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Effect of sulphur dioxide on peroxidase activity in leaves of Weigela rooted cuttings*

INTRODUCTION

The first plant responses to the action of SO_2 that can be measured are the disturbances caused by the gas in physiological and biochemical processes. Determination of changes, direction and intensity of these processes after the action of SO_2 may constitute an indicator of the degree of injury, i.e. the reaction of plants to this gas.

In literature there are various suggestions for the measurement of the degree of plant injury or of the response to the gas absorbed such as the amount of sulphur absorbed, changes in the intensity of photosynthesis, amino acid content and also the activity of enzymes.

There are relatively many reports on the possibility of utilizing changes, both increases and decreases, in the activity of enzymes for the determination of the degree of injurious influence of sulphur dioxide on plants. Pahlich (1972), Pahlich et al. (1972), Jäger and Klein (1977) have shown that SO_2 causes a stimulation in the activity of glutamine synthetase dependent on NAD^+. Soldatini and Ziegler (1979) who have studied the effect of SO_2 on tobacco seedlings have found an increase in the activity of glycolic oxidase. An increase in the activity of glutamate-pyruvate and glutamate-oxaloacetate transaminases in seedlings of *Pisum sativum and Rumex obtusifolius* has been reported by Horsman and Wellburn (1975, 1977). An increase in peroxidase activity in spruce needles treated with SO_2 has been shown by Keller et al. (1976), in needles of Scots pine and leaves of Weigela by Kieliszewska-Rokicka (1979) and in leaves of *Acer negundo* by Nikolaevskij (1968). A decrease in the activity of peroxidases under the influence of SO_2 has been demonstrated by Dässler (1962). Niemtur (1979) has found that there are no

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significant differences in the activity of this enzyme between various progenies of Scots pine subjected to industrial pollution (primarily SO_2).

Under the influence of SO_2 there occurs in plants a release of ethylene (Guttenberger et al. 1978, Bressan et al. 1979, Mudd 1979). From the studies of Osborne et al. (1970) and Ridge and Osborne (1971) it appears that ethylene, which is a natural hormonal inhibitor of rate and magnitude of cell growth, causes a decline in the plasticity of cell walls thereby conditioning tissue maturation. Simultaneously these authors have observed an increase in the activity of peroxidases as tissues age.

As the degree of injury to plant tissues increases under the action of SO_2 there is also an increase in the activity of peroxidase (Keller et al. 1976, Kieliszewska-Rokicka 1979). The studies of Lewington et al. (1967), Lazare and Farkas (1979), and Racusen and Foote (1966) all after Kljačko, Kulaeva (1975) indicate that it is possible to use the increase in peroxidase activity as an indicator of plant ageing.

This would suggest that the action of SO_2 results in an increased intensity of the ageing processes.

Changes in the activity of peroxidase occurring as a result of SO_2 action in relation to the maturation and ageing of plant tissues were the subject of investigations reported here.

MATERIALS AND METHODS

The experimental material consisted of 2-year-old vegetatively propagated plants from the genus *Weigela* (Thunb.), namely *Weigela florida* A. DC and *Weigela*×*wagneri* Bailey cv. 'Van Houtte'. The cultivars were selected from a larger number basing on an earlier investigation (Karolewski and Rachwał 1976). The pots in which the plants grew were tightly covered with polyethene to prevent absorption of SO_2 by the soil and reactions with its components.

The experiments were conducted under controlled laboratory conditions. The plants were exposed to the action of SO_2 in special chambers constructed for the purpose in the Institute of Dendrology in Kórnik (Białobok et al. 1978). The chambers were located inside an air conditioned greenhouse and were connected with a system dosing and measuring the concentration of sulphur dioxide (Fig. 1). The available equipment permitted an automatic dosing of SO_2 supply to the chambers, a continuous measurement of its concentration, a regulation of temperature, humidity and the rate of exchange of the air mixed with gas ($15 \times h^{-1}$).

After exposing plants to the action of SO_2 the degree of their sensi-

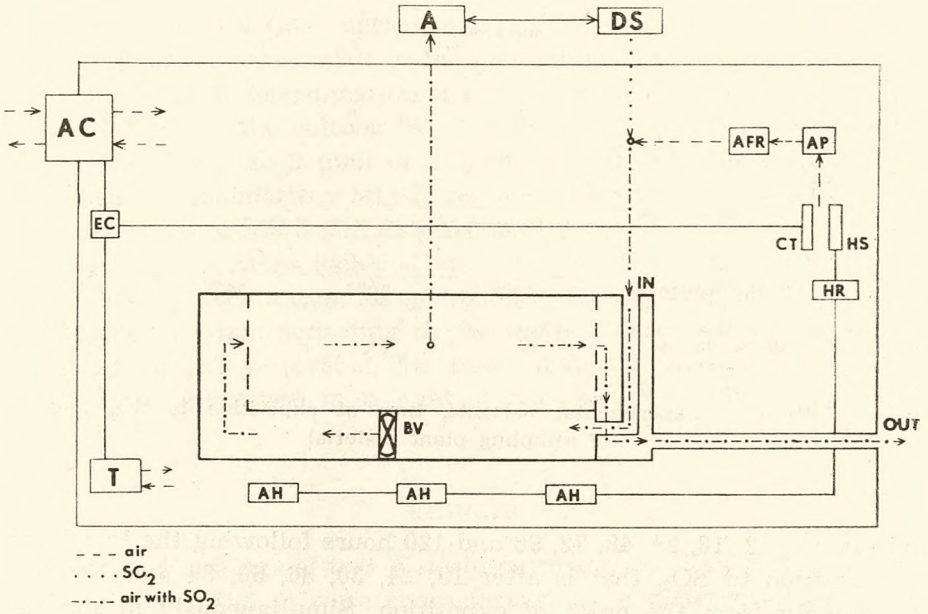

Fig. 1. Scheme for the dosing and measurement of sulphur dioxide in the system *A* — SO_2 analyser, *DS* — dosing system, *AC* — air conditioner, *AFR* — air flow rate, *AP* — air pump, *EC* — electromagnetic conveyor, *CT* — contact thermometer, *HS* — humidity sensor, *HR* — humidity regulator, *IN* — inlet of air with SO_2, *OUT* — outlet of air with SO_2, *BV* — block of ventilators, *T* — thermoventilator, *AH* — air humifier

tivity to the gas was estimated. This was done by a visual determination of the extent of injury to leaf surfaces. When making these estimations a 6 point scale was used (Białobok et al. 1980) in which:

0 — lack of any visible injuries
1 — injuries visible on 1 - 10% of the leaf surface
2 — injuries visible on 11 - 30% of the leaf surface
3 — injuries visible on 31 - 50% of the leaf surface
4 — injuries visible on 51 - 70% of the leaf surface
5 — more than 70% of the leaf surface visibly injured.

Various numbers of replicates were employed depending on the purpose the experiment was to serve.

To study the kinetics of changes in peroxidase activity rooted cuttings of two *Weigela* varieties were used, the relatively sensitive cultivar *W. florida* and the less sensitive one *W.* 'Van Houtte'. The plants were placed in two chambers, with and without SO_2. The pattern of plant exposition to the action of the gas and the time when samples for analyses were taken are shown in Fig. 2. The concentration of SO_2 used was 2 ppm, the relative air humidity 60 - 70%, the illumination 12 - 15 klx and the air temperature 19 - 23°C. The experiments were conducted in June 1980. The plant material was taken for analysis immediately before exposition, after 3, 6, 9 and 12 hours of exposition to SO_2

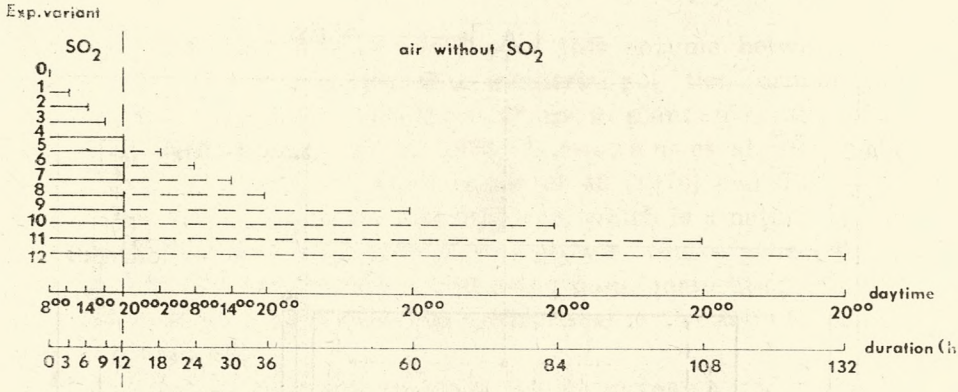

Fig. 2. Pattern of experimental variants, time of exposition to SO_2 and time of sampling plant material

and after 6, 12, 18, 24, 48, 72, 96 and 120 hours following the termination of exposition to SO_2, that is after 18, 24, 30, 36, 60, 84 and 132 hours respectively from the onset of exposition. Simultaneously at the same times leaf samples were taken from control plants held in the chamber that was not treated with SO_2. From the control plants whole leaves were taken for analyses and from the treated plants only the parts of leaves that were not visually affected by the gas. For each *Weigela* variety and for each experimental variant 5 g samples of leaf fresh weight were taken from two plants.

Peroxidase activity was determined in acetone powders previously prepared. The 5 g samples of leaves or discs from them were immersed in 25 cm^3 100% acetone with etanothiol (0.1 $cm^3 \times dcm^{-3}$ acetone) added at a temperature of $-15°C$ and then homogenized. The homogenate was filtered under vacuum on a funnel with Schott G-3 filter and the residue was washed until complete removal of the pigments. The residue was dried with a small quantity of ethyl ether and a stream of air and then stored in an exicator with anhydrous $CaCl_2$ held at about $-15°C$.

The peroxidase activity was determined for various samples (acetone powders) in two fractions:

1. Soluble proteins in a phosphate buffer with Triton X-100,
2. Proteins ionically bound with cell membranes.

The activity of peroxidase has been determined colorimetrically using guaiacol according to the method described by Kieliszewska-Rokicka (1979).

For the studied samples of both protein fractions the activity of peroxidase has been recorded in units of absorption changes (optical density) $\Delta OD \times min^{-1} \times mg^{-1}$ protein. For the colorimetric determination

of protein in an alkaline cupric solution and the presence of the Folin-phenol reagent use was made of the method described by P o t t y (1969).

A histochemical determination of the localization of peroxidase activity was performed on the cultivar *W. florida* sensitive to SO_2, after having exposed the plants to 2 ppm of SO_2 for 6 h in July 1980. After 24 h, when distinct boundaries formed between necroses and visually uninjured tissues, manually 5 mm long sections were out transversely through the leaves. The sections included visually uninjured and necrotic tissues of the leaves. Localization of peroxidase activity was obtained using 3,3'-diaminobenzidine according to the method described by G r a h a m and K a r n o v s k y (1966). The plant material placed in a drop of glycerine was observed in a light microscope. A ×140 magnification was used.

RESULTS

The rooted cuttings of two *Weigela* cultivars, *W.* 'Van Houtte' (Fig. 3) and *W. florida* (Fig. 4) were characterized by a differential sensitivity to the action of the gas. After a 12 h exposition of the plants to 2 ppm SO_2 and 12 h after termination of the exposition, leaves of the less sensitive cultivar *W.* 'Van Houtte' were injured to the level of 1.5 on the adopted scale that is about 15% of the leaf surface had necroses and discolorations, while in the sensitive cultivar *W. florida* leaves were injured to the level 4.0 i.e. had about 60% of the leaf surfaces visibly affected.

The activity of peroxidases in the leaves of *W.* 'Van Houtte' and *W. florida* has been determined for the soluble fraction in a phosphate buffer with Triton X-100 and for the ionically bound fraction before exposition to SO_2, during the 12 h exposition and later for 120 h from the moment of termination of exposition (Table 1). Simultaneously the activity of peroxidases was also determined in leaves of plants not subjected to the action of SO_2 (control). Here in both fractions it was

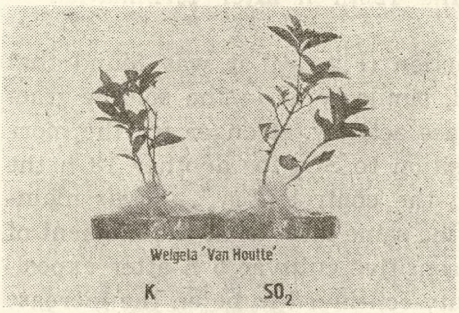

Fig. 3. Control (*K*) and plant exposed to SO_2 (*Weigela* 'Van Houtte')

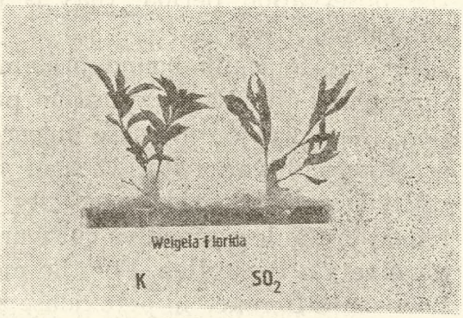

Fig. 4. Control (*K*) and plant exposed to SO_2 (*Weigela florida*)

Table 1

Peroxidase activity Δ/OD × min^{-1} mg^{-1} protein in leaves of *Weigela* 'Van Houtte' and *W. florida*, as affected by treatment with SO_2

Exp. variant defined in fig. 2	Exposition time (h)	Time when taken for analysis	*Weigela* 'Van Houtte'				*Weigla florida*			
			Soluble protein fraction in buffer with Triton		Ionic fraction		Soluble protein fraction in buffer with Triton		Ionic fraction	
			K	SO_2	K	SO_2	K	SO_2	K	SO_2
0	0	0	2.26	2.26	0.12	0.12	3.20	3.20	0.44	0.44
1	3	3	3.05	3.55	0.15	0.21	4.58	6.45	0.32	0.76
2	6	6	2.36	4.00	0.11	0.35	4.76	7.60	0.48	1.19
3	9	9	1.86	5.96	0.10	0.55	3.20	12.45	0.67	1.53
4	12	12	3.75	11.70	0.20	0.95	6.45	21.36	0.58	2.03
5	12	18	3.55	16.15	0.15	1.06	4.73	28.05	0.37	3.11
6	12	24	2.10	7.47	0.11	1.11	4.00	16.60	0.37	2.93
7	12	30	1.65	4.40	0.08	0.75	2.32	16.15	0.27	2.91
8	12	36	2.33	2.95	0.14	0.40	4.48	12.45	0.36	1.90
9	12	60	2.32	2.85	0.14	0.45	3.07	9.93	0.32	1.53
10	12	84	1.15	2.32	0.07	0.37	2.26	8.57	0.15	1.44
11	12	108	2.82	2.00	0.18	0.15	3.05	9.15	0.32	1.72
12	12	132	2.44	1.73	0.19	0.12	4.58	7.63	0.30	1.25

higher in the more sensitive cultivar *W. florida* than in the less sensitive one *W.* 'Van Houtte'.

In the protein fraction soluble in a phosphate buffer with Triton X-100 the activity of peroxidase increased for both cultivars during the 12 h exposition to SO_2 compared to the control (Fig. 5A). A greater increase in activity of the enzyme was manifested in the more sensitive cultivar. An increase in the activity of peroxidase under the influence of SO_2 was observed even 6 h after termination of exposition of the plants of both cultivars. The difference in peroxidase activity (Z_{SO_2-K}) was at that time higher for the sensitive cultivar than for the less sensitive one. Six hours after transfer of the plants to an atmosphere free of SO_2 the level of peroxidase activity started to decline for the less sensitive cultivar almost equalizing with the control after another 12 hours. In the more sensitive cultivar the difference in peroxidase activity with the control (Z_{SO_2-K}) also declined, however it did not reach the control level to the end of the observation period, thas is till the 120th h after termination of exposition.

In the ionically bound protein fraction (Fig. 5B) it was found that throughout the 12 h exposition period, similarly as in the soluble fraction, there was an increase in peroxidase activity, stronger for the more sensitive cultivar. After 12 h of exposition to SO_2 for another 18 h the difference in peroxidase activity with the control (Z_{SO_2-K}) was maintained in the less sensitive cultivar, at the same level as at the moment of termination exposition. For the more sensitive cultivar 6 h after exposition, the difference in peroxidase activity continued to be on the increase and then for 12 h remained at a steady level. After 18 h from the termi-

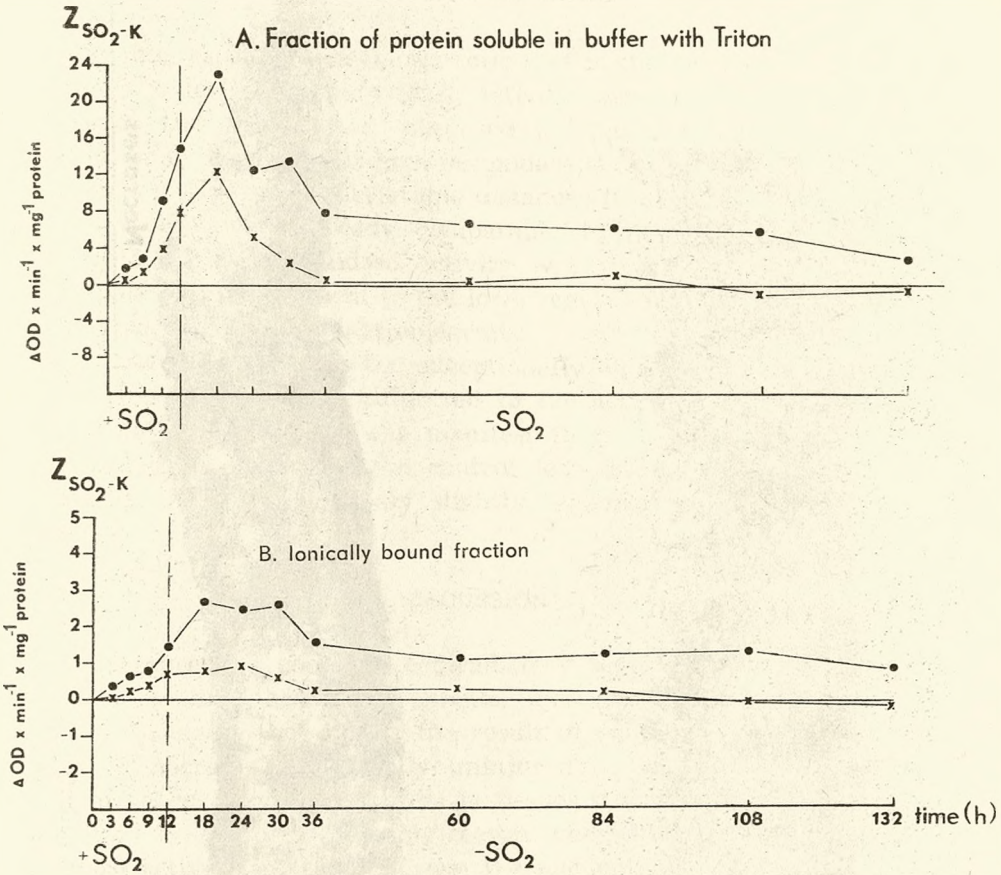

Fig. 5. Changes in peroxidase activity in leaves of *Weigela* 'Van Houtte' (—x—x—) and *W. florida* (—.—.—) exposed to the action of SO_2 during exposition and after, compared to unexposed control

nation of exposition to SO_2 the peroxidase activity in both cultivars started to decline, reaching the control value in the less sensitive cultivar, but not attaining the control level for the sensitive cultivar even 120 h after the termination of exposition to SO_2.

Localization of peroxidase activity in necrotic and visually uninjured parts of leaves of *Weigela florida* subjected to the action of SO_2 has been compared with that in tissues of control leaves not treated with SO_2 (Fig. 6).

The activity of peroxidase in necrotic spots was minimal (the leaf mesophyll was in these places clearly deformed, dried, yellowish-brown in colour; Fig. 6b). The boundary between the necrotic and visually uninjured tissue was very distinct and it clearly differentiated these parts in peroxidase activity. It was highest directly next to the necrotic tissue. Regardless of the type of tissue in which it occurred it was not differen-

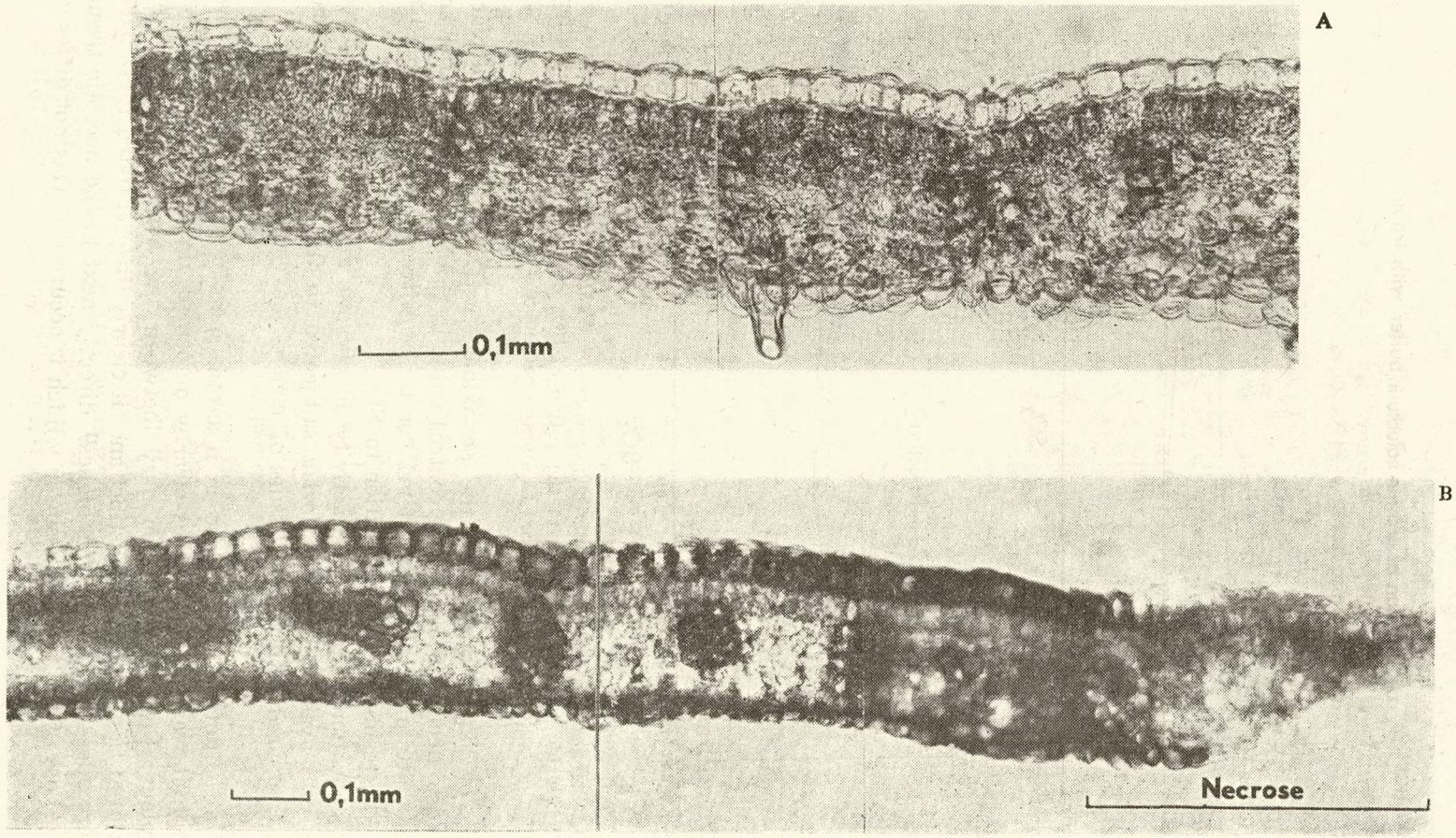

Fig. 6. Localization of peroxidase activity in a transverse section of *Weigela florida*
A — control, B — after treatment with SO_2

tiated in this respect. From the necrotic spot in the direction of uninjured mesophyll a decline in peroxidase activity was observed. A relatively high peroxidase activity was observed in epidermal cells, particularly in the lower epidermis. This high peroxidase activity in the lower epidermal cells was observed considerable distances from necrosis while higher epidermal cells were already comparable to those in control leaves. Similarly a higher peroxidase activity was observable in cells of the spongy parenchyma adjacent to the lower epidermis than in the pallisade parenchyma next to the upper epidermis.

Of particular interest is the exceptionally high peroxidase activity in vascular bundles of leaves subjected to the action of SO_2 compared to the controls. This activity was manifest throughout the length of the observed leaf fragment. In the control leaf the peroxidase activity in the vascular bundles was only slightly higher than in the mesophyll.

DISCUSSION

In literature one can find contradictory reports about the effect of SO_2 on peroxidase activity in plants, increases, decreases or lack of response. I suspect that this is the result of summary determination of activity in necrotic and visually uninjured tissues, and thus they are a joint effect of enzyme activation in tissues still visually uninjured and inactivation in necroses. For this reason when studying the kinetics of peroxidase activity in leaves of two *Weigela* cultivars, *W.* 'Van Houtte' and *W. florida* following treatment with SO_2 I have determined it only in the visually uninjured leaf parts. On the other hand, the histochemical localization of peroxidase activity in tissues of *W. florida* leaves was investigated in uninjured, necrotic and boundary tissues.

The measurements of peroxidase activity made following treatment with SO_2 (Table 1) have shown that it increases more in the leaves of the more sensitive cultivar (*W. florida*) than in the less sensitive one (*W.* 'Van Houtte'). This was found to be true for both the studied protein fractions, soluble in phosphate buffer with Triton and ionically bound. Kieliszewska-Rokicka (1979) has found a higher peroxidase activity in the same fractions and in the fraction of peroxidase covalently bound with proteins for the same more SO_2 sensitive cultivar *W. florida* and a lower one for the less sensitive cultivar *W.* 'Van Houtte', following treatment with this gas. She has also found a higher peroxidase activity in needles of Scots pines more sensitive to SO_2 than in less sensitive ones (Kieliszewska-Rokicka 1979). An increase in the activity of this enzyme has been also found following subjection of plants to other gaseous industrial pollutants (Keller and Schwager 1971, Keller 1974).

The action of SO_2 on plants causes the liberation of ethylene in them (Guttenberger et al., 1978, Bressan et al. 1979, Mudd 1979), a hormonal inhibitor the liberation of which is a manifestation of the ageing of the plant (Aharont and Lieberman 1979, Aharont et al. 1979). As Ridge and Osborne (1971) believe ethylene is a factor increasing the cytoplasmic hydroxylation of proline, participating in the enrichment of cell wall proteins with hydroxyproline. Cell walls particularly rich in hydroxyproline are to be found in mature and ageing tissues (Cleland and Karlsnes 1967, Sadava and Chrispeels 1973, Chrispeels et al. 1974). This would explain the increase in hydroxyproline following SO_2 treatment I have observed (Karolewski 1982).

Ridge and Osborne (1971) have observed that simultaneously with an increase in the content of hydroxyproline in cell walls there is a substancial increase in the activity of peroxidases together with the ageing of tissues. Młodzianowski and Młodzianowska (1978) report that in needles of Scots pine treated with SO_2 the peroxidase activity in the endoderm was associated primarily with the cell walls. The detailed investigations conducted by Ridge and Osborne (1971) on the changes in peroxidase activity and content of hydroxyproline, both in the cytoplasmatic fraction and in the cell walls, have shown however that peroxidase is not an enzyme catalyzing the hydroxylation of proline to hydroxyproline. They suggest however that peroxidase may be one of the group of cell wall proteins rich in hydroxyproline. The level of these proteins determines the extensibility of the cell walls and cell growth.

One can suspect that the increase in peroxidase activity observed by me in leaves of plants subjected to SO_2 has caused an increase in the lignification process of cell walls. In the presence or coniferyl alcohol and H_2O_2 ionically or cavalently bound peroxidase in cell wall proteins participates in the lignification process (Whitmore 1976, 1978 after Łobarzewski 1981). In young developing higher plants, where the intensity of lignification is small it is possible to observe low peroxidase activity. As tissues mature lignin forms in walls of parenchyma cells and simultaneously there is an increase in the activity of isoluble forms of peroxidase (Anzai 1975). Studies which have been conducted by Shannon et al. (1971) indicate that there is a synthesis *de novo* of peroxidase under the influence of ethylene, the liberation of which in plants exposed to SO_2 has been demonstrated by Guttenberger et al. (1978), Bressan et al. (1979) and Mudd (1979). Keller and Schwager (1971) have shown that there is an increase in the activity of peroxidase in leaves of some species of trees as a consequence of the physiological ageing of the leaves.

The action of SO_2 causing in leaves an increase in peroxidase activity appears therefore to be a factor inducing the ageing of leaf tissues.

Histochemical determination of the localization of peroxidase activity in necrotic leaves injured by the action of SO_2 on *Weigela florida* has shown a complete inhibition of the activity of this enzyme in dead tissues (Fig. 6). Godzik (1975) studying the distribution of ^{35}S in leaf blades and needles of some tree species has found that the localization and outlines of spots with larger quantities of ^{35}S occur in places where necrosis most readily occurs. In visually uninjured tissues (Fig. 6) the activity observed by me was high and the higher the closer it was to a necrotic spot, maximal activity occurring on the boundary with the necrotic tissue. One can say here about the increase in peroxidase activity causing ageing of tissues all the way to their death. Of special interest is the high peroxidase activity in vascular tissues, even at considerable distance from necrosis. That this is associated with the action of SO_2 is indicated by the studies of Weigl and Ziegler (1962) in which it was shown that there is a considerable accumulation of ^{35}S in sieve tubes of spinach leaf vascular bundles.

Results presented in this study appear to indicate that differences in the degree of sensitivity of plants to SO_2 may be associated with differences in the rate of ageing processes induced by SO_2 action.

The results indicate also that activity of peroxidase in leaves may be one of the indicators useful in the determination of the negative influences of SO_2 on plants. Tolerance to SO_2 would then be determined by the extent of increase in the activity of peroxidase during fumigation with the gas. After termination of fumigation the rate of return of peroxidase activity to the prefumigation level would be a measure of the ability of plants to regenerate losses.

SUMMARY

Under controlled laboratory conditions studies were conducted on the effect of SO_2 on the kinetics of changes and localization of peroxidase activity in leaves of plants from the genus *Weigela*.

The measurements of peroxidase activity made have shown that it increases in the more sensitive species *W. florida* than in the less sensitive cultivar *W.×wagneri* cv. 'Van Houtte'. After termination of the exposition of the plants to the action of SO_2 a decline in the activity of this enzyme in leaves was observed and the return to the prefumigation level was faster in the more tolerant cultivar *W.* 'Van Houtte'. This regularity was common to both the protein fractions — the soluble one in phosphate buffer with Triton and the ionically bound one.

A histochemical study of the activity of peroxidase in leaves of *W. florida* plants subjected to SO_2 treatment indicated that there is no activity in necrotic tissue. In tissues visually unaffected the activity was high and the higher the closer it was to necrotic tissue.

The obtained results suggest that changes in the activity of peroxidase in leaves of plants may be used as indicators useful for the determination of the degree of injury and ability to regenerate losses following fumigation with SO_2. They show also that differences in plant tolerance to SO_2 may also be the result of differences in the rate of ageing processes in plants induced by the action of this gas.

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Wpływ dwutlenku siarki na aktywność peroksydazy w liściach sadzonek rodzaju *Weigela*

Streszczenie

W kontrolowanych warunkach laboratoryjnych przeprowadzono badania wpływu SO_2 na kinetykę zmian i lokalizację aktywności peroksydazy w liściach sadzonek z rodzaju *Weigela*

Przeprowadzone pomiary zmian aktywności peroksydazy wskazywały na jej większy wzrost w czasie działania gazu u bardziej wrażliwego gatunku *W. florida* niż u sadzonek mniej wrażliwego kultywaru W.' Van Houtte'. Po przerywaniu ekspozycji sadzonek na działanie SO_2 obniżenie aktywności tego enzymu do poziomu kontroli, charakteryzujące proces ich regeneracji, następowało szybciej w liściach sadzonek bardziej tolerancyjnego kultywaru *W.* 'Van Houtte'. Prawidłowości te były zgodne w obydwu badanych frakcjach białkowych: białek rozpuszczalnych w buforze fosforanowym z Tritonem i jonowo związanych białek.

Histochemiczne określenie lokalizacji aktywności peroksydazy w liściach sadzonek z gatunku *W. florida* poddanych działaniu SO_2 wskazało na całkowitą inaktywację tego enzymu w nekrozach. W tkankach wizualnie nieuszkodzonych aktywność była duża i tym większa im tkanki te były bliżej nekrozy.

Otrzymane wyniki sugerują, że zmiany aktywności peroksydazy w liściach roślin mogą być stosowane jako wskaźniki przydatne do określania stopnia uszkodzenia i procesu regeneracji roślin po działaniu SO_2. Wskazują one również, że różnice w tolerancji roślin na SO_2 mogą być wynikiem różnic w intensywności przebiegu procesów starzenia się roślin, indukowanych działaniem tego gazu.

Влияние сернистого ангидрида на активность пероксидазы в листьях саженцев с рода *Weigela*

Резюме

В контролируемых лабораторных условиях проводили исследования влияния SO_2 на кинетику изменений и локализацию активности пероксидазы в листьях саженцев рода *Weigela*.

Проведенные измерения активности пероксидазы указывают на ее более высокий уровень во время действия газа у более чувствительного вида *W. florida*, чем у менее чувствительной разновидности *W.* 'Van Houtte'. После окончания экспозиции саженцев на SO_2 наблюдали уменьшение активности этого фермента по сравнению с контролем, характеризующее процесс регенерации. Этот процесс проходил быстрее в листьях более толерантной разновидности *W.* 'Van Houtte'. Эти закономерности были схожими

для обеих исследуемых белковых фракций: белков растворимых в фосфатном буферном растворе с тритоном и белках с ионной связью.

Гистологическое определение локализации активности пероксидазы в листьях саженцев вида *W. florida* подвергнутых воздействию SO_2 указывает на полную инактивацию этого фермента в некрозах. В тканях визуально неповрежденных активность была большая и увеличивалась по мере приближения к некрозе.

Полученные результаты указывают на то, что изменения активности пероксидазы в листьях растений могут применяться в качестве индикаторов пригодных для определения степени повреждения и процесса регенерации растений после воздействия на них SO_2. Они также указывают, что причиной различий в толерантности растений на SO_2 могут быть вызванные воздействием этого газа различия в процессе старения.