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Visible and invisible injury to Scots pine (*Pinus sylvestris* L.) needles caused by sulphur dioxide

Abstract

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The studies conducted have shown that there are differences in the content of water, free proline, protein bound imino acids: proline and hydroxyproline, ortho-diphenols and in total phenols in the needles of young Scots pine seedlings subjected to the action of SO_2. The level of these compounds depends on the type of injury of the studied needle tissues: visually undamaged ones, necroses and the dark band separating the two zones. This band most probably plays an isolating role protecting the uninjured zone from the necroses, among others against water loss. In the paper the possible role of hydroxyproline and phenolics in the formation of the border zone is discussed. However comparison of the frequency of its occurrence in the needles of Scots pine seedlings being under the influence of imissions of SO_2 and compounds of fluor in field conditions, with the degree of sensitivity and survival ability of the seedlings, did not show any significant correlation.

Additional key words: proline, hydroxyproline, phenols, air pollution

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INTRODUCTION

Gaseous air pollutants, depending on their concentration and duration of action, cause in plants at first only the so called „physiological injuries" and later typical injuries to assimilation organs in the form of chloroses and necroses (Keller 1982).

The most frequently used method in determining the degree of sensitivity of plants to the action of toxic gases is the quantitative observation of visible injuries to leaves (needles). In practice this theoretically simple measurement encounters difficulties even when the so called acute injuries occur. This is caused by unevenness in the nature (shape, colour, quantitative relationships) of a given type of injury (Demeritt et al. 1971). Besides, dead, necrotic tissue, depending on the type of gas acting upon it, has various basic, pigmentation — from white, through beige and reddish-brown to black (Malhotra and Bluel 1980).

At the base of the differences described there lie so far little known relationships associated with the level and turnover of some metabolits. Studies of necroses which originated as a result of the action of gaseous air pollution in the form of oxidants (O_3, NO_2, PAN) indicate that dark-brown or black necroses are caused by oxidation processes leading to more toxic compounds – chinones. These in their turn form complexes with some amino acids and proteins, taking the form of macromolecular polimers (Howell 1970, Howell and Kremer 1973, Tingey et al. 1976). However little is known about the influence of sulphur dioxide on the level of phenolic compounds in plants (Karolewski and Daszkiewicz 1988).

Between a necrose and the visually undamaged (green) part of the leaf there frequently forms, particularily in coniferous plants, a very distinct, dark band. Its occurrence has been observed among others under the influence of O_3 (Demeritt et al. 1971) and HF (Bovay 1971). So far there is no information about the chemical composition and the function of this border zone in plant tissues subjected to the action of sulphur dioxide. One of the causes of differentiation in the necrotisation of tissues by SO_2 may lie in the different intensity of the process of hydroxylation of protein bound proline. This is indicated by results of investigations conducted earlier in plants from the genus *Weigela* (Karolewski 1986). Changes of this

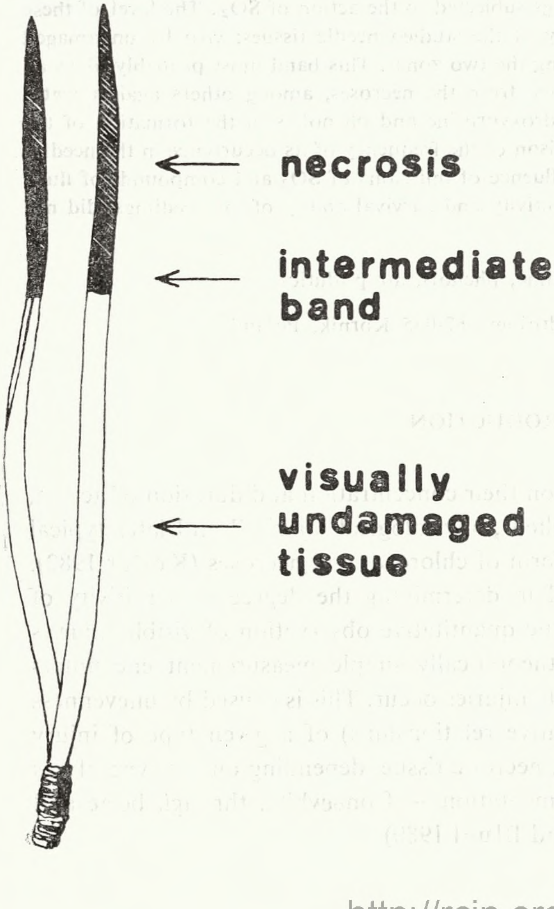

Fig. 1. Diagramatic presentation of injuries observed in Scots pine needles following SO_2 action

type characterize processes associated with the maturation and ageing of plant tissues (Chrispeels et al. 1974).

The aim of the studies described here was to determine the differentiation in the content of proline and phenolic compounds in tissues of Scots pine needles in different condition following SO_2 injury: visually undamaged, necrotic and the band of tissues between the two zones (Fig. 1). An attempt was also made to clarify the relationship between the frequency of occurrence of this intermediate band in the needles of pine and the degree of sensitivity of pine seedlings to the action of toxic gases.

MATERIALS AND METHODS

Two experiments were performed. The first was conducted on 2-year-old Scots pine (*Pinus sylvestris* L.) seedlings originating from seed collected from a seed orchard in the Zwierzyniec Experimental Forest near Kórnik, from clone no. K-11-03. The seedlings grew in greenhouse conditions, singly in pots in a medium composed of forest soil and peat (3 : 1 v/v). The treatment exposition to 0.5 ppm of SO_2 was applied to 72 seedlings, of which 24 were exposed for 2 days (6 h/d) and the remaining 48 for 7 days (6 h/d). Seedlings subjected to the action of the gas for 7 days have been later split into two groups. In this way three variants of the experiment were created. Those exposed for 2 days and half of those exposed for 7 days were analysed immediately after termination of exposition while the third group was analysed 7 days after termination of the 7 day exposition to sulphur dioxide.

The exposition to SO_2 was performed using chambers and measuring and dosing equipment described in the works of Białobok et al. (1978) and Karolewski (1983). During exposition to SO_2 and after its termination the seedlings were placed in chambers at room temperature 18 - 24°C at a relative humidity level of 65 - 80% and under natural illumination of 40 - 70 $W \cdot m^{-2}$.

At the same time in similar chambers and conditions, but without SO_2 there were seedlings representing the control (72 seedlings, 24 for each of the experimental variants).

Within the control and each of the experimental variants with SO_2 the seedlings were divided into 4 groups of 6 each, which were treated as replicates. The use of 6 seedlings as one replicate was caused by there being too little plant material obtainable from one seedling, particularily in the case of needle fragments representing the intermediate zone between necroses and the visually undamaged tissues.

The analyses of needles included the content of free proline, protein bound imino acids: proline and hydroxyproline, water, ortho-diphenols and the total content of phenolics.

The content of free proline was determined colorimetrically using ninhydrine by the method of Bates et al. (1973). The content of proline was determined in fresh weight and it was then converted and presented as $\mu M \cdot g^{-1}$ dry weight (D. W.) of needles.

The percentage water content in the needles was calculated on the basis of the difference in weight before and after drying at 105°C for 24 h.

The imino acids bound in proteins have been determined by first obtained acetone powders and their hydrolysis in 12 N HCl in sealed glass fials for 18 h and then after removal of HCl under vacuum. The level of bound imino acids was determined by colorimetric methods, proline using ninhydrin by the method of Bergman and Loxley (1970) and hydroxyproline using p-dimethylaminobenzaldehyde after Stegmann and Stalder (1967). The content of bound imino acids has been presented in $\mu M \cdot g^{-1}$ of acetone powder of needles dry weight.

The level of phenolic compounds, with a separation of ortho-diphenols and the total phenolic content have been determined in samples of fresh weight of needles after double extraction for 15 and 10 minutes in boiling ethyl alcohol at a concentration of respectively 95 and 80%. Determination of the level of these substances has been performed by colorimetric methods: ortho-diphenols using the Arnova reagent (sodium nitrite + sodium molibdenate) by the method described by Johnson and Schaal (1957), and the total phenols using the Folin-Denis reagent after Swain and Hillis (1959). The content of phenolic compounds has been expressed in μM of chlorogenic acid$\cdot g^{-1}$ needle dry weight.

All the results of this experiment represent averages from 4 replicate measurements. Besides the average values also the standard deviation is shown and the letter symbols indicating significance of differences at a level of 0.05 calculated with the help of *t*-Student's test.

In the second experiment use was made of Scots pine seedlings growing on an experimental area established 2 km from the Luboń Phosphate Fertilizers Factory near Poznań, which emits into the atmosphere sulphur dioxide and compounds of fluor. The seedlings used in this experiment represented 19 European provenances

Table 1

Information about the origin of seed used for the experiment (after Oleksyn et al. 1988)

Provenance no.	Forest District	Country	Latitude N	Longitude E	Altitude (m)
1	Roščinskaya Dača	USSR	60°15′	29°54′	80
2	Kondežskoe	USSR	59°58′	33°30′	70
4	Silene	USSR	55°45′	26°40′	165
5	Miłomłyn	Poland	53°34′	20°00′	110
6	Supraśl	Poland	53°12′	23°22′	160
7	Spała	Poland	51°37′	20°12′	160
8	Rychtal	Poland	51°08′	17°55′	190
9	Bolewice	Poland	52°24′	16°03′	90
10	Neuhaus	Germany	53°02′	13°54′	40
11	Betzhorn	Germany	52°30′	10°30′	650
12	Lampertheim	Germauy	50°00′	10°00′	95 - 100
13	Ardennes	Belgium	50°46′	4°26′	110
14	Haguenau	France	48°49′	7°47′	130 - 180
15	Sumpberget	Sweden	60°11′	15°52′	185
16	Zahorie	Czechoslovakia	48°46′	17°03′	160
17	Pornóapáti	Hungary	47°20′	16°28′	400
18	Maočnica	Yugoslavia	43°10′	19°13′	1200
19	Prušacka Rijeka	Yugoslavia	44°05′	17°21′	800 - 970
20	Çatacik	Turkey	40°00′	31°10′	1380 - 1420

of Scots pine. A detailed characteristics of the provenance experiment and the localisation of the trial is presented in the paper of Oleksyn and Białobok (1986). Information about the origin of the studied provenances is presented in Table 1.

The measurements conducted depended on the determination of the frequency of occurrence of injuries caused by imissions of SO_2 and fluor compounds in the form of intermediate bands between necrotic and visually undamaged (green) tissues. The determination was done in the fall of 1986 immediately after evaluation of the injuries and seedling survival (Oleksyn 1988).

In view of the differentiation in the survival of the seedlings observations were made on various numbers of plants (replicates), from 4 to 60 depending on the degree of sensitivity of the studied provenances.

RESULTS AND DISCUSSION

Results of the first experiment have shown that the action of SO_2 at a concentration of 0.5 ppm for 2 days (6 h/d) does not cause any visible symptoms of injury, nor did it lead to a significant lowering of water content in needles relative to the control (Tab. 2). A significant lowering of water content occurred on the other hand after 7 days of exposition (6 h/d) as well as after the 7 days exposition plus a further 7 days without SO_2. However the changes in the content of water in tissues that were visually undamaged were in both cases small, 1.7% and 2.6% respectively. The inhibition of water loss in the above mentioned tissues may be effected by the darkbrown or almost black band of tissue, which developed over the few days following the 7 day exposition of seedlings to SO_2, compared to the almost twofold lowering of water content over the same period in the necroses.

The content of ortho-diphenols and the total phenolic content increased in the tissues that were visually undamaged together with the extension of the period of seedling exposition to SO_2 from 2 to 7 days (Tab. 2). The level of these compounds in the necrotic zones was significantly lower than in the tissues that were not visibly damaged. The highest content of ortho-diphenols and of the total phenols was observed in the tissues of the dark band separating the necroses from the green tissues. A similar pattern of changes in the content of phenolic compounds was obtained in the experiment of Mireku and Wilkes (1988) in leaves of *Eucalyptus maculata* Hook infected through mechanical injury (cuts) of tissues. They have found that in the period of 7 - 90 days after infection the highest content of phenolics was observed in the intermediate zone of tissue, between tissues not visibly damaged and necroses, the latter having the lowest content. At the same time the tissues of the zone adjacent to the necroses were characterized by the highest activity of one of the enzymes participating in the synthesis of phenols – ALT as well as of enzymes oxidising them – peroxidases (POD) and para-diphenol oxidases (PPO). Besides the activity of POD was higher in visually undamaged parts than in necroses.

Information in the literature is lacking on the influence of SO_2 on the changes in the activity of the complex of enzymes oxidising phenolics. On the other hand

Table 2

Mean water content in %, ortho-diphenols ($\mu M \cdot g^{-1}$ DW), total phenolics ($\mu M \cdot g^{-1}$ DW), free proline ($\mu M \cdot g^{-1}$ DW) and protein bound proline ($\mu M \cdot g^{-1}$ DW) and hydroxyproline ($\mu M \cdot g^{-1}$DW) in needles of Scots pine subjected to the action of 0.5 ppm SO_2 for 2 and 7 days (6h/d) and also after 7 days following the termination of exposition to the gas, and in controls in relation to the type of tissue damage

Compound	2 days		7 days			14 days			
	$-SO_2$	$+SO_2$	$-SO_2$	$+SO_2$		$-SO_2$	7d ($+SO_2$)+7d ($-SO_2$)		
	Control	Undamaged[1]	Control	Undamaged	Necroses	Control	Undamaged	Necroses	Intermediate[3]
Water	78.4±0.3	78.0±0.3	78.1±0.2	a 76.4±0.6	ab 34.6±4.1	77.8±0.4	a 75.2±1.2	ab 18.1±5.3	abc 49.2±3.7
o-diphenols[4]	64.7±4.1	a 92.9±6.2	67.2±2.4	a 110.3±5.8	ab 21.4±3.8	69.3±5.1	a 88.6±6.9	ab 7.0±4.2	abc 186.6±13.8
Total phenols[4]	98.1±7.7	a 146.3±12.4	104.8±9.6	a 288.7±14.8	ab 53.1±8.6	108.4±9.4	a 268.1±11.3	ab 22.3±6.8	abc 348.6±14.7
Free proline	5.7±0.8	a 8.6±1.2	5.3±0.8	a 12.8±1.6	ab 31.8±3.0	6.1±0.6	a 25.4±3.1	ab 4.4±0.4	abc 14.6±2.0
Bound proline	11.2±2.8	a 17.3±3.4	12.6±1.8	a 28.9±5.4	ab 16.7±2.6	11.9±3.0	a 33.4±4.8	ab 14.7±2.2	abc 124.7±30.2
Bound hydroxy-proline	2.3±0.2	a 3.1±0.2	2.6±0.2	a 2.4±0.3	ab 1.9±0.2	2.4±0.1	a 4.2±0.8	ab 1.2±0.1	abc 10.2±3.4

[1] – Undamaged tissue, green with no visible injuries
[2] – Necroses – necrotic tissue
[3] – Intermediate – band of tissue between necroses anp visually undamaged tissue
[4] – Expressed as μM chlorogenic acid$\cdot g^{-1}$ DW of needles
a – Significantly different from control at $\alpha = 0.05$ level
b – Significantly different from visually undamaged tissue at $\alpha = 0.05$ level
c – Significantly different from necroses at $\alpha = 0.05$ level

earlier studies on the changes in the activity of peroxidase in the leaves of seedlings of the genus *Weigela* have shown an increase in the activity of this enzyme in visually undamaged tissues the closer is the sample taken to a necrose, being maximum at she boundary with the necrose and negligible in the necrotic tissue itself (Karolewtki 1983). The role of POD in the processes of oxidation of phenolics is indicated by the results of several studies described in the paper of Łobarzewski (1981).

Measurements of the changes in the content of free proline under the influence of SO_2 have shown an increase in its level in the visibly undamaged parts. It was correspondingly higher after 7 days of exposition than after 2 days of SO_2 action (Tab. 2). The content of imino acids in necroses, measured immediately after 7 daye of exposition of seedlings to the action of SO_2 was significantly higher than in ths tissues that were not visibly damaged. Similarily a higher level of free proline was observed in the necroses than in the tissues that were not visibly damaged by exposition to SO_2 in the studies of Jäger and Grill (1975) and Erickson and Dashei- (1982). However after a period of 7 days from the moment of termination of exposk tion to the gas the mean level of free proline in the necroses declined to a level more than 5 times lower than in the visually undamaged tissues (Tab. 2). This may be caused among others by decomposition of this imino acid or by its elution from this part of the needles. Besides it needs to be stressed that the observed lowering of the content of free proline in the necroses may affect the result of the mean level of this imino acid should the analyses by made for the whole leaf.

The observed changes in the level of proline bound with proteins have shown growing tendencies in all the studied needle fragments subjected to the action of SO_2. However the level of this imino acid in the necroses was significantly lower than in the visually undamaged parts of the leaf (Tab. 2). This may be caused by the influence of SO_2 on the inhibition of protein synthesis (Mudd 1979) or by increased proteolisis (Fischer 1971, Malhotra and Sarkar 1979).

A relatively high content of both the studied imino acids in the protein bound form characterized the tissues of the intermediate zone between the necroses and the tissues not visibly damaged (Tab. 2). Together with an increase in the duration of SO_2 action an increase was found in the level of hydroxyproline in the visibly undamaged tissues and also in the intermediate zone. This is an unfavourable phenomenon leading to a stiffening of cell walls and consequently to an inhibition of the growth and development of cells (Cleland and Karlsnes 1967). Cell walls particularily rich in hydroxyproline are to be found in mature and ageing tissues (Ridge and Osborne 1971; Chrispeels et al. 1974).

On the other hand the changes described above involving hydroxyproline and the mentioned earlier increase in the level of phenolics, compounds which participate in the lignification processes, may contribute to the favourable phenomenon of isolation of the visually undamaged tissues from the necroses.

In the literature there is a lack of data concerning the physiological function of this intermediate zone separating necroses from visually undamaged tissues. The causes of formation of these tissues are not determined. The are observed only after action of some gases and only in some species of plants. Thus for example

Demeritt et al. (1971), who have described also in 2-year old seedlings of Scots pine the nature of injuries effected, have observed this intermediate zone but only after treatment with O_3. On the other hand SO_2 in the conditions of the experiments they conducted (3.5 ppm SO_2 for 4 h and then a long term observation of the plant) did not cause the formation of the intermediate band. Presumably the magnitude of the gas concentration and the duration of exposition determine its manifestation.

There is also no information in the literature concerning the relationship between the occurrence of this intermediate band and the degree of sensitivity of plants to the action of toxic gases. An attempt was to made to answer this question to some extent with the help of the second experiment.

A comparison was made of the degree of needle injury (1) and the survival of seedlings (2), represented by 19 different provenances of Scots pine growing near an industrial plant emitting SO_2 and fluor compounds into the atmosphere, with the number of seedlings of individual provenances in which the needles had the dark intermediate bands of tissue separating the necroses from visually unaffected parts

Table 3

Mean values of needle injury and seedling survival in % for 19 provenances of Scots pine (*Pinus sylvestris* L.) growing near the Luboń Phosphate Fertilizer Factory and the % of seedlings in which the intermediate band of tissue between necroses and visually undamaged tissue appeared, following pollution emitted in the form of SO_2 and fluor compounds

Provenances no.	Needle injuries[3] (%)	Seedling survival[3] (%)	Seedlings with intermediate band[4] (%)
1	–	5.00	100.0
2	4.56	66.25	70.6
3	4.44	73.75	91.7
4	5.62	76.25	94.8
5	7.47	75.00	83.4
6	6.77	60.00	92.9
7	10.02	65.50	86.7
8	16.02	70.00	85.4
10	10.50	13.30	79.2
11	6.35	40.00	92.2
12	8.60	75.00	100.0
13	9.20	25.00	58.4
14	8.80	40.00	91.7
15	5.10	63.00	81.0
16	7.45	47.50	67.2
17	13.40	52.50	97.9
18	6.76	68.80	80.6
19	3.75	13.80	58.4
20	–	7.00	87.5

[1] – a description of the planting site and its localisation is given in the work of Oleksyn and Białobok 1986

[2] – a characteristic of the studied provenances is given in Table 2

[3] – injuries and survival of seedlings determined in the autumn of 1986 (Oleksyn et al. 1988)

[4] – observations made in the Autumn of 1986 after evaluating injuries and survival of seedlings

(3) (Tab. 3). The correlation coefficients obtained, $r_{1,3}=0.244$ and $r_{2,3}=0.257$ proved insignificant with $\alpha=0.4$ and $\alpha=0.3$ respectively.

The results obtained in the conditions of this experiment do not confirm any relation between the frequency of occurrence of the intermediate band of tissue with the degree of injury or survival of the studied Scots pine provenances. However these results do not exclude the possibility that the band of intermediate tissue plays some role in the general sensitivity of plants as one of the contributing factors.

LITERATURE

1. Bates L. S., Waldren R. P., Tears I. D. 1973. Rapid determination of free proline for water-stress studies. Plant and Cell 39: 205 - 207.
2. Bergman I., Loxley R. 1970. New spectrophotometric method for the determination of proline in tissue hydrolizates. Anal. Chem. 42: 702 - 706.
3. Białobok S., Karolewski P., Rachwał L. 1978. Charakterystyka urządzeń służących do badania wpływu szkodliwych gazów na rośliny. Arbor. Kórnickie 23: 239 - 249.
4. Bovay E. 1971. Effects des polluants atmosphérique sur les végétaux. Symp. Problèmes de l'environnement et agriculture. Berne: 123 - 137.
5. Chrispeels M. J., Sadava D., Cho Y. P. 1974. Enhancement of extensin biosynthesis in ageing disks of carrot storage tissue. J. Exp. Bot. 25: 1157 - 1166.
6. Cleland R., Karlsnes A. M. 1967. A possible role of hydroxyproline-containg proteins in the cessation of cell elongation. Plant Physiol. 42: 669 - 671.
7. Demeritt M. E. Jr., Chang W. M., Murphy J. D., Gerhold H. D. 1971. Selection system for evaluating resistance of Scotch pine seedlings to ozone and sulfur dioxide. 19th Northeastern Forest Tree Improvement Conference. Univ. of Maine, Orono, Maine, August 2 - 4, 1971: 87 - 97.
8. Erickson S. S., Dashek W. V., 1982. Accumulation of foliar soluble proline in sulphur dioxide-stressed *Glycine max* cv. 'Essex' and *Hordeum vulgare* cvs 'Proctor' and 'Excelsior' seedlings. Environ. Pollut. 28: 89 - 108.
9. Fischer K. 1971. Methoden zur Erkennung und Beurteilung forstschädlicher Luftverunreinigungen. Chemische und physikalische Reaktionen SO_2-begaster Pflanzen und Blätter. Mitt. Forstl. Bundes-Versuchsanst. Wien 92: 209 - 231.
10. Howell R. K. 1970. Influence of air pollution on quantities of caffeic acid isolated from leaves of *Phaseolus vulgaris*. Phytopathology 60 (11): 1626 - 1629.
11. Howell R. K., Kremer D. F. 1973. The chemistry and physiology of pigmentation in leaves injured by air pollution. J. Environ. Quality 2 (4): 434 - 438.
12. Jäger H.-J., Grill D. 1975. Einfluss von SO_2 und HF auf freie Aminosäuren der Fichte (*Picea abies* (L.) Karsten). Eur. J. For. Path. 5: 279 - 286.
13. Johnson G., Schaal L. A. 1957. Accumulation of phenolic substances and ascorbic acid in potato tuber tissue upon injury and their possible role in disease and resistance. American Potato J. 34: 200 - 209.
14. Karolewski P. 1983. Effect of sulphur dioxide on peroxidase activity in leaves of *Weigela* rooted cuttings. Arbor. Kórnickie 28: 113 - 127.
15. Karolewski P. 1986. The role of proline and hydroxyproline in the sensitivity of *Weigela* to the action of sulphur dioxide. Arbor. Kórnickie 31: 247 - 258.
16. Karolewski P., Daszkiewicz P. 1988. Wpływ dwutlenku siarki na poziom fenoli w liściach topoli o zróżnicowanej wrażliwości na działanie tego gazu. Arbor. Kórnickie 33: 231 - 238.
17. Keller T. 1982. Physiological bioindications of an effect of air pollution on plants. In: Monitoring of air pollutants by plants (eds. L. Steubing and H.-J. Jäger), Dr W. Junk Publishers, The Hague: 85 - 95.

18. Łobarzewski J. 1981. Peroksydazy roślinne. Wiadomości Botaniczne 25 (1): 29 - 44.
19. Malhotra S. S., Bluel R. A. 1980. Diagnosis of air pollutant and natural stress symptoms on forest vegetation in western Canada. Environ. Can., Can. For Serv., North. Res. Cent. Edmonton, Alberta. Inf. Rep. NOR-X-228: 1 - 84.
20. Malhotra S. S., Sarkar S. K. 1979. Effect of sulphur dioxide on sugar and free amino acid content of pine seedlings. Physiol. Plant. 47: 223 - 228.
21. Mireku E., Wilkes J. 1988. Production of phenols in the sapwood of *Eucalyptus maculata* after wounding and infection. Eur. J. For. Path. 18: 121 - 127.
22. Mudd J. B. 1979. Physiological and biochemical effects of ozone and sulphur dioxide. Symposium on the effect of air-borne pollution on vegetation. Warsaw, 20 - 24 August: 80 - 91.
23. Oleksyn J. 1988. Height growth of different European Scots pine *Pinus sylvestris* L. provenances in heavily polluted and a control environment. Environ. Pollut. 55: 289 - 299.
24. Oleksyn J., Białobok S. 1986. Net photosynthesis, dark respiration and susceptibility to air pollution of 20 European provenances of Scots pine *Pinus sylvestris* L. Environ. Pollut. 40: 287 - 302.
25. Oleksyn J., Karolewski P., Rachwał L. 1988. Susceptibility of European Scots pine (*Pinus sylvestris* L.) provenances to the action of SO_2, NO_2, SO_2+NO_2 and HF in laboratory and field conditions. Acta Soc. Bot. Pol. 57 (1): 107 - 115.
26. Ridge I., Osborne D. J., 1971. Role of peroxidase when hydroxyprolinerich protein in plant cell wall is increased by ethylene. Nature New Biology 229 (7): 205 - 208.
27. Stegemann H., Stalder K. 1967. Determination of hydroxyproline. Clin. Chim. Acta 18: 267 - 273.
28. Swain T., Hillis W. K. 1959. The phenolic constituents of *Prunus domestica*. The quantitative analysis of phenolic constituents. J. Sci. Food and Agriculture 1: 63 - 68.
29. Tingey D. T., Fites R. C., Wickliff C. 1976. Differential foliar sensitivity of soybean cultivars to ozone associated with differential enzyme activity. Physiol. Plant. 37: 69 - 72.

Widoczne i niewidoczne uszkodzenia igieł sosny (*Pinus sylvestris* L.) powodowane przez dwutlenek siarki

Streszczenie

Analizowano zmiany zawartości wolnej proliny, związanych z białkiem iminokwasów – proliny i hydroksyproliny oraz o-dwufenoli i sumy fenoli w igłach dwuletnich siewek sosny zwyczajnej (*Pinus sylvestris* L.) poddanych działaniu dwutlenku siarki. Poziomy tych związków były zróżnicowane i zależały od typu uszkodzeń tkanek: wizualnie nieuszkodzonych, uszkodzonych i ciemnozabarwionego pasma tkanek rozgraniczającego te fragmenty. W pracy dyskutowany jest możliwy udział hydroksyproliny i fenoli w procesach prowadzących do izolacji części wizualnie nieuszkodzonej igieł od nekrozy. Przyczynia się on prawdopodobnie do hamowania utraty wody z tkanek wizualnie nieuszkodzonych. Jednakże porównanie przeprowadzone pomiędzy częstością występowania omawianego pasma tkanek i stopniem wrażliwości oraz przeżywalnością 6-letnich siewek reprezentujących 19 proweniencji sosny zwyczajnej, rosnących w terenie skażonym przez SO_2 i związki fluoru, nie wykazało istotnej korelacji.