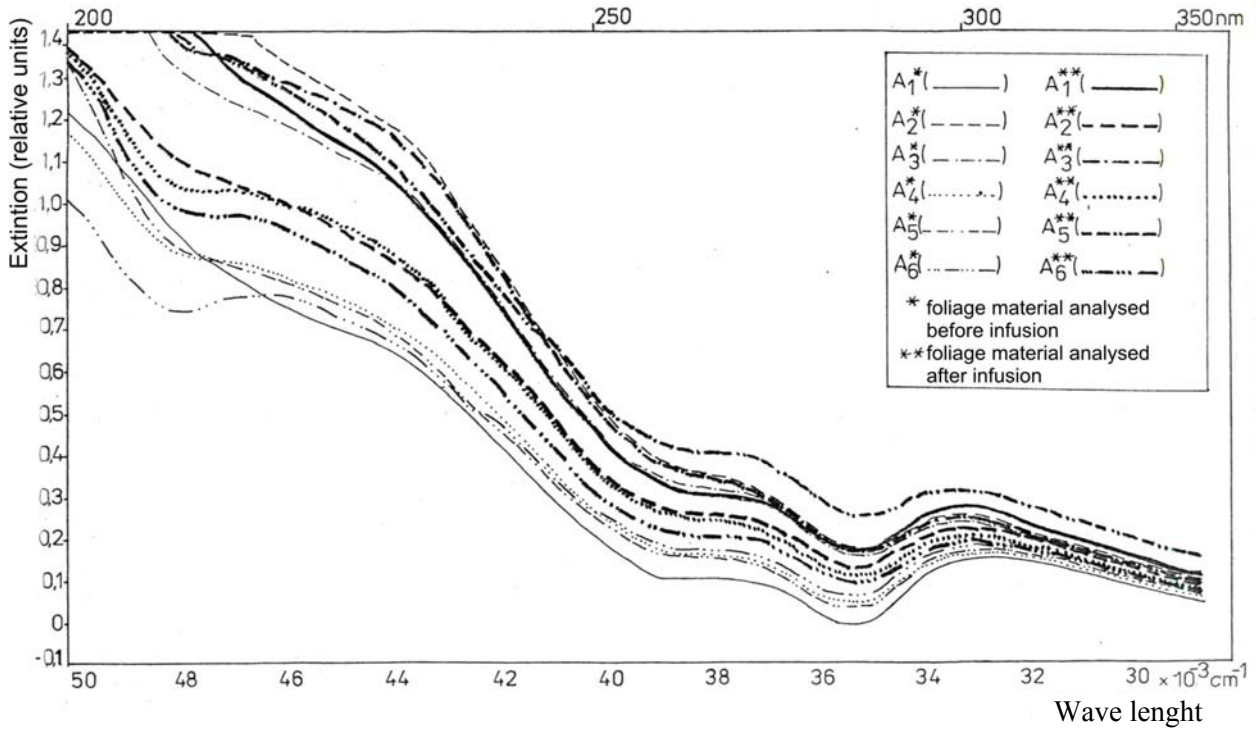

Fig. 1. UV absorbance spectra of foliage materials from sessile oaks seedlings (group A – nonfumigation), "stimulated" by 2 glycoprotein effectors.

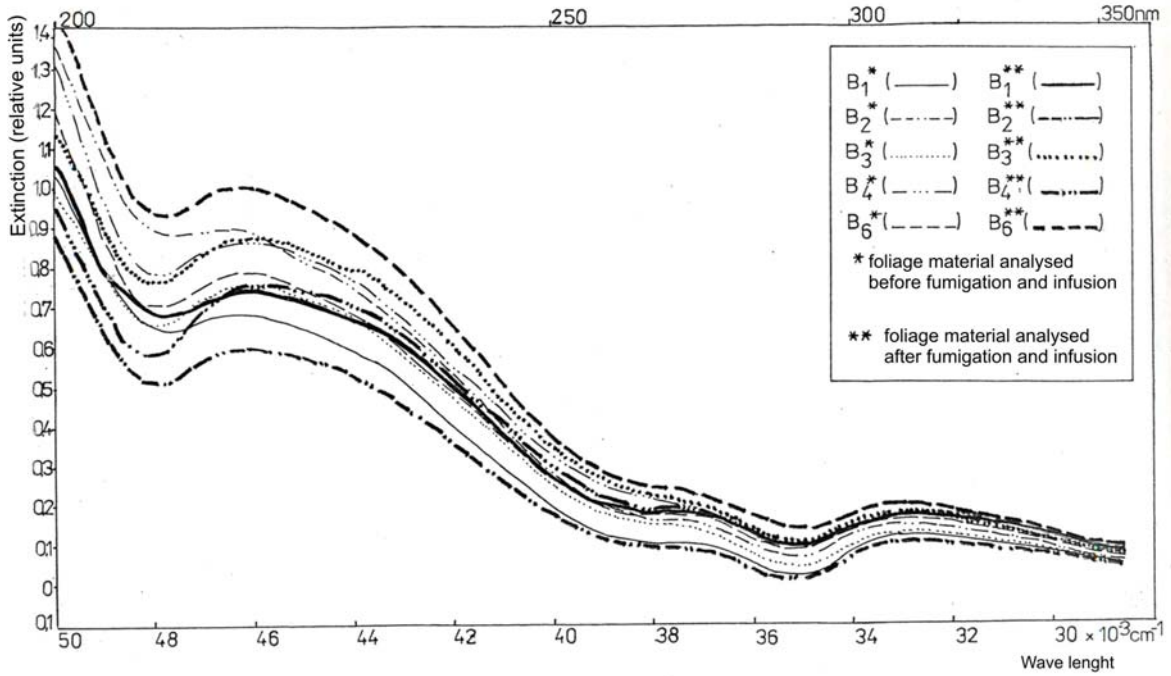

Fig. 2. UV absorbance spectra of foliage matterials from sessile oaks seedlings (group B – used for SO_2 fumigation), "stimulated" by 2 glycoprotein effectors, analysed in controlled SO_2 fumigation chamber.

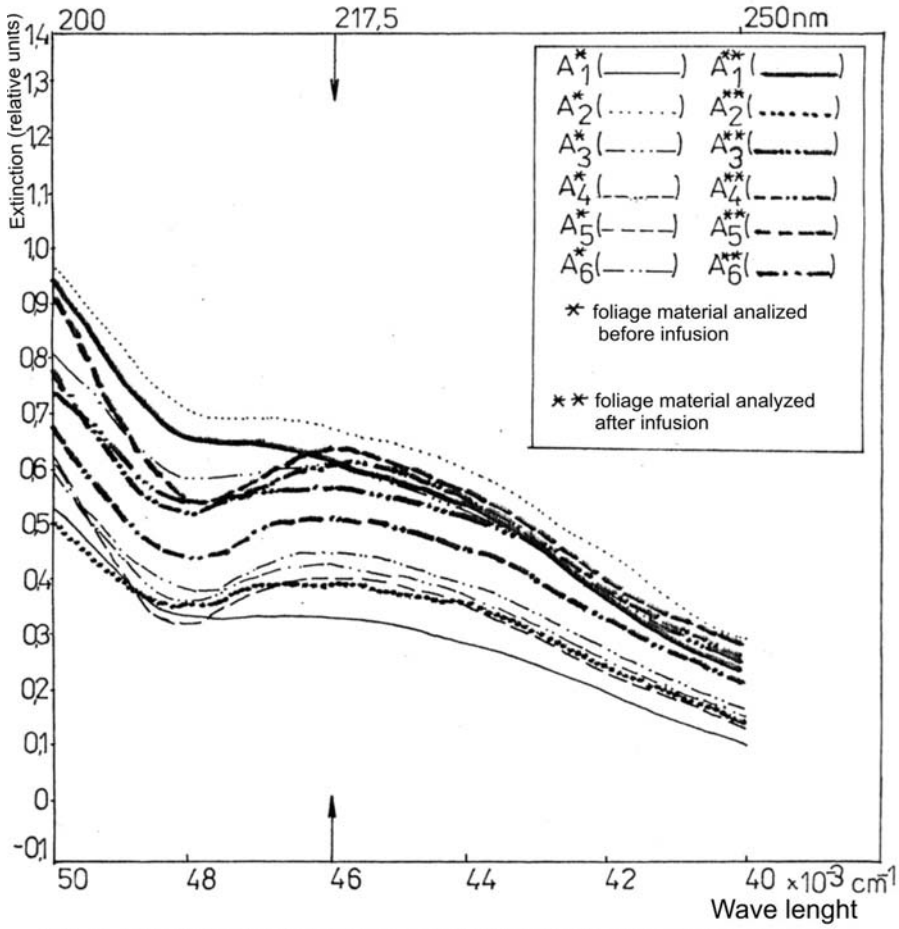

Fig. 3. Variation of UV absorbance at 217,5 nm of sessile oak seedlings' foliage materials (group A – nonfumigation), analyzed in controlled SO_2 fumigation chamber.

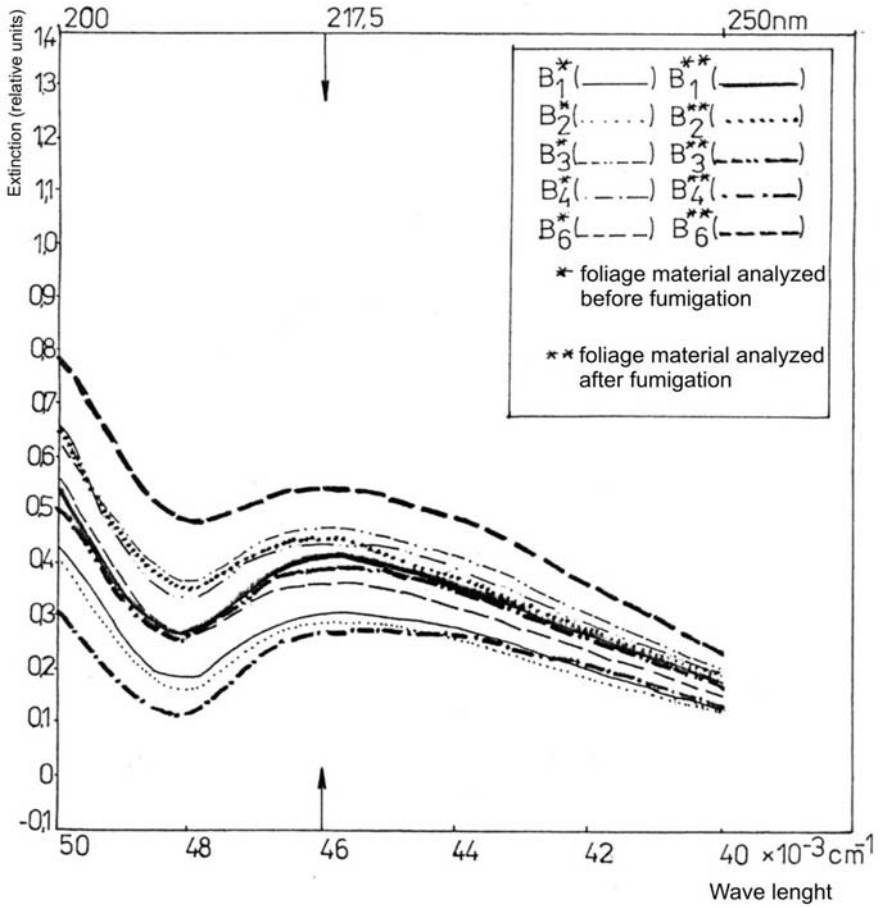

Fig. 4. Variation of UV absorbance at 217,5 nm of sessile oak seedlings' foliage materials (group B – used for SO_2 fumigation), "stimulated" by 2 glycoprotein effectors, analysed in controlled SO_2 fumigation chamber.

CONCLUSION

We can conclude that these glycoproteins, especially G_2, may be involved in the sessile oak resistance towards stress.

More experiments have to be done to elucidate the action of these glycoproteins and their role in the regulation of peroxidase activity as an important factor of trees' environmental stress response.

Based on these results we may be able to evaluate a protection strategy in high polluted industrial areas. It will be also likely to predict the trees response towards climate changes.

REFERENCES

1. Budu C.E., *Rev. Roum. Biochim*., **1988,** 25, 1, 3–7.
2. Budu C.E., Ianculescu M., Seceleanu I., *Further research on peroxidase and two lectins from Fir (Abies alba Mill.). Kinetic Studies*. Presented as poster at the 11th International lectin Conference, **1989,** Tartu, USSR.
3. Castillo, F.J., Greppin, H.C.R., *Seanc. Soc. Phys Hist. Nat*. Geneva, **1980,** 15, 57.
4. Fink, S., AFZ, **1983,** nr. 25 / 27, p. 660–663.
5. Hoefferck, H., *Biochim. Physiol*. **1981,** 176, 170.
6. Ianculescu, M. *et al*., *Cercetări privind dinamica fenomenelor de poluare industrială a pădurilor din zona Copşa Mică*. Manuscris, Referat ştiinţific final, ICAS, **1987,** 182 p.
7. Ianculescu, M., Bândiu, C., Budu, C.E., *Modificări ale principalelor procese ecofiziologice la arborii forestieri ca urmare a influenţei poluării în zona Copşa Mică*, Revista pădurilor, **1989,** nr. 2 (Anul 104), pp. 64–68.
8. Ianculescu, M., *Aspecte ale relaţiilor dintre pădure şi poluare.* In Silvologie, „Pădurile şi modificările de mediu" **2005,** vol. IVA (sub redacţia Victor Giurgiu), Editura Academiei Române, pp. 92–126.
9. Ianculescu, M., Budu C.E. *Indicatori biochimici de evaluare a rezistentei speciilor forestiere la poluarea industriala cu compuşi ai sulfului in acţiune sinergica cu metalele grele in zona Copşa Mică*, Analele ICAS, Editura Silvică, **2008,** vol. 50, pp. 99–119.
10. Kim, Y.H., Lim S., Han, S. H., Lee, J.C., Song, W.K., Bang, J.W., Kwon, S.Y., Lee, H. S., Kwak, S. S., *Plant Physiol. and Biochem*., **2007,** 45, 908–914.
11. Malhotra, S.S., Khan, D., *New Physiol*., **1976**, 79, 227.

12. Mateescu, M. A., Schell, H. D., Budu, C.E., *Rev. Roum. Biochim*., **1979,** 16, 115–120.
13. Morkunas, I., J. Gmerek, J., *Plant Physiol.,* **2005,**164, 185–194.
14. Neamtu, C., *Biochimie ecologica*, Ed. Dacia, **1983,** Cluj Napoca, p. 236.
15. Roldan, A., Vivancos, P.D., Hernandez, J. A., Carrasco, L., Caravaca, F., *J. Plant Physiol*, in press.
16. Rouhier, N., Koh, C.S., Gelhaye, E., Corbier, C., Favier, F. T., Didierjean, C., Jacquot, J., *P. Biochim. Biophys. Acta*, in press.
17. Rosen, P.M., Musselman, R.C., Kender, W.J., *Sci. Hart*., **1978,** 8, 137.
18. Schneider, G. *Ann. Rev. Plant Physiol,* **1970,** 21, 499.
19. Ten, K.H., Swanson, C.A., *Plant. Physiol*, **1982**, 69, p. 88
20. Trinh, T.H., Gaspar, T.H., Van, T.T., Rearcotte, J.L., *Physiol Plant,* **1981,** 53, 53.
21. Van Huytee, R.B., Cairns, W.L., *Phytochemistuj,* **1982,** 21, 1843.
22. Ziegler, I., *Residue Rev*., **1975,** 56, 79.