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INTERIM REPORT ON SYMPTOMOLOGY AND THRESHOLD LEVELS  
OF AIR POLLUTANT INJURY TO VEGETATION, 1978-79

by

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for

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ABSTRACT

The dominant woody boreal forest plant species were fumigated with 0.34 ppm SO<sub>2</sub> under controlled conditions in the laboratory in order to rank their physiological and visual sensitivities to the air pollutant. Deciduous trees and shrubs were much more sensitive than conifers, presumably because SO<sub>2</sub> can enter broad leaves much more easily than needles. Labrador tea was intermediate in sensitivity to SO<sub>2</sub> and so were its leaf resistances to pollutant uptake. Among conifers, jack pine was more sensitive than either black or white spruce, whereas the species within the deciduous group could not be ranked due to inadequate differences between their tolerance levels.

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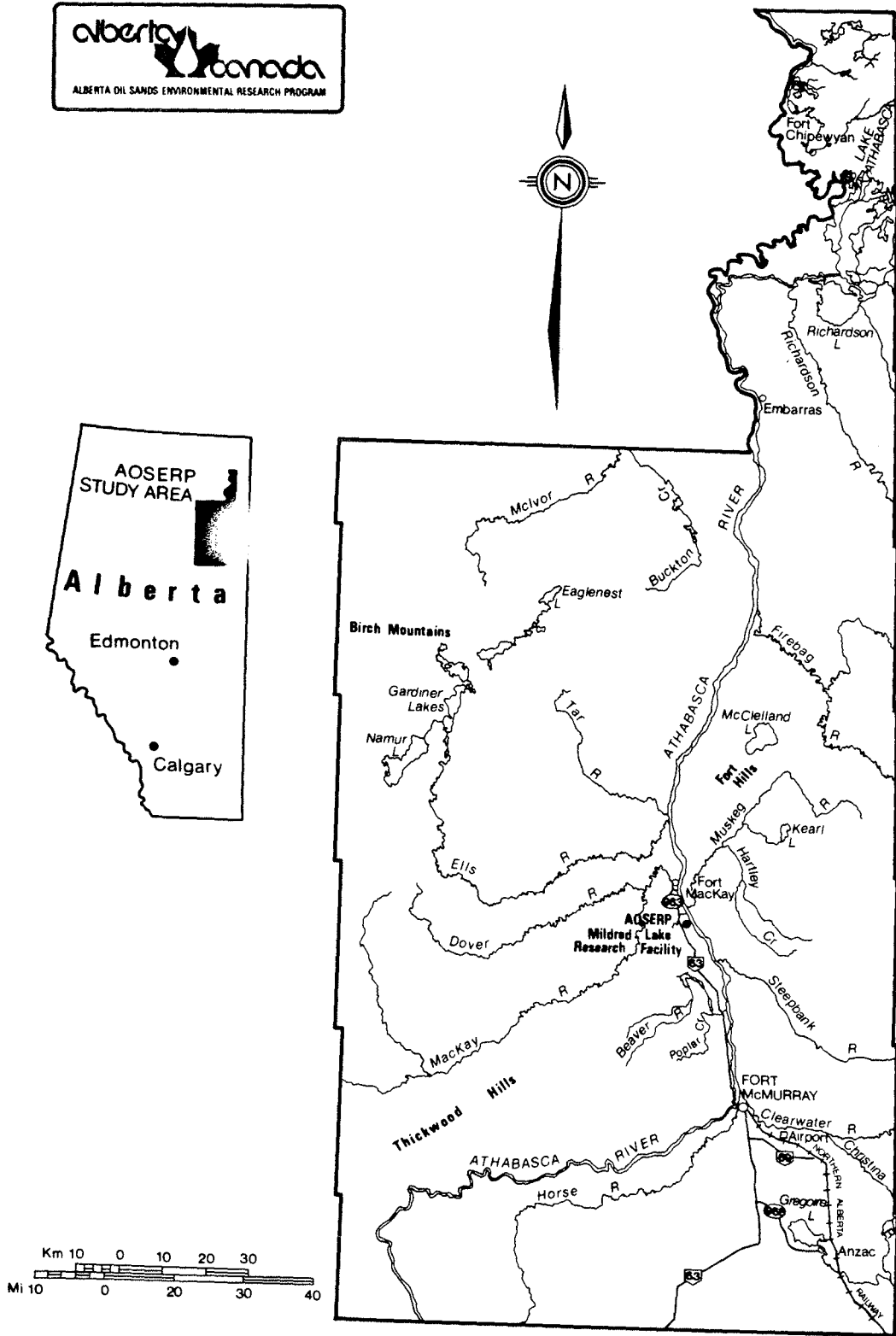


Figure 1. Location of Alberta Oil Sands Environmental Research Program study area.

1.           INTRODUCTION

In the Athabasca Oil Sands area of Alberta, development of petroleum deposits has resulted in emission of SO<sub>2</sub> and other pollutants to the airshed. The potential impact of these emissions on the boreal forest in the area is substantial, and a field program (LS 3.4) was established to monitor any changes that might occur as a result of air pollution. Ranking of species sensitivity to air pollutants is a prerequisite for the interpretation of air pollution injury to vegetation and changes in species composition in the field. This ranking was achieved both on visual and physiological responses of forest species to SO<sub>2</sub> fumigations.

The objective of this study in 1979 was to determine visual and physiological threshold levels of air pollutant injury to species native to the Athabasca Oil Sands region of Alberta. This information will be useful in screening candidate revegetation species for their tolerance to air pollutants characteristic of the oil sands area.

Because of the interim nature of this report, the statements made herein are subject to revision upon completion of the project and release of the final report.



## 2. MATERIALS AND METHODS

### 2.1 PLANT COLLECTION AND GROWING CONDITIONS

Plants were collected from an area greater than 20 km from both pollutant sources at a location approximately 2 km north of Fort MacKay. The plants collected represented the dominant woody species of the area and included a deciduous group of aspen (*Populus tremuloides* Michx.), willow (*Salix* sp.), green alder (*Alnus crispa* (Ait.) Parsh), and paper birch (*Betula papyrifera* Marsh); a coniferous group of white spruce (*Picea glauca* (Moench) Voss), black spruce (*Picea mariana* (Mill.) VSP), and jack pine (*Pinus banksiana* Lamb.); and Labrador tea (*Ledum groenlandicum* Oeder), an angiosperm with persistent leaves. These plants were potted in their native soil, grown in the greenhouse for two to four months, then transferred into a controlled environment chamber for experimentation.

The controlled environment chamber provided an 18 h photoperiod from a combination of high pressure sodium, metal halide, and tungsten filament lamps. Light intensity was programmed to simulate diurnal variations with a maximum of  $500 \mu\text{E m}^{-2}\text{s}^{-1}$ . Temperature was held constant at  $18^{\circ}\text{C}$ , and dew point was also constant at  $15^{\circ}\text{C}$ . Plants were well watered three times a week and fertilized weekly. The thermal light, moisture, and nutrient regimes given ensured that the plants were near maximum in net  $\text{CO}_2$  assimilation rates.

### 2.2 $\text{SO}_2$ FUMIGATION AND MEASUREMENTS OF PHOTOSYNTHESIS AND RESPIRATION

The actively growing plants were held in the controlled environment chamber in clean air for up to 25 days to ensure their stabilization and to determine the pre-fumigation net photosynthetic rates. After this period, plants were fumigated with 0.34 ppm  $\text{SO}_2$  continuously to the end of the experiment.

Net CO<sub>2</sub> assimilation and dark respiration were measured in a controlled environment microchamber at 18°C and a 15°C dew point. Ambient air from outside was mixed in two 200 L drums to provide uniform CO<sub>2</sub> concentrations. An open gas flow system involving a Beckman Model 865 infrared CO<sub>2</sub> analyser was used to differentially measure the CO<sub>2</sub> concentration of the chamber air stream against the reference stream.

During CO<sub>2</sub> assimilation measurements, plants were maintained under a 600  $\mu\text{E m}^{-2}\text{s}^{-1}$  light source (high pressure sodium and metal halide lamps). Dark respiration was measured under reduced air flow with the microchamber completely covered with black plastic. The CO<sub>2</sub> analyzer was calibrated twice daily using standard CO<sub>2</sub> concentrations from precalibrated gas cylinders. Measurements were made every two to seven days, depending upon the response of the species to SO<sub>2</sub>. An average of five replicates was used for each species.

Gas exchange rates were expressed as a percentage of the rate before the pollutant gas was added. An arcsine transformation was carried out on these proportional values to ensure normality of distribution for statistical purposes. A linear regression was then calculated for both the pre- and post-fumigation conditions of each species. The regression coefficients were compared with zero slope and with each other in order to determine if there was a response of the species to SO<sub>2</sub> and to rank the species in order of their sensitivity to SO<sub>2</sub>.

### 2.3 VISUAL SYMPTOM RECORD

A photographic record of visual symptoms of SO<sub>2</sub> toxicity of all species was maintained during the experiments. Photographs were taken during the "control" period in the growth chamber and every two to seven days after the SO<sub>2</sub> fumigation had begun.

#### 2.4 SULPHUR UPTAKE STUDIES

The total sulphur content of plant material was determined before and after fumigation. Samples were digested using an oxygen-flask combustion technique (Chan 1975), and sulphur was determined using a modified Johnson-Nishita  $\text{SO}_4\text{-S}$  technique (Carson et al. 1972).

### 3. RESULTS AND DISCUSSION

#### 3.1 PHYSIOLOGICAL RESPONSES

Maximum, minimum, and average physiological responses of the four deciduous species studied both before and after fumigation with 0.34 ppm SO<sub>2</sub> are presented in Figure 2. The average response is given by the solid line, which is the relative net CO<sub>2</sub> assimilation rate (NAR) regressed against time. Maximum and minimum response, on the other hand, are actual case histories. NAR is defined as the difference between gross photosynthesis and respiration.

The NAR of all deciduous species dropped dramatically when they were exposed to 0.34 ppm SO<sub>2</sub>. All slopes (regression coefficients) of NAR versus time before fumigation were not significantly ( $p < 0.05$ ) different from zero (Table 1). During fumigation, on the other hand, all slopes were significantly ( $p < 0.05$ ) different from zero and were negative, indicating that NAR was decreasing with time.

Examination of the actual case histories presented in Figure 2 gives some idea of the range of response within each species. In general, variability in NAR appeared to be much greater during the fumigation than before in all species (Figure 2). Green alder had the maximum initial divergence between the maximum and minimum NAR responses during SO<sub>2</sub> fumigation. In addition to its great natural variability, green alder also had the smallest number of replicates (three), hence confidence in these data was much less than those for other species.

The response of NAR of Labrador tea and the conifers (Figure 3) was much less than that for the deciduous species. Black spruce had the greatest difference between maximum and minimum responses of NAR with time followed by Labrador tea and white spruce. Jack pine had the most uniform response.

Prefumigation slopes of NAR versus time were not significantly ( $p < 0.05$ ) different from zero except for the slope for jack pine (Table 1). The positive slope of jack pine indicated that NAR

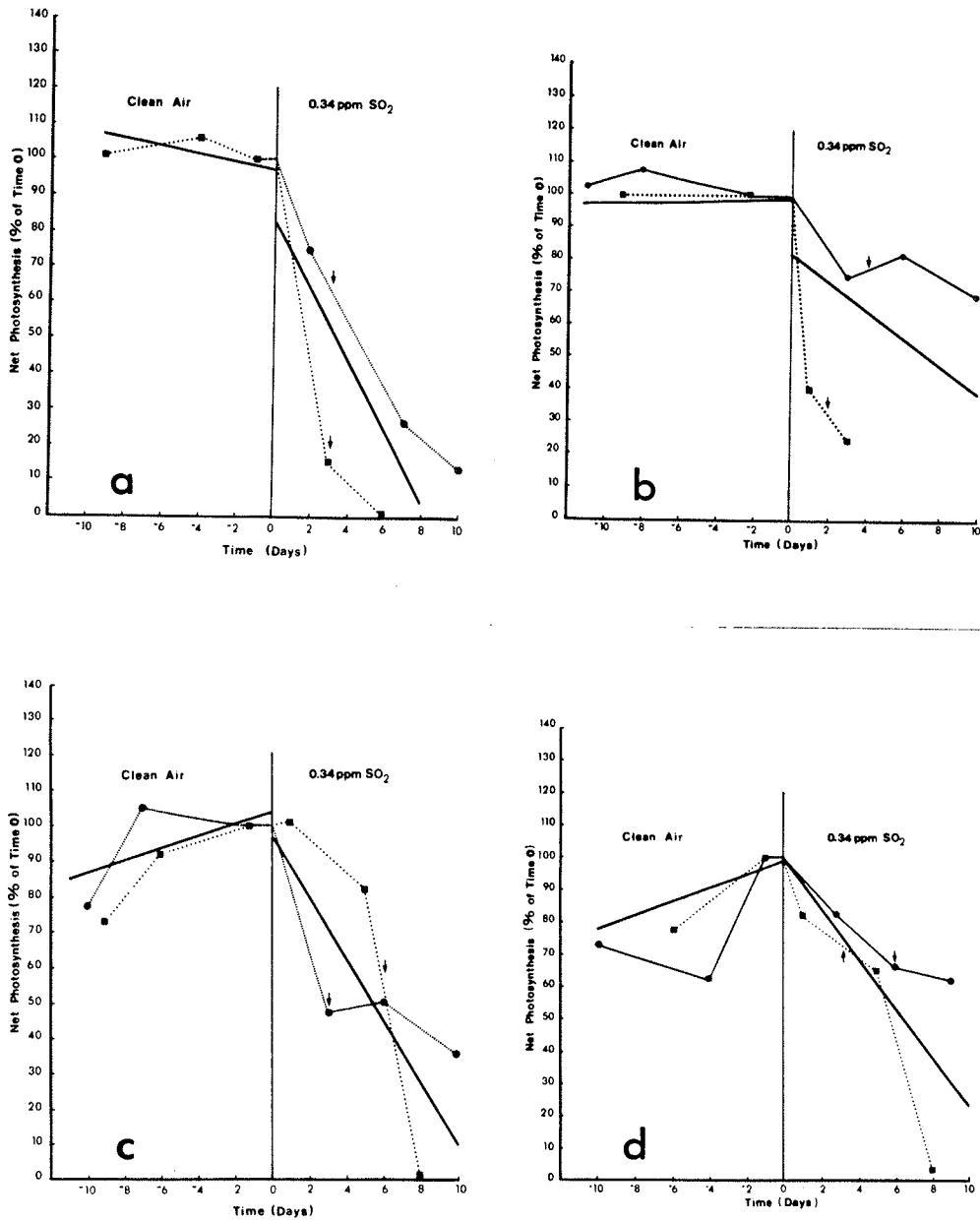


Figure 2. Maximum (·····), minimum (-----), and average (—) responses of relative net CO<sub>2</sub> assimilation rates of willow (a), green alder (b), paper birch (c), and aspen (d) both before and during fumigation with 0.34 ppm SO<sub>2</sub> under controlled conditions. Arrows indicate initiation of visual symptom development.

Table 1. Slope (regression coefficient) of the change in net CO<sub>2</sub> assimilation rate (NAR) with time during fumigation with 0.34 ppm SO<sub>2</sub>. Negative signs indicate a reduction in NAR with time. Units are percentage of maximum NAR decrease per day.

Species	Prefumigation Slope	Fumigation Slope
Willow	-1.09	-9.91 <sup>a</sup>
Paper Birch	+1.70	-8.69 <sup>a</sup>
Aspen	+2.13	-7.68 <sup>a</sup>
Green Alder	+0.19	-4.29 <sup>a</sup>
Labrador Tea	-3.18	-2.15 <sup>a</sup>
White Spruce	+0.01	-2.06 <sup>a</sup>
Black Spruce	+0.48	-1.69 <sup>a</sup>
Jack Pine	+1.51 <sup>a</sup>	-1.30 <sup>a</sup>

<sup>a</sup>Slope is significantly ( $p < 0.05$ ) different from zero.

<sup>b</sup>Vertical lines join slopes that are not significantly ( $p < 0.05$ ) different by the Simultaneous Test Procedure.

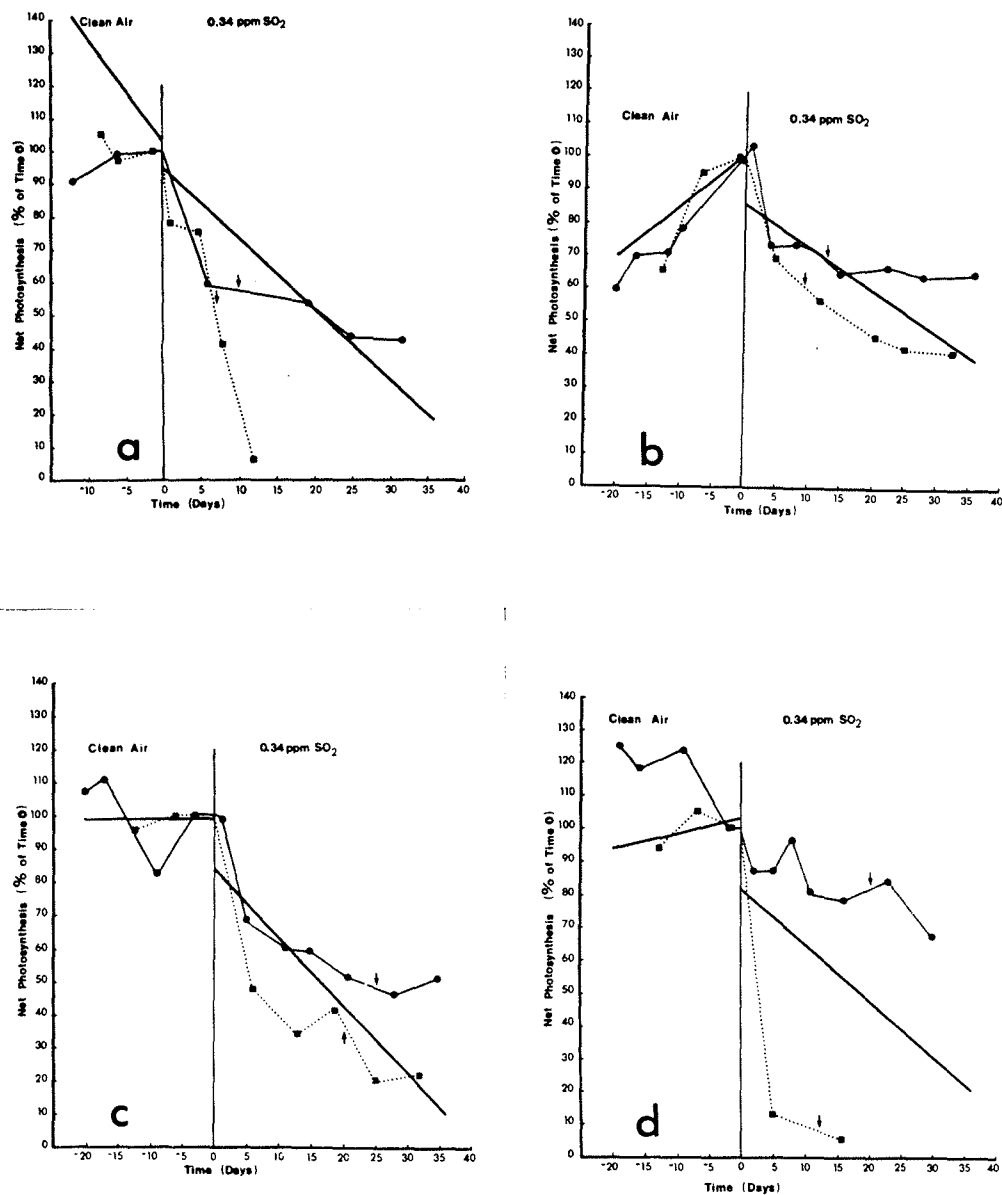


Figure 3. Maximum (.....), minimum (-----), and average (——) responses or relative net CO<sub>2</sub> assimilation rates of Labrador tea (a), jack pine (b), white spruce (c), and black spruce (d) both before and during fumigations with 0.34 ppm SO<sub>2</sub> under controlled conditions. Arrows indicate initiation of visual symptom development.

was actually increasing with time; in this case the increase appeared to be a result of growth of the specimens. All slopes of NAR versus time, after SO<sub>2</sub> was added, were significantly ( $p < 0.05$ ) different from zero, and all were negative.

Apparently, jack pine was the most resistant or least responsive species (Table 1), but if we consider that it had a significantly ( $p < 0.05$ ) positive slope before fumigation, the change in slope between prefumigation and fumigation was actually greater than for other conifers. This would then make jack pine the most sensitive conifer species studied, an observation that concurs with Dreisinger (1965), who studied the effect of air pollution on pine and spruce under field conditions. The position of green alder was also peculiar, since it was expected that its slope would be similar to those of other deciduous species rather than to those of the evergreens. It is felt that the large variability and few replicates were responsible for our inability to demonstrate a significant difference between green alder and the evergreen species.

Figure 3 shows the relative rate of decline of NAR with time for all species studied during fumigation with 0.34 ppm SO<sub>2</sub>. Actual reduction in NAR can be determined by comparing the relative rate of decline with the average NAR before fumigation (Table 2). It should be noted that all values of prefumigation NAR were within normal ranges (Sestak et al. 1971), hence all experiments were run on specimens that were metabolically active and normal.

### 3.2 VISUAL SYMPTOM RECORD

It was possible, through the use of photographs, to determine the time required for 0.34 ppm SO<sub>2</sub> to initiate visible injury to each plant species. The deciduous species developed symptoms of SO<sub>2</sub> injury much more rapidly than did evergreen species (Table 3). There was no significant ( $p < 0.05$ ) difference among the deciduous



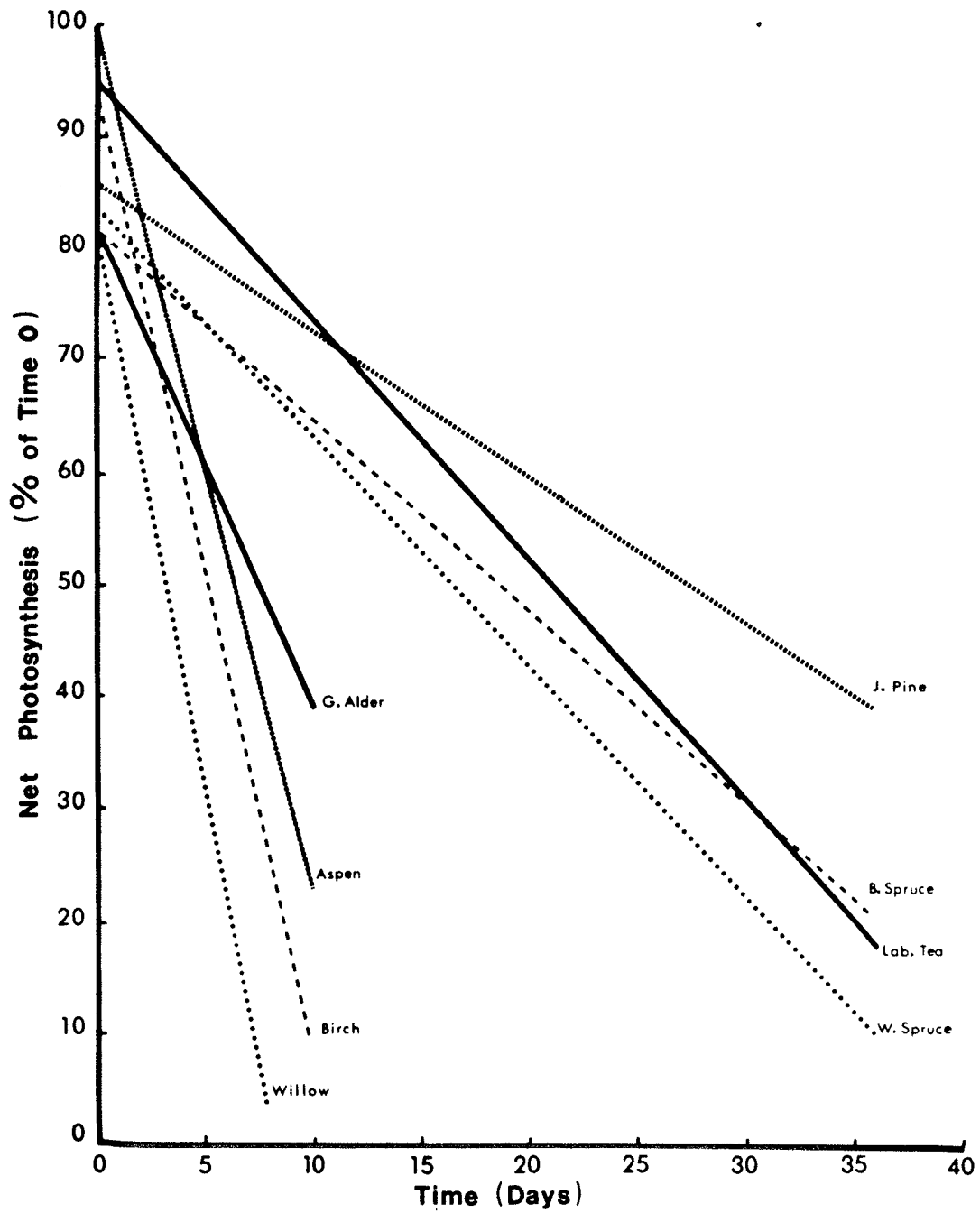


Figure 4. Regression lines of relative net CO<sub>2</sub> assimilation rate versus time of eight boreal forest plant species during fumigation with 0.34 ppm SO<sub>2</sub> under controlled conditions.

Table 2. Net CO<sub>2</sub> assimilation rate (mean ± 95% confidence limits) of several boreal forest plant species before fumigation with 0.34 ppm SO<sub>2</sub>.

Species	NAR mg CO <sub>2</sub> g <sup>-1</sup> hr <sup>-1</sup>
Willow	19.0 ± 11.7
Aspen	17.7 ± 5.5
Paper Birch	14.6 ± 2.8
Green Alder	13.7 ± 2.2
Labrador Tea	7.6 ± 5.0
Jack Pine	6.4 ± 1.5
Black Spruce	5.4 ± 0.8
White Spruce	5.0 ± 1.5

Table 3. Time (mean  $\pm$  95% confidence limits) required for visual symptom development during fumigation with 0.34 ppm SO<sub>2</sub>.

Species	Time (days)	
Willow	1.7 $\pm$ 1.4 <sup>a</sup>	               a
Green Alder	3.2 $\pm$ 1.4	
Aspen	4.3 $\pm$ 1.3	
Paper Birch	4.5 $\pm$ 1.7	
Labrador Tea	8.5 $\pm$ 2.8	
Jack Pine	10.6 $\pm$ 2.4	
Black Spruce	15.2 $\pm$ 5.4	
White Spruce	21.3 $\pm$ 4.0	

<sup>a</sup>Means that are joined by a vertical line are not significantly ( $p < 0.05$ ) different in a Student-Newman-Keuls test.

species themselves. Labrador tea was by far the most resistant angiosperm and was as resistant as the most sensitive conifer (jack pine). The two spruce species took the longest to develop symptoms.

The same pattern of response to  $\text{SO}_2$  was observed for both visual symptoms and physiological response. The four deciduous species responded similarly and were substantially more sensitive than either Labrador tea or the conifers. Black and white spruce were by far the most resistant plant species to  $\text{SO}_2$ . The most important difference between visual and physiological measures was in the speed of the response. It was possible to observe an NAR change before visual symptoms were evident. A reduction in NAR in response to air pollution without visual symptom development has also been observed under field conditions (Legge et al. 1978).

There was very little or no effect of  $\text{SO}_2$  fumigation on respiration, and no consistent pattern of response was observed in any species.

### 3.3 SULPHUR UPTAKE STUDIES

The rate of sulphur (S) uptake (Table 4) provided a third measure of the species with which to estimate plant threshold levels of  $\text{SO}_2$  fumigation. These data were not as accurate as either NAR or visual measures since the initial S concentration of the tissue had to be estimated from similar, but not identical, plant material. There was also a tremendous variability in final S content. Aspen S uptake rates were not significantly ( $p < 0.05$ ) different from the evergreens, whereas paper birch, green alder, and willow all had significantly higher uptake rates (Table 4) than the evergreens. It is expected that some of the differences between the physical uptake of sulphur and the biological responses was probably related to variation among species in their capability to assimilate  $\text{SO}_2$  by transformation of the gas into non-toxic forms. In intact plants, most of the labelled  $\text{SO}_2$  ( $^{35}\text{SO}_2$ ) has been shown to be converted into much less toxic species of  $^{35}\text{SO}_4$  (Garsed and Read 1977).

Table 4. Total sulphur uptake rates (mean  $\pm$  95% confidence limits) of several boreal forest plant species during fumigation with 0.34 ppm SO<sub>2</sub>.

Species	Uptake of Sulphur $\mu\text{g g}^{-1} \text{ day}^{-1}$	
Paper Birch	661 $\pm$ 241	a             
Green Alder	476 $\pm$ 168	
Willow	439 $\pm$ 233	
Labrador Tea	269 $\pm$ 173	
Aspen	263 $\pm$ 81	
White Spruce	146 $\pm$ 63	
Jack Pine	90 $\pm$ 40	
Black Spruce	82 $\pm$ 31	

<sup>a</sup>Means that are joined by a vertical line are not significantly ( $p < 0.05$ ) different in a Student-Newman-Keuls test.

In general, coniferous species were substantially more resistant to SO<sub>2</sub> than deciduous species. Labrador tea appeared to be intermediate in sensitivity. It is felt that a large amount of the difference in both visual and physiological sensitivity of these species was caused by differences in the ease of pollutant entry into the tissue. Conifers have higher stomatal resistances than angiosperm trees--(40 to 50 s·cm<sup>-1</sup> versus 5 to 10 s·cm<sup>-1</sup> (Gates 1966))--and these resistance differences were reflected in sulphur uptake rates (Table 4). Leaf resistances and the response of stomata to SO<sub>2</sub> are currently under investigation at this laboratory.

4. CONCLUSIONS

Sensitivity of woody boreal forest plant species to fumigation with high concentrations of  $\text{SO}_2$  (0.34 ppm Canadian Permissible 1 h Ground Level Concentration Standard) appeared to be related to the ease of entry of the pollutant rate into the leaves. Deciduous species such as willow, paper birch, green alder, and aspen all had similar physiological and visual sensitivities that were greater than either Labrador tea (an evergreen angiosperm) or any conifer studied. Jack pine was more sensitive than either black or white spruce. The information obtained from this study will be utilized in screening candidate revegetation species (oil sands area) for their tolerance to  $\text{SO}_2$ . Methods are currently available to measure both physiological and visual responses of plants to air pollutants in a short period of time. An intelligent decision on which revegetation species will be used can be made, therefore, without the use of long-term and expensive field trials.

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