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EFFECTS OF AIR POLLUTION ON TANNIN BIOSYNTHESIS AND PREDATION DAMAGE IN *CRYPTOMERIA JAPONICA*

TERUTAKA KATOH, MINORU KASUYA, SADANOBU KAGAMIMORI, HIROSHI KOZUKA* and SHOICHI KAWANO†

Faculty of Medicine, Toyama Medical and Pharmaceutical University, Toyama 930-01, Japan; *Faculty of Pharmaceutical Sciences, Toyama Medical and Pharmaceutical University, Toyama 930-01, Japan; †Faculty of Science, Kyoto University, Kyoto 606, Japan

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Key Word Index—*Cryptomeria japonica*; Taxodiaceae; Japanese Cedar; *Castanopsis cuspidata*; Fagaceae; tannins; herbivory; chemical ecology; shikimate pathway; steam power station; air pollution.

Abstract—Decreased levels of foliar tannin was observed with Japanese Cedars growing in the surroundings of a steam power station. Tannin content of the leaves was negatively corrrelated with the levels of foliar soluble sulphate, and a causal association was suggested between air pollution and inhibition of the shikimate pathway. Preliminary observation on predation damage of the Japanese Cedars indicates that increased feeding rate by larvae of a herbivorous moth, *Dasychira abietis argentata*, was associated with low foliar tannin content and the vicinity of the sampling sites to the power station. Considering the physiological function of tannins, e.g. as a defensive factor against insect predation and fungal degradation, it seems that decrease of foliar tannin levels of Japanese Cedars in the polluted areas has relevance to their high susceptibility to air pollution in field conditions.

INTRODUCTION

It has been revealed that most of the sulphur oxides absorbed from stomata do not enter into normal sulphur metabolism and the leaves accumulate them as soluble sulphate. Therefore, foliar soluble sulphate may be a good indicator for the levels of sulphur oxides in ambient air [1, 2]. As an index of air pollution, the authors determined foliar soluble sulphate of Japanese Cedars (*Cryptomeria japonica* D. Don) which grow in the areas surrounding a steam power station [3] (Fig. 1). Tannins were removed from extracts prior to the determination of sulphate, because they form a precipitate with gelatin solution during the turbidimetry procedure and interfere with the determination. It was noted in our preliminary survey that the amounts of precipitated foliar tannins varied over a wide range, i.e. higher levels of tannins were generally detected in leaves collected from control areas, whereas very low levels of tannin precipitates were observed in those collected from polluted areas. This result led us to conduct further critical studies reported in this paper.

Tannins have in common the features of astringency and chemical reducing power. Their biological role is not fully elucidated but protective functions against insect predation, fungal degradation and oxidative environments have been proposed [4, 5]. Therefore, if tannin content in the foliage is reduced, plants may be readily damaged and lose their vigour due to intensive insect predation, fungal degradation, chemical weathering and/or air pollution.

Tannins are classified into two groups according to their chemical characteristics, i.e. hydrolysable tannins and condensed tannins. Woody angiosperms may contain both hydrolysable and condensed tannins but gymnosperms have only condensed tannins [6]. Both categories of tannins are synthesized in foliage from glucose

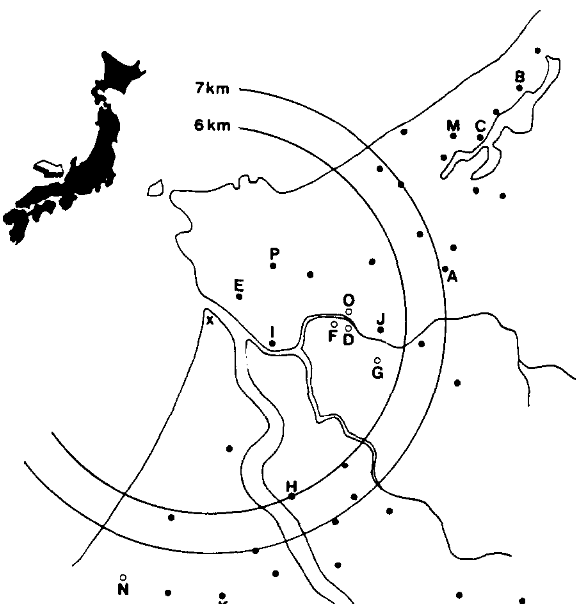

Fig. 1. Study area and sampling sites of leaves. The leaves of Japanese Cedars were collected from 47 trees at 37 sites (●) in June, 1976. Seasonal changes in the levels of foliar tannins were studied for current-year leaves of Japanese Cedars at 10 sites (A–J), in 1977. Predation damage of Japanese Cedar leaves by larvae of a herbivorous moth was also estimated at 10 sites (A–C, E–H, and J–L), in November, 1977. Relative composition of foliar tannins of an evergreen oak tree, *Castanopsis cuspidata*, was determined at 4 sites (M–P), in September, 1977.

via the shikimate pathway [7, 8], but in the case of hydrolysable tannins the importance of an alternative short-cut pathway, via 3-dehydro-shikimic acid, was also revealed [9]. Therefore, if the relative composition of foliar tannins is studied for angiosperms, the site of the inhibition of tannin synthesis may be suggested.

This paper presents data on the inhibition of foliar tannin synthesis of Japanese Cedars in association with slight but continuous air pollution. Foliar tannin composition of an oak tree, *Castanopsis cuspidata*, was also analysed to identify the inhibition mechanisms. A host–parasite interaction, i.e. predation damage of Japanese Cedar leaves by larvae of a herbivorous moth, was also discussed with a special reference to air pollution and foliar tannin content.

RESULTS

Relationship between atmospheric sulphur oxides and the levels of foliar soluble sulphate of Japanese Cedars

To clarify the relationship between air pollution and the levels of foliar sulphate, the levels of foliar soluble sulphate of Japanese Cedars were plotted against the atmospheric sulphur oxides concentration determined by the PbO_2 method. A statistically significant relationship ($p<0.05$) was observed between average atmospheric sulphur oxides and soluble sulphate content of one-year-old leaves collected in July, 1976 (Fig. 2). This is in agreement with those reported in previous study [2].

Foliar soluble sulphate and tannin contents of Japanese Cedars

A negative correlation ($r=-0.48$, $p<0.001$) was observed between foliar soluble sulphate and tannins of one-year-old leaves of Japanese Cedars collected in June, 1976 (Fig. 3). Two-year-old leaves showed a similar result, although the total amount of tannin was considerably higher than one-year-old leaves. Foliar tannin content of the very young current-year leaves (only *ca* one month after bud-burst) exhibited more or less similar low values, ranging from 10 to 30 μmol/g, regardless of soluble sulphate levels (Fig. 4).

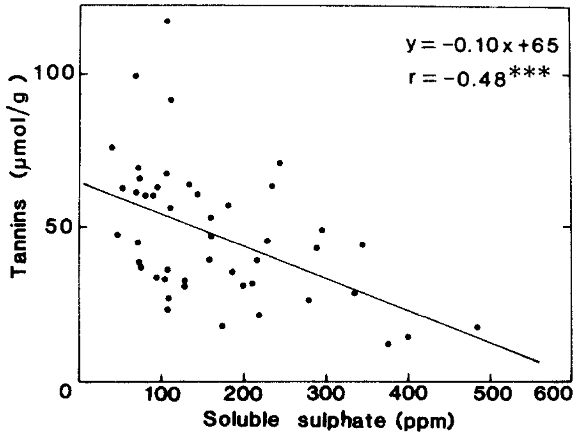

Fig. 3. Correlation between soluble sulphate and tannins of one-year-old leaves of Japanese Cedars collected from 47 trees at 37 sites in June, 1976. Tannins were determined by ferrous tartrate method, using gallic acid as standard. ***, $p<0.001$.

By dividing the sampling sites shown in Fig. 3 into two groups according to the distance from the power station, a significant difference for foliar tannin levels was observed between two groups. The mean foliar tannin level of control area, i.e. in a range of 7–13 km radius from the power station was 51.2 μmol/g, whereas a much lower mean value of 34.0 μmol/g was obtained in those which were collected from the polluted area, i.e. within a 6 km radius (Table 1).

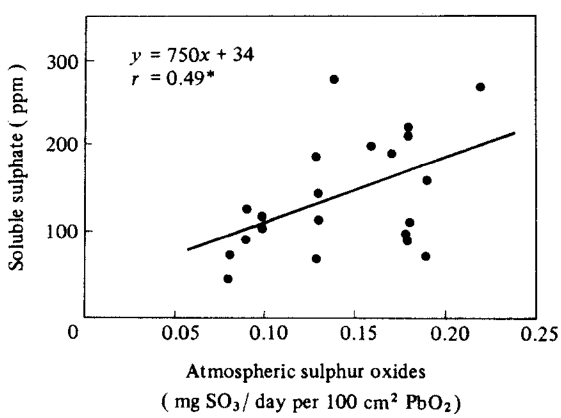

Fig. 2. Relationship between atmospheric sulphur oxides and the levels of foliar soluble sulphate of Japanese Cedars collected in June, 1976. Atmospheric sulphur oxides has been monitored by Fukui Prefecture at 12 sites in the study area. The levels of soluble sulphate of one-year-old leaves of Japanese Cedars growing within 1.5 km radius from these monitoring sites were plotted against average atmospheric sulphur oxides concentration during their growing periods (from May 1975 to May 1976). The levels of soluble sulphate was indicated as sulphur. *, $p<0.05$.

Seasonal changes in foliar tannin content of Japanese Cedar

To study the accumulation profile, foliar tannin content of the Japanese Cedars was determined in June, September and November 1977. The current-year leaves collected from the control area showed a continuous increase in tannin content from June (9.1–13.1 μmol/g) to November (47.5–54.1 μmol/g) [Fig. 5(a)]. On the other

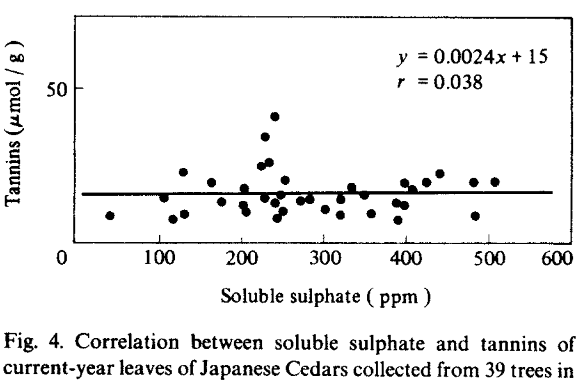

Fig. 4. Correlation between soluble sulphate and tannins of current-year leaves of Japanese Cedars collected from 39 trees in June, 1976.

Table 1. Foliar tannin levels of one-year-old leaves collected from control area and polluted area.

Sampling area	Mean [μmol/g]	s.e. [μmol/g]	*N*
Control area	51.2	4.2	26
Polluted area	34.0	8.3	9

$t = 2.04$ ($p < 0.05$).

hand, those from the polluted area exhibited lower rates of increase. The amount of foliar tannins from the polluted area attained only 30 μmol/g in November, with the exception of one sampling location [Fig. 5(b)].

The levels and relative composition of foliar tannins of Castanopsis

In contrast with the case of the Japanese Cedar, *Castanopsis* showed high tannin content and no significant relationship was observed between foliar levels of soluble sulphate and tannins (Fig. 6). However, fractional determination of foliar tannins (Fig. 7) suggested that *Castanopsis* leaves collected from polluted areas were characterized by low levels of condensed tannin. Scarcely any difference in hydrolysable tannin content was observed between the leaves collected from the control area and those from the polluted area (Fig. 7). In the case of condensed tannins relatively low levels, compared with the control area, were observed in the polluted area.

Relationships between foliar tannin content and predation damage

High values of the predation index of the Japanese Cedar were observed in sites H and J (Table 2). In these sites, defoliation was estimated to be up to about 3–4%. A comparative study was carried out to detect any relationship between the distance from the power station and the predation profile. Japanese Cedars growing in the area within 6 km radius from the power station suffered serious predation damage compared with those grown in the control area ($p < 0.001$) (Table 3).

Predation profiles of Japanese Cedar leaves were also compared with regard to the levels of foliar tannins. Distributions of the predation grades for six Japanese Cedars with low tannin content (less than 30 μmol/g) and four Japanese Cedars with high tannin content (more than 30 μmol/g) are shown in Table 4. This result suggests that predation damage is negatively correlated with levels of foliar tannins ($p < 0.01$).

Relationships between foliar nitrogen content and predation damage

The foliar nitrogen levels of the samples from both control and polluted areas (Table 2) were examined by Mann–Whitney U test, but no significant distinction was detected between the two groups ($p = 0.274$). Furthermore, even if the leaves were classified into two groups according to nitrogen content, i.e. N-rich (above 1.5%) and N-poor (below 1.5%) leaves, again no distinction was

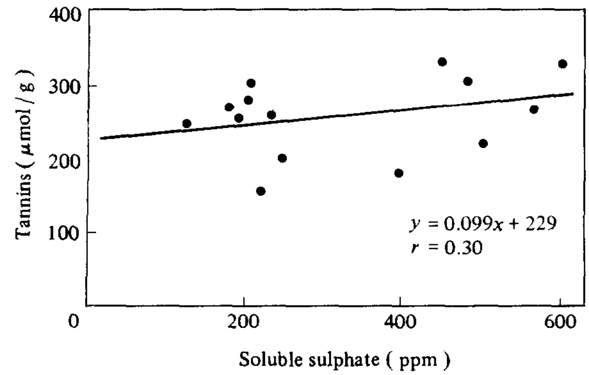

Fig. 6. Correlation between soluble sulphate and tannins of *Castanopsis* leaves collected from 14 trees in September, 1977.

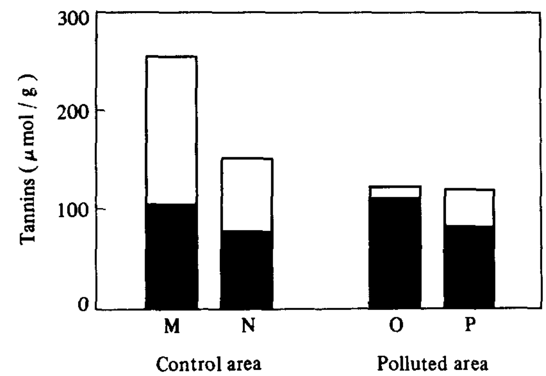

Fig. 7. Relative composition of hydrolysable and condensed tannins of *Castanopsis* leaves. Fractional determination of tannins was carried out by the method of Marigo [34], with some modifications and the Folin–Ciocalteu reagent was used to determine the fractions. Open and closed rectangles represent condensed and hydrolysable tannins, respectively. M–P indicate sampling sites.

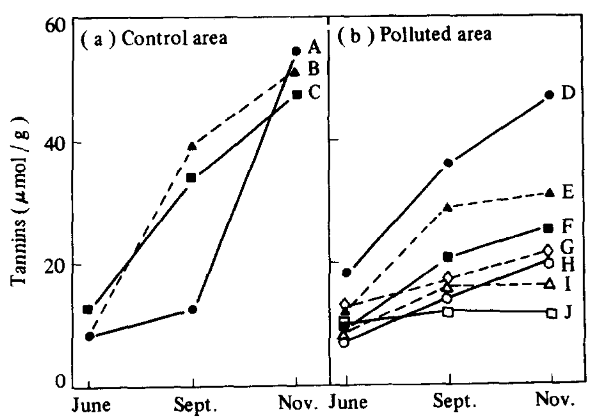

Fig. 5. Seasonal changes in foliar tannin content of Japanese Cedars (current-year leaves). The current-year leaves of Japanese Cedars were collected from marked individual trees from June to November, 1977, at 10 sampling sites (A–J). Fig. 5(a) and Fig. 5(b) exhibit the results in the control area (in a range of 7–13 km radius from the power station) and the polluted area (within a 6 km radius), respectively.

Table 2. Predation profile and tannin content of the Japanese Cedars

Sampling site*	Distance† [km]	Degree of predation‡ (−)	(±)	(+)	(++)	Predation index‡	Tannin content [μmol/g]	Nitrogen content [%]
E	1.2	303	8	10	0	0.75	30.8	1.55
F	3.7	203	6	10	1	1.26	24.4	1.39
G	5.2	262	3	13	3	1.42	21.3	1.58
J	5.2	140	18	18	2	2.97	11.2	1.44
H	6.0	140	19	24	4	3.91	20.6	1.65
A	7.2	272	5	10	0	0.79	54.1	1.64
K	8.7	285	3	2	0	0.19	19.2§	1.51
C	9.2	203	4	11	0	1.11	47.5	1.45
L	10.3	386	2	7	1	0.51	15.5	1.41
B	11.5	249	6	7	1	0.82	51.7	1.32

*Sampling sites E, F, G, J and H are included in the polluted area and A, K, C, L and B are included in the control area.

†Distance from the power station to each sampling site.

‡See Experimental.

§This sample was taken from shady site of the plantation. It is known that light intensity influences the levels of condensed tannins, i.e. the foliage of trees in the shaded site is usually lower than those from the exposed site [10].

Table 3. Distance from the power station and predation profile

Distance*	Degree of predation† (−)	(±)	(+)	(++)
0–6 km (5 sites)‡	1048 (88.3%)	54 (4.5%)	75 (6.3%)	10 (0.8%)
7 km– (5 sites)§	1395 (95.9%)	20 (1.4%)	37 (2.5%)	2 (0.1%)

*Distance from the power station to each sampling site.

†See Experimental.

‡Sampling sites E, F, G, J and H in Table 2 are combined into a total.

§Sampling sites A, K, C, L and B in Table 2 are combined into a total.

Chi-square was calculated from a 2 × 4 tables for the degree of predation. This table yielded a chi-square of 56.7 with three degrees of freedom ($p < 0.001$).

Table 4. Foliar tannin content of the Japanese Cedars and predation profile

Tannin content	Degree of predation* (−)	(±)	(+)	(++)
0–30 μmol/g (6 sites)†	1416 (91.2%)	51 (3.3%)	74 (4.8%)	11 (0.7%)
30 μmol/g– (4 sites)‡	1027 (94.3%)	23 (2.2%)	38 (3.3%)	1 (0.1%)

*, See Experimental.

†, Sampling sites F, G, J, H, K and L in the Table 2 are combined into a total.

‡, Sampling sites E, A, C and B in the Table 2 are combined into a total.

Chi-square was calculated from a 2 × 4 tables for the degree of predation. This table yielded a chi-square of 11.6 with three degrees of freedom ($p < 0.01$).

revealed by chi-square test. A chi-square value of 0.26 was calculated from a 2×4 tables for the nitrogen levels ($p > 0.95$).

DISCUSSION

Visible injury and decrease in productivity of various crop plants have been reported in reference to air pollution in a number of classic studies [11–14]. Recently, it has become evident that sulphur dioxide (SO_2) and other air pollutants show other deleterious effects. For example decreased contact angles of water droplets on the needle surface, i.e. increasing needle wettability, was revealed for Scots pine (*Pinus sylvestris* L.) in apparent association with ambient air pollution [15]. On the basis of the altered fatty acid composition, Grunwald [16] suggested that cell permeability of soybean [*Glycine max* (L.) Merr.] leaves may be increased with fumigation by SO_2. The decreased wax content of eastern white pine (*Pinus strobus* L.) needles which are fumigated by SO_2 has been implicated in the increasing permeability of membranes and lowered resistance to further air pollution [17]. These results imply a mechanism by which acid rain and gaseous air pollutants may penetrate plant tissues, leading to accelerated tissue damage.

Reduced foliar tannin content of Japanese Cedar, in connection with air pollution, was revealed with regard to one-year-old leaves (Figs 2, 3 and Table 1) and also as for two-year-old leaves (data are not indicated) collected in early June. Young foliage leaves may be more sensitive to the changes in atmospheric environments, because metabolic activity is very high in such young leaves. But no conspicuous difference in foliar tannin content was detected in current-year leaves (Fig. 4), which may be due to a rather short exposure of leaves to polluted air for only about one month after expansion. Considering the proposed protective function of tannins as antioxidants [5], susceptibility of Japanese Cedars to oxidative air pollutants, e.g. ozone (O_3) and nitrogen dioxide (NO_2), may be increased by the decrease of foliar tannins.

Tannins are known to have adverse effects on the growth of fungi, by tanning their pectolytic enzymes [18]. Therefore, it is also probable that increased fungal degradation may result from reduced foliar tannin content. Indeed, relationships between air pollution and the incidence of pine needle blight caused by *Rhizosphaera kalkhoffii* BUBAC were revealed in field and experimental studies [19, 20]. The possible involvement of fungi (*Ceratocystis* sp.) was also suggested in widespread deaths of pine trees (*Pinus thunbergii* and *P. densiflora*) in Japan [21].

Tannins have been considered to be defensive compounds acting against herbivores by either interfering with the herbivore's digestive enzymes or lowering the nutritive value of the plant tissue by precipitating ingested proteins [22–25]. The defensive function of tannins against insect predation, however, has been a matter of some controversy [26, 27]. Bernays [26] suggested that the lack of antiherbivore effects of tannins in some species may have an evolutionary explanation, because insects were adapted to cope with tannins at an early stage in evolution. However, tannins themselves are more or less disadvantageous to herbivores. Therefore, if foliar tannin content is reduced to unusually low levels, herbivores are no longer exposed to these unpalatable substances and may increase the extent of their predation pressure by increasing population size. A preliminary observation in which the high predation rate was accompanied by a low foliar tannin content (Table 4) may be interpreted in this context. A full account must also consider the population dynamics and community interactions of leaf-feeding herbivores.

The Japanese Cedar has been revealed to be tolerant to individual air pollutants such as SO_2 [28], NO_2 [29] and O_3 [30] exposed for rather brief period of time in experimental fumigation studies. However, it is generally known to be exceedingly sensitive to ambient air pollution in many field researches [31–33]. This contradiction may be explained by considering the effects of decreased foliar tannin levels of Japanese Cedars growing in the area of air pollution, i.e. intensive insect predation, fungal degradation and lowered tolerance against various oxidative air pollutants.

The biosynthetic pathway of tannins consists of the following five stages [4, 7–9]: (i) biosynthesis of glucose, i.e. photosynthesis; (ii) formation of a six-membered alicyclic carboxylic acid and conversion into 3-dehydroshikimic acid, i.e. the early shikimate pathway; (iii) conversion of 3-dehydroshikimic acid into aromatic compounds, i.e. the later shikimate pathway; (iv) further modification of aromatic intermediates, including hydroxylation of benzene rings; and (v) finally, catechin and gallic acid are converted into condensed and hydrolysable tannins, respectively, by condensation and/or polymerization with sugars.

There is an alternative and more important direct biosynthetic route to gallic acid, i.e. dehydration of 3-dehydro-shikimic acid [9]. Therefore, unlike condensed tannins, synthesis of hydrolysable tannins is not completely dependent on the later shikimate pathway.

The inhibition of tannin synthesis may occur in any of the above mentioned five stages. But, the possible involvement of the stage (v) was ruled out, because both condensation and polymerization are not relevant to the colour reaction of ferrous tartrate reagent. It gives a violet colour for the *o*-dihydroxybenzene moiety of tannins and also low M_r precursors of tannins. Therefore, both low M_r precursors and polymerized tannins were quantitatively determined as 'tannins'.

The inhibition of photosynthetic activity, i.e. stage (i), by air pollution has been well documented in many previous studies. Indeed, we have also confirmed conspicuous negative correlation between foliar soluble sulfate and glucose [34]. This fact suggests that the overall photosynthetic potential of the foliage is evidently lowered in *Cryptomeria* in the polluted area. In addition, the later stage of the shikimate pathway may also be affected. If photosynthesis is continuously inhibited by air pollution, the major portion of the free glucose will be consumed for growth and primary metabolism, then secondary metabolism such as tannin synthesis may also be affected. But, considering the result of the fractional determination of foliar tannins of *Castanopsis* (Fig. 7), the above mentioned secondary effect appears unlikely. If the secondary effect is taking place, then the levels of hydrolysable and condensed tannins would be lowered to a similar degree, because photosynthesis is common process prior to the biosynthesis of tannins. As demonstrated in Fig. 7, only the levels of condensed tannins were conspicuously lowered. Thus, the inhibition of stage (ii) must also be ruled out in this case. Therefore, it is most likely that the later shikimate pathway, i.e. stage (iii), is inhibited by physiological disorder, which is indicated by pronounced decrease of condensed tannins in *Castanopsis* leaves from the polluted area (Fig. 7).

The involvement of stage (iv) is not refuted from the present data. However, reduced contents of aromatic amino acids of buckwheat [35] and Japanese Cedar [36] leaves were reported in experimental fumigation studies with relatively low concentrations of SO_2. These results suggest the inhibition of the shikimate pathway by air pollution.

EXPERIMENTAL

Study area. The study sites were located in an area *ca* 15 km from east to west and 20 km from north to south in Sakai County, Fukui Prefecture, on the Japan Sea side of central Honshu, Japan (Fig. 1). The sites were mainly spread along the downstream of the Kuzuryu River basin, but some isolated sites on the foothills were also selected as controls. A steam power station (350 MW) was constructed on the mouth of the Kuzuryu River in September 1972. An additional thermal electric generator (250 MW) was built in July, 1978 and has operated since then.

As this area has been an agricultural district and no large sources of air pollution existed previously, the power station has considerably altered the atmosphere of this area, although the pollution level remains relatively low [37]. The effects of air pollutants from the power station have been investigated by us [38] since 1974 in this area by using biological indicators, such as necrosis, abnormal defoliation and decreased vitality of the foliage of the Japanese Cedar and several other species of woody plants.

Plant materials. Green healthy leaves of Japanese Cedars were collected for the measurements of foliar levels of soluble sulphate and tannins from 37 sites within a 13 km radius in the area surrounding the steam power station, in 1976 (Fig. 1). *Cryptomeria* normally bears leaves over three years, i.e. new leaves (current-year leaves) initiate in April and complete their expansion by Oct., while those formed in the previous two years (one-year-old and two-year-old leaves) remain on the same branches. The collected leaves were separated into three groups; current-year, one-year-old, and two-year-old leaves, and then dried in a ventilated oven at 60°. Dried leaves were powdered and passed through a 50-mesh sieve. To clarify the seasonal changes in foliar tannin content of Japanese Cedars, the current year leaves were collected at 10 sampling sites from marked individual trees in June, Sept. and Nov. 1977.

In comparison with the Japanese Cedar, an evergreen oak tree, *Castanopsis cuspidata* which also grows abundantly in the study area, was selected for analysis. The methods of sampling and preparation were the same as for the Japanese Cedar.

Chemical analyses. Soluble sulphate in the leaves was determined by turbidimetry with $BaCl_2$ and gelatin solution. The levels of soluble sulphate were indicated as sulphur in the text. Foliar nitrogen was determined by Yanaco CN-corder MT500. Quantitative determination of tannins was carried out by the ferrous tartrate method [39] using gallic acid as standard. To determine relative composition of tannins, i.e. hydrolysable and condensed tannins, the procedure of ref. [40] was used with some modifications.

Estimation of predation rate of Japanese Cedar leaves by larvae of a herbivorous moth. Ca 200 to 400 terminal shoots of the Japanese Cedar leaves were collected in November 1977, from 10 sampling sites. Individual terminal shoots were classified into four grades according to the extent of predation by the larvae of *Dasychira abietis argentata* Butler, that normally feed on Japanese Cedars. Predation free shoots were classified into grade (−). The grades (±), (+) and (+ +) represent the shoots which suffered from needle predation less than 10%, 10 to 30% and more than 30%, respectively, on the basis of the number of needles damaged. The predation index (P.I.) was calculated from the following equation

$$\text{P. I.} = \frac{5 \times N_{(\pm)} + 20 \times N_{(+)} + 40 \times N_{(++)}}{N_{(-)} + N_{(\pm)} + N_{(+)} + N_{(++)}}$$

where $N_{(-)}$, $N_{(\pm)}$, $N_{(+)}$ and $N_{(++)}$ are the numbers of shoots classified into grades (−), (±), (+) and (+ +), respectively. According to the above criteria, the predation index represents a rough approximation of the percentage of leaves damaged by herbivory, based on the number of needles damaged.

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