Response of Rubber Trees to Sulphur Dioxide in the Atmosphere

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To study the effect of sulphur dioxide on the foliage of Hevea brasiliensis, *young trees were exposed in a test chamber to various combinations of SO_2 gas concentrations with times of exposure. Exposures lasting 15 minutes failed to cause foliar injury until a concentration of 75-100 p.p.m. SO_2 was reached. On the other hand, a 4-hour exposure caused leaf damage at a concentration of 0.78 p.p.m. SO_2. An exposure of 3 weeks failed to produce any visible leaf symptom at a 0.30 p.p.m. concentration. Wet leaves were more susceptible to injury than were dry leaves and young leaves were more susceptible than old leaves.*

During the 4-hour fumigations, leaf sulphur content tended to build up as the SO_2 concentration was increased, but not proportionally. A sulphur content in excess of 0.30% was accumulated in the injured leaves. When absorbed over a 3-week period during low-concentration fumigation, the same level of sulphur did not induce injury, indicating the importance of the rate of accumulation. Thus it might be concluded that such low-concentration SO_2 emissions from an industrial operation would not be toxic to the foliage of rubber trees.

The discharge of sulphur dioxide from industrial operations has long been known to damage vegetation. In the past, blast furnaces were the chief source of this effluent, and damage from this source has been the subject of litigation in the United States and many other countries (SCURFIELD, 1960). More recently chemical plants, refineries, and bleacheries have been recognised as potential sources of oxides of sulphur (ELKIN, 1962). Sulphur dioxide injury to forest trees of the Columbia River valley in Washington has been described in detail by KATZ (1952). Among the most sensitive plant species were *Abies lasiocarpa, Pseudotsuga taxifolia, Pinus contorta, Picea engelmanii,* and *Pinus ponderosa,* which were severely injured within a radius of 5 to 8 miles. The most sensitive species suffered some slight damage up to 50 miles from the source.

Modern industrial organisations design their equipment so as to control pollution, ensuring that gaseous emissions will not damage property or vegetation in the vicinity of the plant. One such study is reported here, to ascertain and evaluate the effect, if any, of the SO_2 emissions from two new petroleum refineries at Port Dickson, Malaya, on the rubber tree foliage in the nearby plantations.

MATERIALS AND METHODS

Two-year-old rubber trees (*Hevea brasiliensis*) supplied by the U.S. Rubber Company Plantation Division at Wayne, N.J., were grown in sand culture in the greenhouse. When received, the trees bore three different types of leaves: very small terminal leaves with abundant red pigment, a few characteristically drooping, almost transparent leaves, and finally the more mature leaves which tended to be lighter green towards the top of the tree and darker green toward the base. Since the latter group made up the bulk of the foliage, these leaves were sampled periodically during

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the experiment, the lighter green leaves being designated as young leaves and the darker ones as old leaves. All leaves contained stomata on the lower surface only. The trees were grown in sand culture with a solution containing 96 p.p.m. sulphur, in addition to optimum amounts of nitrogen, calcium, potassium, phosphorus and trace elements.

The trees were fumigated for 4-hour periods at concentrations ranging from 0.13 p.p.m. to 3.30 p.p.m. SO_2 and for 15-minute periods at 15, 25, 50, 75 and 100 p.p.m. SO_2. Longer fumigations, extending from 30 hours to 3 weeks were conducted at concentrations of 0.20 to 0.30 p.p.m. SO_2. From profiles of anticipated ground level concentrations of SO_2 in the Port Dickson area, Esso engineers had calculated the highest possible SO_2 concentration to be slightly less than 0.30 p.p.m., with a maximum duration of 3 minutes. Since even the most adverse weather conditions would probably not persist for 3 weeks, the controlled fumigation at 0.30 p.p.m. for 3 weeks was accepted as a realistic limit of SO_2 exposure.

The rubber trees were fumigated in a glass chamber ($6 \times 6 \times 8$ ft) into which a stream of SO_2 from a cylinder containing 1% SO_2 and 99%N was mixed with a stream of air that had been passed through a charcoal canister. Air exchange within the chamber occurred every 45 seconds. The fumigations were conducted during the months of September through November at 75 to 80% relative humidity and 80° to 90°F temperature in the presence of lights that provided illumination for 12 hours each day. These conditions were considered comparable to those existing in Malaya.

Sulphur dioxide concentration within the chamber was determined periodically by the method of WEST AND GAEKE (1956).

Two days after each fumigation the trees were evaluated for injury symptoms and either separate samples of young or old leaves or a composite (i.e. bulked) sample containing leaves of both ages was taken for total sulphur determinations. The tissues were dried for 24 hours at 70°F, after which they were ground through the 40-mesh screen of a semi-micro Wiley Mill. Total sulphur was determined by the gravimetric method (ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS, 1940), and was recorded as percentage S on a dry weight basis. Soluble sulphate sulphur was determined by the method of FERREIRA (1955) and by the method of MCKEE AND BIEBERDORF (1960).

Respiration studies were conducted on fresh leaf discs in a Warburg respirometer by the method of KLINKER (1950).

RESULTS AND DISCUSSIONS

Injury to the Trees

The results of 4-hour exposures of rubber trees to increasing SO_2 concentrations are presented in *Table 1*. Concentrations up to 0.71 p.p.m. SO_2 caused no visible damage on dry trees, but slight damage occurred at 0.78 p.p.m. At the end of this exposure certain of the fully developed lighter green leaves on the upper half of the tree appeared water-soaked in the interveinous tissue (*Figure 1*); with 24 hours after fumigation the chlorophyll in these areas was completely destroyed and the tissue dried to a light tan colour (*Figure 2*). The injury originated on the upper leaf surface rather than on the lower, despite the lack of stomata in the former. Microscopic examination showed a disruption of the single-layer palisade tissue, similar to that observed in ozone-fumigated plants (HILL *et al.*, 1961). The older leaves were rarely injured. Damage at an SO_2 concentration of 1.50 p.p.m. for 4 hours, was little more than that at 0.78 p.p.m. At concentrations above 3.00 p.p.m. there was a considerable increase in plant injury. All aged leaves except the small terminal leaves developed water-soaked areas before the end of the fumigation period, which on exposure to the sun became bleached. The bleaching pattern (*Figure 3*) varied: at times the marginal tissue remained green and the interior was bleached, and at other times this pattern was reversed (*Figure 4*). The large drooping leaves near the tops of the trees were injured from the base of the leaf to the tip, the bleaching following the midvein out into the leaf. The small terminal leaves appeared

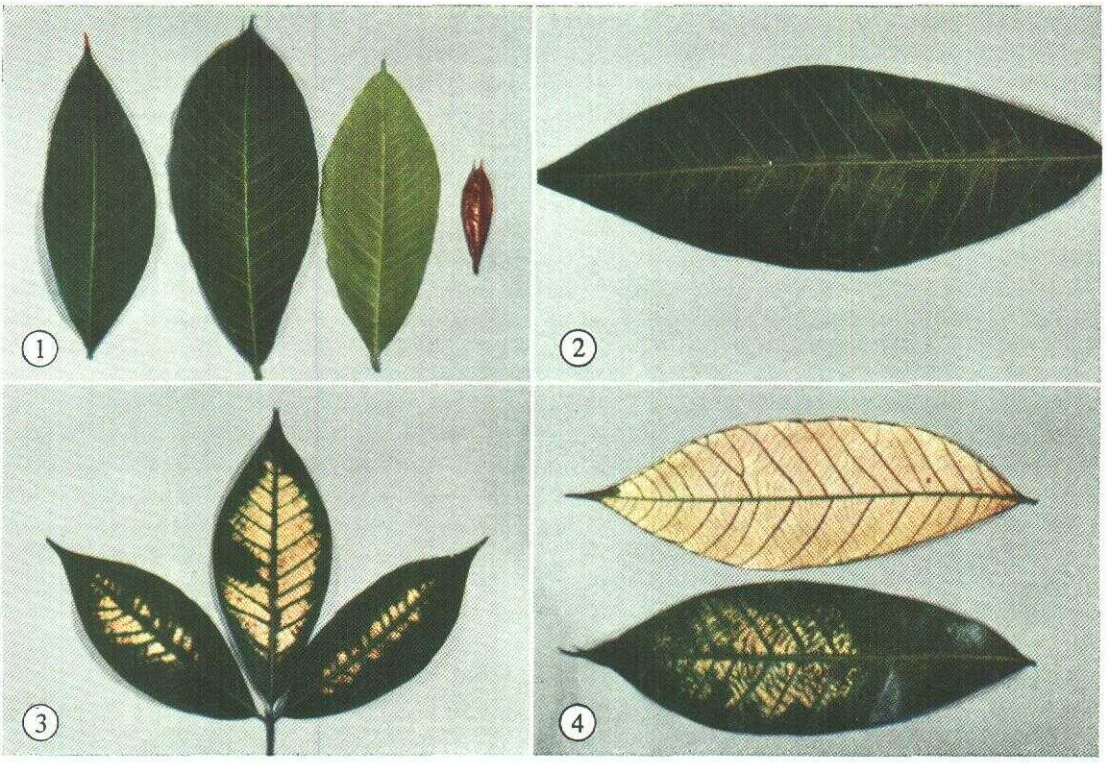

Figure 1. Types of normal leaves present on the experimental rubber trees. Figure 2. An early symptom of SO_2 injury, watersoaking of interveinous tissue, occurring on fully developed lighter leaf from upper part of tree. Figure 3. Slight injury on dry young rubber leaves resulting from 0.78 p.p.m. SO_2 for four hours. Figure 4. Various injury patterns produced on rubber leaves by SO_2.

wrinkled or puckered. Rubber trees fumigated for 21 days at 0.30 p.p.m. SO_2 showed no injury. Trees fumigated for 15-minute periods failed to show injury until the concentration of 75 to 100 p.p.m. SO_2 was reached.

THOMAS *et al.* (1949) present conclusive data that plants endure higher concentrations of SO_2 in relatively dry than in moist air, a finding which is confirmed by our own data (*Table 1*). When the leaf surfaces of the rubber trees were wetted during fumigation, slight injury was induced by 0.40 p.p.m. for 4 hours and moderate injury by 1.40 p.p.m. for 4 hours.

Sulphur Content

The results of total sulphur analyses of rubber leaves exposed for 4 hours to increasing SO_2 concentrations are presented in *Table 2*. These data show a tendency for bulked rubber leaves to increase in total sulphur as the SO_2 content is increased. On statistical analysis by the variable squared method there was, however, no significant increase in sulphur at the three lowest SO_2 concentrations (0.13, 0.18, 0.40 p.p.m. SO_2) over the untreated controls. At the 0.71 p.p.m. exposure the increase over untreated controls was significant at the 5% level, and at all SO_2 concentrations above 0.71 p.p.m. the increase in total sulphur over the untreated controls was highly significant. There was no significant difference between the four highest fumigation levels (0.78, 1.50, 3.07 and 3.30 p.p.m. SO_2). The sulphur content of leaves exposed for 21

days at 0.30 p.p.m. SO_2 showed a highly significant increase over the control, a significant increase (at the 5% level) over 4 hours at 0.71 p.p.m., and no significant increase over 4 hours at any of the four highest levels (0.78 p.p.m. to 3.30 p.p.m. SO_2).

The failure of trees exposed at the three lower fumigation levels to show a significant increase in sulphur was due to the wide variation in sulphur content in the individual trees. Perhaps this variation may have been due in part to the variation in pot size in which the rubber plants were growing at the time they were received by us. It was also noted that the sulphur content of the younger leaves increased as the season advanced. This trend tended to invalidate some comparisons between fumigations. To sum up, the data support the contention of many investigators (SCURFIELD, 1960) that at SO_2 concentrations likely to occur in the atmosphere for short periods, total sulphur analyses are not very reliable in indicating the exposure of rubber trees to SO_2. However, at high concentration levels (>1 p.p.m.) for short periods or as a result of protracted fumigation periods (21 days) of the order of 0.30 p.p.m., leaf sulphur content is indicative of exposure to SO_2 in the atmosphere. Many investigators have found a good relationship between distance from the pollution source and leaf sulphur content of trees (FERREIRA, 1955; KATZ AND MCCALLUM, 1939; MCKEE AND BIEBERDORF, 1960). Obviously the SO_2 concentration close to an industrial plant might well be expected to be of a higher order of magnitude than at increasing distances.

These data also serve to illustrate the contention of Wislicenus and others (SCURFIELD, 1960) that high concentrations of SO_2 applied over short periods could cause the death of a plant without significantly increasing the sulphur content of its leaves. According to *Table 1*, 0.71 p.p.m. SO_2 was the highest concentration which failed to produce any injury to rubber within a 4-hour period. The sul-

TABLE 1. DEGREE OF INJURY PRODUCED ON LEAVES OF RUBBER TREES BY EXPOSURE TO INCREASING SO_2 CONCENTRATIONS IN A FUMIGATION CHAMBER

SO_2 conc., p.p.m.	Dry leaves		Wet leaves	
	young	old	young	old
	4-hour duration			
0.13	−	−	−	−
0.18	−	−	O	O
0.40	−	−	+	−
0.71	−	−	+	−
0.78	+	−	O	O
1.40	+	−	++	−
1.50	+	−	O	O
3.07	+++	+++	O	O
3.30	+++	+++	O	O
	21-day duration			
0.30	−	−	O	O

O Not present in test + Slight injury ++ Moderate injury +++ Severe injury − No injury

TABLE 2. SULPHUR CONTENT OF BULKED RUBBER LEAVES TWO DAYS AFTER EXPOSURES TO INCREASING LEVELS OF SO_2 (FUMIGATED DRY)

SO_2 conc., p.p.m.	% sulphur content†
4-hour duration	
0. 0	0.187
0.13	0.177 n.s.
0.18	0.207 n.s.
0.40	0.249 n.s.
0.71	0.300 *
0.78	0.330 **
1.50	0.336 **
3.07	0.358 **
3.30	0.381 **
21-day duration	
0.30	0.413 **

† Each figure represents the average of 3 replicates ** l.s.d. 0.01 0.144 * l.s.d. 0.05 0.103

phur content produced by this concentration was significantly greater than that of untreated trees. At concentration levels above 0.71 p.p.m. SO_2, injury occurred, but the sulphur content (*Table 2*) was not significantly greater than that of the trees exposed to 0.71 p.p.m. Upon being killed, the cells obviously ceased to absorb SO_2. Trees fumigated for 21 days at 0.30 p.p.m. SO_2 showed a significant increase in sulphur content over those fumigated at 0.71 p.p.m. SO_2 for 4 hours, thus further emphasising the fact that larger quantities of sulphur can be accumulated without injurious effects if the gas is accumulated slowly over a longer period of time. This is in accordance with the findings of Katz and others (KATZ, 1952; THOMAS *et al.*, 1949) in experiments with conifers.

Sulphate sulphur content. To determine whether there might be a better criterion for sulphur absorption, sulphate sulphur was determined by extracting dried tissues with distilled water and following a turbidimetric procedure (AMERICAN PUBLIC HEALTH ASSOCIATION, 1955). The results paralleled those obtained for total sulphur content. Leaves of trees fumigated at 0.13 to 0.70 p.p.m. SO_2 had a soluble sulphur content similar to that of unfumigated material, namely 0.047 to 0.097 sulphate sulphur. Only when the fumigation rate exceed 0.70 p.p.m. SO_2 was there an appreciable increase in sulphate content. At the 1.5 p.p.m. fumigation it increased to 0.149% or 1.5 times greater than the control, and at the 3.0 p.p.m. fumigation it was 0.203% or twice as great as in the control. Since these results were not analysed statistically, they can only be reported as a trend.

Effect of moisture. Table 3 presents the data for total sulphur content of trees which were fumigated simultaneously under wet and dry conditions. Replicate trees were placed

TABLE 3. SULPHUR CONTENT OF BULKED RUBBER LEAVES AFTER 4-HOUR EXPOSURES TO INCREASING LEVELS OF SO_2 (FUMIGATED WET AND DRY)

SO_2 conc., p.p.m.	% sulphur content†	
	dry leaves	wet leaves
0.0	0.187	—
0.13	0.177	0.294 **
0.40	0.249	0.337 **
0.71	0.300	0.390 **
Average	0.228	0.304 **

† Each figure represents the average of 3 replicates ** l.s.d. 0.01 0.047 * l.s.d. 0.05 0.034

on either side of a vinylite partition, which came to within 10 to 12 inches of the baffle in the fumigation chamber, in order not to interfere with uniform distribution of the gas. On one side of the partition the trees remained dry. On the other side, three overhead spray jets were turned on for 1 to 2 minutes every half hour in order to keep the leaf surfaces wet most of the time.

The dry leaves did not show a significant increase in sulphur content over the untreated controls until exposed to a concentration of 0.71 p.p.m. SO_2, whereas the wet leaves showed a highly significant increase even at the lowest fumigation level (0.13 p.p.m. SO_2). In effect, the presence of moisture on the leaves during an SO_2 fumigation actually concentrates the gas on the leaf surface, hydrolysing it to sulphuric acid and causing a higher degree of injury and an increase of almost 50% in sulphur content. This substantiates a point advanced by MEETHAM (1952) in his treatise on atmospheric pollution, and was similar to the effect of free water on the leaf surfaces of tomato leaves exposed to hydrogen fluoride fumigations, as demonstrated in an earlier study by DAINES *et al.*, (1952). It indicates that the presence of dew or mist or very light rain might cause injury to vegetation at a lower level of SO_2 concentration than that required during a dry period. Heavier rains might tend to wash away the gas rather than help to concentrate it on the leaf surfaces.

Effect of leaf age. It has been shown (*Table 1*) that younger fully mature rubber leaves are more susceptible to SO_2 injury than are older leaves. There is also a difference in the total sulphur content of young and older leaves. Even in the untreated control, sulphur content was consistently found to be greater in the older leaves than in the younger leaves, corresponding with a higher tolerance to the gas in the older leaves. In order to give some idea as to the translocation of sulphur, both old and young leaves as well as bulked leaf samples were divided along the mid-rib and the halves divided into two separate aliquots. One aliquot was dried directly and the other was washed for 2 minutes in mildly agitated distilled water before being dried and ground for analysis. After drying, both washed and unwashed leaf samples were analysed for total sulphur content. The data are presented in *Table 4*. Here it can be seen that while bulked samples or older leaf samples did not lose measurable amounts of sulphur on being washed for 2 minutes, there was a considerable loss in the washed younger leaves (up to 30%). This is in accordance with results obtained by LEONE *et al.*, (1956). in relation to hydrogen fluoride absorption in tomato and corn and indicates that sulphur is less firmly attached to young leaves than to old.

TABLE 4. SULPHUR CONTENT OF YOUNG, OLD AND BULKED RUBBER LEAVES AFTER 4-HOUR EXPOSURES TO INCREASING SO_2 CONCENTRATIONS. DETERMINATIONS ON WASHED AND UNWASHED LEAVES

SO_2 conc., p.p.m.	Leaf age	% sulphur content† unwashed leaves	washed leaves
Control	Young	0.121	0.125 n.s.
	Old	0.253	0.253 n.s.
0.78	Young	0.322	0.230 **
	Old	0.357	0.346 n.s.
3.05	Young	0.318	0.255 **
	Old	0.356	0.346 n.s.
3.30	Bulked	0.381	0.374 n.s.

† Each figure represents the average of 3 replicates ** l.s.d. 0.01 0.031 * l.s.d. 0.05 0.022

Effect of Protracted Fumigation on Injury and Sulphur Content

Injury. Since short-term fumigations at concentrations deemed possible in the ambient atmosphere surrounding an oil refinery (0.30 p.p.m.) failed to produce typical injury symptoms on rubber trees, it was decided to conduct a protracted fumigation to determine whether conditions favouring a prolonged stable atmosphere would produce injury at this concentration. For this purpose, six rubber trees were exposed for 3 weeks to an average SO_2 concentration of 0.30 p.p.m. SO_2 under the conditions previously stated. At the end of one-week, two-week and three-week periods the trees were examined for injury symptoms and analysed for total and soluble sulphur. At each of these periods replicate samples of young and old tissues were refrigerated in moist chambers for 2 to 3 hours, after which respiration studies were made.

Results indicated that 3 weeks' fumigation at 0.30 p.p.m. SO_2 failed to produce typical SO_2 injury symptoms (*Table 1*).

Sulphur content. In addition to analysing the leaves for total sulphur at the end of the fumigation, tissue was removed from the trees after 5, 14 and 21 days, and tested by a method outlined by FERREIRA (1955) in Portugal. Fresh tissue, rather than dry, was minced and soaked for 24 hours in distilled water, and, after precipitation as $BaSO_4$, the water-soluble sulphur (*i.e. sulphate*) content was determined gravimetrically. A qualitative test made at the same time showed clearly that the fumigated tissue always contained more sulphate than the control. Quantitative results corroborated the observations (*Table 5*). The rate at which soluble sulphur increased in the fumigated tissue was not constant. At the end of 14 days there was little more than after 5 days. After the 21-day period, however, there was twice as much soluble sulphur as after the 5- and 14-day intervals. After 14 days, 13% of the total sulphur was in the soluble form, whereas after 21 days the figure rose to 27%. The qualitative method can be recommended as a quick one for indicating exposure of plants to abnormal quantities of SO_2 in the air.

Comparative Susceptibility of Hevea and Other Plants

Comparison of the susceptibility of rubber with that of herbaceous plants (*Table 6*) indicated rubber to be considerably more resistant than most of the species tested. Although some of the plants showed no injury within 24 hours at 0.30 p.p.m. SO_2, it was known

TABLE 5. SOLUBLE AND TOTAL SULPHUR CONTENT OF RUBBER LEAVES DURING A THREE-WEEK FUMIGATION AT 0.30 P.P.M. SO_2

Duration of exposure	% soluble sulphur	% total sulphur
Control	0.0159	0.1695
5 days	0.0408	0.2652
14 days	0.0471	0.3492
21 days	0.1122	0.4134

TABLE 6. COMPARISON OF SO_2 INJURY ON RUBBER WITH HERBACEOUS SPECIES AT 0.30 P.P.M. SO_2 FOR 24 HOURS

Species	Degree of injury	Species	Degree of injury
Begonia	++	Periwinkle	–
Dahlia	++	Azalea	–
Aster	+	Swiss chard	–
Zinnia	+	Pepper	–
Cucumber	+	Rubber	–
Violet	+		
Tradescantia	+		

– No injury + Slight injury ++ Moderate injury

from experience that they would become injured within a period of time far shorter than the 21 days during which rubber failed to show injurious effects.

Invisible Injury

Leaf respiration is generally considered a criterion for invisible injury or alterations in the physiology of the plant. In this case oxygen consumption was determined on fresh leaf discs of young and old rubber leaves in a warburg respirometer by the method of KLINKER (*1950*). Data expressed as microlitres of oxygen per milligram of dry tissue per hour are presented in *Table 7*. From these data it can be seen that while there was a significant difference in oxygen consumption between young and old rubber leaves, there was no significance between the control and fumigated tissues of either age, indicating the absence of hidden or internal injury.

Thus it might be concluded that if the predicted maximum of 0.30 p.p.m. SO_2 were to continue for 3 weeks, SO_2, no toxic effects on the dry foliage of rubber trees would be expected. If conditions were to favour moist leaves, injury might occur at this or at a lower SO_2 level. However, an actual downpour might tend to wash the SO_2 off the leaves and thus decrease the possibility of injury.

TABLE 7. OXYGEN CONSUMPTION BY RUBBER LEAVES BEFORE AND AFTER EXPOSURE TO 0.30 P.P.M. SO_2

Duration of exposure	*Microlitres of oxygen per mg dry tissue per hour*			
	Control leaves† young	old	Fumigated leaves† (n.s.) young	old
4 days	2.33 **	1.66	2.67 **	2.28
12 days	2.68 **	1.76	2.53 **	1.58
21 days	2.72 **	2.00	2.75 **	1.82

† Each figure represents the average of 2 replicates (n.s.) No significance between control and fumigated leaves
** l.s.d. 0.01 0.047 between young and old leaves * l.s.d. 0.05 0.37 between young and old leaves

CONCLUSIONS

The results of the tests led to the following conclusions:

1. Four-hour fumigations of dry rubber trees under conditions simulating the climate of Malaya did not produce injury until the concentration of 0.78 p.p.m. SO_2 was reached.
2. Fifteen-minute exposures of the trees failed to cause injury until the concentration of 75–100 p.p.m. SO_2 was reached.
3. When rubber plants having wet leaves were fumigated for a four-hour period, slight injury was produced at 0.40 p.p.m. SO_2.
4. Total sulphur determinations of bulked dry leaves showed no significant increase over that in untreated controls until the concentration of 0.70 p.p.m. SO_2 was reached.
5. Trees which were fumigated when wet showed a significant increase in total sulphur content over dry control trees even at the lowest SO_2 concentration of 0.13 p.p.m.
6. Old leaves consistently had a higher total sulphur content than did younger leaves.
7. In young leaves a considerable part of the absorbed sulphur (up to 30%) could be washed off the surface, whereas old leaves or bulked samples failed to show any decrease in sulphur content on washing. This might be used as a criterion for exposure of foliage to atmospheric SO_2.
8. A three-week fumigation at 0.30 p.p.m. SO_2, the maximum concentration predicted by Esso engineers in the Port Dickson area, failed to produce any injury symptoms.
9. A total sulphur content of the foliage sufficient to cause injury when accumulated at a rapid rate failed to injure when accumulated over a three-week period.
10. Respiration studies failed to indicate the possibility of hidden injury occurring during this prolonged fumigation.
11. Rubber trees respond similarly to other woody species to the presence of SO_2, being injured at much higher concentrations than were most of the herbaceous species tested.

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