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Sensitivity of Polish races of Norway spruce (*Picea abies* (L.) Karst.) to the action of sulphur dioxide and low temperatures*

INTRODUCTION

In recent years significant development of studies on the effect of industrial pollution of air on the growth of coniferous trees from the genera *Pinus* and *Picea* is being observed (Havas 1971, Börtitz and Vogl 1972, Huttunen 1978, Białobok et al. 1980 a, b). Norway spruce from Poland compared to races from western and northern Europe is considered to be characterized by good growth and adaptability to various environments (Giertych 1976). Among the Polish races of spruce there is considerable variability in the dynamics of growth and development (Giertych 1976) as well as in tolerance to low temperatures (Pukacki 1981). It appears important to check which valuable Polish races of spruce are tolerant both to low temperatures and to the action of sulphur dioxide. In natural conditions we are frequently dealing with the complex action of air pollution, low temperatures and other stress factors (Vogl et al. 1972).

Tesche (1979) when studying effects of drought, low temperature and SO_2 on seedlings of Norway spruce has found that as a result of the action of each of these factors there follows and increases in the content of free proline in the needles. Studies of free aminoacid content in plant tissues have often been used when determining frost injuries (Siminovitch et al. 1964, Cordukes et al. 1966). Jung et al. (1967) obtained a highly significant correlation ($r=0.8$) between the degree of resistance of lucerne to low temperatures and the content of soluble proteins, RNA and DNA. It appears that also under the influence of sulphur dioxide in leaves there occurs a substancial accumulation of free amino acids such as glutamic acid, glutamine, ornithine, citruline, proline and γ — aminobutyric acid (Jäger and Pahlich

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1972, Godzik and Linskens 1974, Malhotra and Sarkar 1979). Karolewski (1982) has found that an increase in the content of free proline in Weigela leaves is directly dependent on the degree of their injury by SO_2.

The studies reported in this paper are aimed at a comparison of the tolerance of Norway spruce populations to the action of SO_2 and low temperatures in relation to the changes in free proline content in needles.

MATERIALS AND METHODS

PLANT MATERIAL

Detached one-year-old shoots from 16 years old individuals of Norway spruce (*Picea abies* (L.) Karst.) have been subjected to the action of low temperatures and sulphur dioxide. These trees grow on an experimental area in the Institute of Dendrology (Giertych 1970). For the study 18 provenances of spruce were tested (Table 1).

EXPOSITION OF SHOOTS TO SO_2

The shoots were exposed to the action of sulphur dioxide at 1 ppm for 12 hours (on 2 consecutive days, 6 h daily) starting on August 17th. This was done in laboratory conditions using chambers specially

Table 1

Concentration of proline in spruce needles of different provenance before and after fumigation SO_2 and degree of injury to needles caused by the gas

Provenance no. and name	Proline, conc. (mg/g)			Degree of needle injury
	Before fumigation I	After fumigation II	II - I	
98 Kowary	0.160	0.165	0.005	1.0 a
99 Istebna	0.105	0.189	0.084	1.8 a b
101 Rycerka	0.240	0.310	0.070	2.4 a b
103 Nowy Targ	0.150	0.270	0.120	2.6 a b
104 Wetlina	0.195	0.155	−0.040	1.6 a b
107 Bliżyn	0.175	0.555	0.380	3.4 b
116 Przerwanki	0.185	0.290	0.105	2.8 a b
117 Gołdap	0.180	0.585	0.405	2.8 a b
119 Augustów	0.105	0.145	0.040	2.2 a b
120 Białowieża	0.180	0.105	−0.075	1.0 a
121 Zwierzyniec	0.180	0.255	0.075	1.6 a b
123 Szadek	0.120	0.135	0.015	2.0 a b
124 Suwałki	0.075	0.105	0.030	2.0 a b
125 Stronie Śląskie	0.150	0.165	0.015	2.0 a b
133 Dolina Chochołowska	0.155	0.315	0.160	2.6 a b
180 Gołdap	0.135	0.111	−0.024	1.4 a b
181 Gołdap	0.165	0.175	0.010	2.0 a b
182 Gołdap	0.105	0.270	0.165	1.8 a b

constructed for the purpose with a system dosing and analyzing the SO_2 concentration as described by Białobok et al. (1978). Each provenance was represented by 5 individuals, four shoots per tree. Throughout the exposition period to the gas the shoots were kept with the cut end in bottles with water, additionally covered with polythene in order to prevent absorption of SO_2 by the water. Simultaneously part of the shoots were placed in identical conditions but without SO_2 and these constituted the control. After exposition of the shoots in the fumigation chamber and a 24 h post fumigation waiting period the sensitivity of the shoots to SO_2 was evaluated visually. The degree of injury to leaf surfaces was taken as a measure of sensitivity to SO_2. A six point scale of estimate was used (after Schönbach et al. 1964) in which O indicates lack of any injuries and 5 injury to more than 70% of the leaf surface.

DETERMINATION OF PROLINE CONTENT IN SPRUCE NEEDLES

In order to determine the content of free proline in needles subjected to the action of SO_2 and controls 0.5 g samples of needles were taken from shoots representing one spruce tree. The mean value for a provenance was estimated from an analysis of a 2.5 g sample of needle fresh weight coming from 5 different trees. Determination of the content of free proline has been conducted using ninhydrine and the colorimetric method described by Bergman and Loxley (1970).

DETERMINATION OF THE FROST TOLERANCE OF SPRUCES

The evaluation of tolerance to low temperatures has been performed on September 29th. For the study one-year-old shoots were taken from 4 trees of each of the 18 studied provenances, using 8 shoots for each of the testing temperatures. The shoots were subjected to control freezing for 24 hours. The rate of cooling of these temperatures and of defrosting after termination of the treatment was 3 - 4°C/h. The degree of injury to the frost treated shoots was determined by the method of measuring electrical admittance of shoots according to the description given earlier (Pukacki 1973, Białobok and Pukacki 1974). The measurement of electrical admittance has been made in two positions on the shoot, near the apex and near the base, at a room temperature of $20° \pm 1°C$, using a Radeliks OK-102/1 conductometer at a frequency of 1000 Hz. The results of measurements have been presented as the increase in admittance following freezing of the shoots. As was shown in earlier investigations the value of the differences in admittance of shoots before and after freezing is directly proportional to the degree of sensitivity of woody plants to low temperature (Pukacki 1973, 1978).

STATISTICAL TREATMENT

The differences in injury degree of shoots of various provenances of spruce have been verified statistically using the new multiple range test bringing provenances into groups that are not significantly differentiated within at a confidence level of $\alpha=0.05$ (O k t a b a 1976). Also a matrix of the correlation coefficients r was calculated for all comparisons of the provenances in degree of injury to SO_2, level of free proline in leaves and differences in electrical admittance of shoots before and after freezing.

RESULTS AND DISCUSSION

The selection performed for the sensitivity to SO_2 has shown little differentiation in the degree of spruce needle injury between the provenances, ranging from 1.0 to 3.4 on the adopted scale (Table 1). The two groups of provenances obtained in the *D* test (a and b) which are internally undifferentiated at a significance level of $\alpha=0.05$ substancially overlap. One can only say that two provenances (Kowary 98 and Białowieża 120) are significantly more tolerant to SO_2 than provenance Bliżyn (107). A comparison of tolerance to SO_2 estimated visually as shoot injury with the tolerance to the action of a $-15°C$ temperatures indicates that the two tolerances are correlated (Tables 2 and 3). Absence of correlations following testing with the other temperatures may have been caused by the low differentiation of populations at these temperatures. However, on the basis of the obtained results one can identify

Table 2

Values of difference of electrical admittance (in μS) before and after treatment of Norway spruce shoots with low temperatures

Provenance no.	Incr. in admittance after freezing to		
	−15°C	−20°C	−25°C
98	51.4	84.7	89.3
99	57.0	70.9	94.6
101	49.6	62.7	59.2
103	67.1	64.6	98.0
104	41.5	70.2	71.4
107	49.8	64.1	94.6
116	51.8	84.7	84.6
117	54.1	77.3	90.2
119	43.1	49.2	51.5
120	46.1	64.4	68.1
121	49.7	70.8	81.0
123	62.6	75.3	81.3
124	58.8	70.6	87.3
125	49.8	66.9	77.6
133	53.9	65.1	81.8
180	39.3	53.1	82.8
181	52.7	63.9	92.5
182	42.7	60.7	79.1

Table 3

Matrix of correlation coefficients

	1	2	3	4	5	6
1. Injury degree						
2. Proline level before treatment	0.16					
3. Proline level after treatment	0.76****	0.39*				
4. Incr. in proline following treatment	0.77****	0.01	0.94****			
5. Incr. in proline after −15°C	0.34*	0.19	0.12	0.19		
6. Incr. in proline after −20°C	0.01	0.21	0.17	0.12	0.43*	
7. Incr. in proline after −25°C	0.22	0.18	0.30	0.39*	0.57*	0.66***

**** $\alpha=0.01$, *** $\alpha=0.05$, ** $\alpha=0.1$, * $\alpha=0.2$

two populations, one from northeastern Poland (Gołdap 180) and the other from southern Poland (Wetlina 104) which appear to be more tolerant to low temperatures. The mean value of the difference in electrical admittance for these two provenances is 40.7 μS. The degree of needle injury for the two after fumigation with SO_2 is 1.5. Most sensitive to the action of low temperatures were the lowland provenance Szadek (123) and the montane one Nowy Targ (103). For these two provenances the mean difference in electrical admittance following freezing to −15°C is 64.8 μS. Also the mean value of needle injury following SO_2 treatment is high for these two provenances (2.3). In the light of these data one can say that there appears to be no relation between the region of occurrence of spruce populations and their tolerance to SO_2 and low temperatures.

The action of sulphur dioxide on one-year-old shoots of the studied provenances of spruce has caused an increase of free proline in the needles (Table 1). The populations which are more sensitive to the action of SO_2 were characterized by a significantly greater content of free proline in the needles after fumigation with the gas (Table 3). The high value of the correlation coefficient ($r=0.76$) between the degree of needle injury and the change in the level of free proline indicates that this amino acid is a good indicator of the injury inflicted to the plant by SO_2. On the other hand, the level of free proline measured in the needles before the action of SO_2 does not differentiate the spruce populations in relation to their sensitivity to this gas. This is indicated by the lack of a significant correlation between the level of free proline in needles of control shoots and the degree of their injury as estimated visually following fumigation with the gas (Table 3).

As earlier investigations of other authors have shown, changes in the content of free proline occur also under the influence of low temperatures (Levitt 1972, Tesche 1979). In our studies, a positive correlation was observed between the increase in proline level following treatment with sulphur dioxide and the increase in the level of electrical admittance following freezing to −25°C (Table 2 and 3). This would

indicate that the spruce populations more sensitive to frost are also less resistant to SO_2. After threating them with SO_2 they have a greater increase in proline level.

Subjecting plants to the action of low temperatures or SO_2 we observe that after termination of the injurious conditions there develop distrubances in the water relations of the plants reflected in a water deficit for the leaves. The water deficit in assimilation organs causes a rapid increase in the level of free proline (Britikov 1975). A positive correlation between the degree of tolerance of spruce grafts to the action of SO_2 and the degree of tolerance to a water deficit has been demonstrated by Klein (1980). It was also found that the process of natural winter hardening of spruce is accompanied by an increase in the level of proline in needles and buds (Durzan 1968).

In the light of this data we can assume that there can exist some common mechanisms in the reaction of plants to stress factors such as fumigation with SO_2 or low temperatures. Under the influence of SO_2 there occurs in plant tissues a substancial evolution of toxic ammonia (Godzik and Linskens 1974). Similarly the release of NH_3 has been observed in plant tissues under the influence of low temperatures (Levitt 1972). An increase in the level of free proline in tissues can also be explained as a process counteracting the injuries inflicted on the plant by a stress factor occurring in the environment. The accumulated free proline participates in the rebuilding of chlorophyll and constitutes an effective activator of the Krebs cycle. It also maintains an appropriate colloidal structure of the cytoplasm thanks to its high capacity for binding water (Britikov 1975). At the present stage of investigations it is not possible yet to explain the reaction mechanism to the discussed stress factors.

SUMMARY

The relationship between tolerance to sulphur dioxide and to low temperatures has been investigated for 18 provenances of Norway spruce. It was found that there occurs a correlation between populations in sensitivity to these two stress factors. In needles of spruce after fumigation with SO_2 (1 ppm for 12 h) there occurs an increase in the level of free proline, the greater the more sensitive to the gas is the spruce population. The accumulation of free proline after treatment with low temperatures has been demonstrated by other authors. The possibility that there occur common mechanisms in the response of plants to external stress agents is discussed.

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Wrażliwość polskich ras świerka pospolitego (*Picea abies* (L.) Karst.) na działanie dwutlenku siarki i niskich temperatur

Streszczenie

Na 18 populacjach świerka pospolitego badano współzależność między tolerancją na dwutlenek siarki i niskie temperatury. Stwierdzono, że istnieje korelacja między populacjami we wrażliwości na oba te czynniki stresowe. W igłach świerków z populacji wrażliwych na SO_2, po działaniu tego gazu w stężeniu 1 ppm przez 12 h, zaobserwowano zwiększoną zawartość wolnej proliny, co w przypadku niskich temperatur wykazali także inni autorzy. W pracy przedyskutowano ponadto możliwość istnienia wspólnych mechanizmów w reakcji roślin na zewnętrzne czynniki stresowe.

Чувствительность ели обыкновенной (*Picea abies* (L.) Karst.) польских происхождений к действию сернистого ангидрида и низких температур

Резюме

Взаимосвязь между толерантностью на действие сернистого ангидрида и низких температур исследовали на 18 популяциях ели обыкновенной. Установлено, что существует корреляция между популяциями и чувствительностью к действию обоих стрессовых факторов. В хвое чувствительных к SO_2 популяций ели, после действия на них 2,67 мг/м³ этого газа в течение 12 часов наблюдали увеличение концентрации свободного пролина, что было доказано также исследователями изучавшими влияние действия низких температур. В работе обсуждается также возможность существования общих механизмов реакции растений на внешние стрессовые факторы.