PIOTR KAROLEWSKI

Influence of some chemical compounds on the sensitivity of Scots pine needles to sulphur dioxide treatment*

INTRODUCTION

Some recent papers (Jones 1963, Taylor 1970, Moyer et al. 1974 a, b, Taylor and Rich 1974, Koiwai et al. 1974, Pell 1976, Obydiennyj 1977, Karolewski 1979) informed about the possibility of aplying chemical compounds in order to protect plants against injurious airborne pollution.

Investigation of that kind can, apart from their practical importance, be helpful in identifying the mechanisms determining the toxic effect of the gas or the variable sensitivity of the plants to industrial pollutions.

The aim of the investigation started in 1978 was to estimate the influence of some groups of compounds on changes in the extent of injuries of Scots Pine needles caused by SO_2. Compounds selected in this study will serve future experiments aimed at the identification of defensive mechanisms in plants.

MATERIAL AND METHODS

Plus trees of Scots pine propagated vegetatively in the experimental forest Zwierzyniec near Kórnik were previously selected for their tolerance to sulphur dioxide. A specimen marked K-11-03 considered as susceptible to this gas (Białobok, Karolewski 1978) was chosen for the present investigation. Two-year-old seedlings of that tree proved themselves to be as susceptible as the mother tree was. Experiments were carried out on one-year shoots cut from this tree and put into water as well as on seedlings in pots. Three days before the experiment begun the shoots were placed into vessels with solutions of 26 different chemical compounds and remained there until the end of fumigation. On the other hand seedlings were sprayed 3 times with the above compounds

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during the 3 consecutive days preceding their exposition to SO_2. They were classified into six groups: derivatives of benzimidazole, amino acids, vitamins, derivatives of purine and pirimidine, aminophenols and aromatic diamines and other compounds without nitrogen atoms in their structures. The seedlings were sprayed with solutions of those compounds in the course of three days once a day before they were exposed to the influence of gas. 4 mM solutions of the chemicals were applied to the shoots and 1 mM solutions to the seedlings. There were four shoots and four seedlings treated with each compound. Shoots placed in pure water and seedlings sprayed with water were used as controls. After that all plants were subjected to the influence of SO_2 6 hours daily during three subsequent days. The chambers and arrangements for plant exposition to the SO_2 as well as the system of gas dosage control and of the gas analyzer had been described in one of the previous papers (Białobok et al. 1978).

The sensitivity of plants was estimated 24 hours after exposition to the gas. Visible injuries on the surface of needles from detached shoots (each with 50 needles counting from the top) and from the whole seedlings were classified according to a six-degree scale (Schönbach et al. 1964). This was utilized as a measure of the susceptibility of plants. This estimation was also used in our other works (Białobok and Karolewski 1978). Table 1 presents data about the time of exposition to the gas and of the concentration of solutions applied.

Table 1

Dates and conditions of the experiments

Plant material	Date of the experiment from-to	SO_2 concentration (ppm)	Period of gas treatment (h)	Concentration of the applied solution (mM)	Kind of treatment of plants with the applied compound
Shoots	7 - 9 Sept. 1978	2.0	18	4	Shoots placed into the vessels with chem. solutions
Seedlings	21 - 23 June 1979	0.5	18	1	Seedlings sprayed with the solutions

Results obtained with the experiments were analyzed statistically by means of the Duncan test.

RESULTS AND DISCUSSION

Results of the performed experiments are shown in table 2. The highest, but statistically insignificant, protective activity against SO_2 among the compounds of the first group was found for methyl 1-(butylcarbomyl) -2-benzimidazolecarbamate. This is a well known systematic fungicide

Table 2

Influence of the applied compounds on the degree of injuries caused by SO_2 in the needles of pine shoots and seedlings

Compound applied	Shoots		Seedlings	
	Degree of the injury	Change of the injury as compared to the control	Degree of the injury	Change of the injury as compared to the control
Benzimidazole and benzotriazole derivatives				
Benomyl	1.31	−0.82	1.50	−1.50
Benzimidazole	1.33	−0.80	3.25	+0.25
Benzotriazole	2.00	−0.13	1.75	−1.25
Amino acids				
Cysteine hydrochl.	1.33	−0.80	1.50	−1.50
Histidine	1.52	−0.61	0.50*	−2.50
Tryptophan	1.93	−0.20	2.25	−0.75
Vitamins				
Riboflavin	0.39*	−1.74	0.50*	−2.50
Aneurine hydrochl.	0.54*	−1.59	0.75*	−2.25
Ascorbic acids	1.38	−0.75	1.25	−1.75
Pyridoxine hydrochl.	1.93	−0.20	0.25*	−2.75
Purine and pyrimidine derivatives				
Adenosine	0.84*	−1.29	2.75	−0.25
Guanine	1.32	−0.81	4.75	+1.75
Thymine	1.73	−0.40	5.00	+2.00
Adenine	1.97	−0.16	0.50*	−2.50
Kinetin	2.71	+0.58	3.00	0.00
Aminophenoles and aromatic diamines				
p-phenylenediamine	1.58	−0.55	1.50	−1.50
m-phenylenediamine	1.59	−0.54	0.75*	−2.25
p-amin ophenol	1.71	−0.42	1.75	−1.25
m-aminophenol	2.81	+0.68	0.75*	−2.25
Compounds without nitrogen atoms				
Eugenol	1,35	−0.78	4.50	+1.50
Coumarin	1.97	−0.16	1.50	−1.50
Safrole	2.48	+0.35	3.75	+0.75
Vanilin	2.53	+0.40	2.50	−0.50
4-hydroxycoumarin	2.55	+0.42	1.00	−2.00
Antraquinone	2.55	+0.42	2.50	−0.50
Izosafrole	2.69	+0.56	1.25	−1.75
Water (control)	2.13	–	3.00	–

* Denotes that at a confidence level of 0.05 the degree of damage of needles treated with a given compound differs significantly from the control in Duncan's test.

called „Benomyl". Its protective effect was first found by Taylor (1970). Tobacco plants spread with benomyl powder in the field responsed positively. Injuries caused by ozone were substancially reduced with the help of that treatment. Several other authors confirmed later the protective effect of this compound (Kender et al. 1973, Moyer et al. 1974 a, b, Taylor and Rich 1974, Koiwai et al. 1974). It was also found that the effectiveness of activity of this compound depended on the kind of treatment (added to the soil or spread onto the leaves) and on

its concentration. The mechanism of the influence of benomyl is rather unknown yet, however several studies are in progress. A benzimidazole solution treatment did not cause significant reduction of SO_2 damages on the needles from shoots and seedlings. Earlier experiments (Karolewski 1979) proved that benzimidazole applied to the leaves of Weigela increased damages following SO_2 exposition. One of the reasons for an increased sensitivity of benzimidazole treated plants could be the permeability decrease of primary cell walls.

There was no protective effect of benzotriazole in the present experiment. Koiwai et al. (1974) established no changes in the degree of injuries caused by the exposition of tobacco leaves to O_3 if they were pretreated with benzotriazole.

Treatments with both cysteine hydrochlorides and histidine resulted in marked decrease of SO_2 damage to needles. However, statistically significant decrease of SO_2 damages was recorded only for seedlings sprayed with histidine solution. Positive results were obtained by Dass and Weaver (1968) when seedlings of beans were sprayed with a 10^{-2} M solution of cysteine hydrochloride and thereafter exposed to ozone. A tryptophan treatment did not cause any changes in the degree of injury on SO_2 exposed needles.

Vitamin treatment lowered substantially the degree of SO_2 damages in the needles (pyridoxine hydrochloride applied to the shoots is an exception). Significant protective effect of riboflavin and aneurine hydrochloride treatments was observed on seedlings and shoots. On the other hand, treatment with pyridoxine hydrochloride was effective only in the case of seedlings.

Taking into account all compounds used in this study, riboflavin and aneurine hydrochloride indicated the greatest protective effect against SO_2 injuries. First of these two compounds serves as a component of flavine mononucleotide and flavineadenine dinucleotide taking part in the process of plant respiration. The second one is a component of certain coenzymes related with CO_2 assimilation.

To date there is no information in the literature concerning the role of these two compounds in plant reactions to air pollution. On the other hand there are some reports concerning the influence of air pollution (mainly O_3) on the content of ascorbic acid (Keller, Schwager 1977). The same author indicated a proportional relationship between ascorbic acid content and susceptibility of several Norway spruce clones to SO_2. Hanson et al. (1971) showed the correlation between the degree of O_3 damages and the ascorbic acid content in the leaves of some Petunia varieties. Also Koiwai et al. (1974) found that the injuries caused by O_3 on the tobacco leaves sprayed with the solution of this vitamin were less intensive. Zemlianuchin (1956) proved that ascorbic

acid sprayed on the plants caused an acceleration of their growth, increase in sugars (carbohydrates) and ascorbic acid content, higher respiration and assimilation of CO_2. All this contributed doubtlessly to a lowering of plant sensitivity to the toxic gases.

Among the purine and pyrimidine derivatives used only adenosine, when applied to the shoots and adenine to the seedlings lowered the degree of the SO_2 injuries of the needles. K o i w a i et al. (1974) in similar investigation has found that leaves of tobacco sprayed with adenosine and adenine solutions and exposed to O_3 had two times less injuries. A guanine solution in the investigations of those authors reduced 20% the injuries on plants.

The kinetin in our experiment did not cause a reduction of SO_2 damages on needles. T o m l i n s o n and R i c h (1973) however, gave evidence of a protective effect of kinetin against ozone. In senescing leaves kinetin cased as inhibition of the decrease in chlorophyll, protein and RNA content (W o l i ń s k a 1975) and prevented the degradation of chlorophyll under influence of toxic gases.

Both aminophenols and aromatic diamines displayed a considerable protective influence on the sprayed seedlings, being statistically significant for m-phenylenediamine and m-aminophenol. In the case of shoots the above compounds failed. The same compounds applied in solutions by K o i w a i et al. (1974) for the protection of tobacco leaves against the O_3 proved to be effective.

There were also seven aromatic compounds lacking amines and any nitrogen atoms in their structures used in these experiments, however none of them displayed protective properties against SO_2 injuries. The same compounds used in studies conduced by K o i w a i et al. (1974) also had no protective effect against injuries of tobacco leaves treated with ozone.

It was found in our experiments that among the 26 compounds used 3 applied to the shoots and 7 applied to the seedlings displayed protective effect against SO_2 i.e. caused a statistically significant decrease of injuries to the needles.

They belonged mainly to four groups: vitamins, amino acids, purine and pyrimidine derivatives as well as aminophenols and aromatic diamines.

Considerable differences in reaction between shoots and seedlings treated with solutions of the same compounds and exposed to SO_2 could result from different age plants, methods of treatment as well as from different concentrations of solutions and of gas used.

None of the physiologically active compounds used in the present study as well as in the other experiments indicate a guaranteed protective effect against plant injuries caused by industrial pollution. However,

positive results of experiments confirmed the protective effect of some compounds against the damages caused by toxic gases and could be utilized in the practice.

SUMMARY

Changes in the degree of sensitivity of Scots pine needles to the SO_2 treatment were investigated under the influence of 26 different compounds. The belonged to six groups: benzimidazole and benzotriazole derivatives, aminoacids, vitamins, purine and pyrimidine derivatives, aminophenoles and aromatic diamines and other compounds without nitrogen atomes in their structures.

It was found on the basis of performed experiments that of the 22 the compounds of the vitamine group showed the highest protective influence against SO_2 activity.

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LITERATURE

1. Białobok S., Karolewski P., Rachwał L. 1978. Charakterystyka urządzenia do badania wpływu szkodliwych gazów na rośliny. Arboretum Kórnickie XXIII: 239 - 249.
2. Białobok S., Karolewski P. 1978. Ocena stopnia odporności drzew matecznych sosny zwyczajnej i ich potomstwa na działanie SO_2 i O_3 oraz mieszaniny tych gazów. Arboretum Kórnickie XXIII: 299 - 310.
3. Dass H. C., Weaver G. M. 1968. Modification of ozone damage to *Phaseolus vulgaris* by antioxidants, thiols and sulphydryl reagents. Can. J. Plant Sci. 48, (6): 569 - 574.
4. Hanson G., Thorne L., Jativa C. D. 1971. Ozone tolerance of petinua leaves as related to their ascorbic acid concentration. Proc. 2nd Int. Clean Air Congr., Washington D.C. New York—London, 261 - 266.
5. Jones J. L. 1963. Science. 140 : 1317.
6. Karolewski P. 1979. Effect of the action of some chemical substances on the degree of injury caused by SO_2. Arboretum Kórnickie XXIV; 307 - 312.
7. Keller Th., Schwager H. 1977. Air pollution and ascorbic acid. Eur. J. For. Path. 7 : 338 - 350.
8. Kender W. J., Taschenberg E. F., Shaulis N. J. 1973. Benomyl protection of grapevines from air pollution injury. Hort Science. 8, (5): 396 - 398.
9. Koiwai A., Kitano H., Fukuda M., Kisaki T. 1974. Methylenedioxyphenyl and its related compounds as protectants against ozone injury to plants. Agr. Biol. Chem. 38, (2): 300 - 307.
10. Moyer J. W., Cole H., Lacasse N. L. 1974 a. Reduction of ozone injury on *Poa annua* by benomyl and thiophanate. Plant Dis. Reptr. 58, (1): 41 - 44.
11. Moyer J. W., Cole H., Lacasse N. L. 1974 b. Supression of naturally occurring oxidant injury on azalea plants by drench or foliar spray treatment with benzimidazole or oxathin compounds. Plant Dis. Reptr. 58, (2): 136 - 138.

12. Obydennyj P. T. 1977. Sochranenie lesa v uslovijach promyšlennogo zagraznenija vozducha. Lesnoe chozjajstvo, 6: 35 - 38.
13. Pell E. J. 1976. Influence of benomyl soil treatment of pinto bean plants exposed to peroxyacetyl nitrate and ozone. Phytopathology. 66, (6): 731 - 733.
14. Schönbach H., Dässler H. G., Enderlein H., Bellmann E., Kästner W. 1964. Über den unterschiedlichen Einfluß von Schwefeldioxid auf die Nadlen verschiedener 2 jähriger Lärchenkreuzungen. Der Züchter 34, (8): 312 - - 316.
15. Taylor G. S. 1970. Tobacco protected against fleck by benomyl and other fungicides. Abstr. Phytopathology. 60: 578.
16. Taylor G. S., Rich S. 1974. Ozone injury to tobacco in the field influenced by soil treatments with benomyl and carboxin. Phytopathology. 64. (6): 814 - 817.
17. Tomlinson H., Rich S. 1973. Anti-senescent compounds reduce injury and steroid changes in ozonated leaves and their chloroplasts. Phytopathology. 63. (7): 903 - 906.
18. Wolińska D. 1975. Starzenie się chloroplastów roślin wyższych. Wiadomości Botaniczne. XIX. (!): 165 - 180.
19. Zemljanuchin A. A. 1956. Fiziologičeskie izmenenija v rastenii vyzyvaemye dejstviem askorbinovoj kisloty. Fiziologija Rastenij. 3 (4): 313 - 318.

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Wpływ niektórych związków chemicznych na zmianę stopnia wrażliwości igieł sosny zwyczajnej na działanie SO_2

Streszczenie

Badano wpływ niektórych związków chemicznych na zmianę stopnia wrażliwości igieł sosny zwyczajnej poddanych działaniu SO_2. Badaniami objęto ogółem 26 związków chemicznych sklasyfikowanych w 6 grupach: pochodne benzimidazolu i benzotriazol, aminokwasy, witaminy, pochodne puryny i pirymidyny, aminofenole i dwuaminy aromatyczne oraz inne związki nie zawierające w swojej budowie atomów azotu. Na podstawie przeprowadzonych doświadczeń stwierdzono, że największe właściwości ochraniające przed działaniem SO_2 posiadały związki należące do grupy witamin.

ПЕТР КАРОЛЕВСКИ

Влияние некоторых химических соединений на изменение степени чувствительности хвои сосны обыкновенной к действию SO_2

Резюме

Исследовали влияние некоторых химических соединений на изменение степени чувствительности к действию SO_2 хвои сосны обыкновенной. В общей сложности исследовали влияние 26 химических соединений относящихся к 6 группам: производные бензимидазола и бензотриазола, аминокислоты, витамины, производные пурина и пиримидина, аминофенолы и ароматические диамины, а также соединнеия не содержащие в своем строении атомов азота. На основании проведенных исследований найдено, что лучшими охраняющими от действия сернистого ангидрида свойствами обладают соединения относящиеся к группе витаминов.

12. [illegible] 1977. [illegible]
13. Pell E. J. 1976. Influence of benomyl soil treatment of pinto bean plants exposed to peroxyacetyl nitrate and ozone. Phytopathology 66, (6): 731-733.
14. Schönbach H., Dässler H. G., Enderlein H., Bellmann E., Kästner W. 1964. Über den unterschiedlichen Einfluß von Schwefeldioxid auf die Nadeln verschiedener 2-jähriger Kiefernkreuzungen. Der Züchter 34, (8): 312-316.
15. Taylor G. S. 1970. Tobacco protected against fleck by benomyl and other fungicides. Abstr. Phytopathology 60: 578.
16. Taylor G. S., Rich S. 1974. Ozone injury to tobacco in the field influenced by soil treatments with benomyl and carboxin. Phytopathology 64, (6): 814-817.
17. Tomlinson H., Rich S. 1973. Anti-senescent compounds reduce injury and steroid changes in ozonated leaves and their chloroplasts. Phytopathology 63, (7): 903-906.
18. Wolińska D. 1965. Starzenie się chloroplastów w roślinach wyższych. Wiadomości Botaniczne XIX, (3): 163-185.
19. [illegible] A. A. 1966. [illegible] Fiziologija Rastenij 13, (4): 713-718.

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Исследовали влияние некоторых химических соединений на изменение степени чувствительности к действию SO_2 хвои сосны обыкновенной. В общей сложности исследовали влияние 28 химических соединений, относящихся к 6 группам: производные бензимидазола и бензотиадиазола, аминокислоты, витамины, производные пурина и пиримидина, алифатические и ароматические амины, а также соединения не содержащие в своем строении атомов азота. На основании проведенных исследований найдено, что лучшими охраняющими от действия сернистого ангидрида свойствами обладают соединения относящиеся к группе витаминов.