Center for Air Environment Studies
The Pennsylvania State University

MASTER

1st Annual Progress Report

on

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LIKELIHOOD ESTIMATIONS OF VEGETATIVE ALTERATION NEAR KNOWN OR PROPOSED SOURCES OF AIR POLLUTION

to

U. S. Department of Energy

by

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CAES

DISCLAIMER

THE CENTER FOR AIR ENVIRONMENT STUDIES

Recognizing the interdisciplinary nature of problems involving the interaction of man and his air environment and the necessity for intercollege cooperation in efforts to seek solutions to these problems, The Pennsylvania State University established the Center for Air Environment Studies in 1963. Organized as a unit of the Institute for Science and Engineering within the Intercollege Research Programs and Facilities, it draws on the resources and talents of many departments. Faculty members affiliated with the CAES hold joint appointments with academic departments; such as, Engineering, Plant Pathology, Chemistry, Forestry, Environmental Health, Veterinary Science, and others. There are additional faculty members who are associated with the Center through research projects alone.

The Center for Air Environment Studies fosters intercollege and interdepartmental cooperation and maintains a broad, flexible approach to air pollution research. The nature and direction of this research - supported by federal and state governments, industry, and the University - reflects the personal interests and abilities of the faculty and staff and responds to known problem areas. Typical areas of research are: studies on the biological effects of air pollutants on animals and vegetation; development of species resistant to specific pollutants; studies of combustion processes leading to lower contaminant emissions; determination of best methods of collection and sampling devices; research into small particle behavior; fundamental techniques of particle control; and fundamental research on the chemistry, photochemistry, and atmospheric reactions of airborne contaminants.

Reflecting the demand for atmospheric quality and the need for personnel with varying degrees of education, training, and specialization; several air pollution training programs have been initiated at the University under the auspices of the Center. As an intercollege unit, the CAES does not grant degrees nor offer any courses of its own; however, air pollution courses are taught in many academic departments of the University by CAES associated faculty members. These courses are supported by the Center through the administration of student training funds and through the provision of graduate assistantships. In an effort to expand the offerings in the field, an interdisciplinary Environmental Pollution Control Master's Degree Program was established in 1971 by the Graduate School and a number of the degree candidates conduct research under the direction of faculty associated with the Center. In addition to resident instruction and in response to external requests, public service instructional programs have been initiated through the Center. One example is the Visible Emissions Training Program offered by the Center and coordinated with the Bureau of Air Pollution and Noise Control of the Pennsylvania Department of Environmental Resources. Since federal legislation mandates that smoke inspectors be certified at six month intervals for their ability to estimate visually the density of plumes emitted from stacks or vents, this Program serves as the certification procedure for Pennsylvania Department of Environmental Resources employees as well as for industrial, state, and federal personnel.

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"Likelihood Estimations of Vegetative Alteration Near Known or Proposed Sources of Air Pollution"

Prepared for Submission to

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I. Introduction

There is an increasing trend to locate fossil-fuel electric generating stations in rural areas. These stations emit large quantities of air pollutants capable of causing serious alterations to the surrounding environment. The major phytotoxic air pollutant emitted is sulfur dioxide (SO_2). In our proposition to the (now) Department of Energy we proposed that the best way to predict the potential impact of new or expanded sources in rural areas would be to develop a computer model to simulate and predict SO_2 injury to vegetation. The proposed simulator would provide valuable input information for tasks such as site selection and compilation of environmental impact statements. Such a model would also provide data to management operators for the regulation of emissions; e.g., the reduction of emissions during periods of high hazard conditions and the burning of higher sulfur fuels in low hazard situations. The model would also aid in our basic understanding of the complex interactions which influence plant susceptibility to air pollution. Data for model development and verification would be gleaned from existing literature and from controlled laboratory exposures. Input to the model would consist of biological and physical data and the output would include the probability of SO_2 injuring vegetation near existing or proposed sites. The model would be validated using a field situation. Details of the proposed research may be found in the original proposal.

This report presents the results obtained during the first year of the project (August 1, 1977 to July 31, 1978). Portions of the research were initiated prior to the actual receipt of the Department of Energy (D.O.E.) contract. Upon receipt of the D.O.E. contract, applicable studies were completed using D.O.E. funds and other proposed investigations were initiated.

II. Progress

During the first year our efforts were directed towards completing projects in progress, purchasing and calibrating needed equipment, and initiating proposed research. We are currently evaluating existing models which may be applicable to this project. Because all of the models require input regarding the influence of environmental variables on plant response to SO_2, we have concentrated in this area with our laboratory experiments. Output from the proposed simulation model will not be available until the project nears completion.

A. Predicted ground level SO_2 concentrations. As stated in the original proposal, the input data concerning ground level SO_2 concentrations will be either measured or predicted values. We have access to data from numerous SO_2 monitors in Pennsylvania. However, in order to predict damage around proposed sites, we also must be able to predict ground level SO_2 concentrations. For this reason we considered various diffusion models compatible with our objectives. We currently have a dispersal simulator operable and modified in a manner that its output will drive our computerized vegetation response model.

The atmospheric dispersion program, "STACK," was originally written by the Sun Oil Company and has been modified to run on the computer system at The Pennsylvania State University. STACK estimates 1-hour air pollutant concentrations downwind from a stack or group of stacks. The user has a choice of either the Holland or Moses-Carson plume rise equations and the Sutton or Pasquill-Gifford dispersion models. The program can be utilized to correlate existing pollutant concentrations downwind of a plant; evaluate the impact of new or expanded facilities on ground level concentrations; and predict the effect of pollutant concentrations resulting from the installation of air pollution control equipment. STACK is being incorporated into a computerized system to predict the pollutant damage to vegetation downwind from a source. The input data includes the number of stacks (up to 15), stack height, and diameter; exit gas temperature, velocity, and exit rate; emission rate for each stack; pollutant name and pollutant molecular weight. Weather data used to drive the model includes values for wind speed, wind direction, atmospheric stability class, and ambient air temperature. The desired plume rise and dispersion equations must also be specified.

The computer program outputs the pollutant concentrations downwind and crosswind from the emission source. For flat, level terrain the output consists of a grid of values from 1000 to 30,000 feet downwind of the stack(s) and up to 1250 feet on either side of the plume axis. For hillside terrain, up to 15 point concentrations can be calculated for specified downwind and crosswind distances and altitude. The output may also be plotted as pollutant concentrations vs. crosswind distance and pollutant concentrations vs. downwind distance. An example plot is attached (Fig. 1).

FORTRAN source code and Job Control Language were modified in order that the computer program could be operated at Penn State in conjunction with the Remote Job Entry (RJE) typewriter terminals. The RJE system allows the program and data to be stored on magnetic disk and permits a more flexible operation of STACK. A new plotting routine was added to the program which is compatible with the IBM 370/3330 computer used at this university.

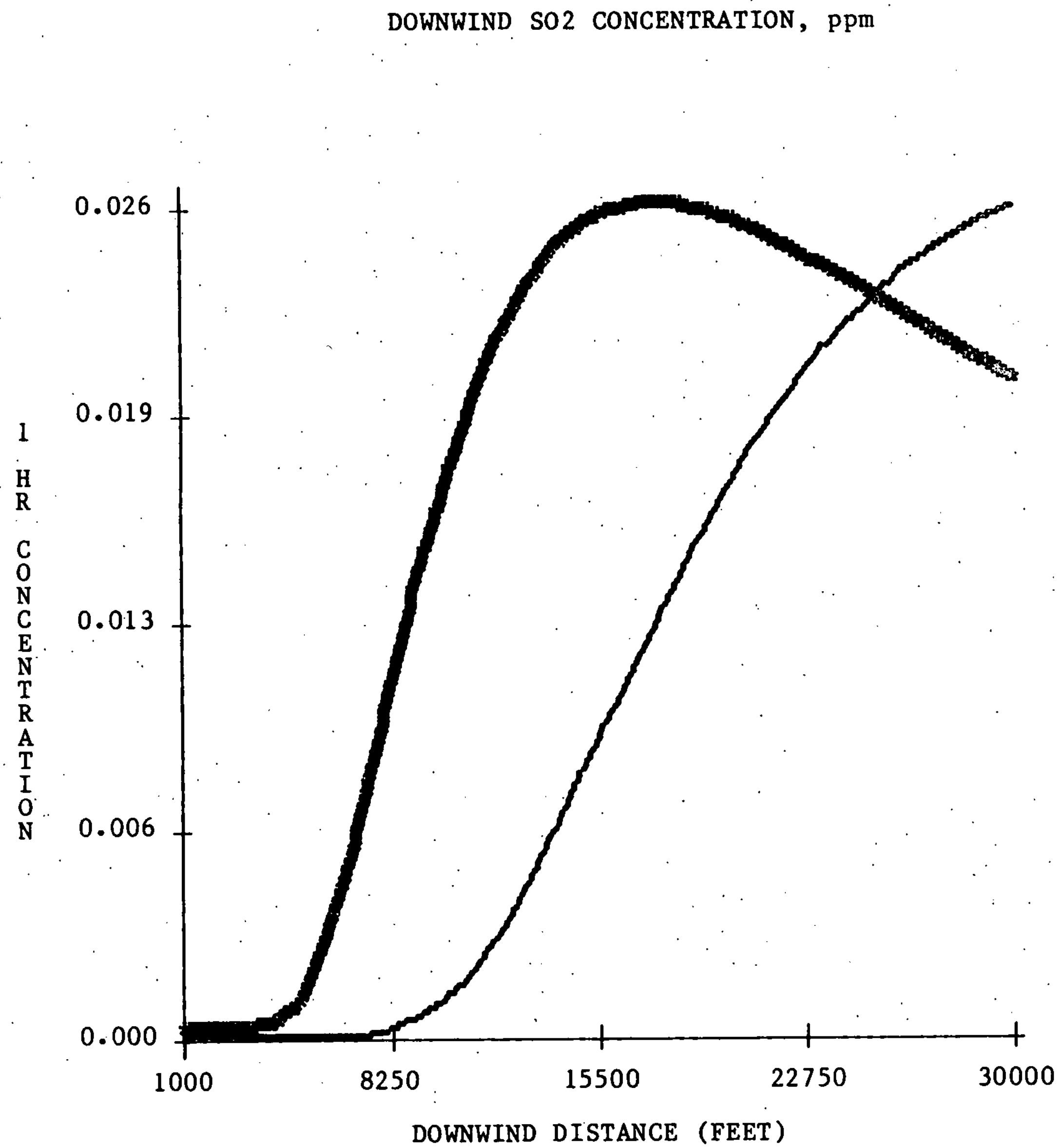

Fig. 1. Computer plot of SO_2 concentration <u>vs.</u> distance based on Sun Oil Company's STACK program.

... 3.0 MPH
*** 10.0 MPH

B. <u>Susceptibility of ten tree species to SO_2.</u> Results from this 3-year study will allow us to numerically rank various tree species, reflecting their susceptibility to SO_2. These rankings will be used within the outlined model. Equipment purchased with DOE funds is currently used for obtaining data to model the plant response to SO_2 and its alteration due to microclimatic influences.

In April 1977, 100 2- to 3-year old seedlings of each of ten tree species (1,000 plants total) were individually planted in plastic pots containing a 2:1 mixture of peat:perlite, fertilized and placed outdoors.

Two weeks after budbreak (May 23, 1977), and every alternate week thereafter for a total of 11 treatment periods, four individuals of each species were brought indoors and exposed to 2,358 $\mu g/m^3$ (0.9 ppm) SO_2 for 2 hr. at 22 C, 75% relative humidity (RH), and 25 klux light intensity in a fumigation chamber. The pollutant was injected into the chamber from a commercial tank of 100% SO_2. The SO_2 concentration within the exposure chamber was continuously sampled and measured with a pulsed fluorescence SO_2 analyzer. The monitor was periodically calibrated using certified SO_2 permeation tubes. After the desired SO_2 concentration was attained, seedlings were placed into the exposure chamber. The seedlings were watered in the early morning preceding each fumigation. After exposure the seedlings were moved to an adjacent controlled environment chamber maintained at 22 C, 75% RH, and 25 klux light intensity with a 12 hr. photoperiod.

Three days after exposure, symptoms were evaluated on each seedling by counting the number of injured leaves and determining the average percentage of leaf area injured. This latter measurement was estimated by using a 0 to 100% scale in increments of 10%; in addition, values of

1, 5, 95 and 99% were included at the extremes. Because the data were not normally distributed, statistical significance of differences among species are currently being evaluated with the non-parametric Mann-Whitney test.

Results

Symptoms. No visible injury was observed on susceptible conifers immediately after exposure; however, distinct areas of tissue injury were observed on the needle tips 72 hr. after exposure. The injury consisted of a reddish discoloration of the distal needle tissue, separated from the uninjured basal portion by a distinct line of demarcation.

River birch (*Betula nigra* L.) was the only broadleaved species to exhibit injury immediately upon removal from the exposure chamber. Foliage of this species exhibited an interveinal, water-soaked discoloration. After 72 hr., the water-soaking developed into brown bifacial necrosis. This symptom type was present on mature leaves of all susceptible broadleaved species; tissue damage rarely occurred on immature leaves. However, the color of the necrotic areas varied from plant to plant and among species. An exception was black cherry (*Prunus serotina* Ehrh.) which exhibited blotchy, undefined, water-soaked necrotic areas on the mature leaves.

Relative Susceptibility. There were distinct differences among the ten species in their relative susceptibility to SO_2 (Table 1). Based on the percentage of plants injured, river birch was most susceptible followed by European white birch (*B. pubescens* Ehrh.). Scotch pine (*Pinus sylvestris* L.), the remaining birch species, and hybrid poplar (Populus trichocarpa X P. maximowizii, Clone 388) were

Table 1. The relative susceptibility of seven broadleaved and three coniferous tree species exposed to 0.9 ppm SO_2 for 2 hours during the 1977 growing season.

Species	Number Plants Exposed	% Susceptible	Average Percent Leaves Injured	Average Percent Leaf Area Injured
River Birch *Betula nigra* L.	48	60	19	32
European White Birch *B. pubescens* Ehrh.	36	31	9	23
Sweet Birch *B. lenta* L.	44	27	6	13
Paper Birch *B. papyrifera* March.	44	20	9	22
Hybrid Poplar *Populus trichocarpa* X *P. maximowizii*, Clone 388	44	20	8	10
White Ash *Fraxinus americana* L.	44	7	13	10
Black Cherry *Prunus serotina* Ehrh.	44	4	2	10
Scotch Pine *Pinus sylvestris* L.	44	27	32	24
Eastern White Pine *P. strobus* L.	44	9	24	10
Austrian Pine *P. nigra* Arnold	44	2	1	30

next in susceptibility, followed by white ash (*Fraxinus american* L.) and eastern white pine (*P. strobus* L.). Black cherry and Austrian pine (*P. nigra* Arnold) were quite tolerant. River birch exhibited the highest percentage of individuals susceptible to SO_2, followed by European white birch, sweet birch (*B. lenta* L.), Scotch pine, paper birch (*B. papyrifera* March.), and hybrid poplar. Eastern white pine, white ash, black cherry and Austrian pine all had less than 10% injured individuals. Severity indices are currently being developed which utilize the percentage of leaves and leaf area injured by SO_2.

Influence of Age. Plants were sensitive to SO_2 for varying periods of time during the growing season, and the age at which sensitivity of the foliage was initiated varied with species (Table 2). All susceptible species were injured at some time during the 6th to 14th week of current growth. Exposure in the 14th week resulted in foliar injury on all species except eastern white pine and Austrian pine. The early sensitivity of Scotch pine during the 4th to 8th weeks contrasts with the later sensitivity of hybrid poplar, white ash, and black cherry. The four birches were sensitive throughout the entire growing period. River birch and paper birch were most sensitive however, in the 10th week and European white birch and sweet birch were most sensitive in the 14th week. River birch, sweet birch, European white birch, and hybrid poplar exhibited two sensitivity peaks, the first occurring between the 4th and 10th weeks and the second between the 14th and 16th weeks of growth.

Table 2. Relationship between current age of foliage and severity index of plants exposed to 0.9 ppm SO_2 for 2 hr.

Species	Age of Current Foliage (weeks)												Mean % Leaf Area Injured
	2	4	6	8	10	12	14	16	18	20	22	24	
River Birch	380[x]	0	25	191	3120	26	24	1188	0	1116	412	58	5.5 a[y]
Scotch Pine	0	2200	101	80	0	0	2	0	0	0	0	0	2.2 bcd
Paper Birch	9	0	371	34	345	0	30	0	0	166	0	–[z]	0.9 b
European White Birch	0	90	25	0	0	0	127	309	21	–	–	–	0.6 c
Sweet Birch	125	90	6	0	1	0	185	0	200	5	0	–	0.6 b
Hybrid Poplar	0	0	144	0	0	0	64	30	42	35	0	–	0.3 bcd
Eastern White Pine	0	240	0	0	0	0	0	0	0	0	0	–	0.2 cd
White Ash	0	0	0	0	0	0	165	45	0	0	0	–	0.2 cd
Black Cherry	0	0	0	0	0	0	80	0	0	0	0	–	0.1 d
Austrian Pine	0	0	0	0	0	38	0	0	0	0	0	–	0.0 d

[x]Severity index, based on an average of 4 individuals, = [(% plants susceptible) (% leaves injured) (% leaf area injured)] ÷ 100.

[y]Different letters nenote significances, p = 0.1.

[z]Indicates no exposure.

C. The influence of SO_2 on foliar sulfur content and stomatal conductance as related to visible foliar injury. An important sub-model within our predictive system is one which accounts for the ability of various plant species to absorb SO_2. Any influence of SO_2 on the stomatal conductance rate is of importance in uptake. Data for obtaining this relationship can be acquired by measuring the sulfur (S) content of leaf tissue before and after exposure to SO_2 and to a degree by measuring stomatal conductance (gas diffusion) rates. The objectives of this portion of the study were to determine whether differences in susceptibility among four birch species were related to leaf tissue S content, and to determine whether the amount of S accumulation within each species was influenced by stomatal response to SO_2.

Stomatal diffusion and percentage S measurements were taken prior to and following the previously described exposures to SO_2. Measurements were made on mature and immature leaves on paper birch, river birch, sweet birch, and European white birch. The data were obtained at 2 week intervals from mid-summer to mid-fall.

Pre-exposure diffusion porometer measurements were taken at 1100 hours, on two leaves on each of four trees of each species. Measurements were performed in a control chamber maintained at 22 C, 75% RH, and 25 klux light intensity. Two or three leaves from each plant were then removed for determination of pre-exposure S content. Immature and mature leaves were removed at the lamina-petiole junction, placed in a paper bag, and dried at 80 C for a minimum of 48 hr. Sulfur content was determined using a LECO sulfur analyzer connected to an automatic titration device.

Four plants of each birch species were exposed to 0.9 ppm (2,358 $\mu g/m^3$) SO_2 for 2 hr. at 22 C, 75% RH as described. Immediately following exposure, plants were removed to an adjacent chamber, maintained at 22 C and 75% RH, where conductance measurements were taken on the immature and mature leaves. Leaves were also removed at this time for S determination.

Measurements pertaining to tree phenology were taken to determine leaf expansion and stem elongation rates. Every two weeks during the season the average leaf and stem length of four plants of each of the ten species was recorded.

Data are currently being analyzed to determine, for each species, the significance of difference among: 1) pre- and post-exposure percent S of the immature and mature foliage and 2) pre- and post-exposure leaf conductance of the immature and mature foliage. Simple correlations were performed to determine if relationships existed between percent injury and leaf conductance, percent injury and S accumulation, and leaf conductance and S accumulation. Preliminary results are as follows:

Results

Phenology. Leaf size of all broadleaved species increased rapidly during the first eight weeks of the 1977 growing season. By mid-July leaf expansion had slowed considerably and by the first week of August expansion had ceased. Stem elongation for all broadleaved species had ceased by mid-August. River birch, sweet birch, European white birch, and white ash were approximately 100 cm in height whereas black cherry, hybrid poplar and paper birch had grown approximately 150 cm during the season. Leaf senescence and coloration, first recorded on 29 August 1977, were observed on all broadleaved species by the first week of September. Needles of coniferous species attained their maximum length by 1 August 1977. Stem elongation of coniferous species did not occur after the first week of July.

Foliar S Content. Non-exposed plants of all four birch species attained greatest foliar S content at 8 to 10 wk after leaf emergence (Figs. 2-5). For the remainder of the season, S content declined significantly ($p = 0.1$), except for mature foliage of river birch and paper birch, then remained fairly constant until leaf senescence. The leaves of river birch exhibited a slight, but significant, increase in S content in late September and early October.

Differences in pre-exposure S content between mature and immature leaves were occasionally significant earlier in the season, although no regular pattern could be discerned. In general, the immature foliage maintained a higher S content than did mature, fully developed leaves.

The seasonal mean S content for each species was determined by summing the weekly foliar S content for the entire season and dividing by the number of exposures. Significant differences in the total seasonal average S content among species were observed (Table 3). Immature foliage of river birch, European white birch, and paper birch maintained the highest seasonal mean S content, whereas the mean S content of immature sweet birch foliage was consistently the lowest. Mature foliage of river birch and European white birch maintained a higher seasonal mean S content than mature leaves of paper birch which, in turn, maintained a higher seasonal mean S content than those of sweet birch. Sweet birch and paper birch were the only species to exhibit a significant difference in seasonal mean percent S between immature and mature foliage prior to exposure.

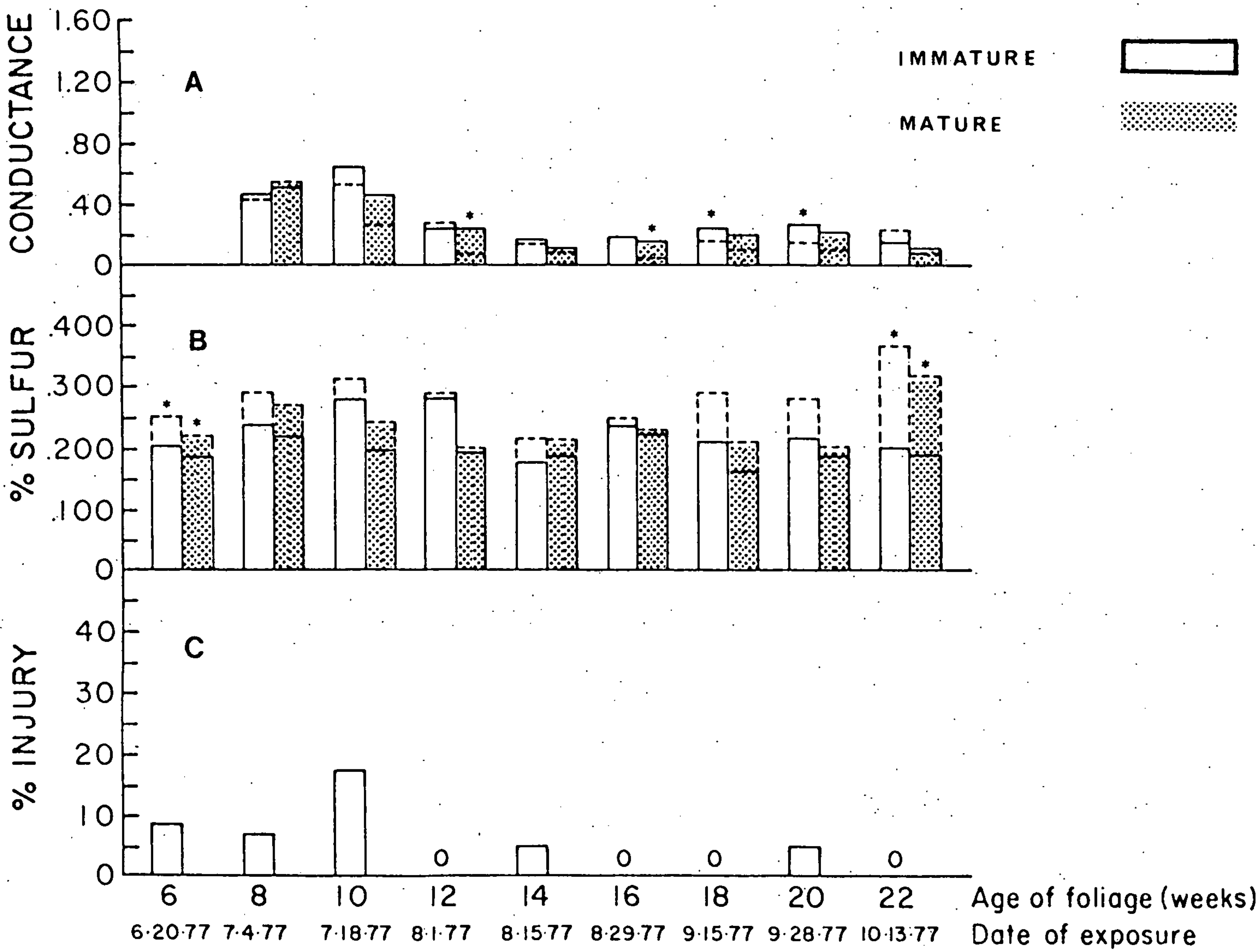

Fig. 2. Leaf conductance (A), percent S content (B), and percent foliar injury (C) of paper birch seedlings exposed to 0.9 ppm SO_2 for 2 hrs. Conductance values and percent S are shown before (solid line) and after (dotted line) exposure, for both immature and mature leaves. The * indicates significant differences between pre-exposure and post-exposure values at $p = .05$. All values are an average of four determinations.

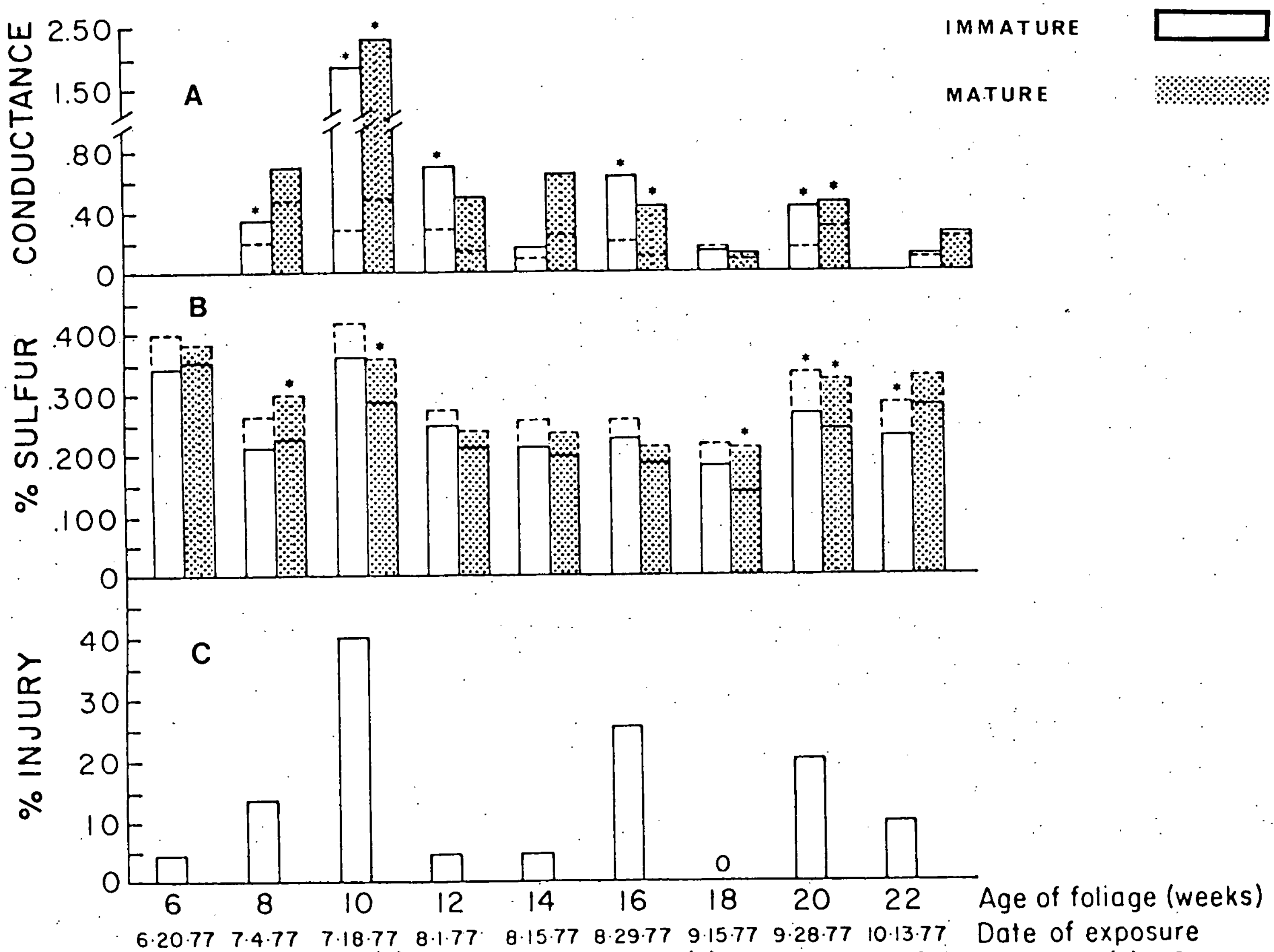

Fig. 3. Leaf conductance (A), percent S content (B), and percent foliar injury (C) of river birch seedlings exposed to 0.9 ppm SO_2 for 2 hrs. Conductance values and percent S are shown before (solid line) and after (dotted line) exposure, for both immature and mature leaves. The * indicates significant differences between pre-exposure and post-exposure values at p = .05. All values are an average of four determinations.

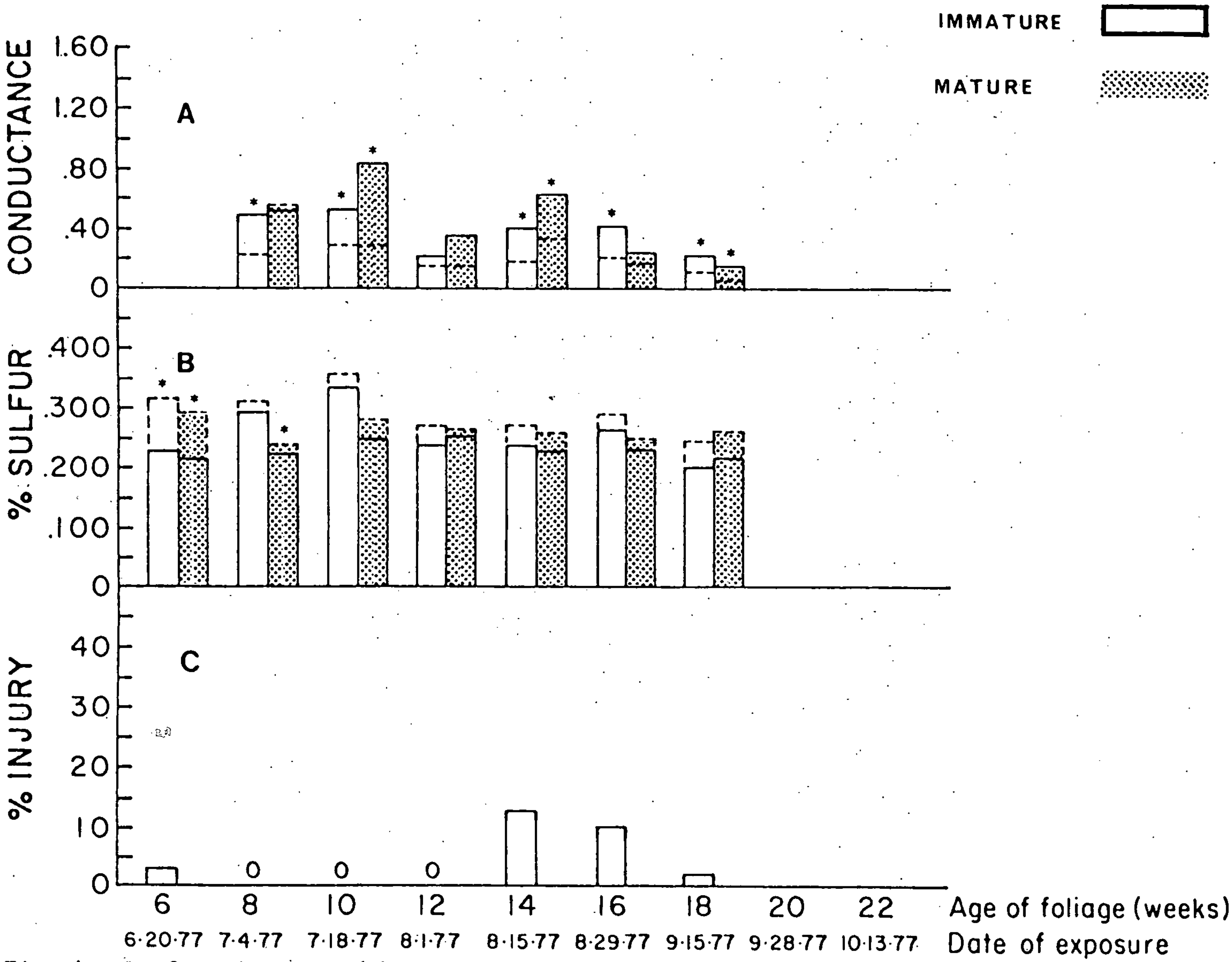

Fig. 4. Leaf conductance (A), percent S content (B), and percent foliar injury (C) of European white birch seedlings exposed to 0.9 ppm SO_2 for 2 hrs. Conductance values and percent S are shown before (solid line) and after (dotted line) exposure, for both immature and mature leaves. The * indicates significant differences between pre-exposure and post-exposure values at p = .05. All values are an average of four determinations.

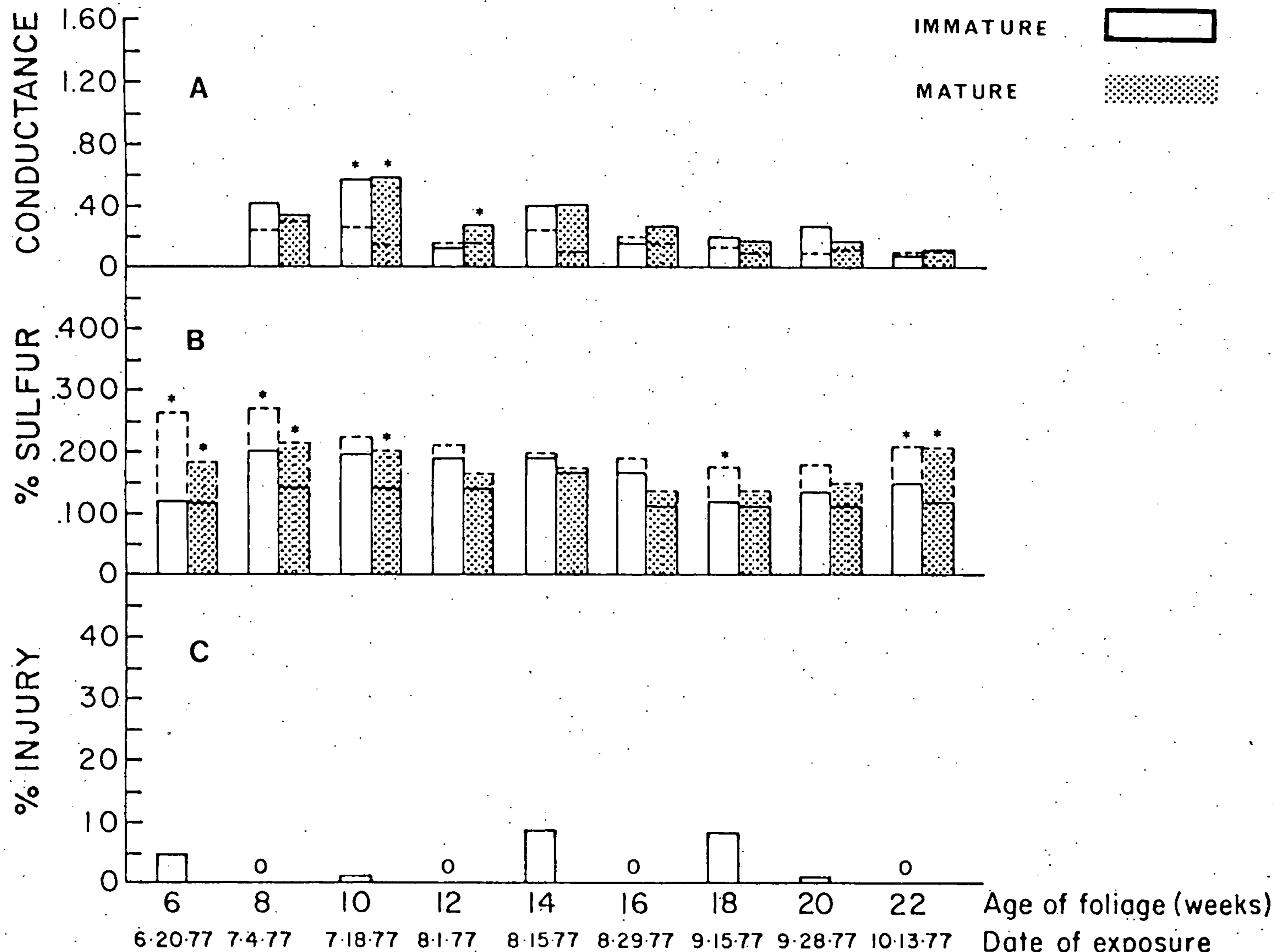

Fig. 5. Leaf conductance (A), percent S content (B), and percent foliar injury (C) of sweet birch seedlings exposed to 0.9 ppm SO_2 for 2 hrs. Conductance values and percent S are shown before (solid line) and after (dotted line) exposure, for both immature and mature leaves. The * indicates significant differences between pre-exposure and post-exposure values at $p = .05$. All values are an average of four determinations.

Table 3. Average percent S content of immature and mature foliage of four birch species before and after exposure to 0.9 ppm SO_2 for 2 hr.[x]

Species	Immature Leaves		Mature Leaves	
	Before Exposure	After Exposure	Before Exposure	After Exposure
River Birch	0.25 a[y] 1[z]	0.30 a 2	0.24 a 1	0.28 a 1 2
European White Birch	0.26 a 1	0.28 a 1	0.23 a 1	0.25 ab1
Paper Birch	0.22 a 1	0.28 a 2	0.19 b 3	0.23 b 1
Sweet Birch	0.16 b 1	0.21 b 2	0.13 c 3	0.17 c 3

[x] Each value is based on 32 samples collected over 16 wks except values for European white birch which are based on 24 samples collected over 12 weeks.

[y] Different letters in columns denote significant differences according to Duncan's new multiple range test difference, $p = 0.10$.

[z] Different numbers in rows denote significant differences according to Duncan's new multiple range test, $p = 0.10$.

Average seasonal S content differences among the four species following exposure to SO_2 were the same as the differences prior to exposure. After exposure, the immature foliage of sweet birch maintained significantly less S than the three remaining species (Table 3). The mature foliage of river birch attained a significantly higher average percent S than did paper birch or European white birch. All three species, in turn, showed significantly greater percent S in the mature tissue than sweet birch.

All four species exhibited high S accumulations following exposure when the foliage was 6 and 8 wks old and again at 20 and 22 wks (Fig. 2-5). There were no significant differences in S accumulation between the mature and immature foliage of any species.

Leaf Conductance. Prior to exposure, all four birch species attained their highest conductance rates at 10 wks after leaf emergence (Figs. 2-5). After the tenth wk, conductance rates of immature and mature foliage declined for the remainder of the season. Differences in pre-exposure leaf conductance rates between immature and mature leaves were occasionally significant although no regular pattern was evident. The mature leaves of river birch, European white birch, and sweet birch occasionally exhibited higher leaf conductance values than their respective immature leaves, but no seasonal pattern could be discerned. Following exposure to SO_2, the seasonal mean leaf conductance of the immature foliage of all four birch species were not different. The seasonal mean leaf conductance for the mature foliage of river birch, although not different from that of European white birch, remained significantly higher than the conductance of both paper birch and sweet birch (Table 4).

Table 4. Average leaf conductance of immature and mature foliage of four birch species before and after exposure to 0.9 ppm SO_2 for 2 hr.[x]

Species	Immature Leaves		Mature Leaves	
	Before Exposure	After Exposure	Before Exposure	After Exposure
River Birch	0.41 a[y] 1[z]	0.18 a 2	0.68 a 1	0.24 a 2
European White Birch	0.35 a 1	0.19 a 2	0.45 ab1	0.20 ab 2
Paper Birch	0.26 a 1	0.21 a12	0.20 b12	0.10 b 2
Sweet Birch	0.25 a 1	0.17 a1	0.28 b1	0.13 b1

[x] Each value is based on 32 samples collected over 16 weeks except values for European white birch which are based on 24 samples collected over 12 weeks.

[y] Different letters in columns denote significant differences according to Duncan's new multiple range test, p = .05.

[z] Different numbers in rows denote significant differences according to Duncan's new multiple range test, p = .05.

The seasonal mean leaf conductance value of immature foliage of river birch and European white birch declined significantly as a result of exposure to SO_2, exhibiting declines in leaf conductance of 62.8%, and 47.7%, respectively. Immature foliage of sweet birch and paper birch did not exhibit significant decreases in conductance rate. The immature foliage of the above species showed significant declines in leaf conductance of 59.1%, 44.5%, 48.3%, and 28.6% respectively. The differences in seasonal mean leaf conductance between immature and mature foliage of river birch were significant both before and after exposure to SO_2.

Correlation Among Foliar Injury, Leaf Conductance, and S Accumulation. The percent foliar injury and pre-exposure leaf conductances of both the immature and mature foliage of river birch and paper birch were significantly correlated (Table 5). The correlation coefficients for European white birch and sweet birch were near zero and were not significant. No significant correlation was obtained when the leaf conductance values were correlated against their respective percent S accumulation values.

D. The stomatal response of three birch species exposed to varying doses of SO_2. To more accurately determine a species' capacity to take up SO_2, or to close its stomata in the presence of SO_2, we must understand how the plant reacts to varying doses of the pollutant. The objective of this experiment was to determine the foliar conductance rates of three birch species as influenced by four SO_2 concentrations, with exposure times varying from 1 to 4 hours.

Beginning 4 October 1977, and every third week thereafter until 1 December 1977, seeds of European white birch, gray birch, and yellow birch were surface sown on a 2:1 mixture of peat:perlite and placed under

Table 5. Correlation coefficients for the correlation among foliar injury and pre-exposure leaf conductance.[z]

Species	Immature Leaves	Mature Leaves
River Birch	0.594 *	0.628 *
Paper Birch	0.638 *	0.454 *
European White Birch	0.120	-0.199
Sweet Birch	0.171	0.176

[z]Correlations derived from 32 injury values and pre-exposure conductance values recorded over 16 weeks. Values for European white birch were derived from 24 values recorded over 12 weeks.

*Indicates significant r value, p = .01.

a clear plastic covering on a greenhouse bench until germination. Approximately 12 wks following seed germination, 120 individuals of each species of uniform development were transplanted, one per cell, into Ball cell placks containing a 2:1 peat:perlite mixture. Approximately 3 wks after transplanting, each plant received 2 g of slow-release 14-14-14 (N-P-K) fertilizer. The transplanted seedlings were subsequently grown for 10 additional wks in the greenhouse with natural daylight supplemented with fluorescent lighting of 6.4 klux intensity applied for 16 hrs each day, beginning at 0600 hours. Malathion was applied weekly to control white flies.

Approximately 26 weeks, after germination, the plants were acclimated by moving them from the greenhouse into the exposure chamber at least 18 hr before each exposure. The seedlings were exposed to 0.3, 0.6, 0.9, or 1.2 ppm SO_2 at 25 C and 75% RH, for 1, 2, 3, or 4 hr. Leaf conductance measurements of 10 plants, (8 plants for yellow birch) were taken at 0800, 0900, and 1000 hours, prior to exposure to SO_2 (baseline measurements). Exposure were initiated at 1030 hours. Measurements were taken immediately following 1, 2, 3 and 4 hr of exposure. Each exposure was repeated twice. Unless otherwise stated, all measurements of conductance were made on the abaxial surface of the fifth leaf from the plant apex. The measurement was taken on the widest part of the lamina, between the margin and the mid-rib. Seventy-two hours after exposure, the percentage of leaf tissue injured by SO_2 was rated visually. To facilitate interpretation of the data, a median baseline conductance rate for each treatment was compared to a median value obtained either 1, 2, 3, or 4 hrs after exposure to SO_2.

Results

European White Birch. After 1 and 2 hrs exposure to 0.3 ppm SO_2, the median conductance rate of European White Birch increased 213 and 82%, respectively, compared to the baseline median. After 3 and 4 hrs exposure, decreases in the median conductance rates of 62 and 71% were apparent (Table 6).

Exposure to 0.6 ppm SO_2 resulted in a 5% increase in conductance after 2 hr exposure and a 2% decrease in conductance after 3 hr exposure. After 4 hr exposure, however, the conductance rate decreased 51% compared to the pre-exposure baseline.

Decreases in conductance rates were also apparent after exposure to 0.9 and 1.2 ppm SO_2. Exposure to 0.9 ppm SO_2 decreased conductance rates by 38, 56, 40, and 39% after 1, 2, 3, and 4 hr exposure, respectively. Exposure to 1.2 ppm SO_2 induced more pronounced decreases in conductance than previous exposures. Reductions of 58, 43, 56, and 75% were observed after 1, 2, 3, and 4 hr exposure, respectively, to 1.2 ppm SO_2.

Seventy-two hr after exposure, visible foliar injury occurred after the following exposures to SO_2: 0.6 ppm for 4 hr, 0.9 ppm for 2 hr, and 1.2 ppm for 2 hr (Table 7).

Gray Birch. Unlike European white birch, the conductance rate of gray birch seedlings did not increase following exposure to 0.3 ppm SO_2. Decreases in conductance rates of 22, 39, 12, and 52%, when compared to the baseline, occurred after the 1, 2, 3, and 4 hr exposures, respectively (Table 8).

Table 6. Median conductance (cm sec^{-1}) values for foliage of European white birch seedlings prior to exposure to various doses of SO_2 and the percent change in conductance after 1, 2, 3 and 4 hrs exposure to 0.3, 0.6, 0.9, or 1.2 ppm SO_2.

Date of Exposure	Conc. SO_2 (ppm)	Baseline Median	Hrs of Exposure 1	2	3	4
			% Change			
5-5, 5-6-78	0.3	1.83[w]	+213[xy]	+82	-62	-71
5-9, 5-10-78	0.6	3.70	**[z]	+ 5	- 2	-51
5-16, 5-17-78	0.9	2.11	- 38	-56	-40	-39
4-14, 4-15-78	1.2	0.53	- 58	-43	-56	-75

[w]All baseline medians were derived from conductance measurements taken on 3 random samples of 10 plants, at 0800, 0900 and 1000 hours, repeated 2 times, for a total of 60 measurements.

[x]All treatment values were derived from conductance measurements taken on 10 different plants at 1130, 1230, 1330 and 1430 hours, after 1, 2, 3, or 4 hr exposure to SO_2, repeated twice, for a total of 20 measurements at each dose.

[y]Percent change = [((median treatment conductance rate ÷ median baseline conductance rate) x 100)-100].

[z]** Indicates no reading.

Table 7. Average percent plants, leaves, and leaf area injured of European white birch and gray birch seedlings following exposure to various doses of SO_2.

	European White Birch			Gray Birch		
Concentration/ Time	% Plants Injured	% Leaves Injured	% Leaf Area Injured on Symptomatic Leaves	% Plants Injured	% Leaves Injured	% Leaf Area Injured on Symptomatic Leaves
<u>0.6 ppm</u>						
4 hr	35	12.1	11.1	0	0	0
<u>0.9 ppm</u>						
2 hr	5	5.9	10.0	0	0	0
3 hr	30	14.3	19.2	5	45.4	80
4 hr	60	14.3	7.8	25	17.3	5.4
<u>1.2 ppm</u>						
2 hr	5	13.3	1.0	0	0	0
3 hr	20	19.8	13.0	10	17.7	1.0
4 hr	35	26.7	39.2	15	10.3	1.0

Table 8. Median conductance (cm sec^{-1}) values for foliage of gray birch seedlings prior to exposure to various doses of SO_2, and the percent change, in conductance after 1, 2, 3, and 4 hrs exposure to either 0.3, 0.6, 0.9, or 1.2 ppm SO_2.

Date of Exposure	Conc. SO_2 (ppm)	Baseline Median	% Hrs of Exposure			
			1	2	3	4
			% Change			
5-2, 5-3-78	0.3	0.70[w]	- 22[wy]	-39	-12	-52
5-9, 5-10-78	0.6	2.26	+248	**[z]	+35	-33
5-16, 5-17-78	0.9	1.49	0	-33	-51	-63
4-18, 4-19-78	1.2	0.37	- 50	-58	-65	-63

[w]All baseline medians were derived from conductance measurements taken on 3 random samples of 10 plants, at 0800, 0900, and 1000 hours, repeated 2 times, for a total of 60 measurements.

[x]All treatment values were derived from conductance measurements taken on 10 plants at 1130, 1230, and 1430 hours, after 1, 2, 3, and 4 hr exposure to SO_2, repeated 2 times, for a total of 20 measurements.

[y]Percent change = [((median treatment conductance rate ÷ median baseline conductance rate) x 100)-100].

[z]** Indicates no reading.

Increases in the conductance rate of gray birch seedlings were apparent after exposure to 0.6 ppm SO_2 for 1 and 3 hr. After 1 hr exposure the conductance rate increased 248% compared to the baseline, and, after 3 hr, the conductance rate increased 35%; a 4 hr exposure reduced the conductance rate by 33%.

One hr exposure to 0.9 ppm SO_2 resulted in no noticeable change in conductance rate of the gray birch seedlings. Continued exposure for 2, 3, and 4 hr was characterized by a steady decline in conductance rates from 33 to 63% of the baseline median conductance rate.

At the 1.2 ppm SO_2 concentration, examination of the conductance rate after 1 hr revealed 50% decrease in conductance when compared to the baseline median. Continued exposure resulted in decreased rates of 58, 65, and 63% after 2, 3, and 4 hr, respectively.

Yellow Birch. Yellow birch seedlings displayed increases in stomatal conductance of 24 and 90% after exposure to 0.3 ppm SO_2 for 1 and 2 hr respectively (Table 9). Additional exposure for 3 and 4 hr resulted in a net decrease in conductance of 65 and 48%, respectively.

Exposure of yellow birch seedlings to 0.6, 0.9, and 1.2 ppm SO_2 for 1 hr were followed by reduced conductance rates of 33 to 38% when compared to the baseline median. After 2 hr exposure to 0.6, 0.9, and 1.2 ppm SO_2, conductance rates continued to decline until, after 4 hr, net decreases in conductance rates were 58, 63 and 58%, respectively.

In summary, there was too much variation in the data to make definite conclusions in this area. However, we feel stomatal conductance increases following exposure to relatively low SO_2 concentrations and

Table 9. Median conductance (cm sec^{-1}) values for foliage of yellow birch seedlings prior to exposure to various doses of SO_2, and the percent change in conductance after 1, 2, 3, and 4 hrs exposure to either 0.3, 0.6, 0.9, or 1.2 ppm SO_2.

Date of Exposure	Conc. SO_2 (ppm)	Baseline Median	Hrs of Exposure 1	2	3	4
			% Change			
5-5, 5-6-78	0.3	1.34[x]	+24[yz]	+90	-65	-48
5-12, 5-13-78	0.6	0.52	-33	-45	-43	-58
5-19, 5-20-78	0.9	0.78	-38	-42	-47	-63
4-18, 4-19-78	1.2	0.14	-35	-23	-37	-58

[x]All baseline medians were derived from conductance measurements taken on 3 random samples of 10 plants, at 0800, 0900, and 1000 hours, repeated 2 times, for a total of 60 measurements.

[y]All treatment values were derived from conductance measurements taken on 10 plants at 1130, 1230, 1330, and 1430 hours, after 1, 2, 3, and 4 hr exposure to SO_2, repeated 2 times, for a total of 20 measurements of each dose.

[z]Percent change = [((median treatment conductance rate ÷ median baseline conductance rate) x 100)-100].

decreases in response to higher concentrations. We plan to repeat this study during the fall of 1978 and winter of 1978-1979. Based on our findings in this study, we should be able to reduce the variation in the data considerably.

E. The influence of exposure temperature on plant response to mixtures of SO_2 and ozone. Exposure temperature has proven to be a major factor influencing plant response to individual air pollutants. We have recently determined the influence of temperature on plant response to SO_2. Temperature will likely be an important input variable in our model. How exposure temperature may affect plant response to a pollutant mixture has not been reported. The objective of this study was to evaluate the influence of exposure temperature on the intensity and character of the macroscopic foliar response of pinto bean (*Phaseolus vulgaris* L. 'Pinto 111') to a mixture of SO_2 and ozone. This plant species was chosen as a model plant based on its known susceptibility to both pollutants and its ease of culture.

Pinto bean seeds were germinated in trays of vermiculite within controlled environment chambers maintained at 24 C and 75% RH. Two days following cotyledon emergence, seedlings of uniform development were transplanted, one per pot, into 950 cc plastic pots containing a 1:1:1 (v/v) peat:perlite:soil mixture. The transplanted seedlings were grown for 5 days in controlled environment chambers at the aforementioned conditions, with a 12-hr. photoperiod of 25 klux beginning at 0600 hours. The plants were fertilized every other day with one-half strength water soluble fertilizer (N20-P19-K18).

Plants were exposed for 3 consecutive hours to either 0.8 ppm (2090 $\mu g/m^3$ @ 24 C) SO_2 alone, 0.25 ppm (490 $\mu g/m^3$ @ 24 C) O_3 alone, or a mixture of O_3 and SO_2 at the aforementioned concentrations at exposure temperatures of 15, 24, or 32 C and 75% RH. Two sets of 15 plants each were exposed to either 0.8 ppm SO_2 or 0.25 ppm O_3 at each exposure temperature. Six sets of 15 plants each were exposed to the combined pollutants at each exposure temperature. This design resulted in a total of 90 plants exposed to O_3 alone, 90 plants exposed to SO_2 alone, and 270 plants exposed to the mixture of the pollutants. Ozone concentration was monitored continuously during the exposures with a chemiluminescent O_3 monitor calibrated using the 1% buffered KI technique. Sulfur dioxide was monitored continuously using a pulsed fluorescent SO_2 analyzer calibrated with SO_2 permeation tubes.

Following exposure, the plants were placed in a controlled environment chamber operating at the previously described pre-exposure conditions. Three to 5 days later, the percentage of visible foliar injury was estimated on the adaxial and abaxial surfaces of the unifoliolate leaves using a 0 to 100% scale in 10% increments with values of 1, 5, 95, and 99% included at the extremes. The arcsin of the square root transformation was performed on the data to equalize the variances. An analysis of variance (AOV) was performed on the transformed data for the adaxial and abaxial leaf surfaces respectively. The adaxial and abaxial treatment means were averaged to determine treatment means for the combined leaf surfaces. An AOV was also performed on these values. The statistical significance of differences between treatment means was determined using Fischer's Least Significant Difference Test, $p = 0.05$. The interaction of the combined pollutants was evaluated statistically by contrast analysis.

RESULTS

Foliage injured by O_3 alone exhibited an interveinal, chlorotic stipple on the adaxial leaf surface. In addition, a less prevalent reddish-brown, interveinal stipple on the adaxial leaf surface was also induced. Leaves on plants exposed to SO_2 alone developed a light tan, interveinal and/or marginal bifacial necrosis. At 32 C, leaves exposed to SO_2 and O_3 exhibited injury resembling that elicited by the latter gas; at 15 C, SO_2 type symptoms predominated. Both symptom-types were apparent at 24 C. Exposure to the combined pollutants resulted in two distinct abaxial symptom-types. The first and most prevalent was a tan interveinal necrosis associated with bifacial injury induced by SO_2. The second symptom-type occurred in conjunction with the first and consisted of a glazing or silvering of the abaxial leaf surface. The latter symptom-type occurred more often following exposures at 15 and 24 C, than at 32 C.

Foliar sensitivity to the individual pollutants was significantly greater at the extreme exposure temperatures of 15 and 32 C, than at 24 C (Table 10). Foliar sensitivity to O_3 alone was greatest at 15 C, whereas foliar sensitivity to SO_2 alone was greatest at 32 C. The combined pollutants caused significantly greater injury on the adaxial leaf surface at 15C than at 24 or 32 C. Injury levels at the latter two temperatures were not significantly different from each other. Exposure to the combined pollutants caused significantly greater injury on the abaxial leaf surface at 15 C than at 24 C, which in turn was significantly greater than that induced at 32 C (Table 11). The combined pollutants caused significantly greater injury to the combined leaf surfaces at 15 C than at 24 or 32 C (Table 12). Injury levels at the latter two temperatures were not significantly different from each other.

Table 10. Visual evaluation of the percentages of foliar injury on the adaxial surface of pinto bean unifoliolate leaves induced by 3-hr exposures to either 0.25 ppm (490 μg/m^3 @ 24C) O_3, 0.8 ppm (2090 μg/m^3 @ 24C) SO_2 or the two pollutants combined at these concentrations at exposure temperatures of 15, 24, or 32 C.

	Temp (C)		
Pollutant	15	24	32
O_3	53.5[w] a[x]	11.5 b	45.1 c
SO_2	32.2[w] a	0.0 b	39.9 c
O_3+SO_2	70.7[y] a	44.3 b	45.9 b
Response	LESS[z] THAN ADDITIVE	MORE[z] THAN ADDITIVE	LESS[z] THAN ADDITIVE

[w]The mean of the percentage of visible foliar injury based on 15 plants per exposure and two repetitions of the exposure.

[x]Means followed by different letters in a row are significantly different at P = 0.05.

[y]The mean of the percentage of visible foliar injury based on 15 plants per exposure and 6 repetitions of the esposure.

[z]LESS THAN ADDITIVE = sum of the % injury induced by each pollutant alone was statistically less (P = 0.05) than the sum of % injury induced by the combined pollutants.

MORE THAN ADDITIVE = sum of % injury induced by each pollutant alone was statistically more (P = 0.05) than the sum of % injury induced by the combined pollutants.

Table 11. Visual evaluation of the percentages of foliar injury on the <u>abaxial</u> surface of pinto bean unifoliolate leaves induced by 3-hr exposures to either 0.25 ppm (490 μg/m^3 @ 24C) O_3, 0.8 ppm (2090 μg/m^3 @ 24C) SO_2 or the two pollutants combined at these concentrations at exposure temperatures of 15, 24, or 32C.

	Temp (C)		
Pollutant	15	24	32
O_3	0.0[w] a[x]	0.0 a	0.0 a
SO_2	32.2[w] a	0.0 b	39.9 c
O_3+SO_2	88.5[y] a	31.6 b	14.6 c
Response	MORE[z] THAN ADDITIVE	MORE[z] THAN ADDITIVE	LESS[z] THAN ADDITIVE

[w]The mean of the percentage of visible foliar injury based on 15 plants per exposure and two repetitions of the exposure.

[x]Means followed by different letters in a row are significantly different at P = 0.05.

[y]The mean of the percentage of visible foliar injury based on 15 plants per exposure and 6 repetitions of the exposure.

[z]LESS THAN ADDITIVE = sum of the % injury induced by each pollutant alone was statistically less (P = 0.05) than the sum of % injury induced by the combined pollutants.

MORE THAN ADDITIVE = sum of % injury induced by each pollutant alone was statistically more (P = 0.05) than the sum of % injury induced by the combined pollutants.

Table 12. Visual evaluation of the percentages of foliar injury on the <u>combined</u> leaf surfaces of pinto bean unifoliolate leaves induced by 3-hr exposures to either 0.25 ppm (490 $\mu g/m^3$ @ 24C) O_3, 0.8 ppm (2090 $\mu g/m^3$ @ 24C) SO_2 or the two pollutants combined at these concentrations at exposure temperatures of 15, 24, or 32C.

	Temp (C)		
Pollutant	15	24	32
O_3	26.8[x] a[y]	5.8 a	22.6 a
SO_2	32.2 a	0.0 b	39.9 a
O_3+SO_2	79.6 a	37.9 b	30.3 b
Response	MORE[z] THAN ADDITIVE	MORE[z] THAN ADDITIVE	LESS[z] THAN ADDITIVE

[x]The average of the means of the percentage of visible foliar injury for both the adaxial and abaxial leaf surfaces.

[y]Means followed by different letters in a row are significantly different at P = 0.05.

[z]LESS THAN ADDITIVE = sum of the % injury induced by each pollutant alone was statistically less (P = 0.05) than the sum of % injury induced by the combined pollutants.

MORE THAN ADDITIVE = sum of % injury induced by each pollutant alone was statistically more (P = 0.05) than the sum of % injury induced by the combined pollutants.

The adaxial leaf surface response to the combined pollutants was antagonistic (less than additive) at 15 and 32 C and synergistic (more than additive) at 24 C. The abaxial leaf surface response and the combined leaf surface response were synergistic at 15 and 24 C and antagonistic at 32 C.

III. Additional Investigations to be Conducted

The simulator for predicting ground level SO_2 concentrations will be verified more thoroughly and analyzed for its sensitivity to each input parameter. Validation of the SO_2 simulator will include data analysis of simulated values and concentrations monitored under actual field conditions.

The data for the individual studies presented in Section II must be further analyzed, compared with existing literature reports, and integrated with the results of previously conducted studies. It will be necessary to repeat those portions of the studies reported herein which exhibit much variation in the recorded data. Replication of these studies may either reduce the variation or identify additional variables which may have an effect on the systems response.

We are currently initiating studies to obtain the required data to model the influence of soil moisture on plant susceptibility. The controlled moisture levels will range from field capacity to the permanent wilting point. The intensity and severity of symptom development will be associated with the corresponding soil water potentials.

The influence of light intensity on plant susceptibility and symptom development must also be considered in future investigations.

Data obtained from the completed phases of this investigation and those studies mentioned above will be analyzed and appropriate simulation models utilized to estimate probable vegetation alteration due to air pollution.

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