AN EVALUATION OF HIGH RESISTANCE IN POLYGONUM CUSPIDATUM TO SULFUR DIOXIDE (SO₂)

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イタドリの二酸化イオウ（SO₂）に対する高い抵抗性について

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Synopsis


The effects of sulfur dioxide (SO₂) on the photosynthesis for Polygonum cuspidatum propagated from shoots sampled near a copper mine at Asio, Tochigi Pref., were compared with those of Helianthus annuus, known as a sensitive plant to SO₂. The percentage inhibition of net photosynthesis and leaf conductance was plotted against the calculated SO₂ absorption rate. The threshold value of SO₂ absorption to photosynthetic inhibition in P. cuspidatum was larger than that in H. annuus. And the photosynthetic inhibition per unit SO₂ absorption rate in P. cuspidatum was smaller than in H. annuus. Furthermore, we studied the effects of SO₂ on the CO₂ concentration in the substomatal cavity. The CO₂ concentration in P. cuspidatum did not increase, but that in H. annuus did. From these data and the change of the extent of inhibition of photosynthesis and transpiration, the photosynthetic decline in P. cuspidatum exposed to SO₂ was primarily due to stomatal closure of the leaf. It was concluded that photosynthetic activity of P. cuspidatum was tolerant to SO₂ firstly because of the small SO₂ absorption rate by leaves resulting from the small leaf conductance, and secondly because of the high resistance to SO₂ of biochemical processes in the photosynthetic pathway.

Introduction

In recent years, sulfur dioxide (SO₂) has attracted attention as a gaseous air pollutant which may cause chronic environmental stress on vegetation growing in urban districts. Several workers reported that long-term exposure to SO₂ could inhibit plant growth and alter the species composition of plant communities in SO₂ polluted areas (ARCHIBOLD, 1978; ASAIO, 1952; FREEDMAN & HUTCHINSON, 1980; GORDON & GORHAM, 1963; HIROI, 1974; HORSMAN et al., 1979; USUI et al., 1975; WAGNER et al., 1978; WINNER & MOONEY, 1980; WOOD & NASH, 1976; YOSHIOKA, 1975).

USUI et al. (1975) reported that in the smoke polluted area along the leeward of the copper mine at Asio, Tochigi Pref., a large section of deciduous broad-leaved forest was almost completely destroyed by fire in 1891, and that the young sprouts which grew afterwards as a secondary succession were probably damaged by the constant attack of SO₂ emitted from the smelter. They also suggested that air pollution by SO₂ might induce a primary dominance of Polygonum cuspidatum population in this area. Likewise, HIROI (1974) also reported that P. cuspidatum was one of the dominant species of herbaceous communities established in copper mine districts such as Asio, Tochigi Pref. and Besshi, Ehime Pref. On the other hand, YOSHIOKA (1975) reported that P. cuspidatum is one of the dominant species of the natural vegetation found at volcanoes such as Mt. Aso, Kumamoto.
Pref. and Sakurajima Island, Kagoshima Pref. in Kyushu. It has been reported that fumarolic gases from Mt. Nakadake (Volcano Aso) contained a relatively high SO$_2$ content of 2.8–12.4% (Iwaki et al. 1962).

The reason for the establishment of peculiar vegetation in SO$_2$ polluted areas may be the difference in resistance to SO$_2$ of the plant species. There are many reports (cf. Japan Society of Air Pollution, 1982) about the responses of plants to SO$_2$, but few about the photosynthetic characteristics of native plants surviving in smoke polluted areas. Therefore, it remains unclear why *P. cuspidatum* populations can survive dominatingly in SO$_2$ polluted areas.

In order to know the response to SO$_2$ of native plants surviving in smoke polluted areas, the effects of SO$_2$ were studied on the leaf photosynthesis of *P. cuspidatum* propagated from shoots collected near the copper mine at Asio, Tochigi Pref. The photosynthetic response was compared with that of *Helianthus annuus* cv. Russian Mammoth, which is known as a sensitive plant to SO$_2$.

**Materials and Methods**

Shoots of *P. cuspidatum* were collected in autumn in the smoke polluted area on the leeward of the copper mine at Asio, Tochigi Pref., about 110 km north-northwest from Tokyo. The shoots were cut off to a length of 5–10 cm. The base of the cut shoots was soaked and rooted in a tray containing water for one month. The rooted plants were transplanted in plastic pots filled with an artificial culture medium composed of peat moss, vermiculite, perlite, fine gravel and Akadamatsuchi (granulated loam) (2:2:1:1:2 on a v/v basis). The plants were grown in an air-conditioned greenhouse at 25°C and 75% R.H. for one year. Seeds of *H. annuus* were sown in 1/5000 a. plastic pots filled with culture medium composed of peat moss, vermiculite, perlite and fine gravel (2:2:1:1 on a v/v basis), and one plant per pot was grown for 4 to 5 weeks in the greenhouse.

The attached mature leaves of the plants were placed in an acrylic assimilation chamber which was 30 cm long, 17.5 cm wide and 2 cm deep. The photosynthetic and transpiration rates of the plant leaves were measured. The conditions in the chamber were regulated to keep 25–27°C leaf temperature, 40–50% R.H. and 64 klx of light intensity at the upper surface of the leaf. The CO$_2$ concentration in the air passing through the chamber was controlled to maintain 341–360 ppm by mixing CO$_2$-free air with a given volume of 4.88% CO$_2$ supplied by a cylinder. CO$_2$-free air was prepared by passing ambient air through tubes filled with soda lime. After the addition of CO$_2$ to the air stream, the water content of the air entering the chamber was controlled by passing it through a humidifier and chilling it with a coiled glass tube placed in the water bath. Water temperature in the bath was controlled using a thermoregulator with an accuracy of ±0.5°C. In order to control SO$_2$ concentration in the air, SO$_2$ from a cylinder was injected through a thermal mass-flow controller into the air stream and mixed with the air by passing it through a 5 m long teflon tube before it entered the chamber. The rate of air flow entering the assimilation chamber was maintained 15 l/min. The average wind velocity across the transverse section of the chamber was 71 mm s$^{-1}$. The concentration of SO$_2$ and CO$_2$ in the air entering and leaving the chamber was measured alternately for 2 min using solenoid valves. CO$_2$ concentration was measured by an infrared gas analyzer (Shimazu Seisakusho Co., Model URA-28), and SO$_2$ concentration was monitored by a flame photometric detector of SO$_2$ (Bendix, Model 830). Leaf temperature was measured by three copper-constantan thermocouples (0.1 mm) attached to three different positions on the undersurface of the leaves. Light was supplied by four 500 W incandescent lamps suspended above the chamber. A water layer of about 10 cm in depth was poured between the lamps and the chamber to filter infrared radiation, and a semitransparent film made of vinyl chloride was used to obtain uniform distribution of light intensity. After the fumigation treatment, leaf area was measured by an automatic area meter (Hayashi Denkoh Co., Ltd., Model AAM-7). The rates of transpiration and photosynthesis were evaluated from the differences in dew point and CO$_2$ concentration of the air at the inlet and outlet of the chamber, respectively. Dew point of the air was measured by two digital humidity analyzers set at the inlet and outlet of the chamber (EG & G, Model 911). Leaf boundary layer resistance to water vapor transfer ($r_b$) in the chamber was obtained by measurements of leaf replicas made of wet blotting.
paper. Leaf conductance to water vapor \(1/(r_a+r_s)\), \(r_s\): stomatal resistance] was calculated with reference to the methods reported by KOH (1981) and by FURUKAWA et al. (1980).

Results

Leaves of *P. cuspidatum* or *H. annuus* were fumigated for 64 min at 1.70 and 0.74 ppm SO\(_2\), respectively. Fig. 1 shows a typical time course response of net photosynthesis and leaf conductance \(1/(r_a+r_s)\) to SO\(_2\) fumigation for both species. Initial rates of net photosynthesis prior to SO\(_2\) treatments were 22.6 mg CO\(_2\)dm\(^{-2}\)hr\(^{-1}\) and 35.5 mg CO\(_2\)dm\(^{-2}\)hr\(^{-1}\) in *P. cuspidatum* and *H. annuus*, respectively. SO\(_2\) fumigation for 60 min resulted in a decline in photosynthesis for both species. The decrease of leaf conductance was in parallel with photosynthesis in *P. cuspidatum* during SO\(_2\) fumigation. But the decrease of leaf conductance in *H. annuus* was slight as compared with that of photosynthesis.

Fig. 2 shows a time trend of net photosynthesis (relative value to that prior to SO\(_2\) fumigation) to SO\(_2\) fumigation for different concentrations. Photosynthesis of *P. cuspidatum* decreased only to 95.6\% at 0.80 ppm SO\(_2\) and to 74.4\% at 1.70 ppm SO\(_2\) at 60 min after the start of the fumigation. On the other hand, photosynthesis of *H. annuus* decreased to 91.5\% at 0.22 ppm SO\(_2\) and to as low as 27.5\% at 0.74 ppm SO\(_2\).

In order to compare the relative sensitivity to SO\(_2\) of the two species, the percentage inhibition of net photosynthesis and leaf conductance \([1-(\text{initial value}/\text{relevant value})\times100]\) determined at 60 min after the initiation of fumigation was plotted against the SO\(_2\) concentration (Fig. 3). The degree of photosynthetic decline increased with increase of the SO\(_2\) concentration. Then, we considered that SO\(_2\) resistance of photosynthesis could be defined 1) by the threshold concentration of SO\(_2\) to induce inhibition of photosynthesis, 2) by the photosynthetic inhibition rate at a unit SO\(_2\) concentration (the slope of the regression line in Fig. 3). The threshold concentra-
The Ecological Society of Japan

Vol. 34, No. 2

Fig. 2. Effects of various concentrations of SO$_2$ on net photosynthesis (Relative Net P.) SO$_2$ concentrations fumigated to P. cuspidatum were 0.80 ppm (○), 1.09 ppm (●), 1.50 ppm (■) and 1.70 ppm (●), and those fumigated to H. annuus were 0.22 ppm (○), 0.37 ppm (●), 0.54 ppm (■) and 0.74 ppm (●). Relative net photosynthesis was expressed as the relative value to the initial value before fumigation.

Fig. 3. Relation between inhibition of net photosynthesis (Inhibition of Net P., circles), leaf conductance (triangles) and SO$_2$ concentrations for P. cuspidatum (closed symbols) and H. annuus (open symbols). The lines in the figure indicate the regression line between inhibition of net photosynthesis and SO$_2$ concentration for H. annuus (- - - -) and P. cuspidatum (-----). Inhibition of net photosynthesis and inhibition of leaf conductance were expressed as the percentage inhibition [(1—relevant value/initial value)×100].

The slope of the regression line in P. cuspidatum was gentler than in H. annuus.

It was considered that the threshold concentration and the slope can be determined by the SO$_2$ absorption rate, SO$_2$ detoxication ability and the susceptibility of the photosynthetic sites attacked by SO$_2$. In order to minimize factors which controlled the threshold concentration and the slope of the regression line in Fig. 3, the percentage inhibition of net photosynthesis and leaf conductance [(1—relevant value/initial value)×100] at 60 min after the fumigation treatment was started, were plotted against the calculated SO$_2$ absorption rate, as shown in Fig. 4. The rate of SO$_2$ absorption in leaves of both species was calculated according to the method reported by OMASA & ABO (1978) on the basis of boundary layer resistance and stomatal resistance under the assumption that SO$_2$ concentration in the substomatal cavity was 0 ppm SO$_2$. This value was ascertained by OMASA & ABO (1978) for H. annuus. The threshold value of SO$_2$ absorption to photosynthetic inhibition in P. cuspidatum was larger than in H. annuus. On the other hand, the slope of the regression line between the photosynthetic inhibition and SO$_2$ absorption rates in the former plant was gentler than in the latter plant. Moreover, the decrease of leaf conductance of P. cuspi-
Fig. 4. Relation between inhibition of net photosynthesis (circles), leaf conductance (triangles) and calculated SO$_2$ absorption rate for _P. cuspidatum_ (closed symbols) and _H. annuus_ (open symbols). The lines in the figure indicate regression lines between the calculated SO$_2$ absorption rate and the inhibition of net photosynthesis for _P. cuspidatum_ (—) and _H. annuus_ (- - - - -). Inhibition of net photosynthesis and inhibition of leaf conductance were expressed as the percentage inhibition [($1 - \text{relevant value/initial value}$) $\times 100$].

datum_ was coincident with that of photosynthesis. These results suggested that photosynthetic inhibition of _P. cuspidatum_ was mainly due to stomatal closure, and that of _H. annuus_ was mainly due to a non-stomatal process, probably the biochemical photosynthetic process.

In order to confirm the speculation mentioned above, the change of CO$_2$ concentration in the substomatal cavity (Ci) was examined using the following equation: $\text{Ci} = \text{Ca} - k (1.37r_a + 1.54r_s) P$, where Ca is CO$_2$ concentration in ambient air (ppm), $P$ is net photosynthetic rate (mg CO$_2$dm$^{-2}$ hr$^{-1}$) and $k$ is a constant (1.544 at 25°C in air at the flow meter). Table 1 shows the effects of SO$_2$ on the CO$_2$ concentration in the substomatal cavity of _P. cuspidatum_ and _H. annuus_. As suggested in Fig. 4, where the inhibition of net photosynthesis in _P. cuspidatum_ mainly depended on stomatal closure, the CO$_2$ concentration in the substomatal cavity of _P. cuspidatum_ did not increase by SO$_2$ fumigation. On the other hand, the value in _H. annuus_ increased.

Fig. 5 shows the measurements of leaf conductance of _P. cuspidatum_ and _H. annuus_ taken prior to the fumigation and at 60 min after the start of fumigation. Leaf conductance of _P. cuspidatum_ was smaller than that of _H. annuus_ in both measurements.

**Discussion**

Several workers (Asai, 1952; HIROI, 1974; USUI et al., 1975) have reported that _P. cuspidatum_ is one of the dominant species in SO$_2$ polluted areas, but causal analysis of the characteristic distribution of _P. cuspidatum_ has not been performed.

Inter- or intraspecific variability in SO$_2$ resistance can be expressed by several indicators. Photosynthesis was a useful indicator for defining SO$_2$ resistance because of its high sensitivity to SO$_2$. But the factors related to the resistance in

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**Table 1. Effects of SO$_2$ fumigations for 60 min on CO$_2$ concentration in the substomatal cavity. Values were expressed as the percentage of those before SO$_2$ fumigation.**

<table>
<thead>
<tr>
<th></th>
<th>Fumigated SO$_2$ conc.</th>
<th>Fumigated CO$_2$ conc. in the substomatal cavity (ppm)</th>
<th>%</th>
<th>Fumigated SO$_2$ conc.</th>
<th>Fumigated CO$_2$ conc. in the substomatal cavity (ppm)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. annuus</em></td>
<td>0.22</td>
<td>104</td>
<td>0.80</td>
<td>106</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.37</td>
<td>129</td>
<td>1.09</td>
<td>96.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.54</td>
<td>140</td>
<td>1.50</td>
<td>96.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.74</td>
<td>159</td>
<td>1.70</td>
<td>85.5</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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Fig. 5. Effects of SO$_2$ on leaf conductance for _P. cuspidatum_ (closed symbols) and _H. annuus_ (open symbols). Circles show the initial value before fumigation. Triangles show the values at 60 min after initiation of fumigation.
terms of photosynthesis have not been discussed in detail. We compared the SO$_2$ resistance in terms of photosynthesis in *P. cuspidatum* and *H. annuus*, which are known to be sensitive to SO$_2$, in order to clarify the affecting factors concerned.

As shown in Fig. 3, the photosynthetic inhibition of *P. cuspidatum* was plotted against SO$_2$ concentration. Also, the photosynthetic inhibition was plotted against the SO$_2$ absorption rate (Fig. 4). It was deduced that the threshold value of SO$_2$ absorption rate to photosynthetic inhibition might be related to SO$_2$ detoxication ability and the susceptibility of the photosynthetic sites attacked by SO$_2$. The slope of the regression line in Fig. 4 was considered to be an expression of the susceptibility of the photosynthetic sites attacked by SO$_2$ and of the formation rate of toxic substances derived from SO$_2$. As shown in Fig. 4, the threshold value of SO$_2$ absorption rate to photosynthetic inhibition in *P. cuspidatum* was larger than in *H. annuus*, and the slope of the regression line was gentler than that of *H. annuus*. Su & Swanson (1974) speculated that stomatal closure could not account for the reduction of photosynthetic rate caused by SO$_2$ exposure in Pinto beans from the results obtained by the simultaneous measurements of photosynthesis and transpiration. Winner & Mooney (1980a, b) reported that when SO$_2$ absorption is 5 $\mu$g SO$_2$ cm$^{-2}$ hr$^{-1}$ or less (0.174 ng SO$_2$ cm$^{-2}$ s$^{-1}$), photosynthetic inhibition for *Diplacus aurantiacus* and *Heteromeles arbutifolia* was due to entirely stomatal closure, and that when SO$_2$ absorption was as high as 15 $\mu$g SO$_2$ cm$^{-2}$ hr$^{-1}$, photosynthetic inhibition for both species was due to non-stomatal factors. As shown in Table 1 and Fig. 4, the present results showed that the decrease of photosynthesis in *H. annuus* was not in parallel with that of leaf conductance during SO$_2$ fumigation, and that CO$_2$ concentration in the substomatal cavity increased. Rashke (1975) reported that DCMU fed to leaves through the transpiration stream caused inhibition of photosynthesis in the mesophyll, and subsequently, an increase in intercellular CO$_2$ concentration. Consequently, it may be suggested that the biochemical photosynthetic process (non-stomatal process) of *H. annuus* was inhibited by SO$_2$ fumigation, as reported by some workers (Furukawa et al., 1980; Ohshima et al., 1973). But the decline of the photosynthetic rate of *P. cuspidatum* was in parallel with that of leaf conductance, and CO$_2$ concentration in the substomatal cavity did not increase. Therefore, the photosynthetic decline was thought to be due primarily to stomatal closure, and photosynthesis was apparently not limited by the biochemical photosynthetic process. From these data, we considered that one of the reasons for the difference between both plants in the threshold value of SO$_2$ absorption rate and that of the slope of the regression line between the SO$_2$ absorption rate and photosynthetic inhibition might be the difference in the susceptibility of the photosynthetic sites which were attacked by SO$_2$.

Considering the assumption that SO$_2$ concentration in the substomatal cavity is 0 ppm, as discussed by several workers (Black & Unsworth, 1979; Omasa & Abo, 1978; Winner & Mooney, 1980a), it can be said that smaller stomatal conductance resulted in smaller SO$_2$ absorption by the leaves. As shown in Fig. 5, the leaf conductance of *P. cuspidatum* was smaller than that of *H. annuus* before and during the fumigation. Therefore, it was considered that the absorption rate of SO$_2$ in *P. cuspidatum* was innately smaller than that in *H. annuus* under the same SO$_2$ concentration.

In conclusion, we summarized the factors affecting SO$_2$ resistance defined by photosynthesis for both plants in Table 2, and postulated that one of the reasons why *P. cuspidatum* could survive in a smoke polluted area was due to the tolerance of its photosynthetic activity to SO$_2$ fumigation because of the small stomatal conductance and the higher resistance of biochemical processes in the photosynthetic pathway, and probably because of its high SO$_2$ detoxication ability.

<table>
<thead>
<tr>
<th>Factors affecting SO$_2$ resistance in terms of photosynthesis</th>
<th><em>H. annuus</em></th>
<th><em>P. cuspidatum</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>SO$_2$ absorption rate</td>
<td>Larger</td>
<td>Smaller</td>
</tr>
<tr>
<td>Threshold value in Fig. 4</td>
<td>Smaller</td>
<td>Larger</td>
</tr>
<tr>
<td>Slope of the regression line in Fig. 4</td>
<td>Larger</td>
<td>Gentle</td>
</tr>
<tr>
<td>Resistance to SO$_2$</td>
<td>Sensitive</td>
<td>Tolerant</td>
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</tbody>
</table>
References


摘 要

1）足尾煙火地より採取し栄養繁殖させたイタドリと二酸化イオウ（SO\textsubscript{2}）に対して感受性が高いとされているヒマツリについて、SO\textsubscript{2}暴露下での光合成反応を比較検討した。

2）気相コンダクタンス [1/(r\textsubscript{s}+r\textsubscript{a})] より推定したSO\textsubscript{2}吸収速度に対して両種の光合成阻害度を図示した結果、イタドリのほうがヒマツリより光合成阻害に対するSO\textsubscript{2}吸収速度の閾値は大きく、単位SO\textsubscript{2}吸収速度当りの光合成阻害度は小さかった。

3）SO\textsubscript{2}暴露による気孔腔内のCO\textsubscript{2}濃度は、イタドリでは増加しないが、ヒマツリでは顕著に増加した。この結果およびSO\textsubscript{2}暴露時の気孔閉鎖および光合成阻害度の変化の結果より、イタドリの光合成阻害は主に気孔閉鎖であることが暗示された。

4）したがって、イタドリが煙火地に生育できる要因の一部は、遺伝的に気相コンダクタンスが小さく、SO\textsubscript{2}が葉内に浸入しにくく、さらに、生理生化学的な光合成過程のSO\textsubscript{2}に対する抵抗性が強いことであると結論される。