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Changes in phenolic compounds content in needles of Scots pine (*Pinus sylvestris* L.) seedlings following short term exposition to sulphur dioxide

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Abstract. Changes in the content of phenolic compounds i.e., ortho-diphenols (o-dPh), meta-diphenols (m-dPh) and total phenols (TPh) was studied in needles of Scots pine seedlings subjected to the action of SO_2. In the first experiment the independent variable was the concentration of gas and in the second the time of exposition. It was confirmed that changes of phenols content are nonlinear in character. After an initial increase there follows a decrease to the control level and next comes another increase. The increases in the content of phenolic compounds may be connected first with the mechanisms of defence and then a stage of degradation. Two provenances differed in sensitivity and natural content of phenols, which may suggest that phenolic compounds determine resistance. The character of changes is in all studied groups of compounds similar.

Additional key words: total phenols, ortho-diphenols, meta-diphenols, resistance, air pollution.

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INTRODUCTION

The influence of different biotic and abiotic factors on changes in phenols content in plants is a broad study (Feldman and Hanks 1968, Howell 1970, Simons and Ross 1971, Scalbert and Haslam 1987, Stenlid and Johanson 1987, Karolewski 1990). There are publications describing changes of phenolic compounds caused by long time exposition to toxic gases in field conditions (Yee-Meiler 1974, 1977; Karolewski and Giertych 1992) but there are few works showing changes immediately after a few hours' action of the toxic factor. Phenolic compounds are important substances

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participating in the defence mechanisms in plants (Hoque 1982), and their levels determine sensitivity to pathogens e.g., to a nematode of some citrus cultivar (Feldman and Hanks 1968). It is interesting to know if the same dependence occurs in sensitivity to abiotic factors. The study of SO_2 influence on two different cultivars of poplar (*Populus*) did not give an answer (Karolewski and Daszkiewicz 1988).

MATERIALS AND METHODS

This paper presents results of two experiments. The first was conducted on 4.5-month-old Scots pine (*Pinus sylvestris* L.) seedlings, originating from seeds collected from clone no. K-11-03 from a seed orchard in the Zwierzyniec Experimental Forest near Kórnik. The seedlings grew in greenhouse conditions, singly in pots in a medium composed of forest soil and peat (3:1 v/v). This experiment was conducted over one week (14.10.91-18.10.91), and each day a subsequent part of seedlings was treated with a different SO_2 concentration (0.25; 0.5; 1; 2; 4 ppm) for 6 hours.

Exposition to SO_2 was performed using chambers and measuring and dosing equipment described in the work of Białobok et al. (1978) and Karolewski (1983). For dosing gas the Interscan LD Continuous Monitoring System Model LD-24 Sulphur Dioxide was used. During exposition to SO_2 the seedlings were placed in chambers at room temperature (18-24°C), at a relative air humidity level of 65-80% and under natural illumination of 40-70 $W \cdot m^{-2}$, with additional lighting on gloomy days.

Seedlings without SO_2, representing the control, were taken only in the first day.

Each experimental variant and control were represented by 5 seedlings, which were treated as replicates.

Analyses of needles included the content of ortho-diphenols (o-dPh), meta-diphenols (m-dPh) and total phenolic (TPh) content. The level of these phenolic compounds has been determined in ca. 1 g samples of fresh weight of needles after double extraction for 15 and 10 minutes in boiling ethyl alcohol at a concentration of 95 and 80% respectively. The extraction was conducted after each day of the experiment, extracts were placed in the refrigerator and further analyses were made after finishing the experiments. Determination of the level of these substances has been performed by the spectrophotometric methods: meta-diphenols using vanillin reagent by the method described by Swain and Hillis (1959), ortho-diphenols using the Arnow's reagent (sodium nitrite + sodium molybdate) and the total phenols using the Folin-Ciocalteu reagent, both methods described by Johnson and Schaal (1957). The content of ortho-diphenols and total phenolic compounds has been expressed as μM of

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chlorogenic acid g^{-1} fresh weight of needles and meta-diphenols as μM of catechin g^{-1} fresh weight.

The second experiment was conducted on 2-year-old Scots pine seedlings, originating from seeds representing two European provenances: no. 9 Bolewice (Poland) and no. 15 Sumpberget (Sweden). A detailed characteristic of the origin of the seed is presented by Oleksyn and Białobok (1986). The seedlings grew singly in pots in a medium composed of forest soil and peat (3:1 v/v) in the greenhouse conditions, and in the periods from June to November first year and from June to March second year they were moved out to open air. Two months before the experiment the seedlings were returned to the greenhouse.

Exposition to 0.75 ppm SO_2 was applied to 48 seedlings. The experiment was conducted in May, over 48 hours, and every 8 hours 4 seedlings following fumigation and 4 controls for each provenance were taken out of the chambers and the extraction was conducted immediately. Treatments and analyses were made in the same way as in the first experiment.

RESULTS AND DISCUSSION

Results of the first experiment (Table 1) have shown that the six hours' action of sulphur dioxide caused significant changes in the level of o-dPh and TPh and nonsignificant changes in the level of m-dPh. The course of these changes was similar in each group of phenols; it was nonlinear and the increase was observed at concentrations of 0.5 and 4 ppm SO_2, while for 0.25, 1 and 2 ppm the content resembled the control (Fig. 1).

Table 1

Mean ortho-diphenols, meta-diphenols and total phenols in needles of Scots pine subjected to the action of 0.25; 0.5; 1; 2 and 4 ppm SO_2 for 6 hours.

SO_2	o-dPh[1]	m-dPh[2]	TPh[1]
Control	28.1 a	17.1 a	95.1 a
0.25	27.9 a	17.1 a	110.7 ab
0.5	32.5 b	18.7 a	125.2 b
1	28.1 a	18.7 a	111.2 ab
2	28.4 a	16.3 a	108.6 ab
4	33.2 b	20.7 a	116.8 ab

[1] Expressed as μM chlorogenic acid g^{-1} f.wt. of needles.
[2] Expressed as μM catechin g^{-1} f.wt. of needles.
Values indicated by the same letter are not significantly different with a confidence level of $P<0.05$.

The above described increase and subsequent decrease of phenolic compounds content in the range of SO_2 concentration 0.25–2 ppm is similar to the results of experiments in which plants were treated with ozone (Howell 1970).

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Howell suggests that the influence of O_3 on activity of enzymes connected with metabolism of phenolic compounds and processes of redox resulting in an initial increase in phenolic compounds level and then a decrease, are caused by an increased activity of biosynthetising enzymes and later by the oxidation, polymerization and utilization in respiration processes of phenols. The utilization of phenolic compounds in respiration processes was shown by the studies of Tomaszewski (1961). However, Ballantyne (1973) and Kozioł and Jordan (1978) point at the great role of respiration in the regeneration processes by plants treated with SO_2.

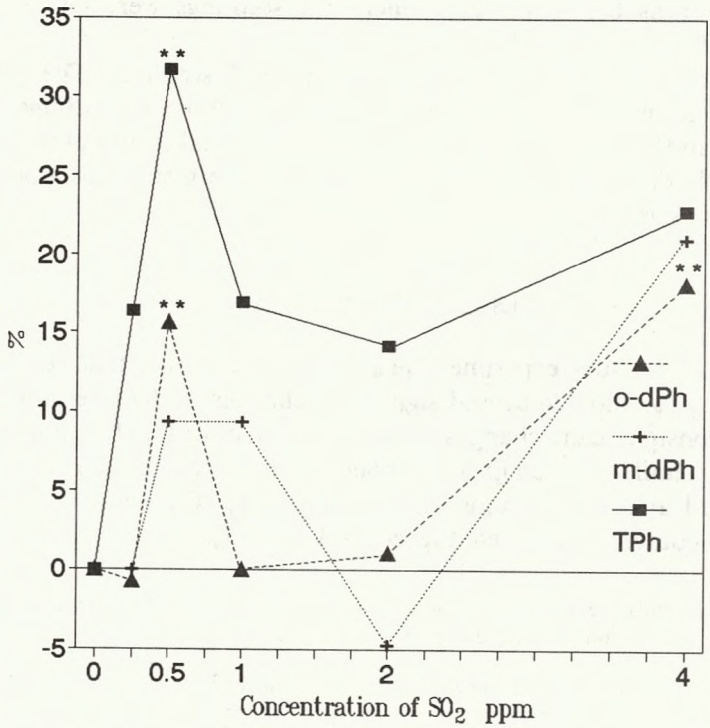

Fig. 1. Changes of ortho-diphenols (o-dPh), meta-diphenols (m-dPh) and total phenols (TPh) content in Scots pine needles subjected to the action of SO_2, expressed as a percentage of the control treated as 0%. ** Significantly different from control at $P<0.05$.

The observed by us increase in phenolic compounds level in seedlings subjected to the action of SO_2 at concentration 4 ppm could have been caused by degradation processes because SO_2 in the higher concentration caused decreased activity of the Krebs cycle enzymes (Pierre and Queiroz 1981) and decreased the intensity of respiration (Karolewski 1989).

Accumulation of phenolic compounds in plants is a negative process and causes inactivation of enzymes with -SH groups (Butt 1972), inhibition ATP synthesis (Dedonder and Van Sumere 1971a), increase respiration

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(Dedonder and Van Sumere 1971b), inhibition of photosyntesis (Ashton and Crafts 1973) and changes in the permeability of membranes (Hess 1958). On the other hand, a high content of phenols in the intermediate zone between necroses and visually undamaged tissues can be associated with defence processes (Scalbert and Haslam 1987; Mireku and Wilkes 1988; Karolewski 1990). The mechanism of response to sulphuric acid treatment seemed to be a "switching on" of a defence mechanism which led to a histologically visible increase in phenolic production after 24 h of exposure to sulphuric acid pH 2.5 and 3 days of pH 3.5 (Zobel and Nighswander 1991). Our result and the described literature data compel one to careful interpretation of the observed pattern. The course and intensity of changes in phenolic compounds content has a complicated character and is dependent on the duration and concentration of toxic gas action and additionally on the type of injury.

Table 2

Mean ortho-diphenols, meta-diphenols and total phenols in needles of two provenances: the less tolerant, no. 9, and the more tolerant, no. 15, of Scots pine subjected to the action of 0.75 ppm SO_2 for 8, 16, 24, 32, 40 and 48 hours.

		o-dPh[1]		m-dPh[2]		TPh[1]	
Time	N[3]	9	15	9	15	9	15
C[4]	24	15.8 cd	19.9 a	4.2 a	5.4 a	47.0 b	52.5 ab
8	4	18.1 a	16.7 b	4.0 a	4.3 a	53.8 ab	41.6 c
16	4	14.7 d	19.7 a	4.1 a	5.2 a	31.3 c	43.8 bc
24	4	15.8 bcd	19.6 ab	4.1 a	5.2 a	51.8 ab	54.8 a
32	4	17.1 abc	21.2 a	4.8 a	5.6 a	62.1 a	58.4 a
40	4	17.7 ab	19.4 ab	4.0 a	5.0 a	58.3 a	59.0 a
48	4	17.1 abc	20.3 a	4.6 a	5.2 a	48.7 ab	50.2 abc

[1] Expressed as μM chlorogenic acid g^{-1} f.wt. of needles.
[2] Expressed as μM catechin g^{-1} f.wt. of needles.
[3] Number of assays.
[4] Control.
Values indicated by the same letter are not significantly different with a confidence level of $P<0.05$.

In the literature there are data connecting natural content of phenolic compounds with sensitivity of trees to the biotic factors action (Feldman and Hanks 1968), but there is lack of such data in the case of toxic gases. In the second experiment we used seedlings of two provenances of Scots pine, differing in sensitivity to SO_2 (Oleksyn et al. 1988) and having naturally different content of phenols (Table 2). The more tolerant provenance (no. 15) had significantly more phenols of all the studied groups than the less tolerant (no. 9). During this experiment, SO_2 did not cause significant changes in the content of o-dPh, m-dPh and TPh in the more tolerant provenance (Fig. 2) (significantly higher level of m-dPh after 24 hours was probably caused by the accidentally low content in the control). Sulphur dioxide had an influence on the content of o-dPh and TPh only in the sensitive provenance (Fig. 3). The character of reaction is similar to that obtained in the first experiment.

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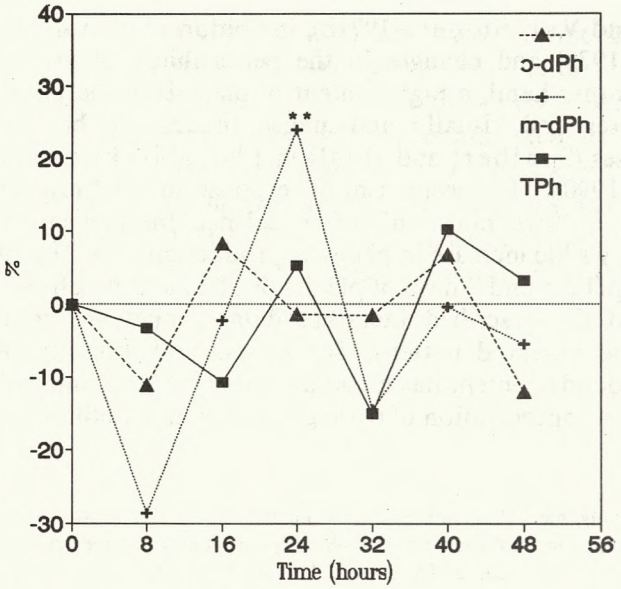

Fig. 2. Changes of ortho-diphenols (o-dPh), meta-diphenols (m-dPh) and total phenols (TPh) content in needles of the more tolerant provenance of Scots pine subjected to the action of 0.75 ppm SO_2, expressed as a percentage of the control treated as 0%. ** Significantly different from control at $P<0.05$.

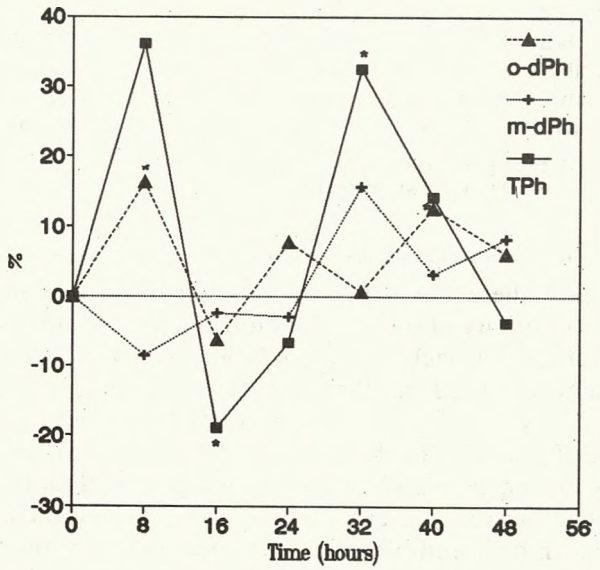

Fig. 3. Changes of ortho-diphenols (o-dPh), meta-diphenols (m-dPh) and total phenols (TPh) content in needles of the less tolerant provenance of Scots pine subjected to the action of 0.75 ppm SO_2, expressed as a percentage of the control treated as 0%. * Significantly different from control at $P<0.1$.

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The significant changes in the level of phenolic compounds only for the less tolerant provenance, which has smaller natural content of these substances, could suggest that this natural concentration of phenolic compounds determines sensitivity of the plant. Confirmation of this thesis would require study on a greater number of provenances.

The obtained results point to:

– nonlinear character of phenolic changes in needles of seedlings treated with SO_2 and express an increase at the beginning, a subsequent decrease and then an increase again,

– similar changes occurring in all the studied groups of phenols,

– a different reaction to SO_2 of two provenances, differing in sensitivity and content of phenolic compounds.

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LITERATURE

Ashton F.M., Crafts A.S., 1973. Mode of action of herbicides. John Wiley and Sons, New York: 1–394.

Ballantyne D.J., 1973. Sulphite inhibition of ATP formation in plant mitochondria. Phytochem. 12: 1207–1209.

Białobok S., Karolewski P., Rachwał L., 1978. Charakterystyka urządzeń służących do badania wpływu szkodliwych gazów na rośliny. Arbor. Kórnickie 23: 239–249.

Butt V.S., 1972. Biochemistry of phenolics compounds. Z. Physiol. Chem. 353: 123–137.

Dedonder A., Van Sumere C.F., 1971a. Effects of phenolics and related compounds on the cyclic and non cyclic photophosphorylation in isolated *Spinacia* chloroplasts. Z. Pflanzenphysiol. 65: 176–182.

Dedonder A., Van Sumere C.F., 1971b. Effects of phenolics and related compounds on the growth and the respiration of *Chlorella vulgaris*. Z. Pflanzenphysiol. 61: 70–80.

Feldman A.W., Hanks R.W., 1968. Phenolic content in the roots and leaves of tolerant and susceptible citrus cultivars attacked by *Radopholus similis*. Phytochem. 7: 5–12.

Hess E.H., 1958. The polyphenolase of tobacco and its participation in amino acid metabolism. I. Manometric studies. Arch. Biochem. Biophys. 74: 198–208.

Hoque E., 1982. Biochemical aspects of stress physiology of plants and some considerations of defense mechanisms in conifers. J. For. Path. 12 (4–5): 280–296.

Howell R.K., 1970. Influence of air pollution on quantities caffeic acid isolated from leaves of *Phaseolus vulgaris*. Phytopath. 60: 1626–1629.

Johnson G., Schaal L.A., 1957. Accumulation of phenolic substances and ascorbic acids in potato tuber tissue upon injury and their possible role in disease and resistance. American Potato J. 34: 200–209.

Karolewski P., 1983. Effect of sulphur dioxide on peroxidase activity in leaves of *Weigela* rooted cuttings. Arbor. Kórnickie 28: 113–127.

Karolewski P., 1989. Rola proliny, poliamin i fenoli u roślin wyższych w warunkach stresowych. Praca habilitacyjna. Instytut Dendrologii PAN, Kórnik: 1–141.

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Karolewski P., 1990. Visible and invisible injury to Scots pine (*Pinus sylvestris* L.) needles caused by sulphur dioxide. Arbor. Kórnickie 35: 127–136.

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.

Karolewski P., Giertych M.J., 1992. Udział fenoli w reakcji sosny zwyczajnej na działanie zanieczyszczeń powietrza związkami fluoru. Symp.: Metabolizm fluoru '92. PTBioch., Szczecin 25 wrzesień 1992: 116–118.

Kozioł M.J., Jordan C.F., 1978. Changes in carbohydrate levels in red kidney bean (*Phaseolus vulgaris* L.) exposed to sulphur dioxide. J. Exp. Bot. 29: 1037–1043.

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.

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.

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.

Pierre M., Queiroz O., 1981. Enzymic and metabolic changes in bean leaves during continuous pollution by subnecrotic levels of SO_2. Environ. Pollut. 25: 41–51.

Scalbert A., Haslam E., 1987. Polyphenols and chemical defence of the leaves of *Quercus robur*. Phytochem. 26: 3191–3195.

Simons T.J., Ross A.F., 1971. Changes in phenol metabolism associated with induced systemic resistance to tobacco Mosaic virus in Samsun NN tobacco. Phytopath. 61: 1261–1265.

Stenlid J., Johanson M., 1987. Infection of roots of Norway spruce (*Picea abies*) by *Heterobasidion annosum*. Eur. J. For. Path. 17: 217–226.

Swain T., Hillis W.E., 1959. The phenolic constituents of *Prunus domestica*. I. The quantitative analysis of phenolic constituents. J. Sci. Food Agric. 10: 63–68.

Tomaszawski M., 1961. Układ fenol-fenolaza i jego związek z procesami oddychania, lignifikacji oraz inaktywacji auksyny w pędach moreli i brzoskwini. Arbor. Kórnickie 6: 169–225.

Yee-Meiler D., 1974. Über den Einfluss fluorhaltiger Fabrikabgase auf den Phenolgehalt von Fichtennadeln. Eur. J. For. Path. 4: 214–221.

Yee-Meiler D., 1977. Phenole als Indikatoren metabolischer Störungen bei fluorexponierten Waldbaumen. Mitt. Eidg. Anst. forstl. Versuchswes., Ber. 53 (4): 203–229.

Zobel A., Nighswander J.E., 1991. Accumulation of phenolic compounds in the necrotic areas of Austrian and red pine needles after spraying with sulphuric acid: a possible bioindicator of air pollution. New Phytoll. 117: 565–574.

Zmiany zawartości związków fenolowych w igłach siewek sosny zwyczajnej (*Pinus sylvestris* L.) wywołane krótkotrwałym działaniem dwutlenku siarki

STRESZCZENIE

Badano zmiany zawartości związków fenolowych z rozróżnieniem na trzy grupy: orto--dwufenole, meta-dwufenole i sumę wszystkich związków fenolowych, w igłach siewek sosny zwyczajnej (*Pinus sylvestris* L.) poddanych działaniu SO_2. W przypadku pierwszego doświadczenia

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zmienną było stężenie gazu, a w przypadku drugiego czas ekspozycji. Stwierdzono, że zmiany zawartości mają charakter nieliniowy: po początkowym wzroście następuje spadek do poziomu kontroli, a następnie ponowny wzrost. Wzrost zawartości związków fenolowych może być związany z procesami obronnymi, a w dalszym etapie degradacyjnymi. Odmienna reakcja u dwóch proweniencji, różniących się wrażliwością i naturalną zawartością związków fenolowych może sugerować determinujący wpływ związków fenolowych na wrażliwość. We wszystkich badanych grupach związków charakter zmian jest podobny.

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