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Peroxidase activity in varieties of *Weigela* and *Pinus silvestris* resistant and susceptible to SO_2*

INTRODUCTION

The injurious effect of SO_2 on plants is a well known phenomenon frequently described in the literature. Already low concentrations of SO_2 affect susceptible individuals causing necroses and chloroses of leaves as the first visible symptoms of injury. However the injurious action of gases may cause metabolic changes in plants long before injuries visible to the naked eye appear. There exists therefore the possibility that an early signal of the presence of toxic gases could be achieved through biochemical methods. The peroxidase activity in leaves proved to be a sensitive test of invisible physiological injuries.

Peroxidase is an enzyme of low substrate specificity catalysing the oxidative reactions in the presence of H_2O_2. Fridorich and Handler (1961) studying seedlings of peas have proven that peroxidase in the presence of hydrogen peroxide can oxidize sulphitex to sulphatex.

Increase in peroxidase activity under the influence of SO_2 has been reported among others by Nikolaevskij (1968) in the leaves of *Acer negundo* and by Horsman and Wellburn (1975) in the seedlings of *Pisum sativum*. Keller and Schwager (1971) and Bucher-Wallin (1976) have demonstrated an increase in the activity of this enzyme in the leaves of several species of forest trees exposed to the action of fluorine. Peroxidase activity increases in plant tissues under various stress conditions as for example following mechanical injuries to plants or attack of tissues by parasitic organisms. Farkas et all. (1964) suggest that an increase in the activity of enzymes under the influence of stress is not a result of de novo synthesis but is associated with destruction of protein cellular structures and the release from them of so far immobilized (latent) enzymatic proteins.

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MATERIAL

The investigations were performed on the shoots of two varieties of *Weigela* and four clones of *Pinus silvestris*. One of the studied *Weigela* varieties was chosen following selection performed by Karolewski and Rachwał (1976) as being sensitive to SO_2 (*W. florida* A. DC 10993) and the other as being resistant to SO_2 (*W. 'Van Houttei'* 2468) Among the four clones of *P. silvestris* I have studied two sensitive ones to SO_2 (K-01-16 and K-11-13) and two more resistant ones (K-08-02 and K-14-12). This material was previously selected from a large group of clones (Białobok, Karolewski and Oleksyn, 1977). The activity of peroxidase in the leaves of detached shoots has been analysed in the following variants: 1. immediately after detaching shoots, 2. controls 6 hours after detaching shoots, and 3. treated with SO_2 at 2 ppm for 6 hours. Each variant was studied in three replicates. In chambers in which the control shoots were placed and treated with SO_2 there existed the following conditions: light intensity 11.5 - 13.8 lux · 10^3, relative humidity 60 - 70%, temperature 21.5 - 23°C, pressure — 760 mg Hg, rate of air change in chamber — 14/hr.

After fumigation on the surface of leaves and needles there were no necrotic changes. From the leaves acetone powders were performed from which proteins were extracted.

METHODS

In order to check the peroxidase reaction to SO_2 treatment at all levels of cell structure acetone powders of *Weigela* leaves have been fractionated into 1) buffer soluble proteins, 2) proteins soluble in 1% Triton ×100, 3) proteins ionically bound with cell membranes, 4) proteins covalently bound with cell membranes. In pine needles only soluble proteins were studied.

Preparation of the enzymatic samples:

— Into the acetone powder a 0.2 M phosphate buffer, pH 6.0, with 0.1% cystein hydrochloride and 5% polyclar AT, was added. After centrifugation for 15 min at 2000 xg the supernatant contained the buffer soluble proteins.

— The remaining residue was washed 3 times in the 0.2 M phosphate buffer, pH 6.0, containg 1% Triton×100 and centrifuged again at 2000 xg. The supernatant contained soluble proteins.

— The residue was washed 20 times with distilled water and centrifuged each time 10 min at 2000 xg. The remaining residue free of soluble proteins was transferred to a 0.2 M phosphate buffer, pH 6.0, containing 1 M NaCl and left overnight in the refrigerator. After

30 minute centrifugation at 10 000 xg the supernatant contained the ionically bound protein fraction.

— The residue was washed twice in a solution of NaCl in the phosphate buffer at pH 6.0 and then placed in an acetate buffer pH 4.0 containing 2.5% pectinase and 0.5% cellulase. After 4 hours of incubation at a temperature of 25°C and centrifugation at 10 000 xg the supernatant contained the covalently bound protein fraction.

Al the fractions were purified 18 hours by dialysis. All the time the preparations were maintained at a temperature of 0 to 4°C.

Measurements of protein content in the enzymatic preparations were made according to Potty (1969).

Measurements of peroxidase activity. Peroxidase activity was measured spectrophotometrically (Vitatron MPS) at 430 nm. The enzymatic reaction was conducted to oxidize guaiacol in the presence of hydrogen peroxide. Use was made of the reaction mixture: 1% quaiacol — 1 ml; 0.2 M H_2O_2 — 1 ml; 0.2 M phosphate buffer, pH 6.0 — 1 ml; enzymatic sample with 10 - 50 ug of proteins.

Electrophoresis. The enzymatic preparations have been separated on a polyacrylamide gel in anionic (Davis, 1964) and cationic (Reisfeld, Lewis and Williams, 1962) systems. As a substrate for peroxidase benzidine was used (Safonov, Safonova and Narbut, 1969).

A densitometric record of absorption by isozyme bands was conducted at 430 nm (Vitatron MPS).

RESULTS

The activity of peroxidase in samples from control shoots analysed 6 hours after cutting did not differ from those analysed immediately after cutting from the trees.

Figure 1 presents the peroxidase activities in four fractions of *Weigela* leaves. A higher peroxidase activity was observed in the leaves of control shoots from the SO_2 susceptible variety (*W. florida*) than in the control leaves of the resistant variety (*W. 'Van Houttei'*). This phenomenon was observed in all four studied enzymatic fractions. In the leaves of shoots exposed to SO_2 there occurred an increase in the activity of peroxidase relative to the levels in control leaves, both in the susceptible and resistant variety. This concerns also all four enzymatic fractions.

The electrophoretic pattern for peroxidases in the leaves of the two *Weigela* varieties do not show any changes in bands following SO_2 treatment. Only the activity of individual bands increased (Fig. 2).

Figure 3 presents the peroxidase activities in samples from control and SO_2 treated shoots of four clones of Scots pine. A higher activity of

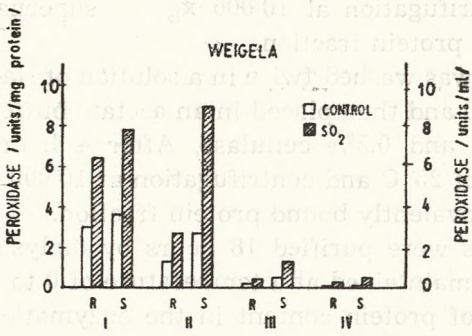

Fig. 1. Activity of peroxidase in four enzymatic fractions from leaves of *Weigela florida* (sensitive — S) and *Weigela 'Van Houttei'* (resistant — R) under control conditions and following SO_2 treatment (2 ppm for 6 hrs.)

I — buffer soluble fraction, *II* — fraction soluble in 1% Triton × 100, *III* — ionically bound fraction, *IV* — covalently bound fraction. Peroxidase activity is expressed in enzyme units. For fractions *I*, *II* and *III* one enzyme unit is defined as a change in OD_{430} per minute caused by 1 mg of protein and for fraction IV as a change in OD_{430} per minute caused by 1 ml of the enzyme preparation

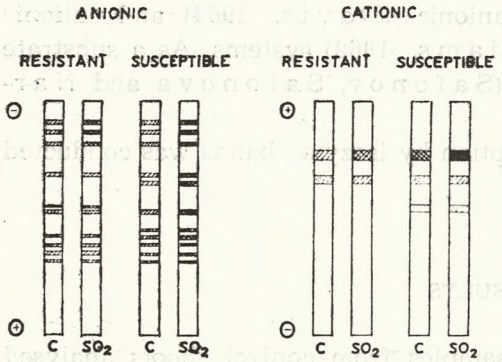

Fig. 2. Electrophoretic separations of peroxidase from control and fumigated with SO_2 (2 ppm for 6 hrs.) leaves of *Weigela florida* (sensitive) and *Weigela 'Van Houttei'* (resistant). Electrophoresis was performed on polyacrylamide gel in an anionic (pH 8.3) and cationic (pH 4.5) system. Peroxidase isozymes was visualized with benzidine — H_2O_2

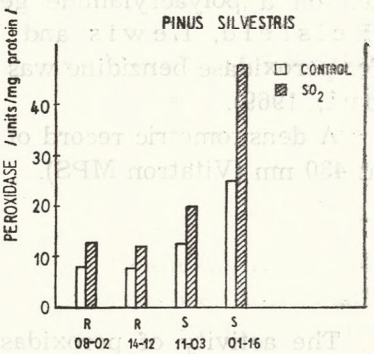

Fig. 3. Peroxidase activity in the soluble fraction of control and SO_2 treated Scots pine needles of four clones (01-16 and 11-03 are sensitive and 08-02 and 14-12 are more SO_2 resistant). Peroxidase activity is expressed in enzyme units. One enzyme unit is defined as a change in OD_{430} per minute caused by 1 mg of protein

peroxidase in the needles of control shoots of the more sensitive clones (K-01-16 and K-11-03) than in the needles of the control shoots of the more SO_2 resistant clones (K-08-02 and K-14-12) was observed. After 6 hours of the SO_2 treatment there occurred a strong increase in the

activity of peroxidase in the needles of all pine clones tested relative to the untreated controls.

The pines did not differ qualitatively in the electrophoretic pattern of bands neither before nor after SO_2 treatment.

DISCUSSION

Sulphur dioxide penetrating into leaves is gradually dissolved into sulphurous acid forming ions of H^+, HSO_3^- and SO_3^{--}. Tager and Rantanen (1955) studying the leaves of spinach have used $^{35}SO_2$ and after 2 or 7 hours 18% or 43% respectively of the label was found in $^{35}SO_4^{--}$. Fridorich and Handler (1961) have proven that in the presence of hydrogen peroxide sulphite may be oxidized to sulphate in the presence of peroxidase. Horsman and Wellburn (1975) in studies on peas have found that the penetration of SO_2 into plants causes a general increase in the oxidizing processes as a result of which ions of SO_3^{--} are oxidized to SO_4^{--}.

In the present study I have found an increase in the activity of peroxidase in the leaves from *Weigela* and *Pinus silvestris* shoots subjected to the action of SO_2 relative to untreated controls. Furthermore I have shown that untreated shoots of *Weigela florida* and of two clones of Scots pine which are deemed more sensitive to SO_2 have a much higher level of peroxidase activity than leaves of *Weigela 'Van Houttei'* and two clones of *Pinus silvestris* considered to be more SO_2 resistant.

Keller and Schwager (1971) have found that an increase in peroxidase activity in the leaves of forest trees takes place not only under the influence of fluor pollution but also as a result of physiological ageing of leaves. Since the oxidative processes lead to maturation and ageing of plants they suggest that the toxic factors arriving at the leaf surface induce the aging of tissues.

Thus it is possible that in the conditions of SO_2 action the ageing processes take place more rapidly and easier in plants with a higher peroxidase activity and it is in these plants that symptoms of injury appear first.

Many authors stress that SO_2 attacks primarily old leaves. Godzik (1976) when studying the absorption of $^{35}SO_2$ by coniferous trees has found that irrespectively of species the smallest quantities of $^{35}SO_2$ per one needle have been absorbed by the needles of the current season. Older needles absorbed more $^{35}SO_2$. In *Pinus silvestris* the greatest amounts of $^{35}SO_2$ have been absorbed by needles from the previous two vegetative seasons. Studies of this author have shown that a ranking of pine species according to the quantities of absorbed $^{35}SO_2$ is almost totally in agreement with the ranking made by Dässler (1965) of the

same species on the basis of the degree of injury to needles by SO_2 (*P. rigida, P. strobus, P. silvestris, P. montana, P. nigra*). *P. strobus* is an exception since it absorbed $^{35}SO_2$ most but its needles are injured much less than those of *P. silvestris*. When comparing some tree species such agreement is not observable. Thus for example birch belongs to species showing most intensive SO_2 absorption from air (Godzik, 1968; Materna and Kohout, 1969) however on the basis of field observations and laboratory studies of many authors it is included among the most resistant species to SO_2.

The present study has shown that under the influence of SO_2 no new electrophoretic bands of peroxidase form. This result does not contradict the suggestion of Farkas et al (1964) that the general increase in peroxidase activity in detached leaves and diseased tissues is a consequence of the liberation of enzymes from inactive forms on destruction of subcellular particles.

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Aktywność peroksydazy u odmian Weigela i Pinus silvestris odpornych i wrażliwych na działanie SO_2

Streszczenie

Aktywność peroksydazy w liściach odmiany *Weigela* wrażliwej na działanie SO_2 jest wyższa niż w liściach odmiany odpornej na ten gaz. Podobną zależność znaleziono w igłach dwóch wrażliwych i dwóch bardziej odpornych na SO_2 klonów *Pinus silvestris*.

Traktowanie SO_2 (2 ppm, 6 godzin) powoduje wzrost aktywności peroksydazy zarówno u odmian wrażliwych jak i odpornych, lecz nie wywołuje zmian jakościowych w układzie pasm elektroforetycznych.

БАРБАРА КЕЛИШЕВСКА-РОКИЦКА

Активность пероксидазы у разновидностей Weigela и Pinus silvestris устойчивых и чувствительных к действию SO_2

Резюме

Активность пероксидазы в листях чувствительной по отношению к сернистому ангидриду разновидности *Weigela* выше, чем у разновидности устойчивой к действию этого газа. Сходная зависимость найдена в случае, когда исследуемым объектом была хвоя двух чувствительных и двух более устойчивых клонов *Pinus silvestris*.

Обработка SO_2 (5,4 мг/м³ в течение 6 часов) вызывала рост активности пероксидазы как у чувствительных так и у устойчивых разновидностей, но этот рост не сопровождался качественными изменениями в расположении электрофоретических полос.