Center for Air Environment Studies The Pennsylvania State University

2nd Annual Progress Report
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LIKELIHOOD ESTIMATIONS OF VEGETATIVE ALTERATION NEAR KNOWN
OR PROPOSED SOURCES OF AIR POLLUTION

Prepared for Submission to
Director, Contracts Management Office
U. S. Department of Energy
Chicago Operations Office
9800 South Cass Avenue
Argonne, Illinois 60439

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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 inter-college 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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I. Introduction

There is an increased interest in the use of coal as a fuel in electric generation stations. The Three Mile Island nuclear accident, rising oil costs, and an "energy self-sufficient" feeling among Americans has made many people demand the return to the use of coal to generate our electricity. However, coal-burning stations may 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 original proposition to the Department of Energy we proposed that the best way to predict the potential impact of new or expanded sources of SO_2 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 represents the results obtained during the second budget period of the project. Work completed during the first budget period of the project was described in detail in the first annual report. Research which was initiated during the 1979 growing season will be presented in the third annual report, as well as in the final report.

II. Progress

During the first year our efforts were directed towards completing related projects already in progress, purchasing and calibrating needed equipment, and initiating proposed research. We also evaluated 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 in the second year. 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 (STACK) operable and modified in a manner that its output will drive our computerized vegetation response model. This program was described in the first annual report.

B. The model

1. General aspects of considered models

The various modeling approaches that have been considered for predicting SO₂ injury to vegetation fall into two broad areas: empirical models and mechanistic models. Empirical models rely completely on correlations derived from field or laboratory data. Modeling consists of manipulating these correlations to reach conclusions. Predictive equations are typically generated by applying statistical methods to the data. Any process by which differing inputs may be seen to cause differing results is beyond the scope of empirical models.

Whereas cause-and-effect logic is foreign to empirical models, it is the backbone of mechanistic models. A theory of how a mechanism functions is translated into the mathematical language of the model. Data are used to provide estimated numerical constants but are not used to suggest the form of the equations. The theory does that. The two broad classes, empirical and mechanistic, are not mutually exclusive; hybrids are possible.

Both classes of models have strengths and weaknesses in any particular application. The ability to write a mechanistic model implies a knowledge of the step-by-step working of the process. A strong confidence in the theorized action of the mechanism is presupposed. If an understanding of the mechanism is incomplete or non-existent, the resulting mechanistic model will be weak. The validity of an empirical model depends upon its data base. If this data set is sparse or non-existent, the empirical model will be weak.

A possible mechanistic model of SO_2 injury to vegetation could be constructed using the physical, chemical, and biotic properties that affect the movement of sulfur into the leaf and the changes the sulfur undergoes therein. The established theory of diffusion of a gas into the leaf through the stomata could be used as a start. Ideally, the kinetics of reactions of SO_2 within the leaf which either render the gas non-toxic or else result in tissue necrosis would be known. Further, the effects of various meteorological and physiological variables both on the stomatal aperture and on the reaction kinetics would be required. Such a model would have several advantages. It would be general in that much of the theory would apply to other air pollutants besides SO_2 . Moreover, adjustments between plant species might be made by only adjusting various constants. Further, transition to predictions of chronic effects would be facilitated. It would be open-ended. Advances in knowledge regarding the action of sulfur in the leaf could easily be incorporated into the model. Unfortunately, such a model is impossible to construct at present. Little is known in detail about the mechanisms by which environmental variables change stomatal aperture. Even less is known regarding the disposition of SO_2 after it enters the leaf. There are competing theories, but none is sufficiently explicit to use in a predictive model. Furthermore, the required knowledge is not the sort that simply requires a set of experiments to generate. The answers to the formidable physiological problems await the development of new approaches and techniques.

Thus, although a mechanistic model might be preferred a priori, an empirical model is the only type currently feasible.

As has been stressed, the outstanding feature of empirical models is their intimate dependence on the data set(s) that sired them. Perhaps the primary problem here is the range of application or the area of inference. For example, extrapolating laboratory data to the field, or extrapolating data gathered using a 'model' species to other species may be viewed with skepticism. In empirical modeling, one is forced to make assumptions in order to step from a circumscribed data set to a more general model. Often such assumptions remain implicit, but the modeler should strive to make them explicit and understand their inherent restrictions. However, as more data is collected and input in this project, the empirical model will become more and more accurate.

2. Specific aspects of selected model

Deciding that empirical models represent the only practicable approach for modeling acute SO_2 injury to vegetation is still far from arriving at a particular model structure. Several types of problems remain. Which of the several dose-response models (discussed in detail in the first annual report) is to be preferred? How are environmental variables to be accounted for? When the existent dose-response models are for square-wave concentration profiles, how are fluctuations in pollutant levels to be allowed for? Granted that empirical models are highly data set specific, in what way may the model be structured to allow a maximum of robustness and flexibility in the face of additional laboratory or field data sets? A model structure which addresses each of these points has been developed and will be implemented as our laboratory data are analyzed.

The proposed model structures has as its basis a dose-response relationship. The Larsen-Heck dose-response equation was judged easiest to use among several competitors and consistently provided a good statistical fit to data sets which were available. Therefore, it was chosen as the keystone for the injury model. However, whereas Larsen and Heck use the damage on the median tree in a set as their injury rating, we have chosen to use the mean value for the set as our rating.

The next task is to devise a way to modify the Larsen-Heck equation to allow for environmental inputs and species differences. One possibility is to add extra terms for environmental variables to the basic dose response regression function. To fit such a function would require an enormous data base where plants were

exposed to SO_2 at varying concentrations for varying amounts of time under all types of different environmental conditions--different levels of temperature, humidity, light, and soil moisture. To circumvent this drawback, we will use a different approach. Dose-response data are taken at a fixed set of standard environmental conditions. The effects of changes in a particular environmental factor are followed at a fixed exposure concentration and duration. An assumption is made that the effect of an environmental variable is proportionately the same at all concentrations. Thus, based on information from experiments on environmental factors, the injury level predicted by a dose-response equation at standard conditions will be scaled proportionately upward or downward to allow for the influence of the ambient environment on response. A similar scaling procedure will allow the species susceptibility classification derived from our screening experiments to be reflected in the predicted response.

Perhaps the most problematical area of the proposed structure is how to make predictions in the face of stochastically fluctuating pollutant levels and environmental conditions. The Larsen-Heck equation assumes a square-wave dose of pollutant. Virtually all the dose-response and environmental factor information from any laboratory is gathered using not only constant SO_2 concentrations but also constant temperature, humidity, and so forth. In fact, using constant levels is the only efficient way to gather information about the role of environmental factors. Nevertheless, since data of this kind must be used to predict injury under fluctuating ambient conditions, the choice of an effective algorithm is critical.

The method devised for the proposed model has not yet been fully implemented or tested but seems promising. The idea is to divide a total period into intervals (to fix ideas, say a day into hourly intervals) and then to compute what might be viewed as 'all possible' injury estimates for the total period. This procedure requires as input hourly average values of pollutant concentration and ambient environmental factors. Also, the scaling procedure is somewhat more complex than indicated previously. For each hour the actual concentration is scaled to an 'effective concentration' based on ambient environmental conditions. The 'effective concentration' is the SO_2 concentration at standard environmental conditions that yields the same damage level as that which the actual concentration under ambient environmental conditions is expected to yield. This concept of effective concentration provides the common denominator of standard environmental conditions. What had originally been hourly fluctuations in both environment and concentration are now hourly fluctuations in effective concentration in a constant environment. In fact, it is precisely the constant environment in which the dose-response equations apply. Now all possible damage estimates are computed. For example, the following series of damage estimates could be generated: Twenty-four estimates for pollutant levels in each single hour, twenty-three estimates for the two-hour-average pollutant level in each adjacent pair of hours (hours 1 and 2, 2 and 3, 3 and 4, etc.), twenty-two estimates for adjacent types of hours, . . . A single estimate for the overall 24-hr. average. This procedure results, in this example, in $24+23+22+\dots+1=300$

separate estimates. Of this large number of estimates the single maximum or perhaps the top five could be reported to give an indication of the worst that might happen during the particular 24-hour time period. The problematical nature of this procedure is clear, but it does appear to be a promising tool for estimating damage in the face of fluctuating conditions when using correlations derived from static conditions. In practice, by eliminating from consideration series of intervals where effective concentration does not exceed a threshold value--for example, in the darkness where stomata may be assumed to be closed--the actual number of calculations may be reduced.

At this point, one must note that the estimating procedure described previously contains another implicit assumption. In order to use environmental data derived from experiments of varying duration (usually 4 hours in the work described later in this report) and to apply it to a modeling interval of varying length (one hour in the example related previously), it is assumed that the effect of an environmental variable is proportionately the same at all exposure durations.

Part of the appeal of this model comes from the ease with which additional experimental results of environmental factors may be incorporated. The scaling of ambient concentrations to effective concentrations may be done by a series of multipliers. There are several possible ways to construct and employ these multipliers and the details (not considered here) are not finalized. Suffice it to say that, initially, each individual environmental factor might have its own multiplier. If experiments

indicate that 2 or more factors interact either synergistically or antagonistically, a single multiplier may be developed for the pair. An additional multiplier may be added to the series if a new environmental factor is found to be important. If the value of a particular environmental factor is unknown during a certain application of the model, the value of its multiplier may be set to unity causing it to have no net effect on the scaling procedure. Thus, the scaling procedure via multipliers, while providing far less flexibility than mechanistic model might achieve, provides a relatively simple structure for adjusting the model in light of new or additional information.

C. Input factors - general

Several series of experiments have been conducted to provide the data required by the model which was outlined in the preceding sections. The results of these experiments, combined with reports from the literature, will provide the information with which a first generation model is formulated.

In order to minimize the problems of comparisons between species, a sensitive angiosperm tree species, river birch, was chosen as the experimental organism. Only in the case where the experimental techniques were being developed was pinto bean used because the time from seeding to exposable size was extremely short. (Many of these experiments will be repeated using a sensitive gymnosperm as called for in the original proposal.)

Many of these experiments are incomplete in the sense that the results are not yet fully analyzed. Others need to have additional replications run. The reports on these incomplete experiments are confined to a brief outline of the method and a summary table showing the average values of the response. Experiments that have been completed and fully analyzed are described in detail.

Please note that in all the experiments using river birch the injury rating reported is the average percent foliage injured per set of trees. For an individual tree, the total number of leaves is counted. Then the number of injured leaves is counted. The quotient of these two counts expressed as a percentage is the percent foliage injured for an individual. This rating system is not an expression of the total necrotic tissue on the trees, rather, it expresses the proportion of leaves on the tree showing any degree of injury.

1. Dosage response of river birch seedlings

As mentioned earlier in this report, the Larsen-Heck dose-response equation is the keystone of the model. In order to accurately establish the co-efficients in the equation, a set of dose-response data is required.

In the dose-response experiment sets of 8 river birch were exposed to each time-concentration combination. Initially, the experiment was designed with four concentration levels (0.3, 0.6, 1.2, 2.4 ppm) and four exposure times (1, 2, 4, 8 hours). Two replications of this design were run on 4 consecutive days in each of two consecutive weeks. In a third week, the two runs at 0.8 ppm were added.

The river birch were 1-year old transplants growing in a peat-perlite mixture in 8" plastic pots in outdoor beds. Approximately 40 hours before exposure, axillary shoots were removed and the trees were brought into growth chamber for pre-conditioning. The growth chamber was set for a 12 hour photoperiod at $500 \mu\text{E m}^{-2} \text{sec}^{-1}$ photosynthetically active radiation (PAR). Temperature and relative humidity remained constant at 24°C, 75%. Immediately before exposure the trees were watered liberally. Eight trays of four trees each were placed in the exposure chamber which had already reached the desired SO_2 concentration. At the end of each time interval two randomly chosen trays were removed. The exposure period generally ran from 0930 to 1730 hours. Exposure conditions were also 24°C and 75% relative humidity, however the light level was $750 \mu\text{E m}^{-2} \text{sec}^{-1}$ PAR. After exposure the trees were returned to the preconditioning environmental regime until they were rated for symptoms approximately 3 days later. Although analysis of the results is incomplete, the average response for each treatment combination is given in Table 1.

Table 1. Percentage foliar injury on river birch seedlings exposed to 0.3, 0.6, 0.8, 1.2, or 2.4 ppm SO_2 for 1, 2, 4, or 8 hr.

Concentration of SO_2 (ppm)	Hours of exposure			
	1	2	4	8
0.3	0.33 ^a	0.24	0.68	1.04
0.6	0.31	0.85	3.51	14.6
0.8	2.10	18.12	40.28	62.78
1.2	14.21	53.44	77.58	88.80
2.4	70.08	84.66	93.64	96.95

^aEach value represents the average percentage leaf injury on 16 seedlings contained in 2 replications (8 seedlings per replication) for a total of 320 plants.

2. Environmental parameters influencing SO₂ damage to river birch seedlings

a. Light intensity during exposure and relative soil moisture content

In this series of experiments sets of 10 river birch trees were exposed to various light intensities and soil moisture levels. The experiment was designed with 4 light levels (750, 500, 260, and 0 $\mu\text{E m}^{-2}\text{sec}^{-1}$ PAR) and three soil moisture levels based on three drying regimes (4, 2, 0 days without water). Soil cores were taken from a random selection of 5 pots in each set of ten in order to ascertain the percent soil water for those pots. The experiment was run as a split-plot design with light the whole plot treatment and soil moisture the subplot treatment. Five replicates have been run. The plants were exposed at 1.25 ppm for 4 hours.

The river birch used in this experiment were raised from seed and grown in the greenhouse until exposure. Seeds were sown in trays of peat-perlite. When the seedlings could be handled they were transplanted into sterilized silt-loam soil in 4" diameter plastic pots. Seeds were sown at intervals of 2 or 3 weeks for a total of 5 sowings. Trees from a single sowing were the experimental material for a single replicate. The plants were exposed between 20 and 24 weeks after sowing.

Approximately 96 hours before exposure 3 sets of trees were watered thoroughly and placed in a growth chamber at the same pre-conditioning environmental regime as described for the dose-response experiments. The trees for the 4 days dry treatment were not watered again until after exposures. Approximately 48 hours before exposure the remaining two sets of tree were watered thoroughly. The set of trees in the 0 days dry treatment was watered again immediately before being fumigated. After exposure, soil cores were taken. Then all trees were watered and returned to the pre-conditioning environment for symptom development. Symptoms were rated approximately 3 days after exposure.

The treatment involving zero light was done at night beginning one hour after the end of the chamber photoperiod. The treatments involving non-zero light levels were run beginning at approximately 0930 hours. The treatments for each replication were run in random order on 3 consecutive days.

Once again the analysis of the results has not been completed. Table 2 gives the average response for each treatment combination based on the first three replicates.

Table 2. Percentage foliar injury on river birch seedlings exposed to 0.9 ppm SO₂ for 3 hr. at 4 levels of light intensity and 3 levels of soil moisture as measured by days without watering. (Data from two additional replications is being analyzed).

Days of drying	Light intensity ($\mu\text{E m}^{-2}\text{sec}^{-1}$) ^a			
	0	260	500	750
0 ^b	5.89 ^c	34.94	28.56	46.59
2	5.15	31.81	34.81	38.44
4	2.04	7.01	4.29	2.04

^athe light intensity values correspond roughly to 0, 15, 30, and 45 klux;

^bdays of drying refers to the length of time that plants went without water while maintained in a growth chamber at 24°C and 75% RH at the specified light intensity. Percentage soil moisture measurements were also determined immediately after exposure but data have not yet been analyzed.

^ceach value represents the average percentage leaf injury on 30 seedlings contained in three replications (10 seedlings per replication) for a total of 360 plants.

b. Atmospheric moisture content and temperature during exposure

1) Relative humidity/temperature

In the relative humidity-temperature experiment sets of 10 river birch were exposed to each treatment combination in each replicate. The experiment was run as a split-plot design. The temperature-humidity combinations represent the whole plot treatments. The plot was split in time--one set of trees was run beginning in the morning, a second set was run beginning in the afternoon after the first set was withdrawn. This allows any difference in response between morning and afternoon exposures to be checked. Each set of trees was exposed for four hours to 0.8 ppm sulfur dioxide.

Nine temperature-relative humidity combinations were planned--all possible combinations of temperatures 15°, 24°, 32°C with relative humidities of 60%, 75%, and 90%. However, the combination 15°-60% proved impossible to maintain successfully. Two complete replicates of the experiment (omitting the 15°-60% combination) have been run. The eight treatment combinations on each replicate were run, one per day, 2 or 3 days per week in three consecutive weeks.

The trees were treated before exposure in exactly the same manner as described for the dose-response experiments. Exposure light level was also $750 \text{ uE m}^{-2} \text{ sec}^{-1}$ PAR. The morning set of trees were generally started around 0930 for REP 1 and around 0830 for REP 2. The 'afternoon' trees were exposed beginning approximately 4 hours later. Symptom development occurred under the same conditions as described in the dose-response experiments.

An additional replication of this experiment on greenhouse grown trees is planned. The average responses for each treatment combination is given in Table 3. Each value represents the average of 4 sets of trees—morning and afternoon trees in each of 2 replicates. The preliminary analysis seems to indicate a trend for morning trees to be more severely damaged than afternoon trees. This data is currently being analyzed and is not reflected in Table 3.

Table 3. Percentage foliar injury on river birch seedlings exposed to 0.9 ppm SO₂ for 3 hr. at 15, 24, or 32°C and 60, 75, or 90% RH. (Data from a fifth replication is being analyzed).

Relative humidity (%)	Temperature (°C)		
	15	24	32
60	- ^a	33.44 ^b	16.32
75	46.28	34.95	32.43
90	40.02	29.82	61.69

^a no data

^b each value represents the average percentage leaf injury on 40 seedlings contained in 4 pots (10 seedlings per set) for a total of 320 plants. Data from morning and afternoon exposures have been pooled.

2) Relative humidity/temperature interactions with soil moisture

A limited number of 1-year old river birch transplants were placed in 8" pots in a silt-loam soil and grown outdoors. These trees were exposed simultaneously with the peat-perlite-grown trees in the relative humidity/temperature study. However, they were pretreated somewhat differently. Also, due to the limited number of trees, only three trees per set were available.

This experiment was designed as a split-split-plot design. The temperature-relative humidity combinations were the whole plot treatments which were split into morning and afternoon subplots. The subplots were further split into wet soil and dry soil sub-subplots.

In the first replicate, axillary shoots were removed and the trees were brought into the greenhouse approximately 64 hours before being fumigated 24 hours later they were moved into a growth chamber with the same preconditioning environmental conditions as prevailed in the dose response experiment. For the second replicate, the trees were pruned and brought to the growth chamber directly approximately 64 hours before fumigation. All trees were watered well when they were first brought inside. Trees of the dry soil treatment were not watered again until after the fumigation. The wet soil trees were watered immediately before exposure. After exposure, a soil core was removed from each pot. Then the trees were watered and returned to the pre-exposure environmental regime for symptom development. The soil cores will be used to determine the percent soil moisture in each pot.

Analysis has not been performed on the results of this experiment.

3. Susceptibility of tree species to SO₂

a. Susceptibility categories

Results from this study will allow us to numerically rank various tree species, reflecting their susceptibility to SO₂. These rankings will be used within the model. A similar study from the 1977 growing season was presented in the first annual report; the 1979 study was initiated during this budget period and will be presented in the third annual report. Results from the 1978 growing season are reported herein in their entirety, rather than to separate out the data gathered only during the budget period, which ended half-way through the growing season.

During the summer of 1978, 100 2- to 3-year old seedlings of each of 20 tree species or clones were individually planted in plastic pots containing a 2:1 mixture of peat:perlite, fertilized and placed outdoors. Two weeks after budbreak and every alternate week thereafter during the growing season, four or five individuals of each species were brought indoors and exposed to 2,358 $\mu\text{g}/\text{m}^3$ (0.9 ppm) SO₂ for 2 hr. at 24°C, 75% relative humidity (RH), and 45 klux light intensity in a fumigation chamber. The pollutant was injected into the chamber from a commercial tank of 100% SO₂. The SO₂ concentration within the exposure chamber was continuously sampled and measured with a pulsed fluorescence SO₂ analyzer. The monitor was periodically calibrated using certified SO₂ permeation tubes which were re-weighed in our laboratory. After exposure the seedlings were moved to an adjacent controlled environment chamber maintained at 24°C, 75% RH, and 36 klux light intensity with a 12 hr photoperiod.

Three days after exposure, symptoms were evaluated on each seedling by estimating the percentage of injured leaves, rating the overall severity of symptoms on a 1 to 5 basis, and recording the percentage of symptomatic plants. The statistical significance of differences among species susceptibility is currently being evaluated using various combinations of the above parameters. Tables 4 and 5 summarize the findings to date. Complete statistical analysis and discussion of results, will be presented in the final report.

b. Stomatal response of selected species

We have conducted considerable research in our laboratory involving stomatal response to air pollutants. Additional knowledge may be useful in developing certain aspects of the model, and also in gaining a more thorough understanding of plant response to SO_2 . Therefore, during the 1978 susceptibility study, stomatal conductance measurements were taken on four tree species or clones: sweet birch, river birch, and two aspen clones. These species or clones were selected based on their known susceptibility to SO_2 , representing both susceptibility and tolerance.

Stomatal conductance measurements were taken, using an electrical diffusion porometer, in the growth chamber immediately before and after exposure to SO_2 . Measurements were taken on the abaxial leaf surface of three different aged leaves per plant: immature, recently mature, and over mature. Conductance rates were determined on the widest part of the leaf lamina between the margin and central vein. A total of 210 pre- and post-exposure and 42 control (unexposed plants) conductance measurements were made on each species or clone in this study. Readings were taken in a manner to insure randomization of species, plants, and leaves.

Table 4. Susceptibility of plants exposed to 0.9 ppm SO₂ for 4 hr at bi-weekly intervals during the summer of 1978.

Common Name ^a	No. Plants Exposed ^b	% Plants Injured	Severity Index ^c	Max. Severity Index ^d
River birch	45	69	27	105
Sweet birch	45	62	16	55
Amer. beech	45	25	5	37
Red pine	35	23	2	8
Eur. Larch	40	33	2	10
Jap. Larch	40	44	1	3
White fir	35	10	1	1
Q. aspen (#10)	40	8	1	8
Red oak	35	6	+ ^e	1
E. hemlock	35	6	+	1
Scotch pine	35	3	+	1
Fuller poplar	35	2	+	1
White pine	35	6	+	1
Black locust	35	0	0	0
Sugar maple	40	0	0	0
Red maple	40	0	0	0
Austr. pine	35	0	0	0
Chestnut oak	35	0	0	0
Norway spruce	35	0	0	0
Q. aspen (#1)	40	0	0	0

^aScientific names will be provided in the final report; all plants except the aspen clones were 2- to 3-yr-old seedlings; the aspen clones were 1-yr-old cuttings.

^bThe most uniform plants were selected from each group of 100, and exposed at approximately bi-weekly intervals throughout the growing season; a different set of plants was utilized in each exposure (see Table 5).

^cBased on [severity factor] x [% foliage injured] x [% population injured]/100.

^dMaximum index recorded during the summer (see Table 5).

^eTrace of injury.

Table 5. Relative susceptibility of 20 tree species or clones exposed to 0.9 ppm SO₂ for 4 hr at various stages of growth during the 1978 growing season.

Common Name	Age of current foliage (weeks)									
	2	4	6	8	10	12	14	16	18	20
	(Severity Index)									
River birch	^a	^b	0.2 ^c	47.5	8.2	105.0	80.9	0.1	3.3	0.7
Sweet birch		0	0	9.2	0.5	43.3	7.8	0.2	55.4	25.5
Amer. beech		3.2	+	0.8	0	0.1	36.6	2.8	0	0
Red pine		7.6	0	0	5.4	0.5	0	0		
Eur. larch		1.8	10.4	0	1.8	0.8				
Jap. larch			0	0	2.6	3.3	0.5	0.5	0	0
White fir		0	0	6.4	0	0	0.1	0		
Q. aspen 10		0	0	7.6	0	0	+	0	0	
Red oak			0	0	0	0	0.5	0	0	
E. hemlock		0	0	+	0	0.2	0	0		
Scotch pine		0	0	0.2	+	0	0	0		
Tulip poplar	0	0	0	0	0	0	0.2			
White pine			0	0	+	0	0	0	0	
Black locust		0	0	0	0	0	0	0		
Sugar maple		0	0	0	0	0	0	0	0	
Red maple		0	0	0	0	0	0	0	0	
Austr. pine	0	0	0	0	0	0	0			
Chest. oak			0	0	0	0	0	0	0	
Nor. spruce			0	0	0	0	0	0	0	
Q. aspen 1		0	0	0	0	0	0	0	0	

^a blank space indicates that the species was not exposed to SO₂ at that time

^b + indicates a trace of injury

^c severity indices given to tenths here for comparison purposes within rows and columns; severity index based on [severity factor] x [% foliage injured] x [% population injured]/100

Data are currently being analyzed. Results and incorporation of data into the model will be discussed in the next report.

4. Soil moisture content, soil water potential, plant water potential, and stomatal conductance of pinto bean

a. Introduction

As described in the previous year's renewal proposal, a study was initiated to determine the influence of soil moisture stress on pinto bean plant response to SO_2 . Pinto bean was used as a model plant because of ease of culture and known susceptibility to SO_2 . In addition, the short time needed for leaves to reach a susceptible age (2 weeks), and small size of plant material made this plant variety an ideal model plant with which to conduct an in-depth study relating soil moisture to SO_2 sensitivity. Data from this experiment have been analyzed, but have not yet been considered for input into the model. This will be accomplished during the proposed budget year.

It is apparent that soil moisture content influences plant sensitivity to SO_2 . However, in many studies, the soil moisture content was not critically controlled or accurately measured. Soil water potential can be maintained at desired levels by using varying concentrations of polyethylene glycol (PEG) in the root zone. However, plants grown hydroponically in this solution may absorb some PEG, altering physiological processes, which in turn may influence their responses to other stresses such as SO_2 . We minimized this problem by separating the soil and roots from the PEG with a cellulose dialysis membrane with a molecular cut off of 12,000 molecular weight (mw) used in conjunction with a 20,000 mw PEG.

The objective of this study was to evaluate the influence of varying soil moisture regimes, and resultant changes in the water status of the plant, on the macroscopic foliar response of pinto bean plants (*Phaseolus vulgaris* 'Pinto 111') exposed to SO_2 . Data presented in this report were collected from December 1978 through May 1979.

b. Materials and methods

1) Plant culture and establishment of varying soil water potentials

Pinto bean seeds were germinated in trays of vermiculite in a pre-exposure growth chamber maintained at 27 ± 0.5 C, $75 \pm 2\%$ relative humidity, with a 12-hr. photoperiod of 33 Klux beginning at 0600 hours. Six days after seeding (2 days after cotyledon emergence), 32 seedlings of uniform development were transplanted into aerated-steam treated Hagerstown silt loam which had been strained through a 7 mm mesh screen and drenched with distilled-deionized water. The soil was contained in tubes of cellulose dialysis membrane which excluded compounds with greater than 12,000 mw. The tubes were 20 cm long, 4.9 cm in diameter, and were sealed at one end.

Sets of eight plants, with roots and soil enclosed in the cellulose membrane, were then placed into one of four 10-liter vessels containing a solution of distilled-deionized water and PEG 20,000. The ratio of PEG:water was varied among vessels to maintain one of four different soil water potentials in each vessel: $-1/3$, -1 , -3 , or -5 atmospheres (atm.) (1 atm. = 1.013 bars). The system allowed for nearly complete interface between the dialysis membrane and the PEG solution. The only area of membrane not in direct contact with the solution was near the top of the vessel, where the tubes were held upright by a 17 mm thick piece of foam insulation. Water used by the plants was replaced to maintain the assigned amount of solution in each vessel.

Excessive nutrient concentrations in the soil or in the PEG solution may lead to rapid deterioration of dialysis membranes. We determined that plant growth and visible response to SO_2 was similar for plants grown in unamended topsoil placed in a mixture of distilled-deionized water and PEG or in Hoagland's solution and PEG mixture. Soil was analyzed and found to be adequate for normal plant growth without the addition of a nutrient supplement. Therefore, to prolong the usefulness of the dialysis membrane, all plants were grown in unamended topsoil surrounded by a water:PEG solution.

2) Description of plant material

Transplanted seedlings were allowed to grow for 4 days in membrane-encased soil surrounded by the PEG solution, after which time the biomass of the roots and shoots was determined for each of the four soil water potentials. Subsets of six plants were removed from the solution at each water potential and the dialysis membrane carefully cut. Roots were washed thoroughly and blotted dry. Fresh weight of the roots, shoots, and leaves was determined and recorded. The plant parts were placed in an oven at 100 C until they reached a constant weight, at which time the dry weight was determined. Leaf area was determined with a portable area meter (Lambda Instruments Corp., Lincoln, Nebraska). This experiment was replicated three times. Data were analyzed by analysis of variance and means were separated at $p=.05$ using Duncan's new multiple range test.

3) SO₂ exposure system

Preliminary experiments were conducted to select the plant age and SO₂ dosage at which high levels of foliar injury would develop on plants growing under the lowest soil moisture stress. Ten days after seeding, plants growing under the four levels of soil water potential were exposed simultaneously to 2.2 ppm SO₂ for 3 hr. at 27 C, 75% rh, and 45 Klux light intensity within a controlled environment exposure chamber (Environmental Growth Chambers Model M-2, Chagrin Falls, Ohio). All exposures began at 1000 hours (10 a.m.). The dosage of SO₂ was selected, based on preliminary studies, to induce maximum foliar injury on plants growing under the least soil moisture stress. Sulfur dioxide concentrations were analyzed with a pulsed fluorescent SO₂ analyzer (Thermo Electron Corp., Hopkinton, Mass.) calibrated periodically with certified permeation tubes which were re-weighed in our laboratory. Following exposure, the plants were returned to the pre-exposure chamber maintained at the same environmental conditions in which they were grown. Twenty-four hours after each exposure, the percentage of injured tissue on each unifoliate leaf was estimated visually on a 0-100 scale in increments of 10. Values of 1, 5, 95, and 99 percent were included at the extremes.

4) Exposure to SO₂ after 1, 2, or 3 days of treatment

Preliminary studies revealed that each desired soil water potential was attained 24 hr after the encased soil was placed in the PEG solution. Therefore plants grown for 4 days in the

PEG were subjected to at least 3 days at the desired water potential. One or 2 days of stress was accomplished by maintaining tubes in distilled-deionized water for 2 days or 1 day, respectively, prior to transfer to the PEG solution.

Prior to exposure to SO_2 , two plants were selected randomly from each treatment in each of four replications. Stomatal conductance was measured at 0940 hours on the abaxial surface of one unifoliolate per plant using an electrical diffusive resistance porometer (Lambda Instruments Corp., Lincoln, Nebraska). The porometer was periodically calibrated using a calibration plate of known resistances. An additional two plants were selected randomly from each treatment, excised with a razor 2 cm below the cotyledons, and plant water potential was measured with the pressure bomb technique prior to exposure. The tubes of the sacrificed plants remained in place until after the vessels were removed from the exposure chamber. At that time, the lowest 10 cm of the tube was excised and approximately 40 g of root-free soil were removed. The soil was dried at 105 C and percent soil moisture determined on a dry weight basis. Six plants at each of four soil moistures (-1/3, -1, -3, -5 atm.) following 1, 2, or 3 days at the former soil treatment were exposed to SO_2 as described above. The percentage of SO_2 injury on the 12 unifoliolates of the remaining six plants in each treatment was estimated as described above.

Correlation coefficients were calculated among percent soil moisture, percent macroscopic injury, stomatal conductance rate, plant water potential, and soil water potential.

Plants were always stressed and treated in groups containing all four soil water potentials ($-1/3$, -1 , -3 , and -5 atm). Four replicates of each treatment were exposed to SO_2 at each of the three stress times (1, 2, 3 days). The design was a nested factorial (treatments within days of stress) utilizing a total of 384 plants. Percent soil moisture and percent macroscopic injury data were transformed taking the arcsine of the square root of the original value. The original and transformed data were examined for heteroscedacity and it was concluded that the original data possessed more suitable variance properties for analysis. Data were analyzed by analysis of variance and means were separated at $p=.05$ using Duncan's new multiple range test.

5) Stomatal conductance prior to, during, and after exposure to SO_2

Results from the previous conductance study revealed that additional studies involving stomatal closure in response to SO_2 were warranted. Plants were grown at the four levels of soil moisture for 3 days. An SO_2 exposure similar to those described above was conducted from 1000 to 1300 hr. Stomatal conductance measurements of one unifoliate leaf per time were made at approximately 30 minute intervals prior to (0930 and 0955 hours), during (1030, 1100, 1130, 1200, and 1230 hours), and after (1305, 1330, 1400, 1430, and 1500 hours) exposure to SO_2 . Percentage SO_2 injury was estimated as described above on both unifoliolates of each plant. This experiment was replicated three times using a total of 96 plants. Stomatal conductance was also measured on 64 nonexposed plants.

c. Results

1) Description of plant material

Pinto bean plants grown in soils at $-1/3$ and -1 atm. of water potential appeared normal in growth and development, whereas those grown in the -3 and -5 atm. potentials were stunted. Leaf area from the plants grown at $-1/3$ and -1 atm. treatments were significantly larger than those grown at -3 and -5 atm. treatments. The mean leaf area was not significantly different (0.05) between the $-1/3$ and -1 treatments, or between the -3 and -5 treatments (Fig. 1).

Fresh and dry weights of roots, shoots, and leaves were greatest on plants grown at $-1/3$ atm. of soil water potential. Weights generally became progressively less at the -1 and -3 atm. treatments. Plant part weights from the -5 atm. treatment were generally similar to those from the -3 atm. treatment. The significance of differences among mean plant part weights at each level of soil moisture are illustrated in Figure 1.

2) Exposure to SO_2 after 1, 2, or 3 days of treatment

The length of treatment had no significant influence in percent macroscopic SO_2 injury, percent soil moisture, stomatal conductance rate, or plant water potential values. Values were not significantly different following 1, 2, or 3 days of stress (Table 6). Therefore, these data were pooled and an overall mean value for each parameter was calculated (Table 7).

Tan interveinal necrosis caused by SO_2 was most severe on those plants grown at $-1/3$ atm., and progressively less necrosis was observed as the soil water potential decreased. However, the amount of foliar injury induced at the -3 and -5 atm. treatments were similar (Table 7).

Figure 1. Fresh weight, dry weight, and leaf area of pinto bean plants grown for 3 days at soil water potentials of $-1/3$, -1 , -3 , and -5 atm. Letters of significance ($p=.05$) refer to the fresh or dry weight of the plant part indicated. The letters should not be compared between plant parts, or between fresh and dry weight.

Table 6. Percent SO_2 injury on unifoliate leaves of pinto bean plants grown for 1, 2, or 3 days at varying levels of soil water potential and exposed to 2.2 ppm SO_2 for 3 hr. Percent soil moisture, stomatal conductance rate, and water potential of plants grown at $-1/3$, -1 , -3 , and -5 atm. of soil water potential for 1, 2, or 3 days. Values within any given row were not significantly different (0.05) from each other.

Soil Water Potential	Parameter	Length of Treatment (Days)		
		1	2	3
$-1/3$ atm	% SO_2 injury	97.1 ^a	98.0	98.3
	% soil moisture	27.9 ^b	27.4	25.8
	conductance rate (cm/sec)	0.235 ^b	0.202	0.237
	plant water pot. (atm)	-1.70 ^b	-0.68	-0.77
-1 atm	% SO_2 injury	69.8	70.8	90.3
	% soil moisture	24.2	22.9	24.2
	conductance rate	0.189	0.195	0.204
	plant water pot.	-1.50	-1.41	-1.31
-3 atm	% SO_2 injury	1.1	0.0	5.8
	% soil moisture	19.8	20.0	20.4
	conductance rate	0.105	0.195	0.110
	plant water pot.	-3.92	-1.88	-2.35
-5 atm	% SO_2 injury	0.0	0.0	0.0
	% soil moisture	19.1	19.6	19.4
	conductance rate	0.080	0.103	0.090
	plant water pot.	-3.64	-3.35	-3.23

^a Each mean in the row is the average of 12 measurements in 4 replications.

^b Each mean in the row is the average of 2 measurements in 4 replications.

Table 7. Mean % SO₂ injury, % soil moisture, stomatal conductance rate, and plant water potential of pinto bean plants grown at -1/3, -1, -3, and -5 atm. of soil water potential.

	Soil Water Potential (atm)							
	-1/3		-1		-3		-5	
% SO ₂ Injury	97.8 ^a	x ^b	77.0	y	2.3	z	0.0	z
% Soil Moisture	27.0 ^c	x	23.8	y	20.1	z	19.4	z
Conductance Rate (cm/sec)	0.224 ^c	x	0.196	y	0.107	z	0.091	z
Plant Water Pot. (atm.)	-1.05 ^c	y	-1.40	yz	-2.72	yz	-3.40	z

^aEach mean is the average of 144 values.

^bValues in a row followed by different letters are significantly (0.05) different.

^cEach mean is the average of 24 values.

The percentage of soil moisture and rate of stomatal conductance were greatest at $-1/3$ atm., and became less with increasing soil water potential. However, mean values from -3 and -5 atm. were not significantly different from each other (Table 7).

Plant water potential values became progressively more negative with increasing soil water potential. However, only the mean plant water potential at $-1/3$ and -5 atm. were statistically different.

Plants grown in soil with a moisture content above 23% (independent of days of stress) were very susceptible to SO_2 (Fig. 2). Those grown in soil with a moisture content below 23% were much more tolerant. There was a significant correlation among all parameters measured (Table 8).

3) Stomatal conductance prior to, during, and after SO_2 exposure

Rates of stomatal conductance were measured at approximately 30 minute intervals from 0930 to 1500 hours. Prior to exposure, at 0930 and 0955 hours, stomatal conductance rates in the pre-exposure chamber were approximately 0.4 to 0.5 cm/sec for plants growing in the $-1/3$ and -1 atm. soil moisture stress, and 0.1 cm/sec for the plants in the -3 and -5 atm. treatments (Fig. 3).

The conductance rates of leaves on plants grown in the $-1/3$ atm. solution initially increased sharply following placement of the plants into the exposure chamber containing SO_2 . Conductance rates in the -1 and -3 atm. treatments also increased, but to a much lesser extent.

As the exposure progressed, conductance rates decreased in the $-1/3$ and -1 atm. treatments, but were largely unaffected in the -3 and -5 treatments. Severe water soaking caused by SO_2

Figure 2. Percent macroscopic injury induced by SO_2 on unifoliate leaves of pinto bean plants grown under various soil moisture regimes.

Table 8. Correlation coefficients among soil water potential, soil moisture content, percent macroscopic injury, stomatal conductance rate, and plant water potential.

	soil water pot.	%SO ₂ injury	% soil moisture	conductance rate
% SO ₂ injury	0.889 ^a			
% soil moisture	0.865	0.905		
conductance rate (cm/sec)	0.801	0.838	0.820	
plant water pot. (atm.)	-0.690	-0.639	-0.591	-0.574

^aEach correlation coefficient is significant at the 0.05 level.

Figure 3. Stomatal conductance rates of the unifoliolate leaves of pinto bean plants grown under the indicated soil moisture stress. A) Pre-exposure conductance rates at 27 C, 75% rh, and 33 Klux light intensity. B) Post-exposure conductance rates at 27 C, 75% rh, and 33 Klux light intensity. Between the dashed vertical lines the plants were exposed to 2.2 ppm SO₂ at 27 C, 75% rh, and 45 Klux light intensity. Each point is the average of 3 replications.

was noted at 1130 hours on the leaves of plants in the $-1/3$ atm. treatment, and slightly less water soaking was observed at this time on plant leaves from the -1 atm. treatment. Water soaking corresponded to a large decrease in stomatal conductance between 1100 and 1130 hours at those two soil moisture regimes (Fig. 3).

The stomatal conductance rate of plants in the $-1/3$ and -1 atm. treatments continued to decrease during exposure until 1400 hours, at which time the leaf tissue was too water soaked to allow measurement to be taken. Following exposure, the stomatal conductance rate of leaves from plants grown in the -1 atm. treatment rose sharply after being returned to the pre-exposure chamber, and then decreased. The stomatal conductance of plants exposed to SO_2 under the -3 and -5 atm. soil moisture stress were not affected by any treatment. The percent foliar injury induced by SO_2 were 96, 83, 0, and 0 percent respectively for the $-1/3$, -1 , -3 , and -5 atm. treatments. The amount of injury is similar to the data presented in the previous study (Table 6).

When control plants were moved from the pre-exposure chamber to the exposure chamber in the absence of SO_2 , the stomatal conductance rate of the plants grown under $-1/3$ and -1 atm. treatments also rose sharply (Fig. 4). The stomatal conductance rate of the $-1/3$ and -1 atm. treatments increased almost six-fold to a peak of over 2.3 cm/sec. The conductance rate of plants in the -3 and -5 atm. treatments did not increase but remained fairly constant.

Figure 4. Stomatal conductance rates of the unifoliolate leaves of pinto bean plants grown under the indicated soil moisture stress. A) Pre-exposure conductance rates at 27 C, 75% rh, and 33 Klux light intensity. B) Post-exposure conductance rates at 27 C, 75% rh, and 33 Klux light intensity. Between the dashed vertical lines the plants were in the exposure chamber without SO_2 present at 27 C, 75% rh, and 45 Klux light intensity. Each point is the average of 2 replications.

The effect of the exposure chamber environment, with and without SO_2 , on stomatal conductance of plants grown at $-1/3$ and -1 atm. of soil moisture was considerable (Fig. 3,4). Exposure to SO_2 decreased the stomatal conductance, whereas the effect of the chamber without SO_2 was to increase the conductance rate. The conductance rate of unexposed control plants decreased when the plants were returned to the pre-exposure growth chamber. The conductance rate of plants exposed to SO_2 remained at a minimum upon movement to the pre-exposure chamber.

These results are currently being incorporated into the model and will constitute an important part of the environmental sub-program. The use of this data as input into the model will be presented in the next report.

III. Additional investigations to be completed

During the proposed budget year, we will concentrate on the following areas. The overall model will be further refined, all possible data will be input into the model, and the model will be brought to operational status on the computer. The 'STACK' model, used to predict ground level SO_2 concentrations, will be interfaced with the overall model. The dosage response study will be replicated one more time using greenhouse grown river birch, and also will be repeated using a greenhouse grown coniferous species, Virginia pine. The relative susceptibility study will be initiated at the beginning of the 1980 growing season, to include an additional 20 species.

Environmental studies to be replicated one more time include the relative humidity study and the light-soil moisture study. Following completion of these studies, all data will be subjected to rigorous statistical examination and utilized as input into the overall model.

We are currently monitoring ambient levels of SO_2 near a coal-burning power plant. In addition we have access to monitoring data from 26 sites in Pennsylvania where ambient concentrations of SO_2 are monitored by the utilities. During the coming growing season, we will conduct field surveys around some of these sites. If vegetation damage from SO_2 occurs, we will utilize the monitoring and meteorological data to validate the model.

It is essential that funding be continued for a full 12 months, and not be discontinued at the end of the project period, which ends directly in the middle of the growing season. This aspect will be considered in the renewal proposal.

IV. Publications and Theses

Research reported in the following manuscripts and theses was supported partially or completely by this grant during the budget period.

A. Theses (M.S.)

1. Biggs, A. R. 1978. Relative susceptibility of ten tree seedlings to sulfur dioxide with emphasis on the sulfur content, stomatal conductance, and pollutant accumulation of several birch species.
2. Miller, C. M. 1979. The influence of exposure temperature on pinto bean macroscopic foliar response to O_3 and SO_2 .
3. Davids, J. A. 1979. The influence of soil moisture on macroscopic sulfur dioxide injury to pinto bean foliage.

B. Publications prepared

1. Biggs, A. R. and D. D. Davis. Foliar response of ten tree species to SO_2 (submitted to Plant Disease Reporter, August 14, 1979).
2. Biggs, A. R. and D. D. Davis. Stomatal response of three birch species exposed to varying acute doses of SO_2 (submitted to HortScience, July 17, 1979).
3. Biggs, A. R. and D. D. Davis. Sulfur dioxide injury, sulfur content, and stomatal conductance of birch foliage (submitted to Can. J. For. Res., September 18, 1979).
4. Miller, C. M. and D. D. Davis. Influence of exposure temperature on plant response to O_3 , SO_2 , or $O_3 + SO_2$ (submitted to Phytopathology, July 18, 1979).

CAES Publications

AIR POLLUTION TITLES is a guide to current air pollution literature and useful as a retrospective searching tool. This publication uses a computer-produced, Keyword-in-Context (KWIC) format to provide a survey of air pollution literature. Pertinent titles are selected from over 1000 journals each year.

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A GUIDE TO AIR POLLUTION RESEARCH [PHS] Publ. No. 981} was prepared in 1969 by the Center for Air Environment Studies under contract to the National Air Pollution Control Administration of the United States Public Health Service. It is available from the Superintendent of Documents of the Government Printing Office, Washington, D. C. 20402. The 1972 edition of the Guide was prepared by the Center under contract to the Office of Air Programs of the Environmental Protection Agency and is also available from the Superintendent of Documents.

HANDBOOK OF EFFECTS ASSESSMENT: VEGETATION DAMAGE was published in 1969. It describes in detail the many various sources of pollution and the effect of these pollutants on vegetation. Included are color slides depicting the characteristic symptoms of plant damage. This publication is available through the Center for Air Environment Studies.

The Information Services of the Center for Air Environment Studies publishes articles, theses, conference and meeting proceedings, and reports by faculty and staff members of the Center for distribution to colleagues in the field. Lists of all CAES publications are available upon request. Write to: Information Services, Center for Air Environment Studies, 226 Fenske Laboratory, The Pennsylvania State University, University Park, Pennsylvania 16802.