United States
Environmental Protection
Agency

Environmental Research
Laboratory
Corvallis OR 97333

EPA-600/3-84-037
February 1984

Research and Development

EPA

A Review and Assessment of the Effects of Pollutant Mixtures on Vegetation— Research Recommendations

Atch
A-1

EPA-600/3-84-037
February 1984

A REVIEW AND ASSESSMENT OF THE EFFECTS OF POLLUTANT MIXTURES ON VEGETATION

-RESEARCH RECOMMENDATIONS-

Vegetation Effects Workshop
April 21-22, 1983
Raleigh, North Carolina

Prepared by

Allen S. Lefohn
Douglas P. Ormrod

January 30, 1984

Project Officers

Eric M. Preston
David T. Tingey
Air Pollution Effects Branch
Corvallis Environmental Research Laboratory
United States Environmental Protection Agency
Corvallis, Oregon, 97333

CORVALLIS ENVIRONMENTAL RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
CORVALLIS, OREGON, 97333

NOTICE

CONTENTS

TABLES

FIGURES

ACKNOWLEDGEMENT

Bruce Jordan and Larry Zaragoza would like to provide acknowledgement to John Bachmann, Kent Berry, John Haines, Pam Johnson, David McKee, and Harvey Richmond for their comments during the preparation of the material that appears in Chapter 1.

Allen S. Lefohn would like to acknowledge the assistance of the following individuals and organizations for providing information that was used in the data analysis described in Chapter 2:

1. Ms. Cel Allard, Center for Data Systems and Analysis, Montana State University, Bozeman, Montana for developing the software programs necessary to access A.S.L. & ASSOCIATES' copy of the SAROAD data base.
2. Dr. Tom Curran and Mr. Jim Reagan of the EPA, Research Triangle Park, North Carolina, for providing insight into the EPA SAROAD and EPRI SURE data bases.
3. The Tennessee Valley Authority for providing its air quality monitoring data on a timely basis, in a readable format.

EPA wishes to acknowledge A.S.L. & Associates for assisting in the integration and editing of this report.

PREFACE

On April 21-22, 1983, a workshop sponsored by the U.S. Environmental Protection Agency Corvallis Environmental Research Laboratory, was hosted in Raleigh, North Carolina to develop research recommendations concerning the effects of pollutant mixtures on vegetation. Prior to the meeting, EPA asked several individuals to develop position papers to a) describe the use of information on plant response to pollutant mixtures in setting ambient air quality standards; b) characterize the spatial and temporal characteristics of air pollutant mixtures in the ambient air; and c) summarize the vegetation effects literature associated with pollutant mixtures. The material was integrated into the first three chapters of this report. The following individuals are acknowledged for the writing of the position papers:

Studies of Combined Exposure Effects on Vegetation:
Role in Establishing National Ambient Air Quality Standards

Bruce Jordan and Lawrence J. Zaragoza
EPA Office of Air Quality Planning and Standards
Research Triangle Park, NC

The Co-Occurrence of Sulfur Dioxide/Nitrogen Dioxide,
Ozone/Sulfur Dioxide, and Ozone/Nitrogen Dioxide
Mixtures in Ambient Air

Allen S. Lefohn
A.S.L. & Associates
Helena, MT

Effects of Pollutant Mixtures on Vegetation

Delbert C. McCune
Boyce Thompson Institute
Ithaca, New York

Douglas P. Ormrod
University of Guelph
Guelph, Ontario, Canada

Richard A. Reinert
North Carolina State University
Raleigh, NC

At the invitation of EPA, eighteen individuals participated in the two-day workshop. Participants were asked to critically review the position papers and 1) summarize the information gaps and assess the significance of the problems associated with those pollutant mixtures exposures that affect vegetation; and 2) identify and recommend activities that would assist the Agency in filling these research gaps.

From these activities (position papers and panel deliberations), EPA's Corvallis Laboratory has produced this document to summarize

- the processes involved in developing ambient air quality standards;
- the spatial and temporal distribution of gaseous pollutant mixture concentrations;
- the effects of gaseous pollutant mixtures on vegetation;
- information gaps; and
- recommendations on research that is required to fill the information gaps.

While the subject was addressed, no attempt was made to prioritize the general research categories because panel members believed pollutant ambient monitoring characterization and vegetation effects research efforts were complementary.

LIST OF PARTICIPANTS

Air Pollutant Mixtures Vegetation Effects Workshop Participants

Thomas C. Curran
U.S. Environmental Protection Agency
Monitoring and Data Analysis Division
Research Triangle Park, North Carolina

J.H.B. Garner
U.S. Environmental Protection Agency
Environmental Criteria and Assessment Office
Research Triangle Park, North Carolina

Walter W. Heck
North Carolina State University
P.O. Box 518
Raleigh, North Carolina

Pam M. Johnson
U.S. Environmental Protection Agency
Strategies and Air Standards Division
Research Triangle Park, North Carolina

Bruce C. Jordan
U.S. Environmental Protection Agency
Strategies and Air Standards Division
Research Triangle Park, North Carolina

Allen S. Lefohn
A.S.L. & Associates
Helena, Montana

Delbert McCune
Boyce Thompson Institute
Cornell University
Ithaca, New York

David Olszyk
Statewide Air Pollution Research Center
University of California
Riverside, California

Douglas P. Ormrod
University of Guelph
Department of Horticultural Science
Guelph, Ontario CANADA

Ronald Oshima
California Department of Food and Agriculture
Environmental Monitoring
Sacramento, California

Eric M. Preston
U.S. Environmental Protection Agency
Environmental Research Laboratory-Corvallis
Corvallis, Oregon

Richard A. Reinert
U.S. Department of Agriculture
Plant Pathology Department
North Carolina State University
Raleigh, North Carolina

George E. Taylor, Jr.
Environmental Sciences Division
Oak Ridge National Laboratory
Oak Ridge, Tennessee

Ted W. Tibbitts
Horticulture Department
University of Wisconsin-Madison
Madison, Wisconsin

David T. Tingey
U.S. Environmental Protection Agency
Environmental Research Laboratory-Corvallis
Corvallis, Oregon

Michael Treshow
Department of Biological Sciences
University of Utah
Salt Lake City, Utah

David E. Weber
U.S. Environmental Protection Agency
Office of Research and Development
Washington, D.C.

Larry Zaragoza
U.S. Environmental Protection Agency
Strategies and Air Standards Division
Research Triangle Park, North Carolina

EXECUTIVE SUMMARY

INTRODUCTION

The Environmental Protection Agency (EPA) is responsible for periodically reviewing and revising all national ambient air quality standards (NAAQS). The Clean Air Act (CAA) requires EPA to establish national ambient air quality standards for ambient air pollutants which may endanger human health and welfare. Secondary ambient air quality standards must be adequate to protect the public welfare from any known or anticipated adverse effects associated with the presence of a criteria air pollutant. To help the Agency develop data that assess the effects of pollutant mixtures on vegetation, the EPA Corvallis Environmental Research Laboratory sponsored a workshop in Raleigh, North Carolina on April 21-22, 1983. Participants reviewed position papers to 1) summarize the information gaps and assess the significance of the problems associated with pollutant mixture exposures that affect vegetation and 2) identify and recommend activities that would help the Agency fill these research needs. This report includes both the position papers and the workshop deliberations.

The ranking of research needs was addressed by the workshop participants. Members believed that no research areas are independent from another and should be treated collectively. For example, the biological research efforts are dependent upon a knowledge of the pollutant concentrations occurring in the field under ambient conditions. Thus, environmental, genetic, and phenological variables should be considered when a study is initiated.

CHARACTERIZING AMBIENT AIR QUALITY EXPOSURES

Different air quality exposure regimes exist for sulfur dioxide (SO_2), ozone (O_3), and nitrogen dioxide (NO_2). These regimes affect the frequency of co-occurrence and sequential exposures that vegetation may experience. An analysis of the 1981 EPA air quality data base (SAROAD), Electric Power

Research Institute (EPRI) 1978 SURE, and Tennessee Valley Authority (TVA) data bases indicates that SO_2, NO_2, and O_3 may co-occur in various concentrations in rural, suburban, and urban areas. Analyses of the data bases show that the frequency of co-occurrence (using 0.05 ppm as the definition of an event) is small for many rural sites. For most of the cases analyzed, events lasted only a few hours and were separated by intervals of weeks or months.

The panel recommends that air quality data be evaluated further to study patterns of occurrence of the combined pollutants to establish guidelines for designing plant interaction research investigations. The three pollutants of primary interest are SO_2, O_3, and NO_2. The effects of these pollutants on vegetation can be evaluated using the available air quality data and the research information dealing with individual effects. In addition, the panel recommends that acidic deposition be considered as a pollutant with the potential for interaction with the above three air pollutants.

The recommendation is to analyze existing air quality data bases (starting with SAROAD) to derive the joint probability distributions of pollutants and the diurnal patterns of exposure for plant exposure experiments. Additional sources of rural air quality data could include the USDA Forest Service, EPRI (SURE), EPA (NCLAN), and permits monitoring programs (e.g., PSD applications). This analysis is to include the following:

1) A search of the data base for locations where either co-occurrence or sequential exposures (starting with a 24-hour time step) occur. This search would include separate listings at several threshold concentrations (e.g., 0.05, 0.04, 0.03, and 0.02 ppm).

2) Once locations are identified, the monitoring data bases at the locations should be presented as joint frequency distributions and as diurnal time series. It is suggested that the utility of spectral analysis (Fourier series) and the Box Jenkins model should be explored.

3) The results of this process should be disseminated to research groups to guide experimental exposures used in interaction experiments.

The panel recommends that potential data displays for individual pollutants include: 1) three-dimensional plots of concentration, duration, and frequency; 2) diurnal plots for individual pollutants in terms of mean concentraiton and frequency greater than particular concentrations. These analyses should be summarized for the growing season (or some relevant time period) and should also serve to identify potential anomalies.

The air quality analyses would provide information that could be used to identify general patterns of exposure that relate to geographic regions or source configurations. It may be necessary to supplement the air quality data around point sources by considering the use of dispersion models to provide information on levels, diurnal patterns, and time between episodic events.

VEGETATION EFFECTS

Pollutant mixtures may induce plants to exhibit different types of responses which are influenced by several variables that are often difficult to predict. The three general categories of responses that may follow plant exposures to mixtures of ambient air pollutants are additive, greater than additive, and less than additive. All three responses are found to some degree in the experimental results using SO_2, O_3, and NO_2. Taken as a whole, the current information on both long- and short-term combined exposure studies provides conflicting results that are difficult to interpret.

The workshop members agree that research efforts should be directed toward important plants (including agronomic, horticultural, and native plants, and tree species). The panel believes that the gaps in knowledge can only be filled by an integrated effort involving growth chambers, greenhouses, and field plots.

Exposure Regimes

The panel is aware that the understanding of the response of plants to various exposure regimes is crucial. Plant effects must be associated with air pollutant peaks, means, length of exposure, and time between exposures that mimic realistic ambient pollutant exposures. Research should evaluate the vegetation effects associated with sequential exposures of pollutant mixtures which duplicate ambient conditions.

Development of Minimum Guidelines for Research Protocols

The panel recommends that a minimum set of standardized procedures be developed to ensure the quality assurance of plant response studies. Generalized guidelines should be proposed for 1) plant growth conditions, 2) environmental and plant monitoring, 3) pollutant exposures, and 4) uniform terminology (describing plant response characteristics). The most efficient experimental designs and analysis procedures available (relevant to a specific experimental goal) should be implemented (e.g., covariate analysis, analysis of variance, and rotatable design). It is proposed that the minimum guidelines be developed through two or three workshops.

Predictive Investigations

One purpose of pollutant interaction research is to develop predictive capabilities for assessing vegetation effects. Predictive models can provide estimates of vegetation effects under a variety of conditions not feasible with direct experimentation. In order to properly generate the information necessary to develop such predictive models, data are needed from research programs involving studies that define 1) the modes of action and 2) the sources of biological variation.

The following research activities are recommended and considered instrumental in the development of this predictive capability:

1) Modes of Action: The objective of this research activity is to understand how air contaminants influence biological processes and in doing so, determine whether their actions may cause significant ecological alterations. It is necessary that studies address modes of action for pollutants singly and in combination. The research effort should include both sequential and co-occurrence exposures and should be conducted with an appreciation of realistic exposure regimes. The biological level of organization to be investigated should focus on processes at all levels of plant organization (i.e., the cell, whole-plant, population, and ecosystem). The panel believes that there should be two major areas of interest

a) The relationships between different mechanisms of pollutant response.

b) The varying biological responses attributed to different levels and combinations of air pollutant exposure.

2) Sources of Variation: The plant response to a given exposure regime varies significantly with specific environments, environmental changes, and the stage of plant development. The panel recommends research that focuses on each of the following:

a) environmental factors-the significance of edaphic (e.g., soil water availability, soil nutrients), climatic (e.g., temperature, light, relative humidity, elevated carbon dioxide, etc.) and biotic factors (e.g., pathogens, symbionts, competition, etc.).

b) genotype factors-the significance of intra- (e.g., cultivar, population) and interspecific genotypes. This includes phenology as a source of variation.

3) Modeling-The development of data that describe the process and mechanistic activities associated with air pollutant mixture vegetation effects should allow for the development of conceptual and quantitative models that describe observed biological response.

CONCLUSION

It is the opinion of the workshop participants that the position paper focusing on ambient exposures was an initial attempt to identify realistic exposure regimes that exist in the ambient air. The panel believes that additional efforts should be made to supplement the existing analysis and that they should simultaneously proceed as the biological vegetation effects research is implemented. It was recommended that the results of the air quality characterization should feed directly into the design of the pollutant mixture experimental protocols.

The process and mechanistic research activities should involve two stages: 1) a biological effects screening exercise to prioritize which air pollutant mixture exposures are most likely to be significant, 2) a more detailed investigation that is performed under field and laboratory situations for the purpose of quantifying the significance of the major factors affecting plant response.

It was the conclusion of the panel members that the conceptual models should combine existing models of joint action with the data that describe the modes of biological action. The quantitative models should be capable of providing accurate and precise estimates of plant response. In addition, the models should be able to complement the physical and/or biological processes that are responsible for producing the observation.

1. ROLE OF POLLUTANT MIXTURE STUDIES IN ESTABLISHING NATIONAL AMBIENT AIR QUALITY STANDARDS

INTRODUCTION

The Clean Air Act (P.L. 95-95) requires that the Environmental Protection Agency establish national ambient air quality standards (NAAQS) for certain air pollutants that, if present in the ambient air, may endanger human health and welfare. Primary NAAQS are established to protect human health while secondary NAAQS are established to protect public welfare. Section 302(h) of the Act specifies that the effects of air pollution on crops and other vegetation are among the effects that must be considered in establishing secondary NAAQS.

The original NAAQS were established in 1971 and included secondary standards set at levels designed to protect public welfare including effects on crops and other vegetation. No detailed discussion of the rationale for the secondary standards was provided in the original proposal and promulgation notices (36 FR 1502, 36 FR 5867, 36 FR 8186). The secondary standard for ozone (O_3), which the original criteria document associated with damage to vegetation, was set equal to the primary standard. Based on comparison of the original sulfur dioxide (SO_2) standards and supporting documents, it appears that support for the SO_2 standard levels was based upon a limited number of studies and that the issue of the importance of pollutant mixtures was not raised (DHEW 1970). Available information does not suggest that the effects of nitrogen dioxide (NO_2) on vegetation played a role in setting the original NO_2 standard.

The potential for increasing plant sensitivity by the presence of multiple pollutants is significant because emission sources often release different pollutants within an area. However, there is often considerable controversy over the characterization of plant response in the presence of even a single pollutant. The assessment of plant response to pollutant mixtures is a more difficult task.

This chapter describes how information on pollutant mixtures is being considered and provides suggestions that should increase the utility of studies on plant response to pollutant mixtures in the standard review process for NAAQS. Focus is placed on evaluations developed during ongoing standard reviews, with discussion of past review practices included.

The Standard Review and Development Process

The complex standard review and development process is designed to solicit the best available scientific information and public comment. The use of welfare information and those factors that have appeared to be most influential in the ultimate decision-making process are described by Bachmann and Zaragoza (1983). More general discussions of the process are provided elsewhere (O'Connor 1980, Padgett and Richmond 1983, Zaragoza 1982). In order to better understand the role of scientific information in this process, this section highlights those activities that most pertinent to the present subject area.

The standard review and development process combines scientific review and assessment with the judgment of EPA's Administrator. As Figure 1-1 shows, the first part of this process involves an in-depth scientific review, including the collection of relevant information and review by specialists within each scientific area. When available scientific information can resolve questions so that uncertainty is small, the degree of judgment required of the Administrator for a given degree of protection is reduced.

The criteria document development process is the Agency's means of conducting an unbiased and public review of all literature used in support of a particular standard. Once a study is included in the criteria document, the characterization of that study will play a major role in determining its potential utility in the standard-setting process. Those investigators who employ reliable methodologies and who design experiments so that their results

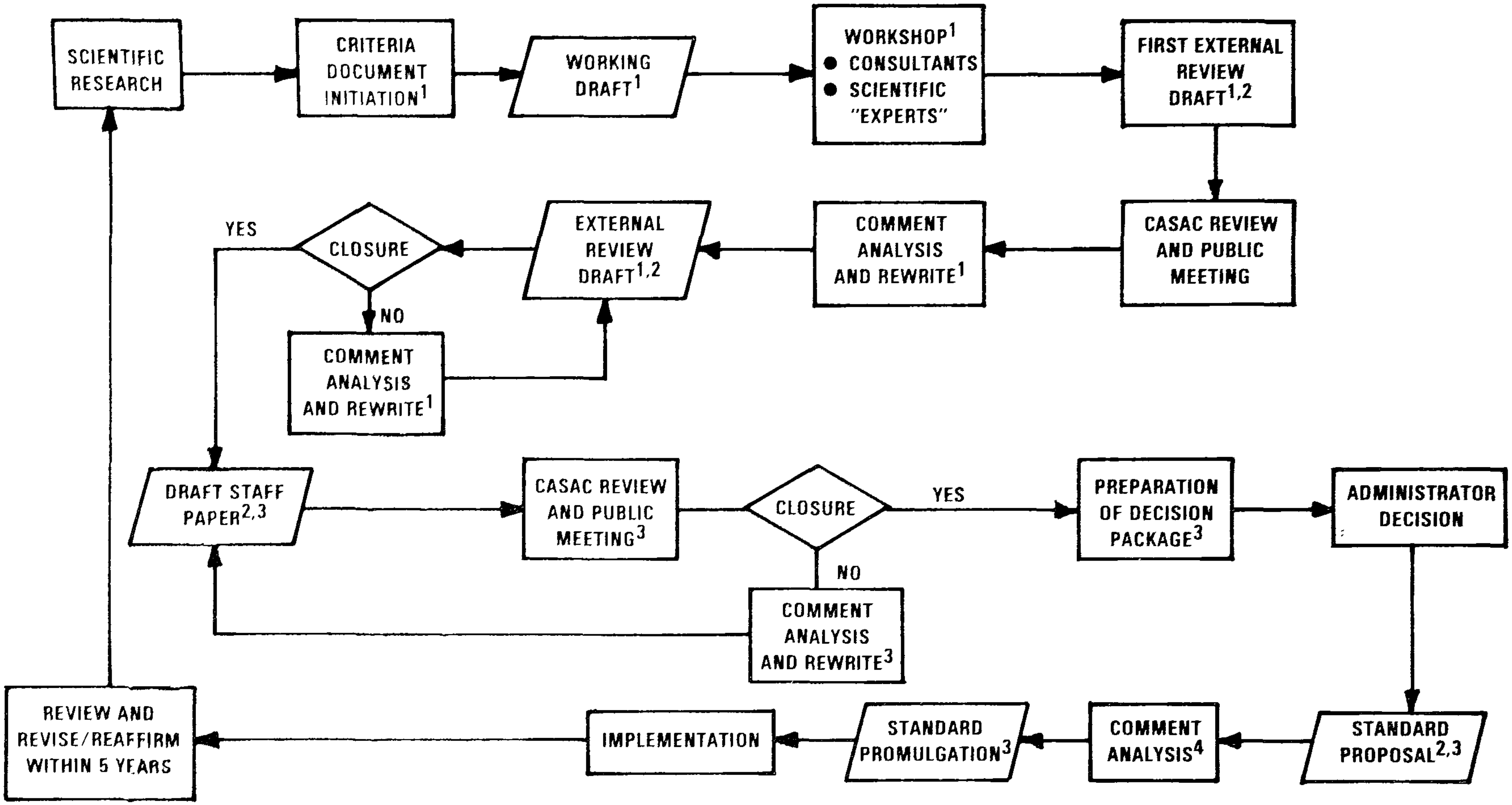


Figure 1-1. The Standard Review and Development Process (reproduced with permission from Zaragoza 1982)

[1] Office of Research and Development assumes primary responsibility for these activities. The Environmental Criteria and Assessment Office (ECAO) plays a major role in preparation of the criteria document.

[2] Public comment is requested at this stage.

[3] Managed by the Office of Air, Noise, and Radiation.

[4] This phase of the process includes one or more public meetings, receipt and formal review of public comments, the preparation of a revised regulatory decision package, formal Agency review, and a final decision by the Administrator.

will be relevant to ambient situations usually produce studies that prove to be of greatest relevance in standard-setting.

As Figure 1-1, illustrates, the next step in the standard review process is the development of a "staff paper." The staff paper evaluates and interprets available scientific and technical information most relevant to the standard review, and presents recommendations on alternative approaches to revising or maintaining standards.

Both the criteria document and staff paper are reviewed by the Clean Air Scientific Advisory Committee (CASAC). The CASAC is an independent scientific review committee established by the Clean Air Act to provide the Administrator with advice on the scientific issues related to NAAQS. The Clean Air Act specifies that CASAC be composed of seven members, including at least one member of the National Academy of Science, one physician, and one person representing State Air Pollution control agencies. Formal review on each document is complete when a "closure" memorandum, which indicates committee endorsement of the document, is sent from the CASAC chairman to the Administrator.

The effects of pollutant mixtures are currently assessed as modifying influences on plant sensitivity for a pollutant under review. This is consistent with section 108 of the Clean Air Act, which specifies that the criteria for a pollutant shall include information on "the types of air pollutants which, when present in the atmosphere, may interact with such pollutant to produce an adverse effect on public health or welfare." In ongoing reviews, the concern for pollutant mixtures arises when exposure to pollutant mixtures appears to be greater than the effects that would be expected from exposure to the individual pollutants. If it is determined that a combination exposure could produce effects that are greater than the effects of exposure to an individual pollutant, then adjustments in the level of the standards for the individual pollutant may be the appropriate response. While consideration could be given to establishing a combined standard, the details of specifying and complications associated with implementing such a standard would have to be carefully evaluated.

Secondary ambient air quality standards must be adequate to protect the public welfare from any known or anticipated adverse effects associated with the presence of a criteria air pollutant. Given the mandate in the Clean Air Act to protect public welfare from any known or anticipated adverse effect, it is incumbent upon the Agency to consider the effects of pollutant mixtures on vegetation. The task of deciding which or at what point welfare effects become adverse is a difficult one. Because there is usually no sharp demarcation between a level where effects of uncertain significance are reported and a level where clearly adverse effects occur, the Act explicitly requires the Administrator to exercise judgment in setting a standard. Though relying heavily on scientific advisors for technical evaluation of data and for those judgments that are scientific in nature, the Administrator alone is responsible for considering risks and determining at which point effects should be regarded as adverse.

RECENT VEGETATION STANDARD REVIEWS

Pollutant mixture studies become most important for standard review exercises when the presence of additional pollutants causes effects that are greater than the effects of exposure to the pollutant alone. Although Tingey et al. (1971a, 1973a) have reported such results, these studies have not provided compelling support for either a combination standard or a more stringent standard based on pollutant mixture effects (EPA 1982a, 1982b).

Plants exposed to pollutant mixtures may exhibit different types of responses that are influenced by several variables that are often difficult to predict. The general kinds of responses that may follow plant exposures to mixtures of ambient pollutants are described in Chapter 3. All these kinds of responses are found to some degree in the experiments using SO_2, O_3, and NO_2.

Available information, taken primarily from laboratory studies of foliar injury, report the following responses to pollutant mixtures (EPA 1982a, 1982b):

a. At lower concentrations (e.g., 0.10 ppm NO_2 and 0.05 ppm SO_2 for a few hours), little, if any, foliar injury is observed from either mixtures or single pollutants alone.

b. At higher concentrations (e.g., 0.10 ppm O_3 and 0.50 ppm of SO_2 for a few hours), foliar injury may be greater than the amount of foliar injury that could be predicted by adding the amount of foliar injury produced by either pollutant alone.

c. At still higher concentrations, usually not observed in the ambient air, the amount of foliar injury produced may be equal to or less than that predicted by adding the amount of foliar injury produced by each pollutant alone.

If these generalizations are correct, greater than additive responses should have a greater potential for occurring in the ambient air than additive or less than additive responses.

The interpretation of the results of pollutant mixture studies is also complicated by the exposure regimes used. Although studies employing unrealistic exposure regimes may contribute to our understanding of plant response, they are very difficult to use in assessing impacts of air pollution on vegetation. For example, it is reasonable to expect concurrent SO_2 and NO_2 exposures for peak exposures near a coal-fired boiler (Table 1-1). However, it is unlikely that high O_3 concentrations would co-exist with peak SO_2 and NO_2 levels because nitric oxide (NO) would titrate the O_3, increasing NO_2 and reducing O_3 concentrations. Such information suggests that experimental designs employing sequential exposure to pollutants would be a more realistic approach for simulating exposure regimes.

Table 1-1. Plant Exposure to Criteria Pollutants

Pollutant(s)	Sources	Exposure Pattern Characteristics	Comments
O_3[*]	Not a primary emission; results from photochemical reactions involving reactive hydrocarbons, nitrogen oxides and oxygen.	- Peak O_3 concentrations at some sites can exceed 0.2 ppm over an hour. - Average (yearly) O_3 concentrations can range between 0.025 and 0.07 ppm.	Peak O_3 levels may occur in the same region as elevated SO_2 concentrations, but peak levels of each pollutant would be expected to occur at different times.
NO_2[**]	Results from combustion processes from mobile and industrial and domestic (e.g., oil furnace) sources.	Concentrations tend to reach short-term peaks near sources (0.06 to about 0.5 ppm for peak hourly averages). Long-term yearly averages range between 0.01 and 0.08 ppm in urban areas and are about 0.001 in rural areas.	If nitric oxide is released in the presence of O_3, then the O_3 will be titrated increasing NO_2 and decreasing O_3 concentrations.
SO_2[†]	Emitted primarily from combustion or processing of sulfur containing fossil fuels and ores.	Modeling results indicate that the current 24-hour standard would not prevent 1-hour peaks in the range of 0.5 to 0.75. Seasonal averages occurring over large regions tend to be higher in the northeastern U.S. (~0.01 to 0.02 ppm) than in other parts of the U.S.	Peak SO_2 levels near sources (e.g., coal-fired power plants) are strongly affected by meteorology. It is likely that short-term peak concentrations of SO_2 and NO_2 would have considerable overlap (some displacement of the peak NO_2 level relative to the peak SO_2 level is expected due to possible conversion of NO to NO_2).[††]

---

[*] Source: EPA 1978.

[**] Source: EPA 1982a.

[†] Source: EPA 1982b.

[††] Source: Personal communication from H. Cole to L. Zaragoza.

Use of Mixture Studies in Ongoing Standard Reviews

Taken as a whole, the cumulative information on both long- and short-term combined exposure studies provides conflicting results that are difficult to interpret. Biological responses to pollutant mixtures do not show consistent patterns. The situation is further complicated by the use of exposures in both acute and chronic exposure studies that are not representative of exposure patterns, distributions, and levels of pollutants observed in ambient air.

The criteria documents and staff papers recently have been completed for both SO_x and NO_x. Based on its review of the criteria documents, EPA staff concluded that the available data on combination exposures indicate that plant responses to NO_2 and SO_2, either together or in various combinations with O_3, are highly variable. For example, one study reported that exposure of a commercial crop species to equal concentrations (0.20 ppm) of NO_2 and SO_2 caused less injury in five of six species tested (Tingey et al. 1971a). In the review of the SO_2 standard, studies examining effects of SO_2 near point sources included some NO_2 and higher O_3 before and after the SO_2 fumigation events. Injury attributed to SO_2 under these conditions resembled typical SO_2 foliar injury; it is not possible to determine whether plants in this study responded to different levels of SO_2 from those reported in studies of SO_2 alone.

IDENTIFICATION AND TREATMENT OF UNCERTAINTIES

Additional research is necessary to reduce uncertainties associated with assessing regulatory alternatives. This section presents major areas of uncertainty that are identified in recent EPA staff assessments of information for recent NAAQS reviews (EPA 1982a, 1982b). A more explicit treatment is being developed for handling uncertainties in the biological information that is used to support regulatory alternatives. The relative prioritization of these research needs from a regulatory perspective is not directly addressed. As the discussion of plant response to pollutant mixtures in earlier sections indicates, response can vary greatly. Interpretation and comparison of results from different studies is complicated by variability in plant response, which is influenced by a number of factors, including: experimental exposure regime, exposure situation, biological endpoint, fundamental response triggering mechanisms, genetics, and environment.

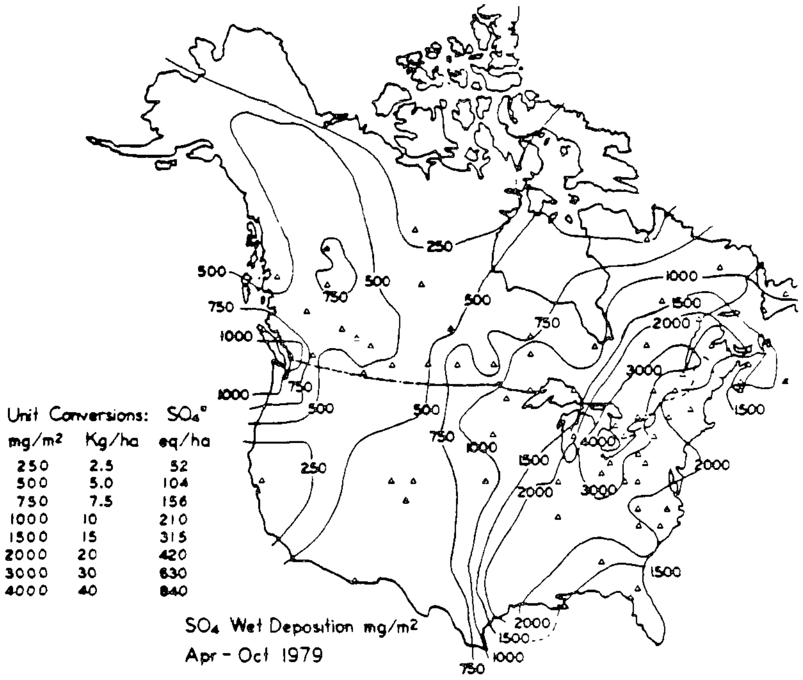
Perhaps the greatest impact of pollutant mixtures lies in the potential effects of the complex mix of pollutants that is associated with acidic deposition. Here SO_2, acid aerosols, O_3, and other pollutants may cause or promote ecosystem effects. Figure 1-2 shows regional wet deposition of sulfates for "summer" and "winter" seasons, with wet sulfate deposition occurring over relatively large areas. If available data from the Electric Power Research Institute's Sulfate Regional Experiment (SURE) are representative of regional SO_2 and sulfate levels, then SO_2 levels may be expected to be substantially higher than sulfate levels (Mueller et al. 1980). Moreover, ozone and other photochemical oxidants and other acidic aerosols (e.g., nitrates, organics) also occur, in these same regions.

Genetics and Environment

Both genetics and environment can affect plant sensitivity to pollutants (EPA 1982a, 1982b). Different species vary in their sensitivity to pollutant exposure, even when environmental conditions are identical. Moreover, the influence of environment, especially light and water stress, have been shown to produce profound effects on plant sensitivity to air pollutants.

Exposure Situations

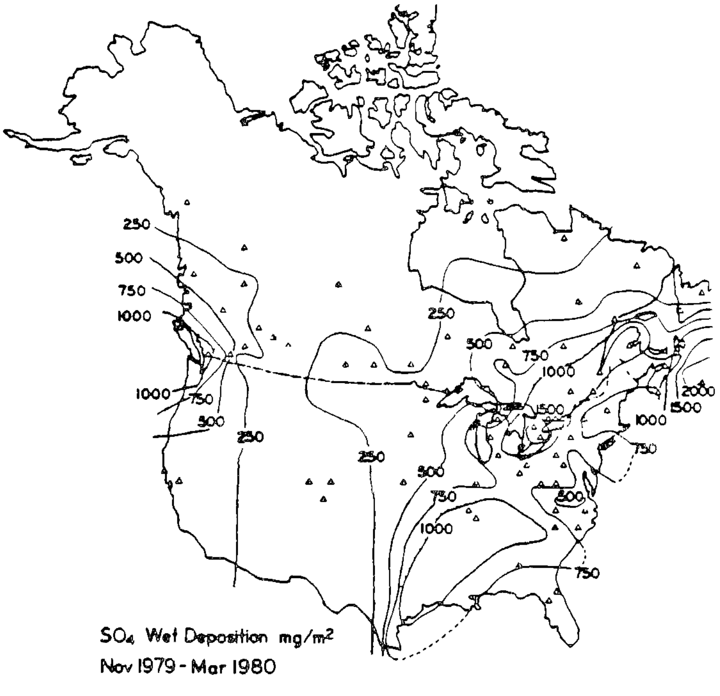
The air quality information used to supplement the standard review and development process focuses on defining and characterizing exposure to populations that might be impacted. In the case of vegetation, air quality information is usually separated into one of three exposure situations: point, area, and regional exposures. The conditions of plant exposure for each of these situations differ, as do the concentrations associated with different



Unit Conversions: $SO_4^{=}$

mg/m²	Kg/ha	eq/ha
250	2.5	52
500	5.0	104
750	7.5	156
1000	10	210
1500	15	315
2000	20	420
3000	30	630
4000	40	840

SO_4 Wet Deposition mg/m²
Apr - Oct 1979



SO_4 Wet Deposition mg/m²
Nov 1979 - Mar 1980

Figure 1-2. Seasonal SO_4^{2-} wet deposition (mg/m^2) for North America, "Summer" April through October 1979 and "Winter" November 1979 through March 1980. Sites (Δ) reporting data are from the NADP and CANSAP precipitation monitoring networks (reproduced with permission from Glass and Brydges 1981).

averaging periods, the distribution of concentrations within an averaging period, and the spatial distribution of concentrations. Table 1-1 summarizes information related to plant exposure including: emission sources, characteristics of plant exposure patterns (e.g., peak and mean concentrations), and potential for combined exposures.

Experimental Exposure Regimes

Studies of plant exposure are separated into two basic categories: plant responses to controlled exposure and plant response to uncontrolled exposures in the field. In general, controlled exposure studies have not used exposure regimes representative of those expected to occur in the ambient air. Interpretation of some earlier controlled exposure studies is not only complicated by unrealistic exposure regimes but also by growing conditions that were unusually favorable for plant growth, which probably increased the sensitivity of plants (EPA 1982b).

The differences in the distribution of pollutants in time, space, and concentration can be shown by comparison of regional and point source situations. Figure 1-2, illustrated regional concentrations over seasonal averaging periods. Although pollutant concentrations may be elevated to some extent, over relatively large regional levels, the changes in pollutant concentration are typically gradual and extend over large distances. The situation is markedly different in the case of point sources. Figure 1-3 shows a fumigation event near a coal-fired power plant; changes in pollutant concentration are most strongly influenced by wind direction, windspeed, emissions, and mixing level. Fumigation events in this situation tend to last only a short time. During these fumigations, plants are typically exposed to both SO_2 and NO_2 in SO_2/NO_2 ratios ranging from 3 to 15. As concentrations of these pollutants decrease, the concentrations of SO_2 and NO_2 may show convergence (Noggle and Jones 1981). Although SO_2 and NO_2 may be present simultaneously, it is unlikely that peak O_3 concentrations would occur simultaneously. This situation tends to occur because nitric oxide (NO) titrates O_3, elevating NO_2 concentrations and reducing O_3 concentrations. Such

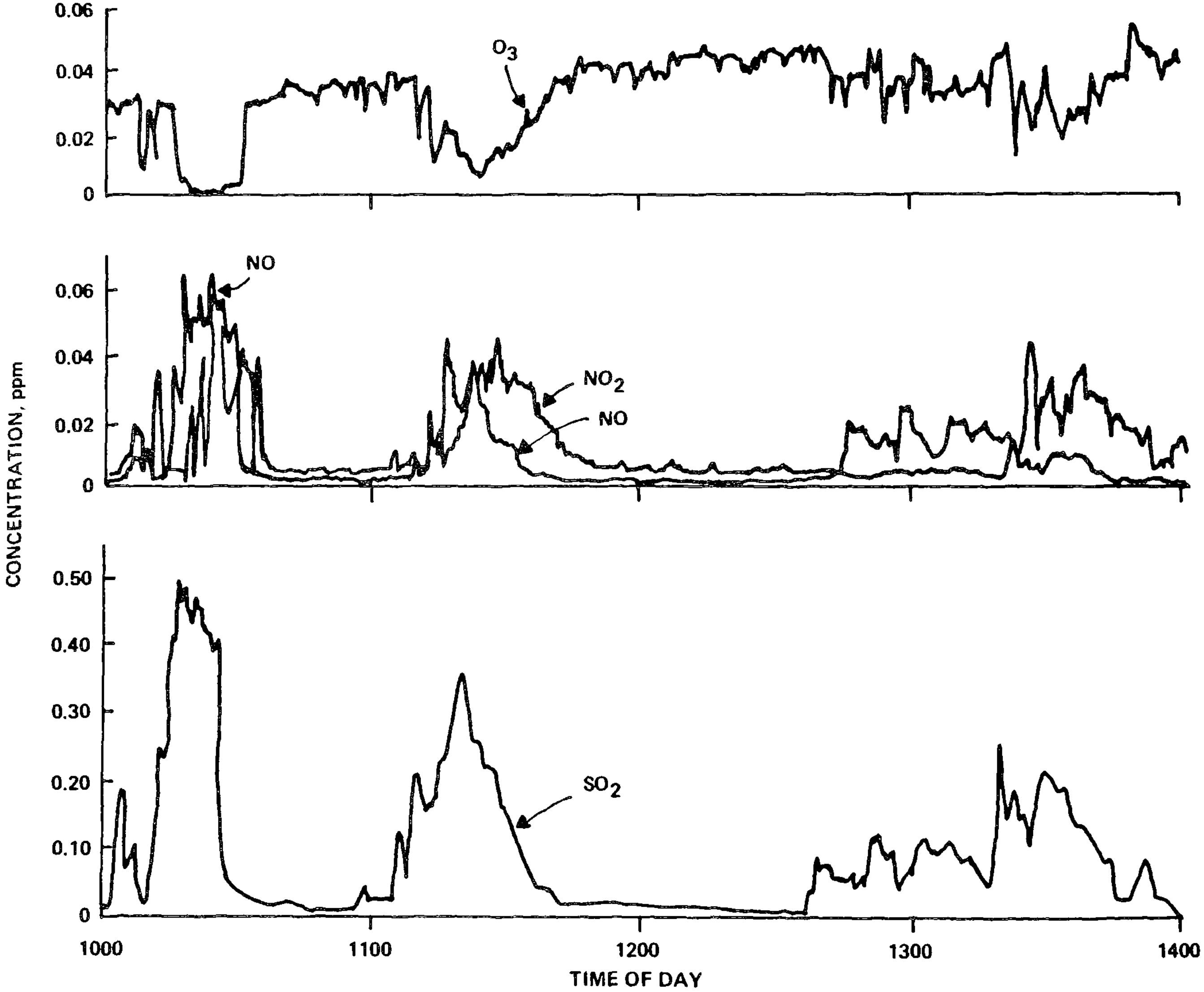


Figure 1-3. Temporal changes in ground level concentrations of SO_2, NO_2, and O_3 during a fumigation event near a coal-fired power plant (modified with permission from Noggle and Jones 1981).

information suggests that experimental designs employing sequential exposure to pollutants could be a more realistic approach for simulating exposure regimes (see Chapter 2 for further discussion).

Observations of plant response in the field are needed to confirm observations in controlled exposure studies. However, the lack of control of environmental variables affecting plant sensitivity, including the presence of other pollutants, reinforces the need for these studies to be complemented by controlled exposure studies.

Mechanisms of Action

The weaknesses in our understanding of the mechanisms of damage preclude the use of mechanistic models as a predictive tool. Available studies on the mechanisms by which pollutants cause effects have focused on single pollutants. Basic information on the physiology, growth, and development of plants eventually should be useful in the development of mechanistic models.

Even studies that have used reasonable concentrations for the averaging periods selected, they have not generally reflected the distribution of air pollutants within the averaging period that might be expected for the situation. Studies by McLaughlin et al. (1979) demonstrate that the relative distribution of air pollutants within an averaging period can be an important determinant of plant response to SO_2.

Biological Endpoint

The evaluation of plant response for purposes of setting NAAQS is complicated by consideration of different biological endpoints. Depending on the objective of the study, researchers have employed a variety of endpoints in examining the effects of air pollutants on vegetation. However, those endpoints that can be used as a measure of the intended use of the plant are most useful.

As the standard-setting process has evolved, the use of information in the process has changed. Available information suggests that foliar injury played a prominent role in the setting of the original NAAQS. However, in 1979, one of the primary reasons for the relaxation of ozone secondary standard was the lack of data showing reductions in growth and yield in agricultural crops or native vegetation at exposures below the level at which the primary standard was set. Currently, major emphasis is placed upon the characterizing impacts on intended use of the plant. Using this approach, foliar injury is of greater importance in ornamentals, native vegetation, and crops whose leaf appearance can be an important consideration in marketability (e.g., spinach).

Because a number of studies have employed foliar injury as the endpoint, the associations between foliar injury and yield have been sought by some researchers as a means of estimating possible effects on yield from foliar injury data. Although increases in foliar injury and decreases in growth and yield tend to occur simultaneously when pollutant exposures are sufficiently high, foliar injury is an imprecise measure of the effect of pollutants on growth and yield parameters. Growth and yield reductions may occur with minimal or no accompanying foliar injury (Reinert and Weber 1980) and it is possible to have foliar injury with no apparent effect on crop yield (Heagle et al. 1974). It is possible that effects on growth and yield are most consistently related to increases in foliar injury when development is limited by photosynthetically active surface area or leaf area.

SUMMARY

Conflicting results from both short- and long-term mixture studies are difficult to interpret. Although these studies indicate that pollutant mixtures can produce effects that are greater than additive, especially at low exposure levels, additional research is needed to resolve reported differences in biological responses. In addition to resolving these differences, information in the following areas would be useful for improving the scientific basis of regulatory activities designed to protect vegetation:

a. Differences in peak pollutant concentrations associated with the temporal and spatial patterns should be reflected in the design of exposures employed for pollutant mixture studies.

b. Exposures should be representative of peak and mean concentrations occurring in the ambient air.

c. The mechanisms by which plants respond to pollutant stress should be elucidated.

Current studies involving pollutant mixtures are still characterizing the types of biological responses to pollutant exposure. Methodologies have evolved sufficiently to develop reasonable models of plant response to pollutant mixtures in the field.

2. THE CO-OCCURRENCE OF SO_2/NO_2, O_3/SO_2, AND O_3/NO_2 MIXTURES IN AMBIENT AIR

INTRODUCTION

A great deal of the air pollution vegetation effects literature deals with the direct impacts associated with O_3, SO_2, and NO_2 air pollutants acting as independent phytotoxic agents; there is a dearth of information that describes the effects associated with their mixtures. Critical to the development of relevant dose-response data is the identification of NO_2, SO_2, and O_3 exposure regimes that adequately describe the concentration, frequency of events, length of occurrence, and time between events. A review of the U.S. EPA's air quality data information base, SAROAD, the Electric Power Research Institute's Sulfate Regional Experiment (SURE) data, and the Tennessee Valley Authority's (TVA) air quality monitoring data was undertaken to characterize the exposure of pollutant mixtures at specific sites across the United States. Air quality information reported by EPA for 1981, EPRI SURE data for May through September 1978, and TVA data for May through September 1978, 1979, 1980, and 1981 were reviewed.

In developing estimates of plant exposure to pollutants, consideration should be given to characterizing exposures that are similar to ambient conditions. For the purposes of this analysis, we have utilized hourly averaged air quality data because the short averaging time provides important information to those scientists interested in developing pollutant exposure regimes for vegetation effects research.

Co-occurrence is defined as the simultaneous occurrence of hourly averaged concentrations at 0.05 ppm or greater for pollutant pairs (SO_2/NO_2, O_3/SO_2, or O_3/NO_2). A 0.05 ppm concentration was selected because minimum biological responses have been shown to exist at these levels. Tingey et al. (1971a) reported that a 4-hour exposure of several crops to levels up to 2 ppm NO_2 and 0.5 ppm SO_2, caused no injury when administered singly. Slight foliar injury was observed at 0.05 ppm NO_2 and 0.05 ppm SO_2. Ashenden (1978, 1979a) and Ashenden and Williams (1980) reported growth and yield suppression from combined exposures of 0.1 ppm NO_2 and 0.1 ppm SO_2, using a constant fumigation exposure for 103.5 hours per week for 20 weeks. These exposures caused significant reductions in the growth parameters of all four grass species tested. Because these exposures were based on a constant fumigation regime greater than one hour, it was believed that a one time hourly co-occurrence of 0.05 ppm represented a conservative definition for an event.

Based on a review of available data, EPA has previously concluded that there is inadequate evidence to determine a yield reduction relationship associated with vegetation effects for various ambient exposure combinations of pollutant gases (EPA 1981a, 1982b). This chapter explores the characteristics of co-occurring air pollutant mixtures and identifies exposures that may be considered typical of several rural monitoring sites across the United States.

THE POLLUTANTS

Sulfur dioxide is one of a number of sulfur-containing compounds found in the atmosphere. Although SO_2 enters the air primarily from the burning of coal and oil, it is also produced by other industrial and natural processes. EPA reports (EPA 1981b) that nationally, the urban SO_2 problems have diminished so that only a few urban areas now exceed the air quality standard. Pollutant peaks appear to be controlled by emissions and the topographical and meteorological conditions associated with air monitoring sites (EPA 1981b).

Nitrogen dioxide is one of a family of nitrogen oxides. Nitrogen dioxide plays a major role in the atmospheric reactions which produce photochemical oxidants (EPA 1981b). Two major factors that affect NO_2 concentrations are mobile source emissions and photochemical oxidation; both contribute to the observed diurnal variation in NO_x concentrations. EPA (1981c) reports that such a variation is described by a rapid increase in NO_2 in the morning as the result of NO emissions and photochemical conversion to NO_2. This is followed by a decrease of NO_2 in the midmorning due to advection and increasing vertical dispersion and loss of NO_2 in various atmospheric chemical transformation reactions. Peaks in the NO_2 concentration are often observed during other times (EPA 1981c); elevated NO_2 levels usually occur between 7 PM and 6 AM.

Unlike other gaseous criteria pollutants, O_3 is not emitted directly by specific sources. It is a secondary pollutant, formed in the air by photochemical chemical reactions between nitrogen oxides and volatile organic compounds, such as gasoline vapors, chemical solvents, and the combustion products of various fuels. Because the chemical reactions necessary to produce O_3 are principally controlled by sunlight, in most parts of the country O_3 reaches peak levels during the late spring and summer months between 11 AM and 4 PM. Year-to-year variations are associated with factors such as meteorology, measurement and calibration techniques, and quality control procedures (EPA 1981b).

THE DATA BASES

In accordance with the requirements of the Clean Air Act and the Environmental Protection Agency's regulations for State Implementation Plans (SIPs), ambient air quality data resulting from air monitoring operations of state, local, and Federal networks must be reported to EPA each calendar year. The SAROAD base is the established medium for the information distribution. Ambient observations reported to EPA must satisfy minimum summary criteria--sampling interval (e.g., continuous, noncontinuous) and period of coverage (e.g., quarterly, annually). The criteria (EPA 1982c) for continuous observations, with sampling intervals of less than 24 hours, are

1. Data representing quarterly periods must reflect a minimum of 75 percent of the total number of possible observations for the applicable quarter.
2. Data representing annual periods must reflect a minimum of 75 percent of the total number of possible observations for the applicable year.

The SURE program is an EPRI-sponsored investigation of air quality in the northeastern United States. The program is directed primarily at regional definition of the relationships between emissions of SO_2 and the distribution and concentrations of its reaction product, sulfate. The ultimate objective is to develop a regional-scale air quality model capable of predicting sulfate levels as a function of sulfur dioxide emissions. Investigators have collected SO_2, NO, NO_x, O_3, and total suspended particulate matter (TSP) air quality data for nine sites that EPRI identified as removed from major local emissions of the above pollutants and their precursors (EPRI 1982). The EPRI remote sites provide a valuable data base from which the frequency of co-occurrence of pollutant mixtures can be evaluated. In 1978, the nine SURE sites were located at

Montague, Massachusetts
Scranton, Pennsylvania
Indian River, Delaware
Duncan Falls, Ohio
Rockport, Indiana
Giles County, Tennessee
Ft. Wayne, Indiana
Research Triangle Park, North Carolina
Lewisburg, West Virginia

Eight sites were included in the analysis because they were considered rural. For the purpose of this study, sites located near agricultural land and containing a point source were identified as rural. Thus, Scranton, Pennsylvania and Indian River, Delaware, with SO_2 point sources nearby, were considered rural and included in the analysis. Similarly, sites influenced by automobile emissions (diurnal NO_x fluctuations) such as Rockport, Indiana and Ft. Wayne, Indiana, were categorized rural because they were surrounded by agricultural land.

The TVA provided printouts of hourly averaged ambient air quality data for those sites where simultaneous monitoring occurred for O_3, SO_2, and NO_2. For this analysis, data from seven sites were used:

Allen 7 (Shelby County, Tennessee)	1978 - 1980
Paradise 21 (Muhlenberg County, Kentucky)	1978 - 1982
Paradise 23 (Muhlenberg County, Kentucky)	1980
Giles County (Tennessee)	1980 - 1982
Land Between the Lakes (Trigg County, Tennessee)	1982
Murphy Hill (Marshall County, Alabama)	1980
Saltillo (Harden County, Tennessee)	1979 - 1980

The Allen 7 site is in an urban location near downtown Memphis and is a point source monitor for the Allen Steam Plant (6.6 km away). TVA reports that there are various types of heavy industry located 0.8 km to the northwest, an oil refinery 0.8 km to the south, and an interstate highway 400 m to the east. Paradise 21 and 23 are rural sites used to monitor the Paradise Steam Plant. Paradise 21 and 23 are 7.0 km and 6.0 km from the point source. There are no other major pollutant sources nearby. Most of the surrounding area is cultivated or in pasture. The Giles County site is rural, serving as a regional air quality background monitor, remote from any major sources of air pollutants. The Land Between the Lakes site is also a background monitor; virtually all the surrounding area is forested. The Murphy Hill and the Saltillo monitoring locations are PSD background sites. Thus, except for Allen 7, all TVA sites used in this analysis are located in remote areas.

RESULTS

To identify and characterize pollutant distribution at specific sites, the 1981 hourly averaged SAROAD air quality data were reviewed to identify all sites with a maximum NO_2, SO_2, or O_3 concentration equal to or greater than 0.05 ppm. Each site was then evaluated by determining whether one of the other two pollutants was co-monitored and also experienced an hourly averaged concentration equal to or greater than 0.05 ppm. After identifying those co-monitoring sites, the data base was evaluated with the following criteria:

1. Identify those sites where co-monitoring of SO_2 and NO_2 occurred and where 0.05 ppm was measured for each pollutant at least once during the 1981 sampling period.

2. Identify those sites where co-monitoring of O_3 and SO_2 occurred and where 0.05 ppm was measured for each pollutant at least once during the 1981 sampling season.

3. Identify those sites where co-monitoring of O_3 and NO_2 occurred and where 0.05 ppm was measured for each pollutant at least once during the 1981 sampling season.

Using the site identification code and the above criteria, a computer listing of the hourly averaged concentrations, by day and by month, was obtained for each of the 1981 SAROAD ozone, nitrogen dioxide, and sulfur dioxide monitoring sites for the months May through September 1981. Data from each identified site were reviewed for the possibility of the co-occurrence of 0.05 ppm SO_2/NO_2, O_3/SO_2, or O_3/NO_2 at least once during the months May through September. At least three months of data during the five-month period had to be available. Using the SAROAD site identification coding (EPA 1976), the selected monitoring locations were segregated into "rural" and "non-rural" categories. Rural sites are considered by EPA to be those monitoring locations that have not been designated as center city, suburban, or remote (far enough from any activity to measure geophysical background levels).

SO_2/NO_2

Most sites experienced fewer than 10 co-occurrences of SO_2/NO_2 during May through September. Usually there were weeks, sometimes months, between co-occurrences. Figure 2-1 illustrates the frequency site distribution for the SO_2/NO_2 co-occurrences. All of the rural sites sampled experienced less than 50 co-occurrences during the five-month season. This amounts to less than 1.5% of the total hours available (3,672) during the period. Only 6 of the 32 rural site years had more than 10 co-occurrence events; most non-rural sites experienced more than 10 events. Philadelphia experienced 123 co-occurrences during 1981.

The Indian River, Delaware rural site (located near the Indian River Power Plant) experienced a series of co-occurrence events (each lasting several hours) on May 26 and 27, and September 13, 19, and 23, 1978. Figure 2-2 shows the distribution of sulfur dioxide and nitrogen dioxide during the

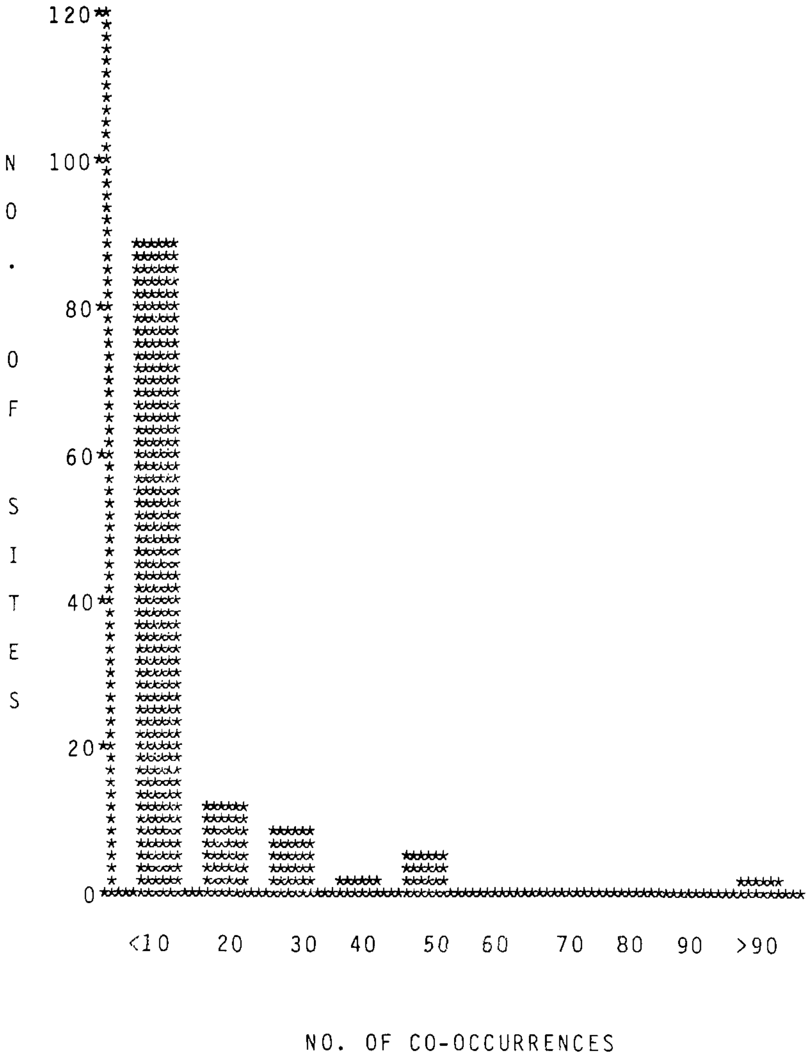


Figure 2-1. SO_2/NO_2 Co-Occurrence, Frequency Site Distribution

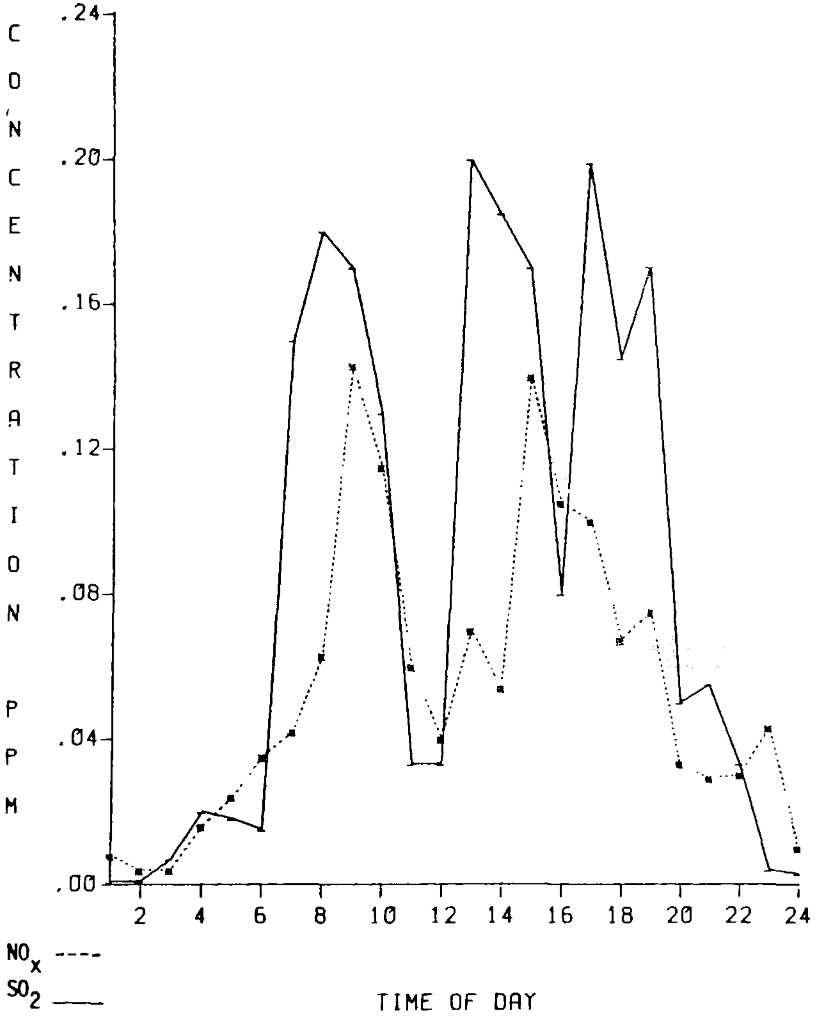
24-hour period of September 13, 1978. The numbers of events during the day began in early morning and lasted for several hours during the daylight. The episode disappeared the following day.

During 1979, the Paradise #21 TVA rural site experienced six co-occurrence episodes. The May 15 episode is presented in Figure 2-3. The one co-occurrence during that day was a typical pattern of episodes monitored during the season.

During 1980, the Allen Steam Plant No. 7 TVA site had 29 episodes. Figure 2-4 illustrates the concentration of SO_2 and NO_2 over the period May through September. The number of co-occurrences during the day was small and the time period between episodes was large. Figure 2-5 shows the episode for July 30.

Figure 2-6 illustrates the June 27, 1981 episode for Fontana, California. The site has been designated by EPA as rural industrial. During the five months monitored in 1981, the site experienced a large number of occurrences of nitrogen dioxide concentrations equal to or above 0.05 ppm. The frequency of occurrence was so great that when an SO_2 concentration above 0.05 ppm occurred, there was a high probability of the simultaneous occurrence of SO_2 and NO_2 concentrations above 0.05 ppm. Figure 2-6 shows the presence of the large number of nitrogen dioxide concentrations above 0.05 ppm.

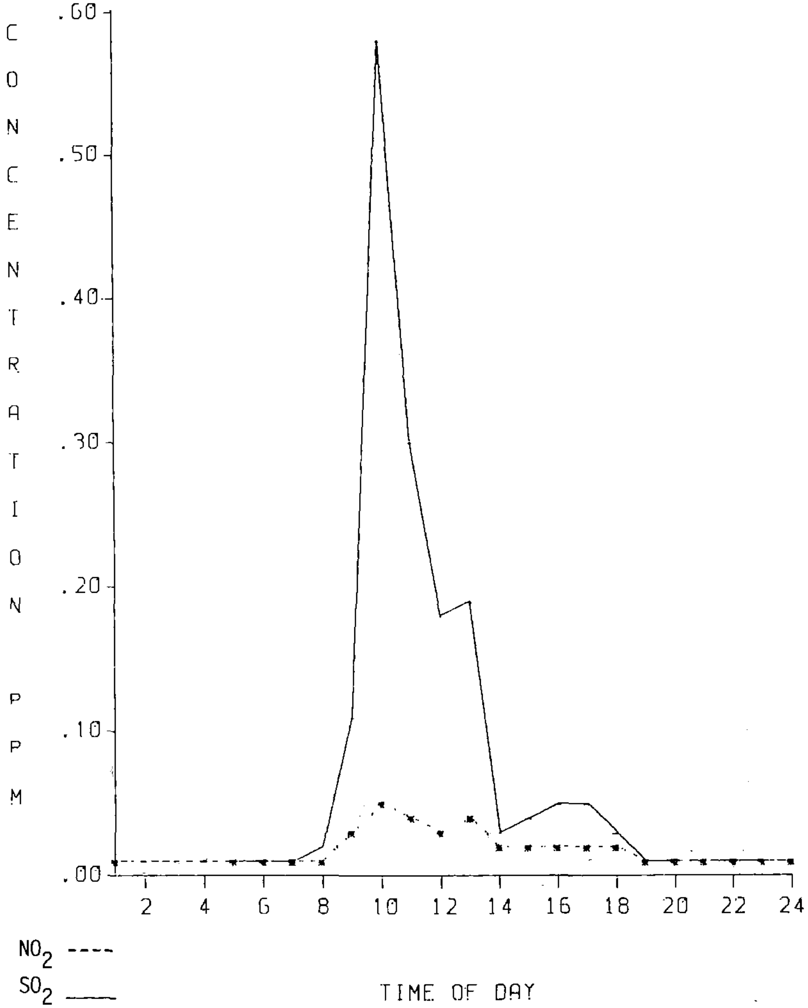
In contrast, the Kansas City, Kansas monitoring site experienced a small number of NO_2 occurrences above 0.05 ppm (94 $\mu g\ m^{-3}$) during the five-month period. The frequency of SO_2 concentrations equal to or greater than 0.05 ppm (131 $\mu g\ m^{-3}$) during the period was typical of the data produced for many of the rural sites analyzed. Figure 2-7 shows the exposure regime of SO_2 and NO_2 on September 4, 1981. On that date, at 9 PM, the sulfur dioxide concentration was 0.06 ppm (134 $\mu g\ m^{-3}$) and the nitrogen dioxide concentration was 0.05 ppm (102 $\mu g\ m^{-3}$). The simultaneous readings equal to or above 0.05 ppm lasted for only two hours.



Indian River, Delaware

September 13, 1978

Figure 2-2. Indian River, Delaware, SO_2/NO_x Co-Occurrence.



Paradise, Kentucky

May 15, 1979

Figure 2-3. Paradise, Kentucky, SO_2/NO_2 Co-Occurrence.

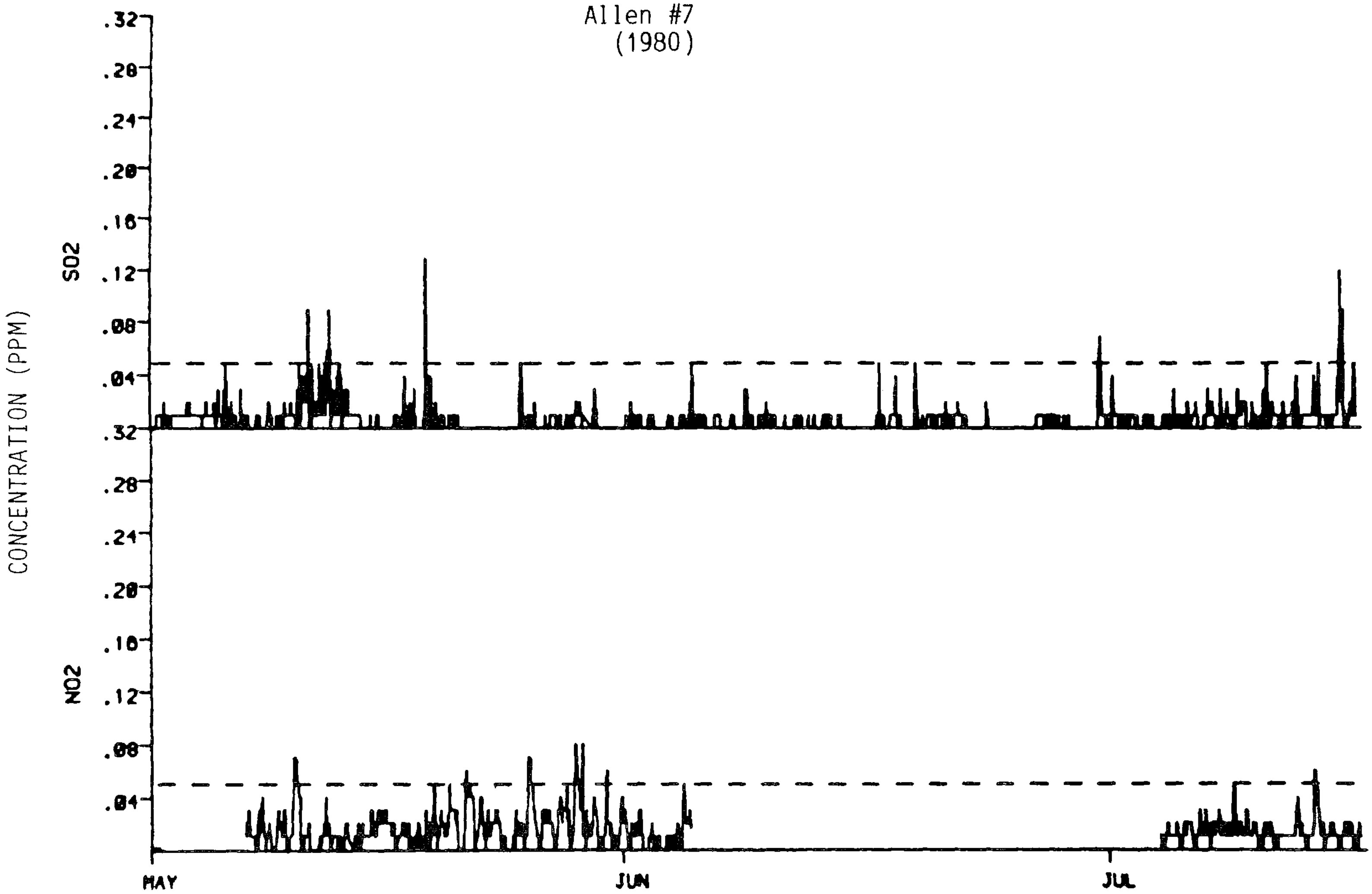


Figure 2-4. Allen Steam Plant, Tennessee, SO_2/NO_2 Concentration Over Time.

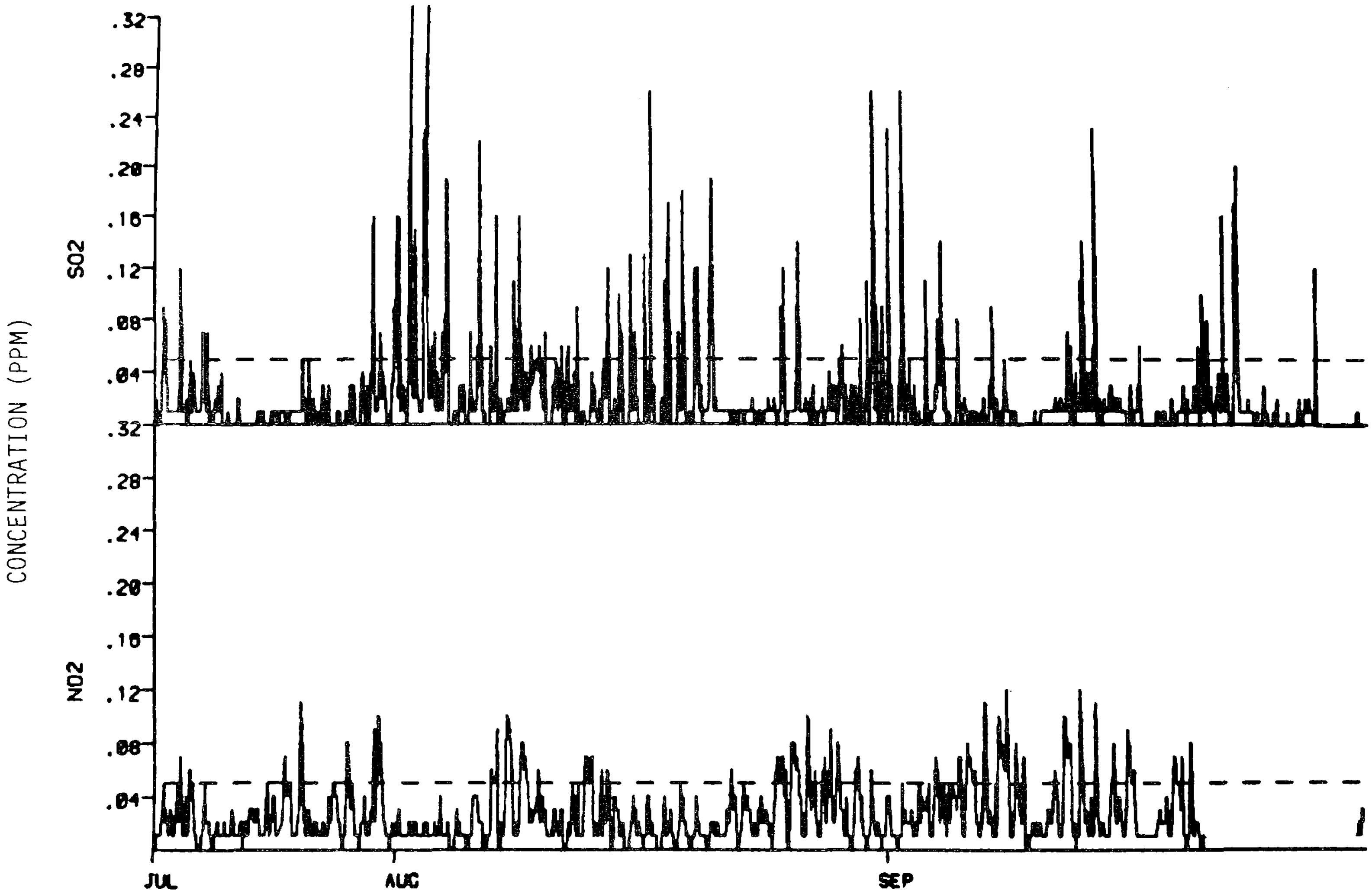
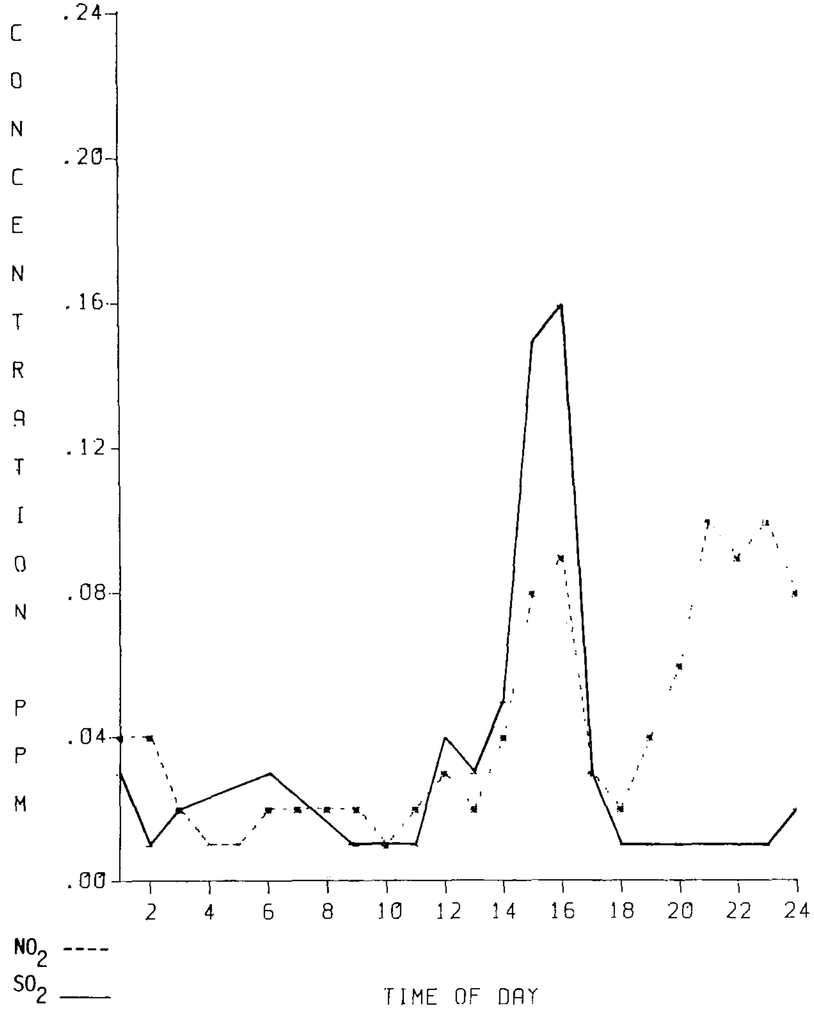
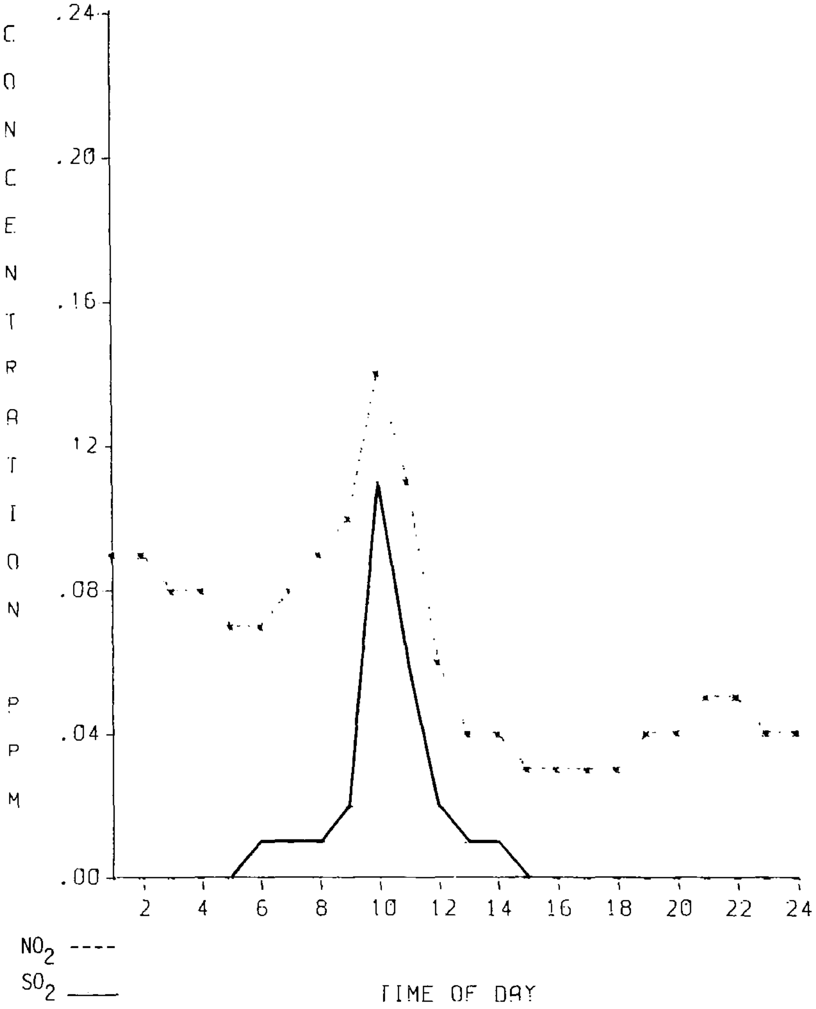


Figure 2-4. Allen Steam Plant, Tennessee, SO_2/NO_2 Concentration Over Time (Cont.).



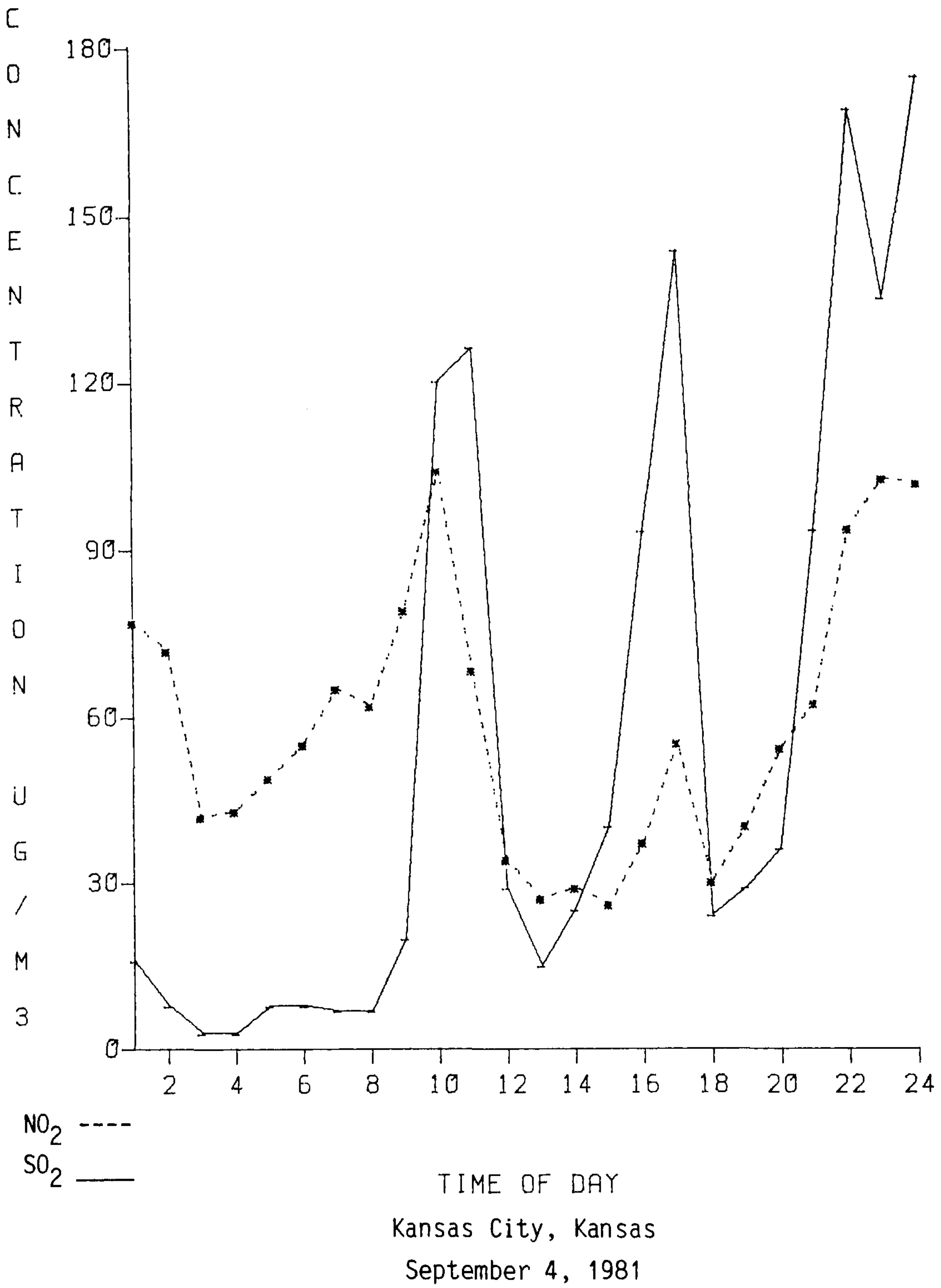
Allen Steam Plant, Tennessee
July 30, 1980

Figure 2-5. Allen Steam Plant, Tennessee, SO_2/NO_2 Co-Occurrence.



Fontana, California
June 27, 1981

Figure 2-6. Fontana, California, SO_2/NO_2 Co-Occurrence.



1ppm = 1960 µg/m^3 at 25°C and 1 ATM pressure.

Figure 2-7. Kansas City, Kansas, SO_2/NO_2 Co-Occurrence.

O_3/SO_2

Figure 2-8 shows the frequency site distribution for the number of occurrences of O_3 and SO_2 for the monitoring locations analyzed. The majority of sites (135) experienced less than 10 co-occurrences during the season. Only the Rockport, Indiana and Paradise No. 21 sites had more than 40 co-occurrences during the season (48 and 45, respectively), a small number when compared with the total number of sites that measured SO_2/NO_2 and O_3/NO_2.

The Fontana, California site experienced numerous occurrences of ozone episodes above 0.05 ppm. Therefore, there was a high probability that when the sulfur dioxide hourly averaged concentration rose above 0.05 ppm, both pollutants would be present at levels equal to or greater than 0.05 ppm. Events of co-occurrence, lasting a few hours each, were present in June, July, August, and September 1981. Figure 2-9 presents the July 23 data for the site. A large number of ozone episodes above 0.05 ppm is evident. However, only a few SO_2 hourly values above 0.05 ppm were present.

The Madison County, Illinois site has been coded as rural. Events of co-occurrence were present in May, June, and September 1981. The controlling variable for determining co-occurrence was sulfur dioxide with ozone events above 0.05 ppm present in sufficient amounts so as not to be the limiting factor. As in previous examples, the number of events during the entire period was small. Figure 2-10 shows the exposure pattern for a co-occurrence episode for May 27, 1981. The number of SO_2 concentrations above 0.05 ppm is small.

The ozone hourly data for Scranton, Pennsylvania were missing for the Pennsylvania site (May, June and July 1978). Available data did show sufficient amounts of ozone concentrations above 0.05 ppm to indicate that the co-occurrences were controlled by SO_2 events. Co-occurrences were present in

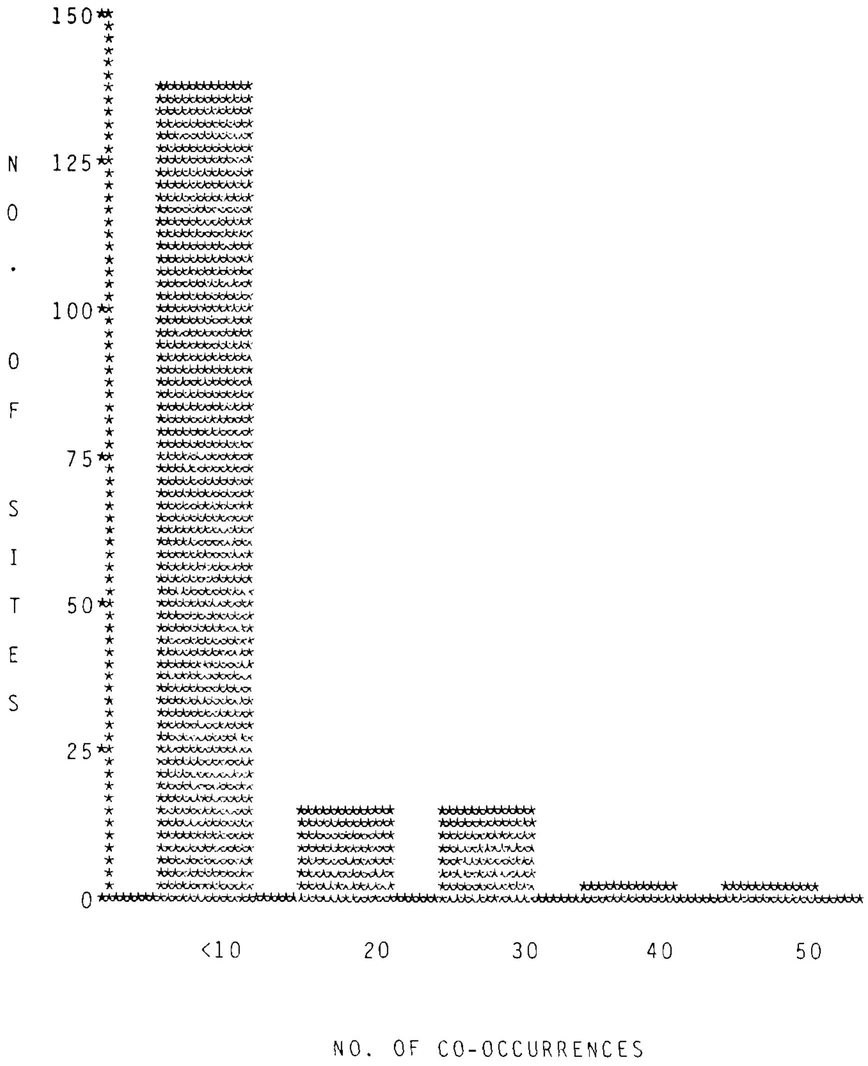
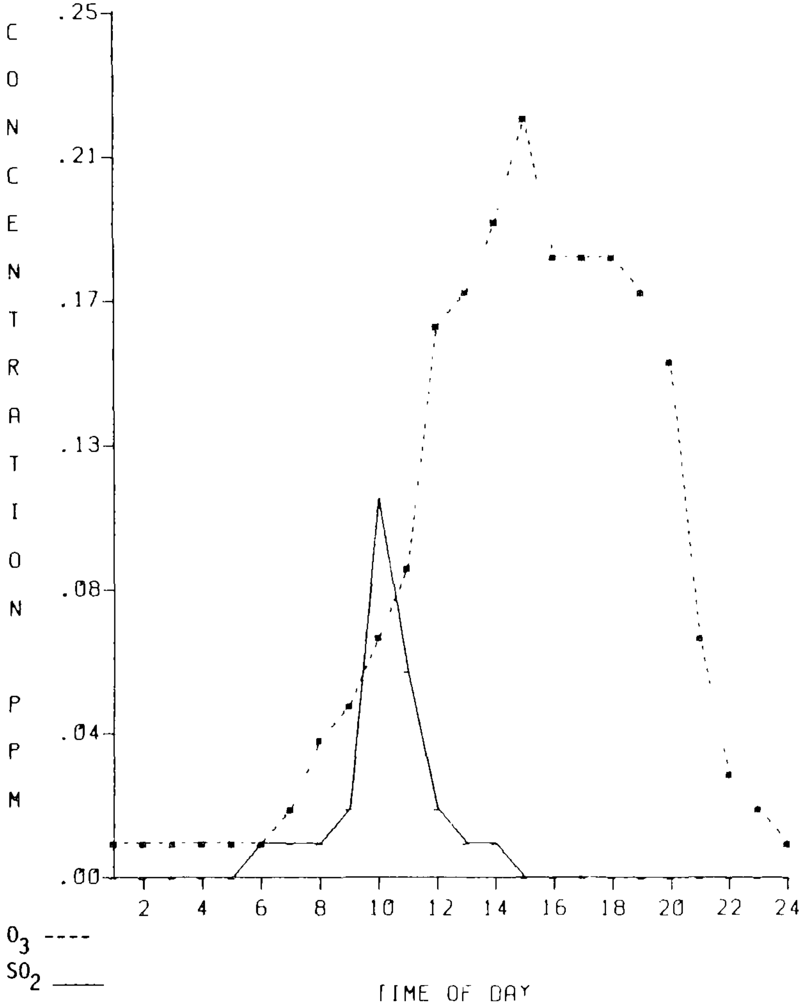
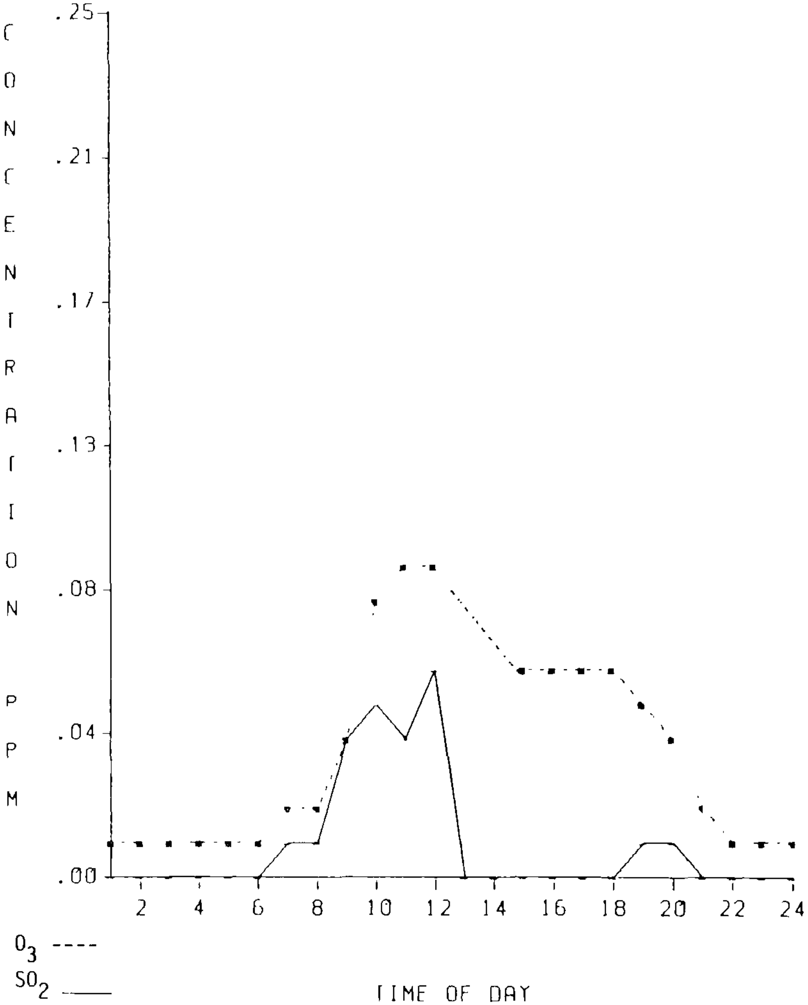


Figure 2-8. O_3/SO_2 Co-Occurrence, Frequency Site Distribution



Fontana, California
July 23, 1981

Figure 2-9. Fontana, California, O_3/SO_2 Co-Occurrence.



Madison County, Illinois
May 27, 1981

Figure 2-10. Madison County, Illinois, O_3/SO_2 Co-Occurrence.

July, August, and September. Figure 2-11 presents data for August 1, 1978. Ozone levels were above 0.05 ppm during mid-day when sulfur dioxide exposures were high. The sulfur dioxide concentrations above 0.05 ppm decreased in the early afternoon and the co-occurrences disappeared.

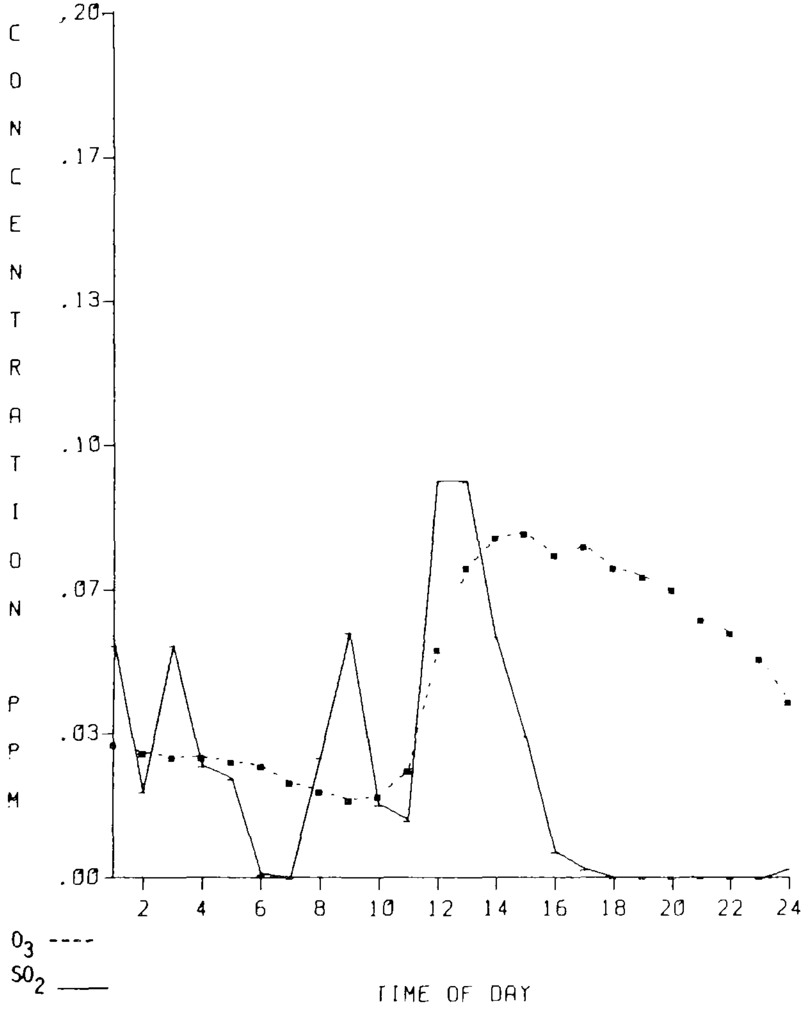
The Rockport, Indiana, site experienced co-occurrences in June, July, August, and September. An event would last for a few hours, then be followed by several days or weeks of no co-occurrence before another event. The number of ozone events was great and therefore, co-occurrence was controlled by the SO_2 events. Figure 2-12 describes the co-occurrence episode for August 25, 1978. The ozone remained above 0.05 ppm between 9 AM and 4 PM and the sulfur dioxide concentrations above 0.05 ppm defined the number of co-occurrences.

In 1978, the Paradise #21 TVA rural site experienced a large number of co-occurrences. Figure 2-13 shows the concentration of hourly averaged O_3 and SO_2 concentrations from May through September. Figure 2-14 illustrates the exposure regime during an episode on September 21, 1978. The ozone levels remained fairly constant during daylight, and the sulfur dioxide concentrations defined the number of co-occurrences.

O_3/NO_2

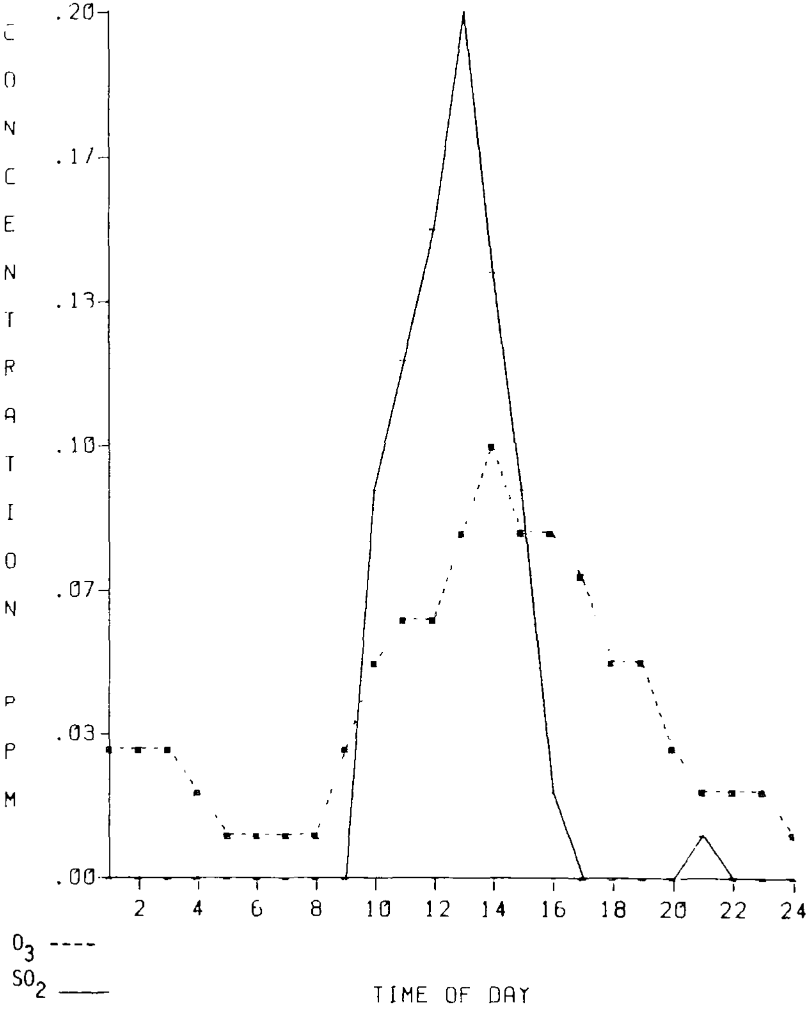
Figure 2-15 presents the summary of the frequency distribution for those sites that were analyzed for O_3/NO_2 co-occurrences. For the three pairs of air pollutants, the ozone-nitrogen dioxide combination showed, by far, the greatest number of co-occurrences. Several sites in the South Coast Air Basin of Southern California experienced more than 450 co-occurrences. The rural sites of Riverside, Fontana, and Rubidoux, California had more than 100 co-occurrences. However, most of the analyzed sites (143) experienced fewer than 10 co-occurrences. Denver, Colorado and San Jose, California did experience more than 100 co-occurrences.

Rubidoux, California, located in the South Coast Air Basin, is designated as a rural commercial site. There were ozone and nitrogen dioxide co-occurrences in May, June, July, August, and September 1981. Because nitrogen dioxide concentration maxima tended to peak in the evening or early



Scranton, Pennsylvania
August 1, 1978

Figure 2-11. Scranton, Pennsylvania, O_3/SO_2 Co-Occurrence.



Rockport, Indiana
August 25, 1978

Figure 2-12. Rockport, Indiana, O_3/SO_2 Co-Occurrence.

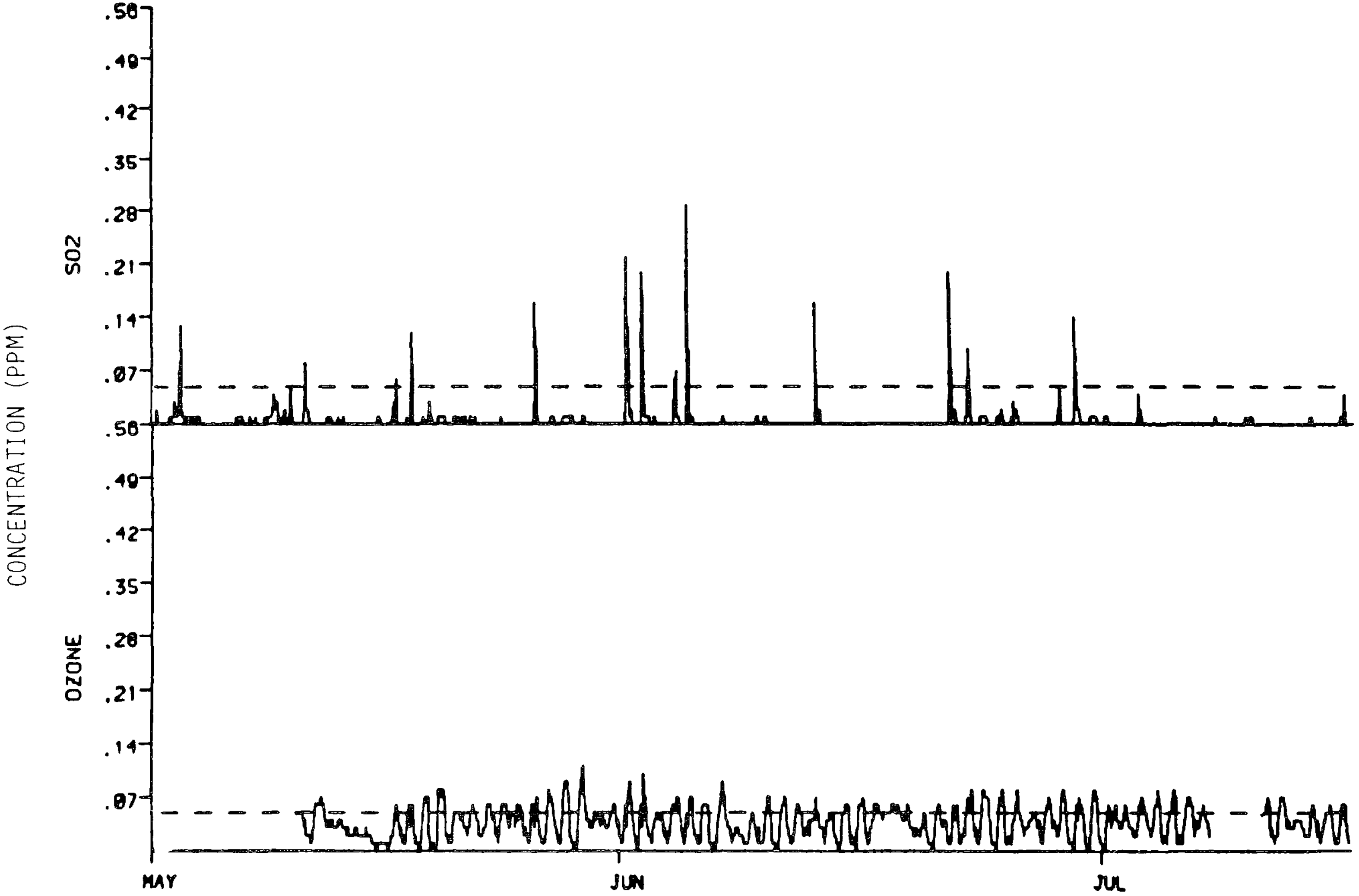


Figure 2-13. Paradise, Kentucky, O_3/SO_2 Concentration Over Time.

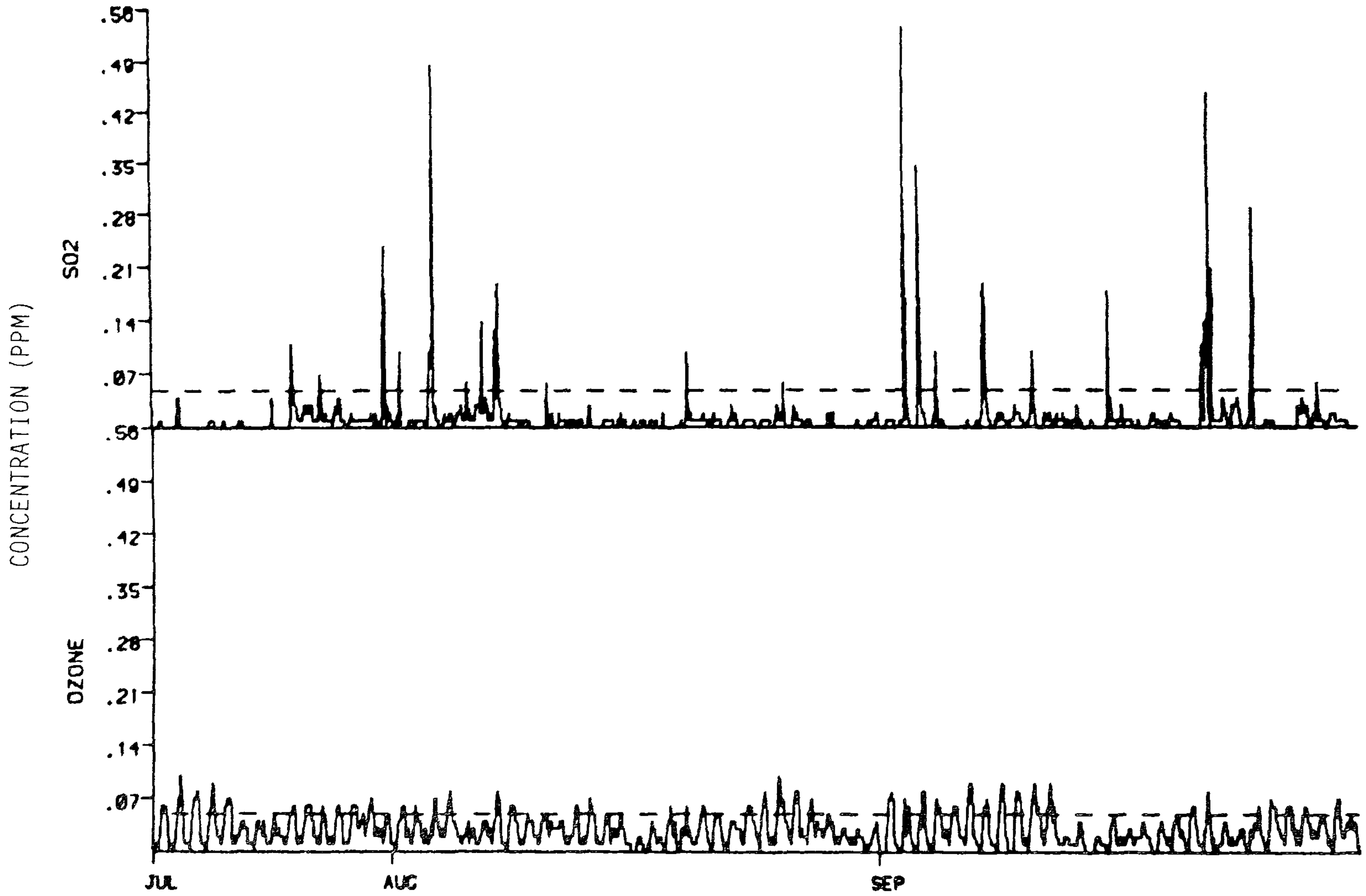
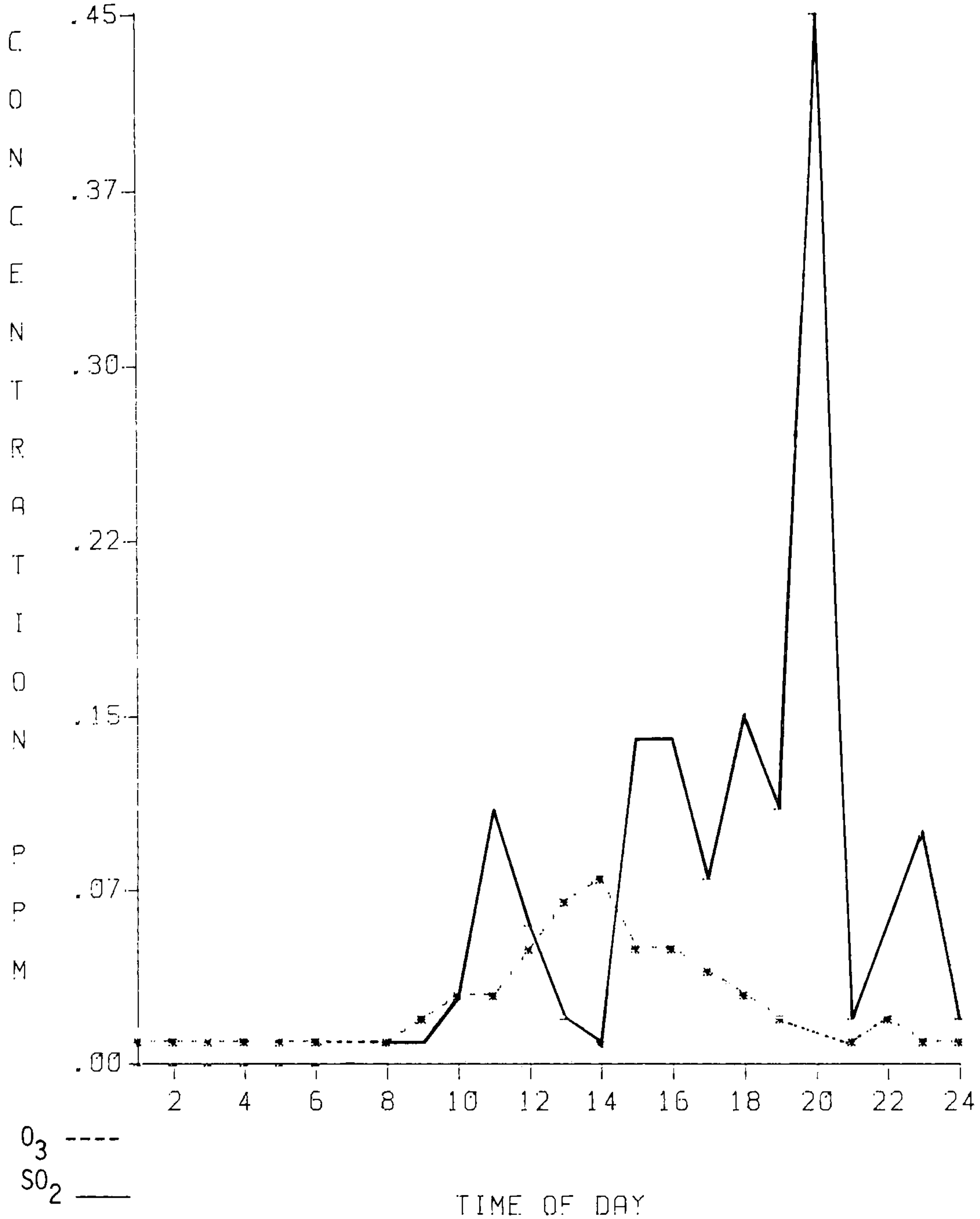


Figure 2-13. Paradise, Kentucky, O_3/SO_2 Concentration Over Time (Cont.).



Paradise, Kentucky
September 21, 1978

Figure 2-14. Paradise, Kentucky, O_3/SO_2 Co-Occurrence.

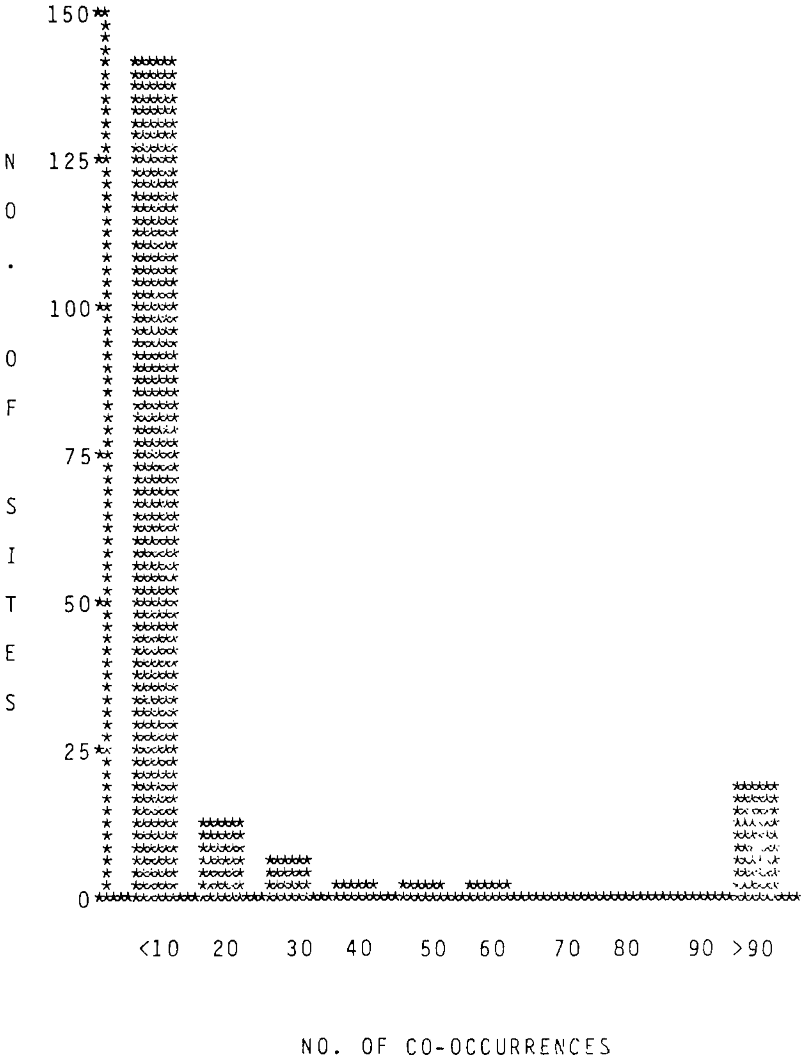


Figure 2-15. O_3/NO_2 Co-Occurrence, Frequency Site Distribution

morning, the co-occurrences were present at these times. Figure 2-16 illustrates the monitoring data for June 26, 1981. Because ozone concentrations were mostly above 0.05 ppm, the number of co-occurrences was large.

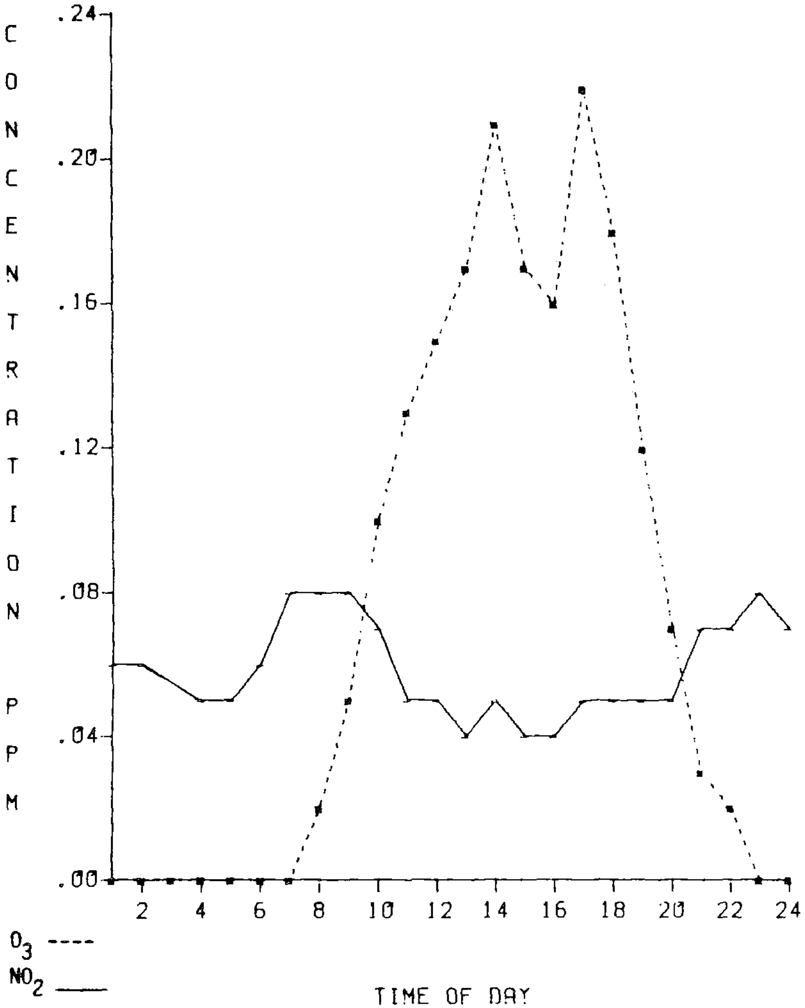
The Indian River, Delaware site experienced its only two co-occurrences on June 3, 1978. There were NO_x events on May 26 and September 13, but they did not match the dates of the many ozone events. Figure 2-17 shows that the co-occurrences appeared in the late afternoon and early evening.

During 1979, the Paradise No. 21 site experienced the only O_3/NO_2 co-occurrence that was measured from 1978 through 1982. Figure 2-18 shows the exposure regime on July 17, 1979. The co-occurrence was measured at 11 AM in the morning.

The Allen Steam Plant No. 7 TVA site experienced nine O_3/NO_2 co-occurrences during the 1978 season. Figure 2-19 describes the episode that took place on July 6. Ozone concentrations were fairly high during the daylight hours. In the early evening, the nitrogen dioxide concentrations rose above 0.05 ppm, resulting in the co-occurrence events. Figure 2-20 shows the hourly averaged concentrations for O_3 and NO_2 for May through September.

DISCUSSION

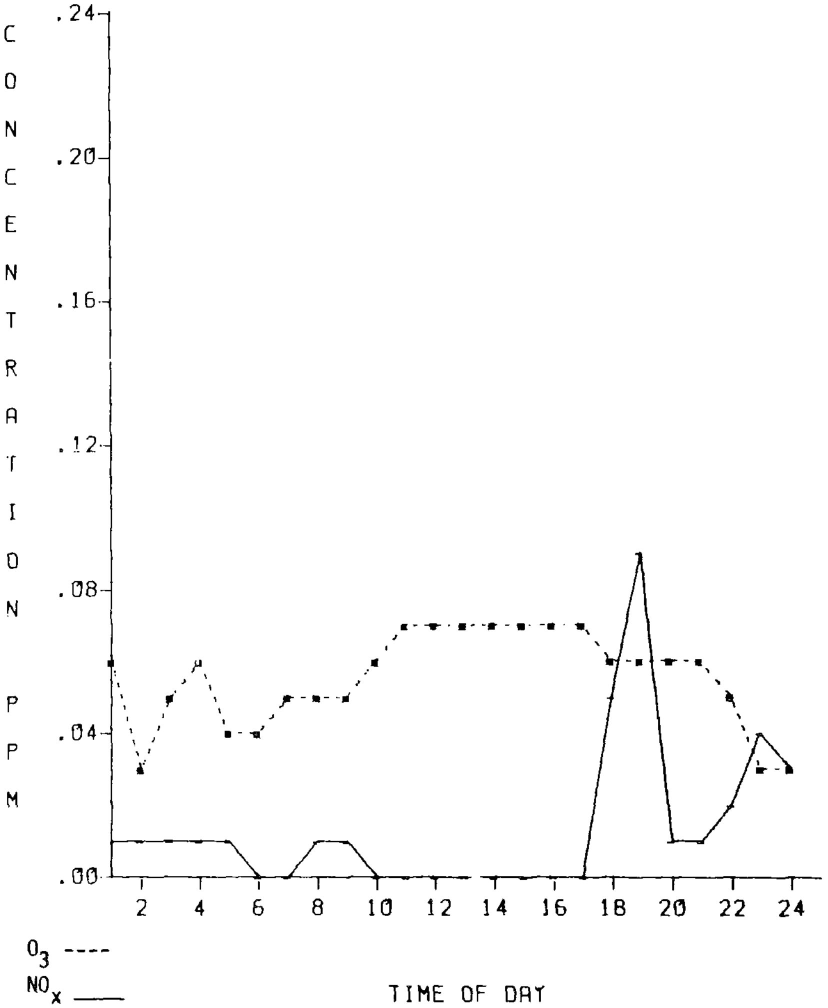
The seasonal variation in specific pollutants was evident. For all 1981 SAROAD sites that measured ozone above 0.05 ppm, 92% of the maximum hourly readings were observed during the May through September period. Less than 20% of the NO_2 hourly maxima were recorded during the same period. While the daily ozone hourly peak concentration usually occurred in the late morning and early afternoon, the daily nitrogen dioxide peak usually occurred in the early morning or late evening. Many of the sulfur dioxide peaks occurred during the daylight hours.



Rubidoux, California

June 26, 1981

Figure 2-16. Rubidoux, California, O_3/NO_2 Co-Occurrence.



Indian River, Delaware

June 3, 1978

Figure 2-17. Indian River, Delaware, O_3/NO_x Co-Occurrence.

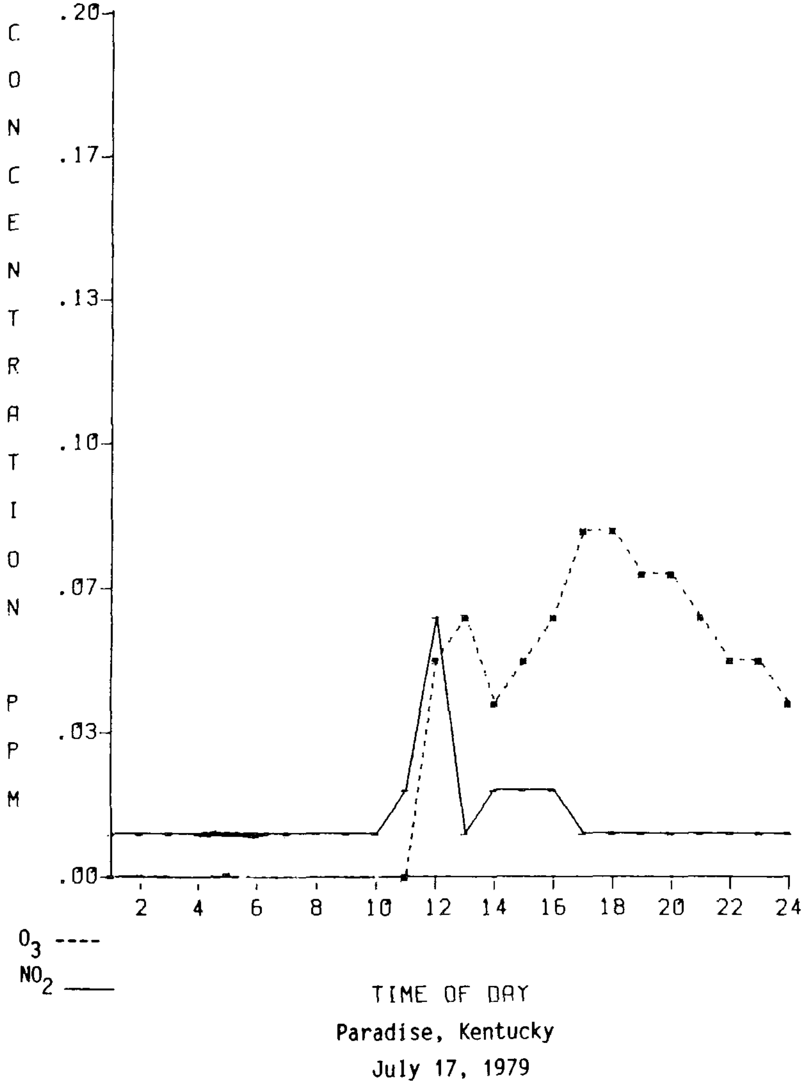


Figure 2-18. Paradise, Kentucky, O_3/NO_2 Co-Occurrence.

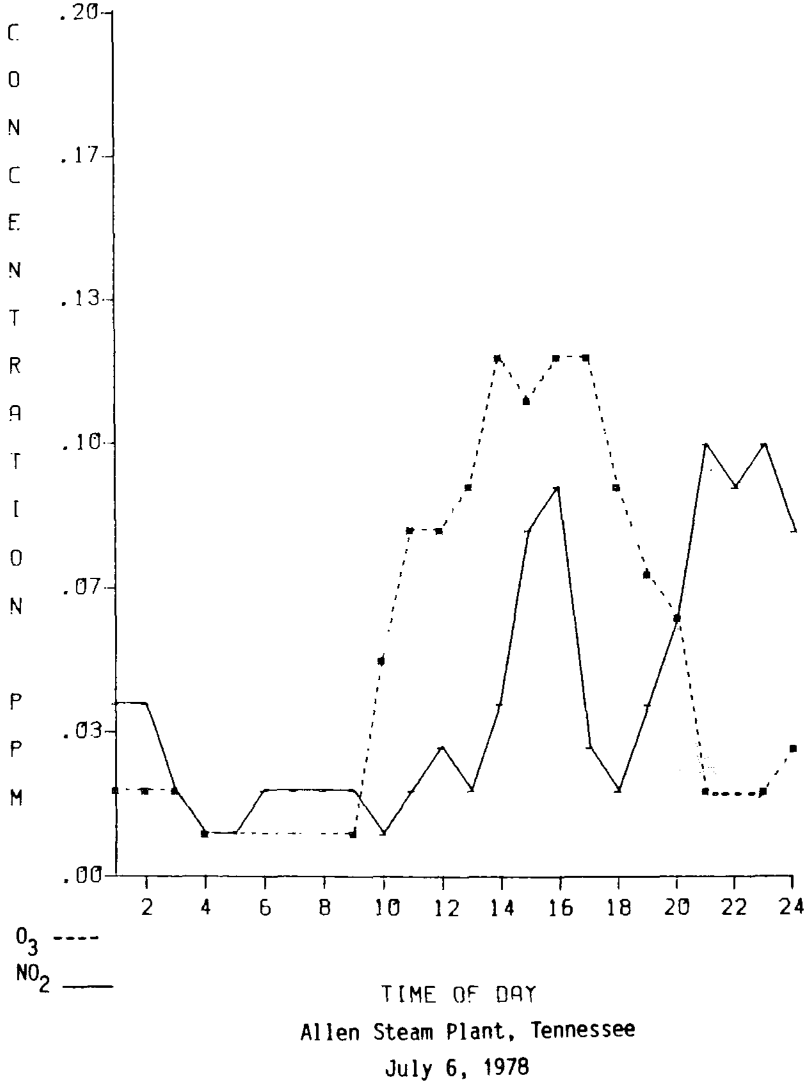


Figure 2-19. Allen Steam Plant, Tennessee, O_3/NO_2 Co-Occurrence.

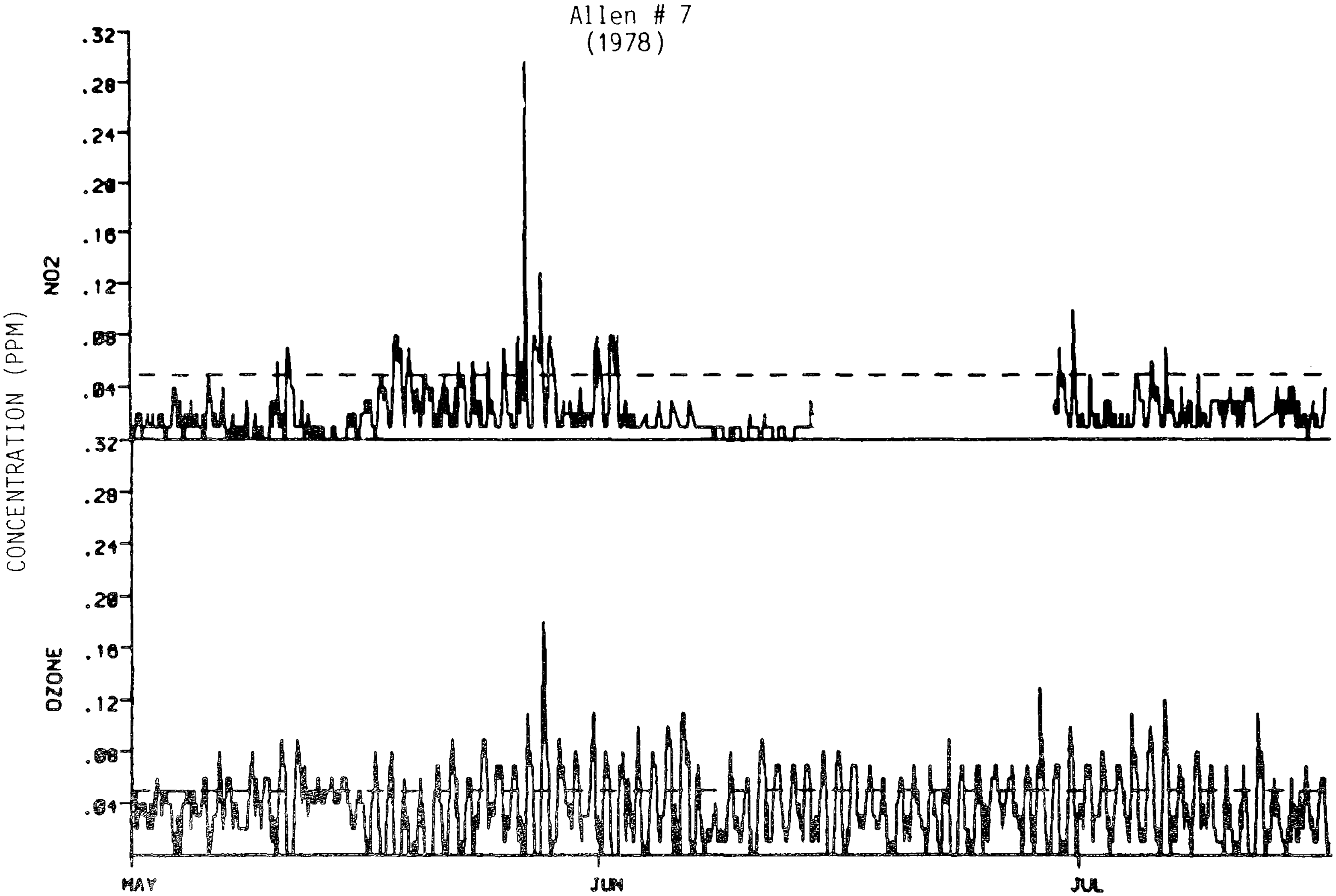


Figure 2-20. Allen Steam Plant, Tennessee O_3/NO_2 Concentration Over Time.

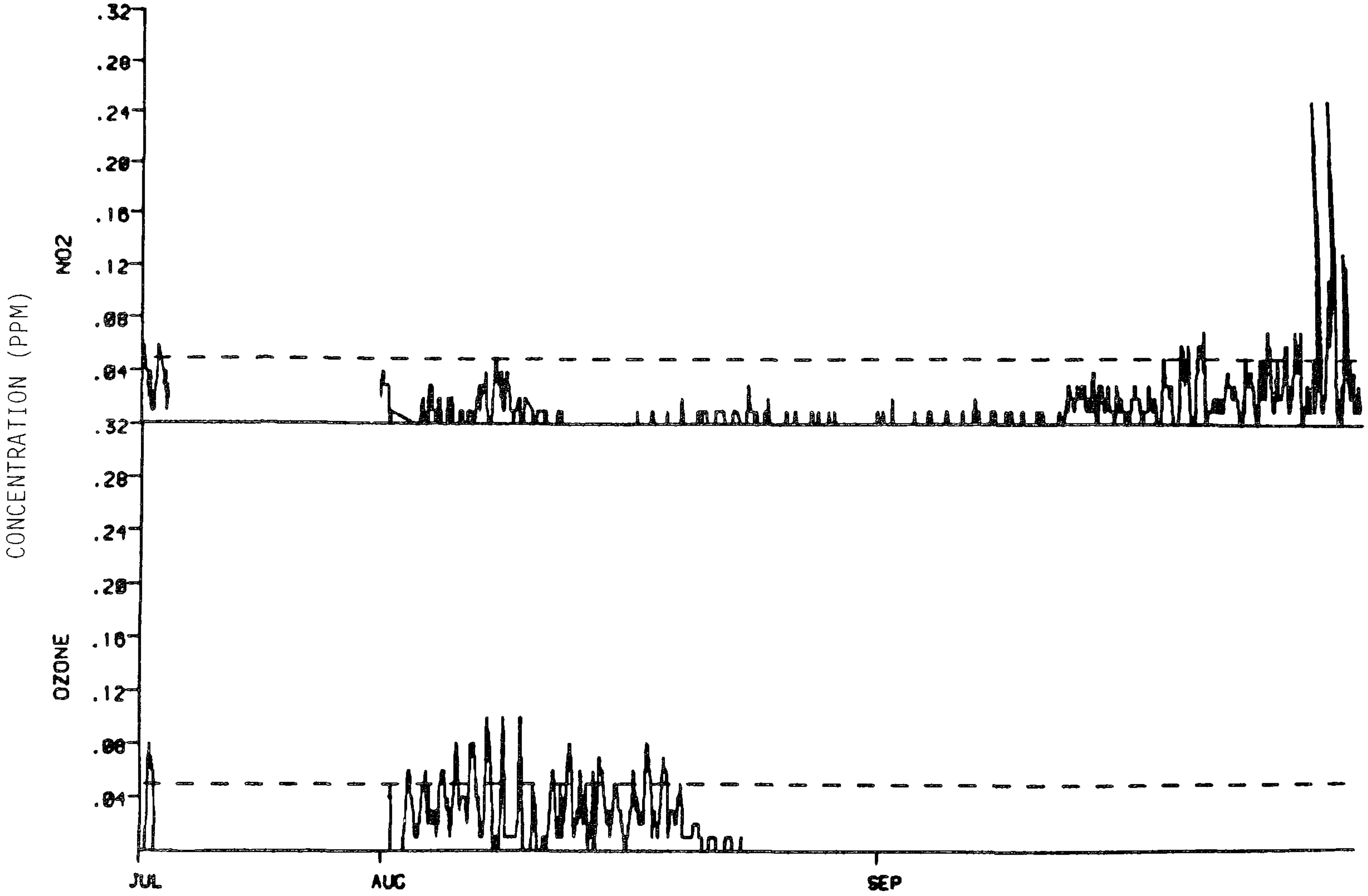


Figure 2-20. Allen Steam Plant, Tennessee O_3/NO_2 Concentration Over Time (Cont.).

Using rural and non-rural 1981 SAROAD data and the criteria previously mentioned, 66 percent of the sites that co-monitored SO_2 and NO_2 experienced a co-occurrence at least once during the five-month period. Of the O_3/SO_2 sites, 63 percent experienced a co-occurrence at least once; 71 percent of those sites that co-monitored O_3 and NO_2 experienced at least one co-occurrence during the period.

The EPRI SURE hourly averaged air quality data for SO_2, O_3, and NO_x were analyzed for co-occurrence events. Because the EPRI data were reported in NO_x, SO_2, and O_3 concentrations, it was assumed for this study that the NO_x could serve as a surrogate for NO_2 concentrations; using NO_x as a surrogate results in an overestimate of the co-occurrences of NO_2 with either SO_2 or O_3. Eight EPRI sites were reviewed for the co-occurrence of pollutant mixtures where the concentrations were equal to or greater than 0.05 ppm. Only one site (Indian River, Delaware) experienced at least one co-occurrence of SO_2/NO_x. Four EPRI sites experienced at least one co-occurrence of O_3/SO_2 (Scranton, Pennsylvania; Duncan Falls, Ohio; Rockport, Indiana; and Giles County, Tennessee). The Indian River site was the only one that experienced at least one co-occurrence of O_3/NO_x during the period May through September.

The TVA provided hourly averaged air quality data for seven sites that had monitored the three pollutants since 1978. Of the 14 site years reported for SO_2/NO_2 co-monitoring, eight reported co-occurrence at least once. Of the 14 site years reported for O_3/SO_2, 11 showed co-occurrence. For the 12 site years recorded for O_3/NO_2, only 4 showed co-occurrence.

Table 2-1 summarizes the results of the SAROAD, SURE, and TVA data analysis. For ozone, sulfur dioxide, and nitrogen dioxide, there were 752, 921, and 345 total site years monitored, respectively. Of the total monitored, 370 (O_3), 321 (SO_2), and 291 (NO_2) site years were identified for those locations where co-monitoring occurred. Using the monitoring data produced from EPA, EPRI, and TVA, 32, 36, and 34 rural monitoring site years were evaluated for SO_2/NO_2, O_3/SO_2, and O_3/NO_2 co-occurrences, respectively.

Table 2-1 Summary of Site Years Analyzed
(EPA SAROAD, EPRI SURE, and TVA Data)

Pollutant	Total # Site Years Monitored	# Sites Yrs. Where Pollutant > Threshold and Co-Monitoring Occurred	# Rural Site Yrs. > Thresh. & Co-Monitoring Occurred
O_3	752	370	40
SO_2	921	321	37
NO_2*	345	291	37
SO_2/NO_2*	-	91	32
O_3/SO_2	-	124	36
O_3/NO_2*	-	146	34

* EPA provided the EPRI SURE monitoring data in the form of NO_x, SO_2, and O_3 concentrations. EPA and TVA reported NO_2, SO_2, and O_3 concentrations.

CONCLUSION

Analysis of selected ambient air quality data collected by EPA, EPRI, and TVA shows that, for most cases, the constant artificial fumigation exposures do not mimic actual exposures. Since co-occurrence may be infrequent, researchers may want to focus on the sequential pollutant exposures characteristic of the rural sites analyzed. For example, while the daily ozone hourly peak concentration usually occurred in late morning and early afternoon, nitrogen dioxide typically peaked in the early morning or late evening. Similarly, sulfur dioxide episodes mostly appeared during the same daylight hours that ozone concentrations reached their maximum.

The monitoring data used in this analysis support the conclusion that 1) co-occurrence of pollutant mixtures lasts only a few hours per episode and 2) the time between episodes is great (weeks, sometimes months). The analysis of rural air monitoring data, generated by three different organizations, represented a first-step effort in characterizing rural sampling sites. Air quality data from a subset of the data bases were used to identify the distributions for SO_2/NO_2, O_3/SO_2, and O_3/NO_2 co-occurrences, respectively. Many of the sites were located in rural agricultural areas.

The lack of a comprehensive rural air monitoring data base has made it difficult to judge the representativeness of those sites used in the analysis. However, by 1) defining hourly averaged concentrations of 0.05 ppm and above as an event, and 2) combining air quality data bases (SAROAD, SURE, and TVA), the analysis shows a consistent exposure pattern, suggesting that the use of sequential exposure regimes should receive more attention and that researchers may want to reconsider their importance relative to co-occurrence exposure regimes.

3. EFFECTS OF POLLUTANT MIXTURES ON VEGETATION

INTRODUCTION

The first comprehensive reviews of literature on mixture effects were published eight years ago (Reinert et al. 1975, Williams and Ricks 1975). Much of the research concerning air pollutants and pollutant mixtures has since been summarized in several books (Heck et al. 1982, Ormrod 1978, Unsworth and Ormrod 1982), recent review articles (Ormrod 1982, Wellburn 1982), and research reports (Fujiwara 1973, Fujiwara and Ishikawa 1976, Ishikawa 1976, Reinert and Heck 1982, Yamazoe and Mayumi 1977). These reviews and summaries reveal that only a few combinations of pollutants have been studied and that little attention has been given to environmental and biological factors that influence vegetation responses. Few studies have dealt with any aspects of the responses of major plant species to mixtures of air pollutants at ambient concentrations administered under typical ambient environmental conditions.

When two pollutants occur in combination, there is the possibility of visible injury totaling more than the sum of visible injury caused by each pollutant alone. This concept, which has encouraged research, has been extended to changes in growth and yield as well as to biochemical and physiological changes in plants following exposure to pollutant mixtures. The concept is only one of the possible categories of plant response to pollutant mixture but it is the one which may be of greatest concern in vegetation effects assessment. When one pollutant has no effect on the plant response to the other pollutant, the category is termed no joint action. The category joint action implies that both pollutants have some effect on the plant response. This latter category is frequently divided into the subcategories additive response, when effect_{12} equals effect_1 plus effect_2, and interaction, when effect_{12} is not equal to effect_1 plus effect_2. There are two possible types of interaction: synergism (greater than additive action) when effect_{12} is greater than effect_1 plus effect_2, and antagonism (less than additive action) when effect_{12} is less than effect_1 plus effect_2. It is the concept of synergism that has been of greatest interest and concern. It would be difficult, using the present experimental knowledge, to fully characterize the nature of the joint action of two or more pollutants on major species in environments typical of ambient conditions.

Joint action of major gaseous pollutants has been by far the most studied kind of mixture response. This is because the more phytotoxic air pollutants are gaseous (e.g., O_3, SO_2, NO_2 and HF); there is a greater knowledge of their atmospheric chemistry and occurrence; and they are easily generated and monitored in experiments. However, there has been little or no research on mixtures involving minor gaseous pollutants (e.g., H_2S, HCl, Cl_2, NH_3, and C_2H_4). Air pollutants occur also as aerosols or as dissolved or suspended material in precipitation. Some information is available on gas-aerosol joint action (Krause and Kaiser 1977, Singh 1980). Aside from problems created by the physical and chemical heterogeneity of aerosols, deposited material remains on plant foliage after joint exposure has ceased and thus constitutes a virtually continuous source of exposure. For this reason, the interaction of gases with ions of trace elements or heavy metals (e.g., Cd, Ni, Cu, and Zn) on or in plants may be important (Czuba and Ormrod 1974, Krause and Kaiser 1977, Lamoreaux and Chaney 1978, Ormrod 1977, Toivonen and Hofstra 1979).

The intent of this chapter is to summarize and interpret existing data concerning plant responses to pollutant mixtures. Since O_3, SO_2, and NO_2 have had most attention to date in mixtures research, much of the discussion will focus on paired combination mixtures ($O_3 + SO_2$, $O_3 + NO_2$, $SO_2 + NO_2$) and on combinations of $O_3 + SO_2 + NO_2$. Even though most research has focused on the above noted mixtures, studies of mixtures of SO_2 + HF (Mandl et al. 1975, Mandl et al. 1980, Matsushima and Brewer 1972, McCune 1983) and NO_2 + HF (Amundsen et al. 1982) have also been reported. Also there have been recent reports concerning interactions between acid rain and SO_2 (Irving and Miller 1981) and acid rain and O_3 (Troiano et al. 1981).

EXPERIMENTAL METHODS AND INTERPRETATION OF DATA

The species of concern, stage of growth, and other plant factors, together with the need for control, separate and combined treatments, and considerable statistical precision, have dictated certain requirements for methodology in mixture studies. Experimental protocol appropriate for mixture studies has been developed to increase the validity and comparability of air pollutant research (Heck et al. 1979). Studies of mixture effects have taken place in controlled environment chambers, in greenhouses, and in field facilities. Each system has its particular merits. Fully controlled environments provide relatively high precision and repeatability but response patterns may not correspond well with field responses. Field facilities provide the most direct comparison with open field conditions but results may not be replicable because of changing weather patterns outdoors.

Most studies to date of mixture effects have been conducted in controlled environments or greenhouses. The utilization of continuous stirred tank reactor chambers by Heagle and Johnston (1979), Le Sueur-Brymer and Ormrod (1983), Reinert and Nelson (1980), and others has been a recent innovation in mixtures research. A few mixture studies have been conducted entirely in field facilities and a few have included both field evaluations and controlled exposures in an attempt to allow direct comparisons. Menser and Hodges (1970) compared tobacco cultivar sensitivity to O_3 + SO_2 in the field with that determined with controlled exposures in the greenhouse. There was a major shift of sensitivity of one cultivar. Hodges et al. (1971) also reported comparisons of chamber responses of tobacco to O_3 + SO_2 with field responses. Beckerson et al. (1979) compared bean cultivar sensitivity to O_3 + SO_2 in controlled environments with injury development following ambient outdoor exposure. Outdoor responses correlated more strongly with sensitivity to O_3 than with sensitivity to mixtures.

Outdoor exposure chambers, with environmental conditions more characteristic of ambient conditions, have been used by some researchers. Heagle et al. (1974, 1983) used open-top outdoor chambers for long-term O_3 + SO_2 treatment of soybean. Mandl et al. (1980) used similar chambers to study SO_2 + HF responses of sweet corn and Heggestad and Bennett (1981) used such chambers to observe SO_2 enhancement of O_3 injury to snap beans. Hill et al. (1974) used a portable field chamber to expose desert plants to SO_2 + NO_2 at concentrations measured downwind of a large coal-fired power plant, while Thompson et al. (1980) used open-top chambers for their studies of desert plant responses to SO_2 + NO_2. Other field chambers have been utilized by

Foster et al. (1983) and Oshima (1978). Bennett et al. (1980) grew snap beans in the field and exposed them to O_3 + H_2S through a plastic duct assembly. A linear gradient exposure technique was used by Reich et al. (1982). Ashenden et al. (1982) have described a system for exposing plants to SO_2 + NO_2, using hemispherical greenhouses having good air circulation and near-ambient temperatures.

Numerous studies exist which allow only individual comparison of treatments because there were not sufficient experimental units available to permit a full concurrent examination of mixture effects. In all these studies, the interaction or dependency of the effect of one pollutant on the level of another could not be fully evaluated due to missing treatments. An important consideration was whether or not there were enough exposure chambers to run all treatments at the same time. If not, considerable confounding may have developed when treatments could not be evaluated under similar conditions. This might have resulted in apparent pollutant interactions which did not reflect the true plant response.

Most researchers have used factorial experiments and analysis of variance for interpretation of combined effects of pollutants. A statistically significant interaction of two pollutants has been regarded as evidence for synergistic or antagonistic effects. Such techniques were used by Ashenden (1979a, 1979b), Bull and Mansfield (1974), Gardner and Ormrod (1977), Heagle and Johnston (1979), Tingey and Reinert (1975), Tingey et al. (1973b), Wellburn et al. (1976), and others.

More information may be obtained concerning the ability of each pollutant to produce a biological response by averaging effects over the presence and absence of other pollutants. One type of factorial design involves using one concentration of each of three pollutants, alone and in all two- and three-way mixtures, plus a charcoal-filtered air control treatment. Thus, eight treatments are involved and the main and interaction effects of each pollutant factor can be assessed through treatment component contrasts (Heck et al. 1979). Experiments using this design have been reported in which pollutant concentrations were either high (Reinert and Gray 1981, Reinert and Heck 1982) or low (Reinert and Heck 1982). The exposure durations varied from

3-6 hours and the number of exposures from one (Reinert and Gray 1981) to many (Reinert and Sanders 1982, Sanders and Reinert 1982a, 1982b). Plant species (Reinert and Sanders 1982, Sanders and Reinert 1982b), as well as cultivars within species (Sanders and Reinert 1982a), have been compared using this design.

Additional information on the nature of interactions can be obtained by determining the effect of increasing concentrations of one pollutant on plant growth and other responses in the presence of more than one concentration of a second pollutant. When three or more concentrations of one or both pollutants are used in the experimental design, the dose-response relationships may be evaluated. The responses may be described as linear or curvilinear and further serve to interpret some of the complexities of additivity, synergism, and antagonism found when only one concentration of each pollutant is used. Such a mathematical/statistical approach to O_3 + SO_2 + NO_2 interactions has been used by Reinert et al. (1982) to study the influence of sub-injury threshold concentrations of SO_2 + NO_2 on plant responses to O_3. A numerical evaluation of dose-response surfaces in terms of linear and curvilinear components was also presented.

Ormrod et al. (1983a) utilized quadratic polynomial equations, three-dimensional response surfaces, and contour plots to evaluate the effects of O_3 and SO_2 on one cultivar each of lettuce, radish, and pea, using a rotatable experimental design. The use of the rotatable design decreased the number of required treatments, compared with a full factorial design. For this study, plants were grown in a controlled environment chamber and exposed to seven combinations of O_3 and SO_2. Injury was evaluated on the basis of visible chlorosis and necrosis and growth was evaluated as leaf area and dry weight. The contour graphs in Figure 3-1 are two-dimensional representations of three-dimensional surfaces. The concentrations of SO_2 and O_3 form the abscissa and the ordinate, respectively, and the response is shown as a series of isoeffect or contour lines. The shapes of the isoeffect lines illustrate cross-sections of the surface, while their spacing shows the rate of change or curvature of the surface. These contour plots indicated the diversity of response patterns and particularly that some response variables demonstrated

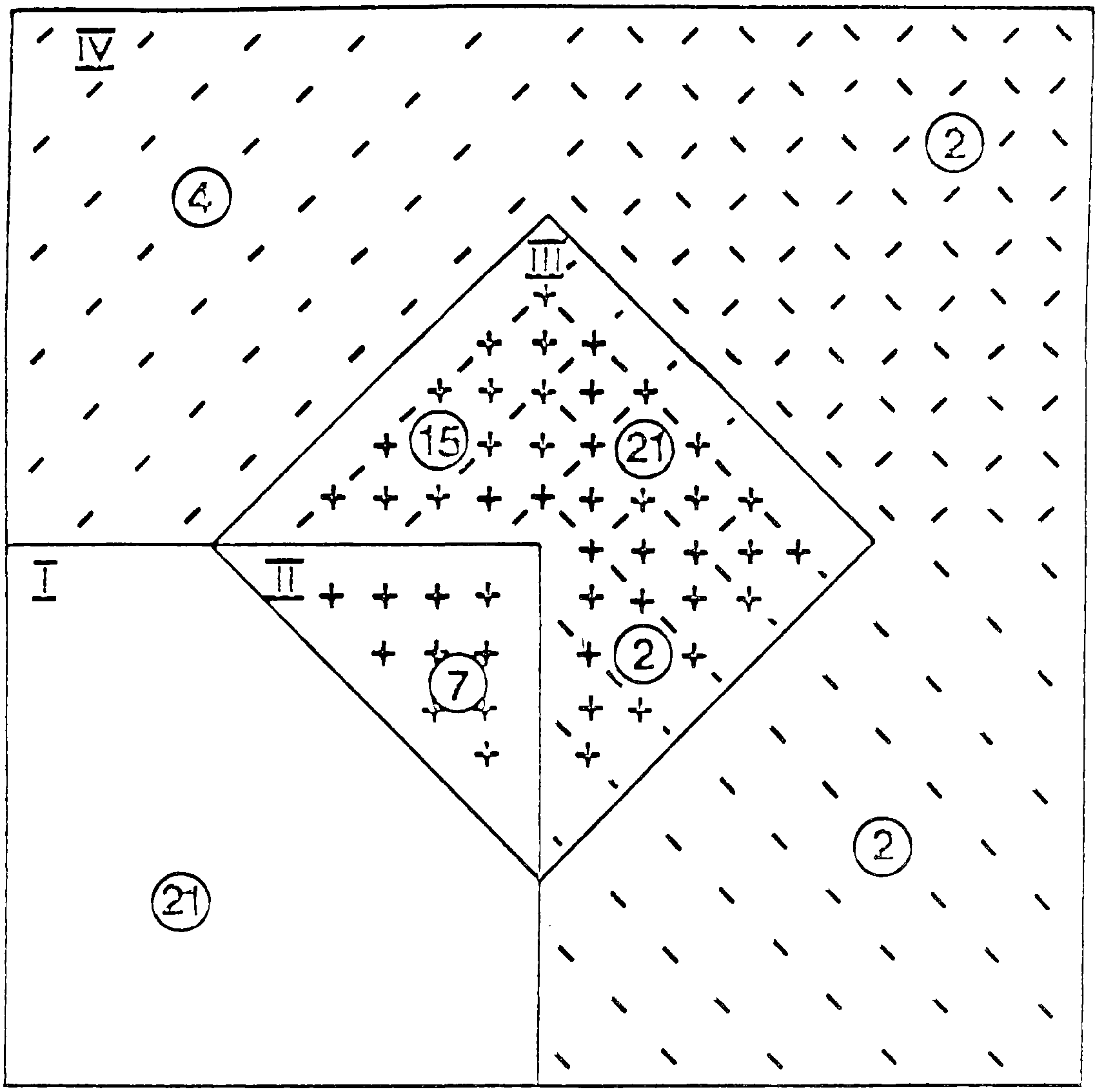


Figure 3-2. Graphical representation of the four response regions of practical interest when two pollutants are combined. For explanation of I, II, III, and IV, see text. The blank portion in the area in which no effects occur, ▨ designates the area with pollutant A effects, ▧ designates the area with pollutant B effects, and ⊞ designates the area with combined effects of A and B. The number of circles indicate numbers of results with each kind of outcome in the research on effects of SO_2 + NO_2 on native desert plants reported by Thompson et al. (1980).

additive effects at low concentrations of O_3 + SO_2 and antagonistic responses with increasing concentration of both gases in combined treatments.

Other statistical methods have also been used. White et al. (1974) used tests of non-additivity of pollutant effects. A statistical test of synergism has been made with models derived by polynomial analysis of injury index data (Macdowall and Cole 1971). Chi-squared analysis was used by Jacobson and Colavito (1976). Probit analysis has been used to interpret antagonism and synergism, by determining median effective doses (Macdowall and Cole 1971, Jacobson and Colavito 1976). To increase precision, Ormrod et al. (1983b) used covariate measurements to account for significant within-treatment variation in plant growth. Some differences that were not significant in conventional analysis of variance were significant when tested by analysis of covariance.

Most reports of studies involving mixtures have included, or implied, an assessment of the joint action of the pollutants in terms of additive effects, greater than additive (synergistic) effects, or less than additive (antagonistic) effects, using the terminology of Tingey and Reinert (1975). Responses have been expressed in terms of amount of injury or changes in threshold concentrations causing injury. Additivity has been suggested for a number of diverse plant responses (Ormrod 1982), but many reports have suggested synergistic responses. Reports of antagonistic responses have been more limited. Many of the reports on additivity, synergism, and antagonism indicated that the nature of the interaction was dependent on factors such as pollutant concentration and exposure duration, as well as on the species and gases studied.

The threshold phenomenon may be an important component of response patterns to mixtures. In general, when pollutant concentrations are below or at the threshold for individual visible injury responses, synergism (in terms of reduced growth and plant yield) is more frequently observed. As the concentrations of both pollutants increase in mixture above their individual injury thresholds, weight loss may only be additive. When the concentrations of the pollutants are relatively high, antagonism often develops and further weight loss may be minimal. This threshold phenomenon resulting in apparent synergism will be particularly important in 2 X 2 factorial experiments for the case when neither pollutant alone produces a response, but their combination does. When a threshold exists, it is possible that an apparent interaction results because no injury occurs until the weighted sum of the two components exceeds a certain value. The weighting would give a measure of the relative effectiveness of each component. The sum of individual effects, near the threshold, may not be the appropriate term for comparison with joint effects to determine interaction.

For a particular set of combinations of two pollutants, the responses can be graphically presented to illustrate the four regimes of practical interest: (I) where neither an effect of pollutant A, pollutant B, nor combined effects of A and B occur; (II) where combined effects occur but an effect of neither A nor B occurs; (III) where two or more of the three effects occur; and (IV) where effects of A or B occur but combined effects of A and B do not. These four possible outcomes for a single and mixed two pollutant study are diagrammed in Figure 3-2. The frequencies of such outcomes are illustrated for the data of Thompson et al. (1980) who exposed several desert species to a wide concentration of single and mixed SO_2 and NO_2 for several weeks. An effect was considered to be a decrease in some measure of weight, linear growth, or reproduction to less than or equal to 90% of the unexposed controls. The effects were grouped into the four possible outcomes as indicated by the Roman numerals in Figure 3-2. This illustrates that all types of outcomes were found with these exposure regimes used in this research project, but that the most frequent responses were either no effects or effects of both combined and single gases.

Most research with mixtures has been conducted in controlled exposure facilities using certain concentrations of gases for a specified duration. While the need for systematic testing of a range of mixture concentrations has been recognized (Heagle and Johnston 1979, Mandl et al. 1975), few investigators have described the exposure in terms of dose (the combination of pollutant concentration and duration), or have manipulated the components of dose. The concept of pollutant flux or uptake rate has barely appeared in the literature on effects of air pollutant mixtures even though the amount of pollutant sorbed may be much more closely related than concentration to the response.

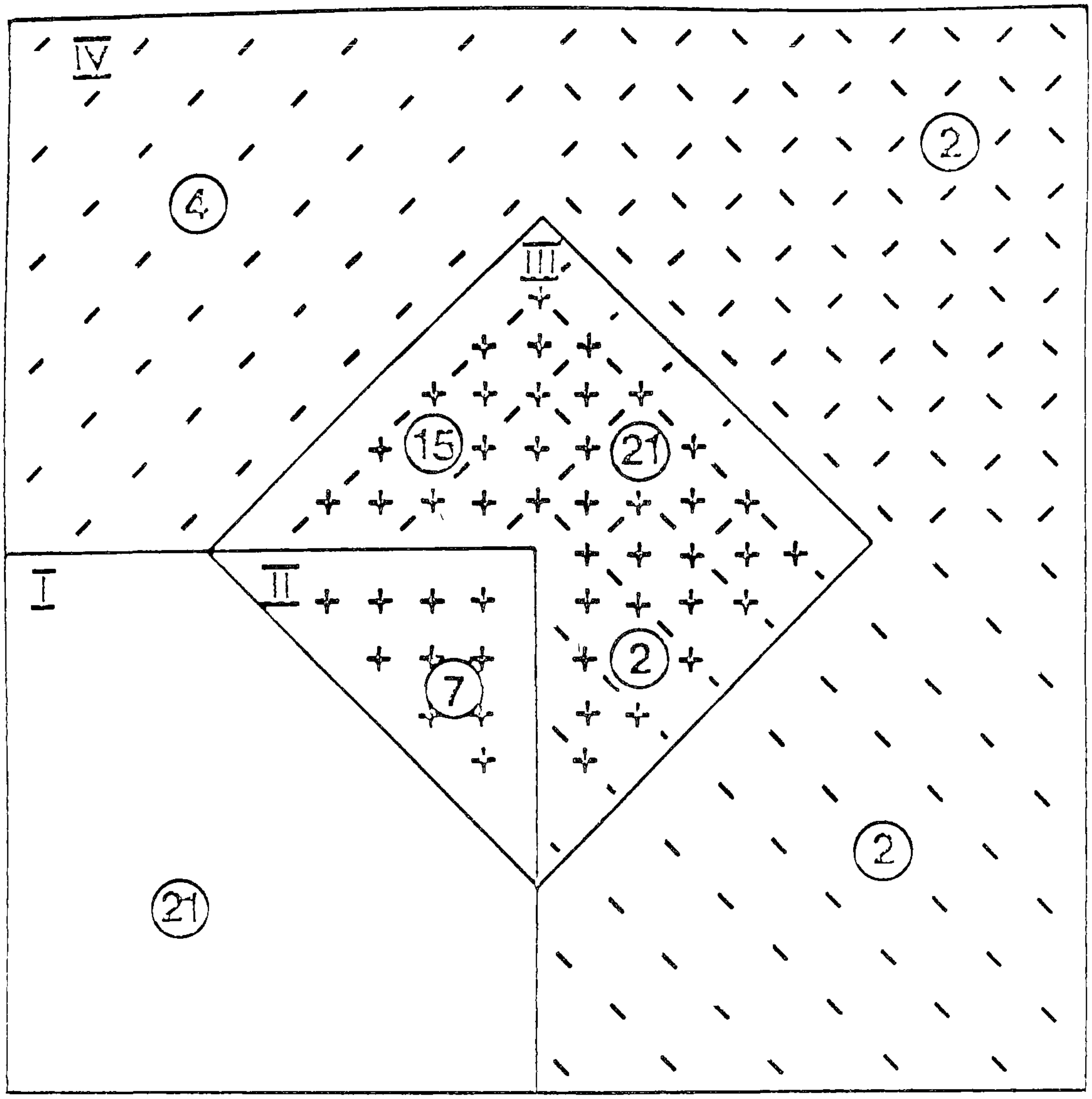


Figure 3-2. Graphical representation of the four response regions of practical interest when two pollutants are combined. For explanation of I, II, III, and IV, see text. The blank portion in the area in which no effects occur, ▨ designates the area with pollutant A effects, ▧ designates the area with pollutant B effects, and ⊞ designates the area with combined effects of A and B. The number of circles indicate numbers of results with each kind of outcome in the research on effects of SO_2 + NO_2 on native desert plants reported by Thompson et al. (1980).

Most investigators to date have used simultaneous exposures to nonvarying concentrations. In the natural environment, peak concentrations of pollutants may occur at different times for different pollutants. Such patterns may have considerable impact, with the preconditioning of plants by one pollutant affecting their response to another pollutant. Matsushima and Brewer (1972) investigated sequential reciprocal exposures of orange to SO_2 + HF to determine whether one gas influenced the subsequent response of the plants to the other gas, but found little effect. Costonis (1973) found a sequence of O_3 followed by SO_2 to be more toxic to eastern white pine than exposure to both gases simultaneously. White et al. (1974) found that neither SO_2 nor NO_2 pretreatment of alfalfa affected response to a subsequent exposure to SO_2 + NO_2. Exposure to SO_2 before exposure to O_3 + SO_2 markedly influenced bean, cucumber and tomato sensitivity (Hofstra and Beckerson 1981). Many investigators have subjected plants to intermittent treatments within overall long-term exposures. Tingey et al. (1971b, 1973b) noted that this might allow plants to repair injury and regain normal metabolic functions during the non-exposure period.

In some cases where many processes are involved, such as in components of yield, there may be a multiplicative, rather than additive, effect. Each process may have a different dose-response function. For example, Reich et al. (1982) showed different dose-response relationships for three components of soybean yield. In such a case the dose-response relationship may be approximated by a third- or higher-degree polynomial or at least a four component function. Sometimes a multivariate approach may help to elucidate the nature of the effects or at least serve to simplify the nature of the responses. For example, in chronic exposures to O_3 and/or SO_2 of alfalfa, treatment-induced changes in foliage and root dry weight were so closely associated that a weighted sum of the two could serve as a single measure of response (Tingey and Reinert 1975). On the other hand, with acute exposures of radish, O_3 and SO_2 appeared to have opposite effects on a weighted sum of root and leaf dry weight, but no interaction. However, with reference to a weighted contrast between roots and leaves, their effects alone were similar (decreasing the difference between root and shoot), but opposite (increasing differences) when both were present.

Whether the concern is about visible injury on foliage or growth effects, one should not expect response to be linear with dose nor should one expect a single mode-of-action as pollutants may induce increases, as well as decreases, in growth (Bennett et al. 1974) or other responses. It may well be that linearity is adequate over narrow ranges, but not over the entire range of interest. When a linear dose-response function is found, as with the total dry weight of radish plants (Tingey et al. 1971b), the dose can be expressed as a weighted sum of SO_2 and O_3 concentrations. Other responses also appear to approximate what would be expected from a linear relationship to a weighted sum (e.g., K-efflux from 'White Cascade' petunia leaf discs [Elkiey and Ormrod 1979a]). When a quadratic function is found (Tingey et al. 1973b), a weighted sum of SO_2 and O_3 concentrations, which implies a constant relative effectiveness, may also be used as a dose-variable.

CHARACTERISTICS OF PLANT RESPONSE

Responses to pollutants and pollutant mixtures have been detected in several ways: visible symptoms of injury; altered growth and development; physiological and metabolic imbalances; and accumulations of certain elements. Growth and yield are often the most important response variables and it is probably this concept that effects may be greater than additive (synergistic) that has been the predominant concern of combined pollutant studies.

Foliar Symptoms

The practical significance of visible foliar response has been mainly in the diagnosis of effects. Kinds of symptoms, and their distribution on a plant or between species, have provided the investigator with inferences as to the kinds of air pollutants (qualitative factors). The degree of symptom development has allowed inferences as to amount of pollutant or exposure (quantitative factors). Degrees of symptom development has also served as a measure of the likelihood of effects on growth and reproduction. Exposure-effect relationships derived from visible injury data have allowed inferences to be made as to the nature of the dose-response relationship, the effects of biologic and environmental factors upon it, and the mode of action of air pollutants in the plant. However, if the combined effect of pollutants of air pollutants in the plant. However, if the combined effect of pollutants alters the qualitative or quantitative foliar symptom characteristics, errors in diagnosis could have occurred.

Many investigators have devised a leaf injury rating system and have used the data obtained for interpretation of mixture effects. Few have assessed differences in appearance by providing descriptions of the injury. In some cases, experiments did not last long enough to permit the development of stable injury symptoms. In general, ozone injury symptoms have dominated in studies of mixtures of O_3 + SO_2 (Menser and Heggestad 1966, Menser and Hodges 1970, Hodges et al. 1971, Tingey et al. 1971a, 1973b, Heagle et al. 1974, Elkiey and Ormrod 1979b). There were exceptions and, in some cases, different symptoms developed in response to the combination. Grosso et al. (1971) found different symptoms of O_3 + SO_2 on tobacco leaves than when O_3 alone was the pollutant. Combined O_3 + SO_2 injury to apple leaves differed from either O_3 or SO_2 injury (Kender and Spierings 1975, Shertz et al. 1980a). Undersurface glazing, a symptom usually attributed to PAN, was found on petunia leaves exposed to O_3 + SO_2 (Lewis and Brennan 1978). Cucumber exposed to O_3 + SO_2 had an additional symptom of interveinal chlorosis, compared with O_3 alone (Beckerson and Hofstra 1979a). Visible injury to three woody species, caused by exposure to O_3 + SO_2, included symptoms of each pollutant alone (Carlson 1979). Acute responses of eastern white pine to O_3 + SO_2 had different symptoms from responses to either O_3 or SO_2 alone (Costonis 1973), but Dochinger et al. (1970) reported that O_3 and SO_2 separately or in combination produced the same initial symptoms on eastern white pine.

Duration of exposure is important. Jacobson and Colavito (1976) indicated antagonism of O_3 + SO_2 in navy bean and attenuation of visible injury with short term exposure. This contrasted with Hofstra and Ormrod (1977) who found unique and severe injury symptoms on navy bean leaves by O_3 + SO_2 after several days' exposure. Symptoms of injury from mixtures of SO_2 + NO_2 differed greatly, in several species, from those caused by single gases (Tingey et al. 1971b). In contrast, Hill et al. (1974) found the symptoms of SO_2 + NO_2 on many species to be the same as those for SO_2, and Matsushima and Brewer (1972) found SO_2 + HF -induced chlorotic patterns to be the same as for individual gases. Kohut and Davis (1978) found an interaction of O_3 + PAN which affected lower and upper leaf surfaces. On the lower surface, the two gases were antagonistic in causing injury; on the upper surface, they were additive or synergistic.

$SO_2 + O_3$

The synergistic action of $SO_2 + O_3$ first noted on tobacco in 1966 (Menser and Heggestad 1966) encouraged all subsequent research on pollutant mixtures. During the first few years that followed, the concept of greater than additive or synergistic amounts of foliar injury, following exposure to pollutant mixtures, became accepted as a frequent occurrence. However, Tingey et al. (1973a) reported that not all visible-injury response to mixtures of $SO_2 + O_3$ was synergistic. They concluded that foliar injury responses for six plant species could be additive, greater than additive, or less than additive depending on the species, and on the concentration and exposure duration of both pollutants. Visible foliar injury has been widely used to evaluate variable species responses to $SO_2 + O_3$. Examples of research reports illustrating the nature of joint effects on visible injury are presented in Table 3-1. The diversity of effects obtained in such research serves to illustrate the importance of subtle or controlled factors in addition to the more obvious species and dose differences.

The experimental concentrations, durations, and frequencies of gaseous pollutant exposure have varied widely (see Tables 3-1 and 3-2). The longest concentrations studied could occur in the ambient environment (see Chapter 2) but no other features of the exposure doses reflected the ambient polluted environment. Thus, it is not possible, at this stage, to estimate the probability of occurrence in the ambient environment of such experimental conditions. The experimental durations, frequencies, and constant concentrations used in the artificial fumigations do not represent the real temporal patterns in ambient polluted environments. Also most mixture experiments to date have not been designed to study effects of sequential exposures.

Table 3-1

Visible foliar injury on various plant species in response to the joint action of $SO_2 + O_3$

Species	Dose			Effect(s) obtained[c]	Reference
	O_3 conc[a]	SO_2 conc[a]	Duration[b]		
Apple	0.8	0.8	4 h	antagonism	Shertz et al. 1980a
Grape	0.8	0.8	4 h	synergism, antagonism	Shertz et al. 1980b
Navy bean, tobacco	0.8	1.6	6 h/5 d	antagonism, no effect	Jacobson and Colavito 1976
Navy bean, soybean	0.15	0.6	6 h/5 d	antagonism	Hofstra and Ormrod 1977
Soybean	1.0	1.5	0.75-3 h	synergism, antagonism, additive	Heagle and Johnston 1979
Radish, cucumber	0.15	0.15	6 h/5 d	synergism	Beckerson and Hofstra 1979a
Soybean	0.15	0.15	6 h/5 d	antagonism	Beckerson and Hofstra 1979a
Pinto bean	0.25	0.8	3 h	synergism, antagonism	Miller and Davis 1981a
Begonia	0.3	1.8	4 h/5d	synergism	Gardner and Ormrod 1977
Pea	0.13	1.23	4 h	synergism	Olszyk and Tibbitts 1981
Poplar	0.05	0.2	3 h	synergism	Karnosky 1976
Tobacco	0.03	0.28	4 h	synergism	Menser and Heggestad 1966
Tobacco, alfalfa	0.1	1.0	4 h	synergism, no effect	Tingey et al. 1973a
Broccoli, cabbage, radish	0.1	1.0	4 h	synergism, additivity no effect	Tingey et al. 1973a
Tomato	0.03	0.28	4 h	antagonism, additivity no effect	Tingey et al. 1973a
Eastern white pine	0.1	0.1	8 h/8 w	synergism	Dochinger et al. 1970
Eastern white pine	0.05	0.05	2 h	antagonism	Costonis 1973
Eastern white pine	0.025	0.05	6 h	synergism	Houston 1974

---

[a] Maximum concentrations (ppm) used in the research. A range of concentration was used by some investigators.

[b] h=hours, d=days, w=weeks, 6h/5d=6 hours per day for 5 days, 8h/8w=8 hours per day for 8 weeks

[c] compared with effects of single gases, as presented by the authors. Different methods may have been used to arrive at the effects statements.

Table 3-2

Growth and yield of various plant species in response to the joint action of $SO_2 + O_3$

Species	Dose				Reference
	O_3 conc.[a]	SO_2 conc.[a]	Duration[b]	Effect(s) obtained[c]	
Radish	0.45	0.45	4h	additive, root most responsive	Tingey and Reinert 1975
Radish	0.05	0.05	8h/5d/5w	additive root most responsive	Tingey et al. 1971b
Tobacco	0.05	0.25	8h/5d/4w	synergistic root, additive shoot	Tingey and Reinert 1975
Alfalfa	0.05	0.05	8h/5d/12w	antagonistic on plant wt.	Tingey and Reinert 1975
Eastern white pine	0.025	0.5	6h	antagonistic on needle length	Houston 1974
Navy bean	0.15	0.6	6h/5d	antagonistic on plant wt.	Hofstra and Ormrod 1977
Snap bean	ambient	0.3	6h/5d/4w	synergistic on fruit yld.	Heggestad and Bennett 1981
Soybean	0.1	0.1	6h/133d	additive on seed yield	Heagle et al. 1974
Soybean	1.0	1.5	0.75-3h	synergistic, antagonistic additive	Heagle and Johnston 1979
Soybean	0.05	0.05	8h/5d/3w	synergistic on plant wt.	Tingey et al. 1973b
Soybean	0.25	0.25	4h/3d/11w	additive on plant wt.	Reinert and Weber 1980
Begonia	0.3	1.2	4h	synergistic on shoot wt.	Gardner and Ormrod 1977
Poplar	0.25	0.5	12h/24d	antagonistic on plant wt.	Noble and Jensen 1980
Apple	0.4	0.4	4h	synergistic on shoot gr.	Shertz et al. 1980a
Turf grasses	0.15	0.15	6h/10d	synergistic or additive	Elkiey and Ormrod 1980

---

[a] Maximum concentrations (ppm) used in the research

[b] h=hours, d=days, w=weeks, 8h/5d/5w=8 hours per day for 5 days per week for 5 weeks.

[c] Compared with the effects of single gases, as presented by the authors.

$SO_2 + NO_2$

Studies concerning mixtures of NO_2 and SO_2 have been few. Tingey et al. (1971a) found that mixtures of NO_2 and SO_2 at concentrations ≤ 0.25 ppm following one 4-hour exposure, caused visible injury in six plant species, whereas there was no visible injury to plants exposed to NO_2 (2.0 ppm) or SO_2 (0.5 ppm) alone for the same exposure duration. Decormis and Luttringer (1977) also have reported synergistic injury responses in tomato, geranium, and petunia from exposure to a mixture of SO_2 (0.3 ppm) and NO_2 (0.5 ppm). The resulting injury symptoms differed from those caused by NO_2 or SO_2 alone. Nearly all of the published visible injury responses to mixtures of $NO_2 + SO_2$ have occurred at concentrations at which NO_2 or SO_2 alone did not injure plants, using the same exposure duration.

Growth and Yield

The quantitative characteristics of plant response, unlike the visible injury responses, are measured on a continuous scale and no qualitative distinction may be available with respect to which response is characteristic of one pollutant or another. Measures of growth or yield that can be translated into economic terms are generally of primary significance. Other measures of growth response may be viewed as useful in explaining or predicting characteristics of plant response that can be extrapolated to yield. Several studies of yield responses to mixtures have been reported. Such studies have usually involved long-term exposures.

While visible injury may be a useful variable in determining how plants might be expected to respond to mixtures, the magnitude of visible injury does not always correlate well with other responses such as plant weight and yield changes. For example, if the visible injury response is greater than additive, the changes in foliage weight compared with the control and the pollutants alone may be only additive or not different at all. This is especially true when the magnitude of injury is extremely small or large. Also, close relationships between plant growth and foliar injury are not necessarily to be expected, because plant growth is a composite of many reactions, any of which may be limiting (Tingey et al. 1973b). Substantial growth reductions, with or without visible leaf injury, may be the result of more than reduction of leaf area; they may also be attributable to decreases in the formation of plant parts (Ashenden 1979b). There are published examples of plant growth reduction without foliar injury (Kress et al. 1982a, 1982b) and foliar injury without substantial growth reductions (Mandl et al. 1975). Also photosynthesis rates can be reduced without visible symptoms (Carlson 1979).

Root growth has seldom been measured in gas mixture studies. Although it is unlikely that the pollutants penetrate rooting media and exert a direct effect on roots (Tingey et al. 1971b), root growth may be a sensitive indicator of the physiological status of plant shoots because reductions in root growth may indicate diminished photosynthesis or interference with translocation. Also, reductions in root growth in response to gas mixtures may have a secondary effect on the whole plant as a result of decreases in the absorption of water and nutrients. Root growth was reduced more than top growth in soybeans by O_3 + SO_2 mixtures, probably as a result of reduced translocation of photosynthate to the roots (Tingey et al. 1973b). Greater reduction in root growth than in top growth of soybean indicated that root growth was more sensitive than top growth (Tingey and Reinert 1975). Radish root (technically part hypocotyl) growth has been used to probe leaf/root relationships. Fresh weight of radish leaves was reduced by a smaller percentage than was root fresh weight after the same O_3 + SO_2 treatment, indicating either that available photosynthate was sufficient for normal leaf growth but insufficient for normal root growth, or that there had been a change in partitioning (Tingey et al. 1971b). Reinert and Gray (1981) found the root weight of radish to be decreased by NO_2, SO_2 and O_3 combinations, even though foliage was the direct receptor of the pollutant stress.

Reproductive ability may be changed by mixtures as a result of direct action on generative tissues or some indirect effects. Deleterious effects of combined SO_2 + F on reproduction in Scots pine have been reported by Rogues et al. (1980). Mixtures also interfered with reproduction in lily; SO_2 + NO_2, O_3 + NO_2 and NO_2 + formaldehyde markedly inhibited pollen-tube elongation (Masaru et al. 1976).

$SO_2 + O_3$

Researchers realized some of the limitations of studies based solely on visible foliar injury in the early 1970s and initiated studies to evaluate the effects of $SO_2 + O_3$ mixtures on growth and yield of numerous plant species. Examples of these studies are presented in Table 3-2. Two additional reports have presented effects of SO_2 and O_3 alone and in mixture on the parasitism of nematodes on soybean (Weber et al. 1979) and tomato (Shew et al. 1982). These reports have resulted in a more meaningful understanding of how $SO_2 + O_3$ influences plant growth and development. There are some limitations to the use of the data. There is little information concerning the effects of SO_2 and O_3 mixtures at various stages of development, since usually plant growth or yield at final harvest have been the only response variables measured. Another limitation is that species sensitivity determinations generally involved only one cultivar. There is a wide genetic variation in response to O_3 among cultivars within a species. Sensitivities of various cultivars to SO_2 and O_3 alone and in combination have shown differential responses which suggest that antagonism or synergism may result from differential sensitivity to each of the pollutants alone (see Modifiers of Plant Response-Genetic Factors).

$SO_2 + NO_2$

There have been several recent reports on $SO_2 + NO_2$ interactions leading to greater-than-additive growth reductions. Two grass species were exposed to weekly means of 0.06-0.08 ppm of NO_2 and SO_2 alone and in combination (Ashenden 1979a). The mixture of $NO_2 + SO_2$ and SO_2 applied alone caused significant reduction in leaf area and plant dry weight of *Dactylis glomerata* L. and *Poa pratensis* L. There were also reductions in the number of tillers and leaves of both species exposed to $NO_2 + SO_2$ but senescence was not enhanced (Ashenden 1979b). Similar effects were found on *Lolium multiflorum* Lam. and *Phleum pratense* L. (Ashenden and Mansfield 1978; Ashenden and Williams 1980; Mansfield and Ashenden 1978). It was concluded that NO_2 and SO_2 in combination were more toxic to the grass species than was predicted by summing their individual effects on growth.

Irving et al. (1982) dispensed SO_2 and NO_2 through a system of aluminum pipes suspended over a canopy of field-grown soybeans. The soybeans were fumigated on ten occasions, with mean concentrations of SO_2 during fumigation ranging from 0.13-0.42 ppm, while NO_2 ranged from 0.06-0.40 ppm. Results from the 2-year study showed that exposures to NO_2 alone had no effect on soybean seed yields. Exposure to SO_2 alone decreased yield by 6% the second year. Mixtures of SO_2 and NO_2 in both years of the study resulted in yield decreases ranging from 9-25%. These losses, however, were obtained in the presence of 7-hour average ambient O_3 levels ranging from 0.006-0.095 ppm. Thus, O_3 may have caused some of the NO_2 x SO_2 interaction on soybean yield. Amundson and Weinstein (1980) exposed soybean to SO_2 (0.0, 0.1, and 0.3 ppm) and NO_2 (0.1 ppm), singly, and in combination for 4 hours daily for a period of 14 days during pod fill in open top field chambers. Sulfur dioxide (0.3 ppm) + NO_2 (0.1 ppm) caused early senesence and reduced yield, compared with plants exposed to SO_2 (0.3 ppm) alone. Nitrogen dioxide (0.1 ppm) in the presence of SO_2 (0.1 ppm) had no effect on soybean yield. Klarer (1982) exposed soybean in the vegetative growth stage to single and mixed NO_2 (0.0, 0.1. and 0.2 ppm) and SO_2 (0.0, 0.2 and 0.3 ppm) 15 times, every other day for three hours per day. Leaf, stem, and root dry weights were significantly less (18, 12, and 32%, respectively) when the four mixture treatments were averaged together and compared with the mean of the four treatments of NO_2 and SO_2 alone. The response of soybean to NO_2 and SO_2 was nearly linear.

Nitrogen dioxide and SO_2 mixture studies have not been limited to herbaceous plants. A 37% reduction in the growth of poplar was reported following continuous 8-week exposure to 0.06 ppm SO_2 + 0.06 ppm NO_2; no growth reductions were observed following exposure to SO_2 or NO_2 alone (Whitmore et al. 1982).

NO_2 + O_3

The effects of NO_2 + O_3 generally have not been studied. However, in recent reports of two- and three- pollutant mixture treatment comparisons, the NO_2 + O_3 treatment has been included. Kress and Skelly (1982) have studied the response of seven tree species to NO_2 (0.1 ppm) and O_3 (0.1 ppm) alone and in mixture for six hours per day, for 28 consecutive days. Virginia pine and loblolly pine height were significantly suppressed by $NO_2 + O_3$ treatment. There were significantly less than additive suppressions of sweetgum root and total dry weight and white ash root weight.

$SO_2 + NO_2 + O_3$

Only a few studies of three-gas interactions have been conducted to date and response patterns are complex. In some cases the experimental design has limited the interpretation of the research. For example, Elkiey and Ormrod (1980) exposed 18 turfgrass cultivars, representing six species, to NO_2, SO_2, and O_3 singly at 0.15 ppm of each pollutant and to a mixture of all three pollutants (0.15 ppm of each gas). The three-pollutant mixture treatment caused more leaf injury and greater reduction in leaf area in most cultivars when compared with the additive effects of the pollutants singly. The lack of two-pollutant mixture treatments was a limitation to the full interpretation of this research. Similarly, Yamazoe and Mayumi (1977) exposed sweet corn and rice for one 24 hour period to NO_2 (0.5 ppm) + O_3 (0.15 ppm), alone and in mixture, and to a mixture of NO_2 (0.5 ppm) + SO_2 (0.15 ppm) + O_3 (0.15ppm). The mixture of $NO_2 + O_3$ caused additive injury in rice but not in sweet corn, while injury in both sweet corn and rice from the $NO_2 + SO_2 + O_3$ suggested an antagonism by SO_2. The lack of certain treatment combinations prevented full interpretation of this research.

The $NO_2 + SO_2$ dose/response relationship was studied with snap bean exposed to NO_2 (0.0, 0.05, and 0.1 ppm) + SO_2 (0.0, 0.1 and 0.15 ppm), in the presence of 0.05 ppm O_3 under greenhouse exposure conditions (Reinert and Heck 1982). Nitrogen dioxide at 0.1 ppm in the presence of 0.05 ppm O_3 and the absence of SO_2, caused a 10% loss of snap bean fruit fresh weight. When SO_2 was held constant at 0.1 ppm, there was a 15% and 11% weight loss in bean fruit as NO_2 was increased to 0.05 or to 0.1 ppm, respectively. These data suggest a significant effect of NO_2 in the presence of SO_2 and O_3 at ambient air concentrations of all three pollutants.

Kress et al. (1982a) found substantial growth suppression in loblolly pine by using mixtures of O_3, NO_2, and SO_2 at concentrations below the national ambient air quality standards for each pollutant singly. They also found greater growth suppression of American sycamore with O_3 + SO_2 + NO_2 than with two-gas mixtures, without foliar injury (Kress et al. 1982b).

The use of full factorial designs to evaluate 3-way mixtures by Reinert and colleagues (Reinert and Gray 1981, Reinert and Sanders 1982, Sanders and Reinert 1982a, 1982b) has led to the emergence of several concepts. Nitrogen dioxide in mixture with SO_2 and/or O_3 has significantly reduced growth and yield in crop plants. In nearly every instance, the three-pollutant mixture, NO_2 + SO_2 + O_3, caused more loss in weight and yield than the single gases or two-pollutant mixtures. If the plant is capable of developing repair mechanisms against stress by O_3, it appears that under the simultaneous stress of NO_2, SO_2, and O_3, repair mechanisms may not be able to function, and plant productivity is reduced.

Other Mixtures

Mixtures of 0.8 ppm SO_2 + 2.5 to 13 ppb HF for 23 days decreased linear growth and leaf area of orange in the research of Matsushima and Brewer (1972). Mandl et al. (1975) found no significant effects of 0.15 or 0.3 ppm SO_2 + 0.6 to 0.9 ppb HF for 7 days on growth of several species even though there were visible symptoms of injury. Gas-precipitation interactions have been studied concerning acidity of rain and the occurrence of SO_2 or photochemical oxidants. In soybeans, Irving and Miller (1981) found 0.19 or 0.79 ppm SO_2 in 17 or 24 4-hour exposures to have deleterious effects that were not affected by simulated acid precipitation of pH 3.1 every 5 days. Troiano et al. (1982) used open-top chambers to study the interaction of ambient O_3 and simulated acid rain of pH 4.0, 3.4, or 2.8 on soybean seed quality.

Physiological and Metabolic Responses

Exposure to pollutant mixtures may result in physiological and metabolic responses which in turn result in growth and yield reduction. The means by which these responses occur are not well known. One predominant mechanism is the physiological or metabolic alteration induced by one pollutant which then increases or decreases the susceptibility of the plant to another pollutant.

This would appear to be a most likely means of explaining the effects of two successive exposures to different pollutants (Hofstra and Beckerson 1981, Masaru et al. 1976). A change in sensitivity would be the most likely explanation for some observations of the joint action of pollutants on foliage (Miller and Davis 1981a) where the effects on uptake were not similar or where new kinds of symptoms occurred (Mandl et al. 1980, Lewis and Brennan 1978).

Little is understood concerning the metabolic and physiological action of pollutants in mixture. Many experiments have established dependent or independent relationships among pollutants through statistical interpretations. However, further physiological and/or biochemical mechanisms associated with photosynthate production, growth regulation, water relations, changes in metabolic pathways, metabolite and nutrient allocation, enzyme function, and those processes associated with energy production and utilization at the cellular level have not been studied thoroughly.

Studies of enzyme activity have indicated that, in some cases, the threshold concentration of a pollutant required to produce a change in enzyme activity was lowered when combinations of pollutants were used (Horsman and Wellburn 1975). For combinations of 0.1 or 1.0 ppm NO_2 and 0.2, 1.0, 1.5, or 2.0 ppm SO_2 synergistic interactions were found in pea seedlings in terms of increased activities of peroxidase, glutamate-pyruvate transaminase (GPT), and glutamate oxaloacetate transaminase (GOT), and decreases in chlorophyll content and ribulose 1,5-diphosphate carboxylase (RuDPC) activity. These changes occurred in the absence of visible injury. The inhibition of RuDPC may be due to an accumulation of sulfite ions in the chloroplasts. The increases of GPT and GOT activity indicate a disturbance in amino acid metabolism. If these types of metabolic activities continued, the plant will eventually develop some visible evidence of injury. However, these observations do not necessarily explain additive or synergistic effects of mixtures. Examination of numerous other enzyme responses also indicated different responses to the combination gas than to single gas exposures (Wellburn et al. 1976).

Nitrite reductase (NiR) activity due to NO_2, SO_2, or NO_2 + SO_2 has been investigated (Wellburn 1982, Wellburn et al. 1981). Sulfur dioxide had little effect on NiR, but NO_2 increased its activity. The mixture of SO_2 and NO_2 reduced NiR activity. Thus, Wellburn (1982) proposed that the presence of SO_2 prevents the induction of additional NiR by NO_2 which would normally lead to ammonia and amino acid synthesis.

Studies of metabolic function and biochemical changes in plants do not presently provide any definite evidence of a specific site of action for SO_2 + NO_2 + O_3 synergism. It is possible that (as shown by changes in permeability, Elkiey and Ormrod 1979b, Beckerson and Hofstra 1980), O_3 or SO_2 could alter the permeability of cellular membranes to other dissolved pollutants or their reaction products in the aqueous phase. The findings of Wellburn and others (Horsman and Wellburn 1975, 1976, Wellburn 1982, Wellburn et al. 1976, 1981) concerning the interactions of NO_2 and SO_2 may support the concept that membrane integrity is damaged rapidly by the inability of the plant to detoxify NO_2 in the presence of SO_2. With increasing SO_2, sulfite accumulates (Malhotra and Hocking 1976) and the dual impact of sulfite and nitrite on membrane integrity in the presence of O_3 may allow O_3 to enter the cell freely on a continuing basis. Ozone could then cause injury while SO_2 and NO_2 were impairing specific enzyme functions leading to energy utilization, as well as impairing transport and allocation of needed cell repair components to counteract the stress from further exposure to all three pollutants.

Studies of the influence of mixtures on the chemical composition of plants have been largely concerned with chlorophyll concentration. Horsman and Wellburn (1975) found decreased chlorophyll with SO_2 + NO_2 even though NO_2 alone increased chlorophyll. Olszyk and Tibbitts (1981) established that near-threshold injury by O_3 + SO_2 on peas could be evaluated by chlorophyll concentrations of expanded leaves, as well as by surface area of expanding leaves.

Research concerning mixtures has also involved an examination of gas exchange between plants and polluted atmospheres. Black (1982) suggested that a pollutant-induced change in stomatal aperture and function would result in important consequences by altering a) photosynthetic CO_2 uptake and transpirational water loss, b) the rate at which the pollutant enters the plant and arrives at the metabolic sites, and c) metabolism, resulting in growth and yield change. Stomata may be induced to either open or close in response to SO_2 depending on the species, SO_2 concentration, duration of exposure, and environmental conditions at the time of exposure (Black 1982). There are reported instances of SO_2 enhanced stomatal opening in 16 plant species and SO_2 enhanced closure or depressed transpiration in 24 plant species. In fact, enhanced opening and closure have been reported to occur within the same species (Black 1982).

This realization that SO_2-induced effects on physiological processes in plants are exceedingly variable may offer an explanation for many reported incidences of synergistic and antagonistic responses of plants to pollutant mixtures. When more visible injury develops from a mixture of SO_2 + O_3 than from either pollutant alone, some investigators have reasoned that SO_2 in mixture decreases stomatal resistance, allowing more O_3 to enter. Beckerson and Hofstra (1979a, 1979b) have tried to develop an experimental basis for understanding antagonism and synergism of visible injury response and stomatal resistance by using species such as navy bean and soybean, which respond antagonistically, and cucumber and radish, which respond synergistically to SO_2 + O_3. White beans were exposed to 0.15 ppm SO_2 and 0.15 O_3 singly and in combination six hours per day for five days (Beckerson and Hofstra 1979a). The SO_2 + O_3 mixture increased stomatal resistance (depressed stomatal opening) about 30% more than O_3 alone during the first three days of exposure and the amount of injury was 50 times less than for O_3 alone (Beckerson and Hofstra 1979a). Sulfur dioxide alone decreased stomatal resistance (enhanced stomatal opening). Thus, it was concluded that any protective action of SO_2 against O_3 injury (antagonism) was not completely explained on the basis of stomatal response of white bean.

Investigations were continued using radish, cucumber, and soybean. In all three species, SO_2 decreased stomatal resistance, O_3 increased resistance, and the SO_2 + O_3 mixture increased resistance much more than O_3 alone (Beckerson and Hofstra 1979b). Hofstra and Beckerson (1981) also found that SO_2 pre-treatment of white bean and cucumber did not prevent increased stomatal resistance following exposure to SO_2 + O_3 and O_3 alone, at concentrations used in previous studies (Beckerson and Hofstra 1979a). They also reported that the presence of SO_2 in mixture with O_3 reduced the O_3-induced increase in membrane permeability in white bean and soybean, but not in radish and cucumber (Beckerson and Hofstra 1980). This suggested differential modes of action of SO_2 + O_3 alone or in mixture, acting at membrane sites within the plant. In summary, Beckerson and Hofstra concluded that stomatal function can be disregarded as having any major influence on SO_2 + O_3 interactions and as a mechanism for understanding synergistic, additive, or antagonistic plant responses to SO_2 + O_3, at least for the species studied. They further proposed that in the case of synergism in radish and cucumber, SO_2 may have altered the nutritional or enzymatic status of the plant, thus increasing sensitivity to O_3 (Hofstra and Beckerson 1981). Nevertheless, further research is warranted on stomatal responses to SO_2 + O_3, utilizing techniques having greater resolution and more statistical strength. In addition, stomatal responses need to be related to visible injury and to air pollutant flux into leaves.

Stomate function also has been studied in attempts to explain the additive and frequently greater than additive response of plants to NO_2 + SO_2. Amundson and Weinstein (1981) found high leaf resistance in soybean plants exposed to SO_2 (2.0 ppm) + NO_2 (0.5 ppm), partially accounting for antagonistic effects of the two gases. Ashenden (1979a) found that NO_2 (0.1 ppm) and SO_2 (0.1 ppm) alone caused short-term increases in bean leaf transpiration rates, while NO_2 + SO_2 in mixture decreased transpiration. Stomate function was apparently not involved in the synergistic foliar injury response resulting from mixtures of NO_2 + SO_2. However, Ashenden gave a possible explanation for the synergistic foliar injury response to mixtures of NO_2 + SO_2, by proposing that the stomata were closing in response to physiological injury within the leaf. Since NO_2 and SO_2 interfere with respiration, there is a possibility that increased CO_2 concentrations could arise within the leaf and subsequently decrease stomatal opening (Ashenden 1979a). Amundson et al. (1982) found a HF (0.6 and 1.9 ppb) and NO_2 (0.6 and 1.2 ppm) interaction, with less HF injury on sweet corn in the presence of the higher of the two NO_2 concentrations. Leaf resistance was higher in the combined treatments. There was greater stomatal closure of snap beans with H_2S + O_3 than with H_2S alone (Coyne and Bingham 1978). Williams et al. (1971), in evaluating the interaction between SO_2 and particulates at naturally polluted sites, noted that particles accumulated in stomatal pores, probably keeping them open, increasing permeability, and admitting more SO_2. When pollutants are sorbed or deposited on foliar surfaces and then penetrate through the cuticle, reciprocal effects on uptake are unknown. Thus, interactions of pollutants might be explained readily, in some cases but not others, on the basis of direct effects on stomatal opening. However, there is considerable evidence that other factors in the plant, in addition to stomata, have an important role in determining responses to pollutants.

Impairment of photosynthesis by mixtures has been studied by several investigators. Carlson (1979) found that photosynthesis rates of sugar maple and white ash leaves exposed to O_3 (0.5 ppm) + SO_2 (0.5 ppm) decreased more than additively. The reduction in photosynthesis was least when irradiance was optimal. Ormrod et al. (1981) found joint action of concentrations of O_3 (0-0.25 ppm) + SO_2 (0.04 ppm) in suppressing net photosynthesis in broad bean, but there was recovery if visible leaf injury did not occur (Black et al. 1982). Similarly, Le Sueur-Brymer and Ormrod (1983) found mixtures of O_3 (.067 ppm) + SO_2 (0.3 ppm) suppressed net photosynthesis of fruiting soybeans when single gases did not. However, the photosynthesis apparatus apparently adapted readily to the stress because net photosynthesis was no longer decreased by the mixture after two successive six-hour daily exposures. Substantial reduction in photosynthesis of peas by SO_2 (0.0 to 0.25 ppm) + NO_2 (0.0 to 0.25 ppm) was noted by Bull and Mansfield (1974). White et al. (1974) found greater than additive effects of SO_2 (0.15-0.35 ppm) + NO_2 (0.1-0.2 ppm) on net photosynthesis of alfalfa. Net photosynthesis rates of sunflower leaves were depressed by all combinations of O_3 (0.2 ppm), SO_2 (0.2 ppm), and NO_2 (1.0 ppm) (Furukawa and Totsuka 1979). At a higher than ambient CO_2

concentration (645 ppm), the inhibition of net photosynthesis by SO_2 (~0.8 ppm) + NO_2 (~0.3 ppm) was less than half that at an ambient concentration (315 ppm) (Hou et al. 1977). Addition of 0.072 ppm O_3 to 0.74, 3.25, or 5.03 ppm H_2S resulted in greater reductions in net photosynthesis rates of snap beans than those caused by H_2S alone (Coyne and Bingham 1978).

While many anatomical studies of tissue and cell injury have been conducted with single pollutant gases, very little has been done with mixtures. Solberg and Adams (1956) found no differences in microscopic injury of apple leaves between single and mixed SO_2 (0.5 ppm) + HF (5 ppb) for 4 hours per day for 2 days. However, Evans and Miller (1975) found different sites of injury for single or mixed O_3 (0.45 ppm) + SO_2 (0.45 ppm) for 9 hours in pine needles. Krause and Jensen (1978) found inclusions in poplar leaf cells exposed to O_3 (0.15 ppm) + SO_2 (0.25 ppm) for 12 hours per day for 21 days that were not in leaves exposed to single gases. Leaf surfaces were injured by the mixture but single gases had no effect (Krause and Jensen 1979).

Recovery from mixture-induced injury to plant processes to normal levels of functional activity has been reported. Net photosynthesis, impaired by O_3 (0.05-0.30 ppm) + SO_2 (0.04 ppm) for 4 hours recovered if there was no visible injury to broad bean leaves (Black et al. 1982). Bennett et al. (1980) found recovery of bean growth and yield following O_3 (0.046 to 0.127 ppm) + H_2S (0.3 to 7 ppm) exposure. Kress et al. (1982b) reported recovery in growth of American sycamore after injury by O_3 (0.05 ppm) + SO_2 (0.14 ppm) + NO_2 (0.10 ppm) for 6 hours per day for 28 days. The mechanisms of recovery from pollutant injury have been described by Tingey and Taylor (1982). The extent and speed of re-establishment of a normal metabolic state following pollutant mixture injury will be an important determinant of economic loss from exposure to mixtures, but economic losses have not been estimated on these bases.

A possible explanation of apparently diminished injury from O_3 + SO_2 mixtures was the chemical combination of these gases in exposure chambers (Costonis 1973). However, no evidence was obtained for reactions that would lower the concentration of either gas (Jacobson and Colavito 1976). A direct reaction between O_3 and SO_2 may occur in plant tissue and the direct aqueous oxidation of SO_2 by O_3 has been described (Heagle and Johnston 1979).

Accumulation and Uptake

Accumulation may be considered a major concern for several reasons. First, with reference to HF and certain airborne particulate compounds, accumulation of a toxicant by the plant constitutes a potential hazard to consumers of the plant. Second, uptake and accumulation of a pollutant can be viewed as the first links in a chain of events leading to some altered state or process that may be detrimental. Third, tissue levels of pollutants are often used as diagnostic measures to assess exposures. As discussed by McCune (1983), many factors influence the effect of SO_2, NO_2, or O_3 on the accumulation of fluoride from HF. One of the major difficulties in the interpretation of effects arises when the levels of exposure are too few to determine whether accumulation of F is linear with exposure. If the plant is exposed successively while it is growing, exposures during the latter periods should be weighted more heavily than those during the earlier periods to compensate for growth dilution. If one pollutant affected growth during a series of exposures to HF, wherein the concentration of HF varied, the result would be an apparent effect on F-uptake itself. Another aspect of this problem appears when pollutant mixtures affect the uptake of minerals from the rooting medium and their distribution within the plant. Increased cadmium concentration in young leaves of cress during O_3 exposure was noted by Czuba and Ormrod (1981). Immediately after ozone exposure, there was stimulated uptake of cadmium and redistribution of cadmium between the leaves and stem.

The actual uptake of pollutant gases, rather than the concentration or dose, would be expected to relate most closely to biological responses. There is little information available on pollutant uptake rates from gaseous mixtures. The amount of pollutant sorbed by plants is the product of the uptake rate or flux and the duration of exposure. Also, sorption includes both absorption through stomata or cuticle into the mesophyll tissue of leaves, while it may be metabolically active, and adsorption on surfaces which may injure the surface materials, but not penetrate into areas of metabolic and physiological significance. Elkiey et al. (1982) exposed shoots of ten shade tree species to O_3 (0.25 ppm) + SO_2 (0.4 ppm) + NO_2 (0.4 ppm) for 6 hours and measured individual uptake rates. Sorption from the mixture was consistently less than from single gases in species that did not close stomata at night, while single and mixed gases had similar sorption if stomata closed at night. There was less absorption of each gas from the 3-gas mixture than from single gases by Kentucky bluegrass plants (Elkiey and Ormrod 1981a).

In this study, plants of nine Kentucky bluegrass cultivars were exposed to O_3, SO_2, NO_2, or a mixture of the three gases for three days to determine absorption and adsorption rates of each gas. Absorption rates into stomata differed among cultivars and generally decreased with longer exposure. Leaves of insensitive cultivars generally absorbed less of the single gases than the leaves of sensitive cultivars. Adsorption rates on leaf surfaces, determined with stomata closed, were substantial and varied with pollutant gas and cultivar. Absorption of O_3 (0.25 ppm), SO_2 (0.4 ppm) and NO_2 (0.4 ppm) by petunia plants from single gases was generally greater than from the mixed gases (Elkiey and Ormrod 1981b). Absorption rates tended to decrease gradually throughout the day and from day to day with continuous exposure. Accumulation of tissue sulfur and nitrogen in petunia plants did not agree well with uptake rates. Tissue analysis of petunia plants exposed to O_3 + SO_2 + NO_2 mixtures indicated less accumulation of sulfur from mixed than single SO_2, and less total nitrogen in plants exposed to any NO_2 compared with those not exposed (Elkiey and Ormrod 1981c). This suggests that sulfur- and nitrogen-containing volatiles may be released by exposed plants or that nutrient uptake and distribution or re-distribution may be affected by the pollutant treatments.

There is considerable information available on pollution uptake, based upon studies of single pollutants. Those studies provide conceptual models which should be useful in considering the uptake of pollutant mixtures by plants. The collection and interpretation of uptake data should help resolve the conflicting results of experiments conducted in different environments with differing exposure regimes.

MODIFIERS OF PLANT RESPONSE

Genetic Factors

Resistances to pollutant mixtures have genetic and environmental components. The most useful comparisons of species sensitivities to mixtures are those based on studies in which more than one species was exposed at the same time in the same facilities under the same environmental conditions. There have been several reports of research of this kind (Table 3-3) indicating large differences in species sensitivity to various mixtures.

Most investigators have used one cultivar or line to represent a species, even though there is ample evidence, from single-gas and mixed-gas studies, that there can be wide variation in cultivar response within species (Ormrod 1978). There have been several studies reported in which cultivar responses to mixtures of $SO_2 + O_3$ were directly compared at the same time (Table 3-4). In forest species, Kress et al. (1982a) found differential sensitivity to $O_3 + NO_2 + SO_2$ among lines of loblolly pine.

The degree of association of sensitivity to SO_2 and O_3 among cultivars within species has been examined by means of a distribution-free measure of association, Kendall's Tau statistic. Its value was 0.455 for 17 cultivars of Kentucky bluegrass (Murray et al. 1979); 0.2018 for 19 cultivars of soybean (Miller et al. 1974); and 0.1346 for 33 cultivars of bean (Beckerson et al. 1979). However, sensitivities to a mixture of SO_2 (0.15 ppm) + O_3 (0.15 ppm) for 6 hours per day for 5 days in the bean cultivar study were associated with both SO_2 and O_3 sensitivity (tau - 0.3570 and 0.4227, respectively). Karnosky (1976) found some association of SO_2 and O_3 sensitivity in five lines of trembling aspen. In *Picea abies*, resistance to HF and to SO_2 appeared to be positively correlated (Halbwachs and Kronberger 1979). When the joint distribution of sensitivities has a positive correlation, a population of

Table 3-3

Direct comparisons of species sensitivity to $SO_2 + O_3$, $SO_2 + NO_2$, $O_3 + NO_2$, and $HF + SO_2$

Mixture	Species compared	Reference
$SO_2 + O_3$	Tobacco, radish, alfalfa, cabbage, broccoli, tomato, onion, bromegrass, spinach	Tingey et al. 1973a
	Tobacco, alfalfa, radish	Tingey and Reinert 1975
	Navy bean, tobacco	Jacobson and Colavito 1976
	Radish, cucumber, soybean	Beckerson and Hofstra 1979a
	Navy bean, soybean, cucumber, radish	Beckerson and Hofstra 1980
	Sugar maple, black oak, and white ash	Carlson 1979
	Ginseng, radish, tobacco	Proctor and Ormrod 1981
	Lettuce, radish, pea	Ormrod et al. 1983b
$SO_2 + NO_2$	Tomato, radish, oats, tobacco, pinto bean, soybean	Tingey et al. 1971a
	Several native desert species	Hill et al. 1974
		Thompson et al. 1980
	Radish, swiss chard, oats, peas, orchard grass, annual ryegrass, timothy, perennial ryegrass,	Ashenden and Mansfield 1978
	Orchard grass, perennial ryegrass	Ashenden 1979b
$O_3 + NO_2$	Forest tree species	Kress and Skelly 1982
$HF + SO_2$	Sweet orange, mandarin	Matsushima and Brewer 1972
	Bean, barley, sweet corn	Mandl et al. 1975

Table 3-4

Direct comparisons of cultivar sensitivity within species to $SO_2 + O_3$

Species	Number of cultivars compared	Reference
Tobacco	3	Menser and Heggestad 1966
Tobacco	9	Menser and Hodges 1970
Tobacco	3	Tingey et al. 1973a
Soybean	2	Tingey et al. 1973b
Navy bean	2	Jacobson and Colavito 1976
Strawberry	6	Rajput et al. 1977
Petunia	3	Elkiey and Ormrod 1979c
Bean	33	Beckerson et al. 1979
Soybean	2	Heagle and Johnston 1979
Begonia	5	Reinert and Nelson 1980

plants should be less affected by a mixture than would be expected should the distribution of sensitivities to each pollutant suggest an assumption of an independent distribution.

While most investigators have not reported the extent of plant-to-plant variation in sensitivity to mixtures, Skelly et al. (1972) reported high variability among eastern white pine trees in response to ambient SO_2 + NO_2. Although it might be expected that an exposure to SO_2 would affect the mean tolerance of plants to O_3 (Jacobson and Colavito 1976, Macdowall and Cole 1971), the latter investigation showed an effect of SO_2 on dispersion of tolerance as indicated by changes in slopes of probit regressions.

The use of highly sensitive species or cultivars as test plants to indicate the presence of single pollutants is widely practiced, but only two recommendations of plant indicators of mixtures have been made. Menser and Hodges (1970) suggested 'Burley 49' tobacco and Grosso et al. (1971) suggested *Nicotiana glutinosa* for detection of O_3 + SO_2 effects.

Phenological Factors

Plant development stage may also be an important determinant of sensitivity to pollutant mixtures. Few studies of mixture effects have considered the impact of leaf age or growth stage on sensitivity to pollutants, since usually plant yield and biomass at final harvest have been the only response variables measured. Menser and Heggestad (1966) noted that older tobacco leaves were more sensitive to O_3 (~0.03 ppm) + SO_2 (~0.26 ppm) for 2 or 4 hours than younger leaves. The midshoot leaves of grape and apple were most sensitive to O_3 (0.4 ppm) + SO_2 (0.8 ppm) for 4 hours (Shertz et al. 1980a, 1980b). Alteration of the susceptibility of leaves by the stage of development is another area in which distributional aspects of the combined effects of pollutants have not been considered. One obvious example is where younger leaves tend to be susceptible to one pollutant and older leaves to another. If the exposures are consecutive or separated in time for plants with determinate growth, the sequence of exposures will determine the effects.

Environmental Factors

There may be a strong environmental component in determining plant sensitivity to mixtures, as well as in modifying genetically determined sensitivity. Irradiance, temperature, water supply, and other environmental factors are known to affect plant responses to air pollutants and have been studied extensively for single gases (Ormrod 1978). However, studies of environmental effects on mixture responses have been more limited. Carlson (1979) found much more visible injury and growth suppression of sugar maple and white ash by O_3 + SO_2 at high than at low irradiance. Miller and Davis (1981b) found that exposure temperatures affected the type of O_3 + SO_2 visible injury symptoms in beans, as well as the amount and location of injury.

The water status in each component of the soil-plant-atmosphere continuum may alter response to mixtures as there may be an interaction with CO_2 concentration. More injury was noted after exposure of eastern white pine to O_3 + SO_2 during periods of high humidity compared with low humidity (Jaeger and Banfield 1970). Humidity was found to have a marked effect on the diffusive resistance response of petunia leaves to O_3 + SO_2. Exposure of petunia to SO_2 (0.8 ppm) + O_3 (0.4 ppm), for 4 hours at 50% relative humidity, caused an increase in stomatal resistance, regardless of cultivar sensitivity to O_3. However, at 90% relative humidity, there was an increase in stomatal resistance only in the O_3 sensitive cultivar, 'White Cascade' (Elkiey and Ormrod 1979a). Although changes in relative humidity, leaf water potential (Elkiey and Ormrod 1979a), and membrane permeability (Elkiey and Ormrod 1979b) may be demonstrated among cultivars of differing O_3 sensitivity, these phenomena did not completely explain differences in petunia cultivar sensitivity to SO_2 and O_3 alone and in mixture.

Plant water status not only affects the responses to mixtures but also is affected by plant exposure to mixtures. Plant water potential decreased quickly on exposure of petunia to O_3 + SO_2 (Elkiey and Ormrod 1979c). Leaf diffusive resistance changes are an indication of altered stomatal action.

Adequate soil water, optimal mineral nutrition, high relative humidity, and sufficient irradiance will lead to full stomatal opening, subjecting the leaf tissue to maximal pollutant entry initially (Carlson 1979).

The CO_2 research of Hou et al. (1977) was based on the recognition that CO_2, as well as SO_2 and NO_x, is included in the exhaust gases of many industries. The ratio of SO_2:NO_2:CO_2 concentrations occurring downwind from a power plant burning coal (1:0.33:326) was used in controlled exposure studies. Doubling the CO_2 concentration increased net photosynthesis of alfalfa in SO_2 + NO_2, even though this mixture decreased net photosynthesis in ambient CO_2.

Another factor that could vary and exert an effect in the field is mineral nutrition. Elkiey and Ormrod (1981a) exposed turfgrass plants growing at different nitrogen and sulfur nutrition levels to O_3 (0.1 ppm, 6 hours per day) + SO_2 (0.15 ppm continuously) + NO_2 (0.15 ppm continuously) for 10 days. Low sulfur or low nitrogen usually enhanced the effect of SO_2 or NO_2, respectively. Misting with deionized water increased severity of visible injury. In the compilation of effects of mineral nutrition and responses of plants to pollutants by Cowling and Koziol (1982), one can see that the nutrient-determined tolerances of plants are positively or negatively correlated, depending upon the crop, nutrient element, and set of pollutants. For example, in tomato, tolerance to HF decreased but tolerance to O_3 increased with P-deficiency; in tobacco, tolerance to O_3 or to SO_2 increased and then decreased as the supply of N increased; in barley, tolerance to HF decreased with deficiencies of P, K, or Ca and tolerance to SO_2 decreased with Ca- or K-deficiency, but increased with P-deficiency. An important part of this review was the attempt mechanistically to reconcile and explain the effects of nutrients. Some could be attributed to increased or decreased uptake of pollutants and others to effects on the inherent, metabolic susceptibility. Whether the presence of one pollutant alters the nutrient-determined response of a plant to another pollutant is unknown. To the extent that the effects of other environmental factors can be similarly partitioned, one could also predict their likely effects on the joint action of pollutants. For example, the effects of temperature were not concordant with effects on gaseous exchange (Miller and Davis 1981b).

The only report of chemical protection against mixture injury indicated that benomyl protected pinto beans from the two oxidants, O_3 + PAN (Pell 1976).

SUMMARY

The available literature indicates that most pollutant mixture research has been confined to the use of various combinations of O_3, SO_2, and NO_2 on major species. Other combinations of gases have had much less attention and almost no research on interactions of gaseous, aerosol, and precipitation pollution has been reported. Many species of considerable economic or ecological importance have had little or no attention. Visible injury has been the most frequently reported response variable but concern for growth and yield effects has increased in recent years. The visible injury may not adequately reflect growth and yield responses. Discovery of additive and synergistic responses to two and three pollutant mixtures has provided the impetus for further study and for concern about impacts of mixtures.

Suitable experimental methods and statistical analyses are available for effective studies of mixtures but some of the most appropriate experimental designs and analyses are not widely used. Experiments have been conducted in controlled environments, chambers, greenhouses, and field facilities; each of these approaches has strong and weak points, and the effectiveness will depend on the purpose of each experiment. Some disparities in results between experimental approaches have been reported. Diversity and ambiguity of terminology have created some difficulty in interpretation, but clear definitions of joint action, additivity, synergism, and antagonism are available.

Exposure of plants to pollutant mixtures affects visible injury development, growth, yield, physiological processes, biochemical activities, and plant anatomy with responses often differing from those due to the single components of the mixture. Photosynthesis, transpiration, pollutant uptake rates, enzyme activities, stomatal function, tissue elemental concentration, and other response variables may be differentially affected by mixtures compared with single gases. Much of the information on physiology and biochemistry is fragmentary and, while stomate function and pollutant uptake have been most extensively studied, the data are difficult to interpret as these responses are extremely sensitive to environmental changes.

Species differ widely in sensitivity to pollutant mixtures and there may be large cultivar differences within species, as well as plant-to-plant variation in response. Plant and leaf development stages may be important determinants of sensitivity to mixtures but there have been few studies of this concept. Environmental factors, including irradiance, CO_2 concentration, temperature, water status, and nutrition may affect mixture responses. Little is known of the effects of pollutant mixtures on plant hardiness, reproductive processes, competitive ability and interactions in ecosystems. It is clear, from the few studies conducted in which appropriate follow-up measurements were made, that plants can recover from mixture stresses and even adapt to them. The relationship of concentration and duration in determining doses of mixtures has had little attention nor have the flux rates of pollutants from mixtures to plants, even though actual uptake is likely to be an important determinant of injurious effects. There has been little study of the effects on plants as a result of changing pollutant mixture composition and concentration patterns that may occur in nature.

4. RESEARCH NEEDS

AMBIENT AIR QUALITY EXPOSURE

Based upon a review of the scientific presentations and their knowledge of the scientific literature, the panel members determined that a series of technical gaps exists in defining the effects of air pollutant mixtures on vegetation.

The panel concluded that the ambient air quality air pollution review described in Chapter 2 represented a first step in understanding the characterization of co-occurrence and sequential exposure regimes present under ambient conditions.

- *Additional evaluation is required to determine the important exposure sequences of air pollution.*

VEGETATION EFFECTS

The identification of regimes of pollutant mixtures representative of ambient exposures is important because these regimes define concentration peaks, means, varying times of exposures, and time between events to be used in vegetation experiments. Access to the existing data bases facilitates the design of experiments that mimic the exposures representative of ambient conditions.

- *At present, there are extensive knowledge gaps on effects on major crop species, garden and amenity plants, and native herbaceous and tree species. Current data are based on experiments conducted with different methodologies, environments, experimental designs, and interpretation.*

- *The panel believed that the effects on vegetation resulting from the interaction of pollutant gases and acid deposition, aerosols and heavy metals on soils need further study.*

- *Added information is required to describe the mechanistic and plant processes at realistic exposure regimes for pollutant mixtures.*

- *Little data exist to explain the assimilative allocation, physiological, and biological responses that relate to vegetation dose-response studies. Little data are available on relating uptake rates to pollutant mixtures.*

- *The current data base does not provide sufficient information to understand the genetics of vegetation response to mixtures. Species, cultivar, and individual plant variability have not been well-characterized. The heritability of mixture sensitivity and its relationship to single pollutant sensitivity have not been characterized adequately.*

- *For vegetation effects research involving air pollutant mixtures, as well as for singular pollutant exposures, there exist a paucity of data describing environmental effects (e.g., temperature, soil moisture, humidity, wind velocity, nutrition, and age).*

The panel strongly urged the selection of specific research investigations that were applicable to address high priority research questions.

- *The panel believed that additional information was needed to develop modeling and predictive capabilities. Sources of information about phenology, environmental, and genetic (intra- and interspecific) variation need to be quantified to describe interdependent effects.*

- *The changes in plant sensitivity to gas mixtures with changing leaf age and development stage have had little study. Information is especially lacking on sensitivity during the fruiting period.*

- *There is little or no information on effects of mixtures on plant hardiness, reproduction, nutritional value, and other characteristics affecting adaptation and utilization of plants. While there is now some evidence for the existence of plant recovery and adaptation processes, little is known of the nature of such homeostatic processes and mechanisms.*

- *The panel believed that there have been few attempts to fully utilize alternative statistical designs and analyses. Such methods could include covariate measurements, rotatable designs, and response surface presentations.*

Following the identification of research requirements, the panel focused on recommending the specific direction in which air pollution mixture vegetation research should follow. Chapter 5 presents the panel's findings.

5. RECOMMENDATIONS

INTRODUCTION

The potential value of prioritizing research needs was addressed by the panel. The members believed that research areas were not independent of each other. For example, the biological research efforts described later in this chapter are dependent upon a knowledge of the pollutant concentrations occurring in the field under ambient conditions. The panel has concluded that environmental, genetic, and phenological variables should be considered when research studies are initiated.

AIR QUALITY

An analysis of the EPA SAROAD, EPRI SURE, and TVA data bases indicates that SO_2, NO_2, and O_3 may co-occur in various concentrations in rural, suburban, and urban areas. For many rural sites, co-occurrence (using 0.05 ppm as the definition of an event) is infrequent. For most cases analyzed, events lasted for only a few hours and were separated by weeks or months.

The panel recommends that

- o *air quality data be further evaluated using patterns of occurrence of the combined pollutants to establish guidelines for designing plant interaction research investigations. The primary pollutants of interest are* SO_2, O_3, *and* NO_2; *they can be evaluated using the available air quality data and research information dealing with the individual effects on vegetation.*

- o *acidic deposition be considered as a pollutant with potential for interaction with* SO_2, O_3, *and* NO_2.

- *an analysis of existing air quality data bases (starting with SAROAD) be instituted to derive the joint probability distributions of pollutants and the diurnal patterns of exposure for plant exposure experiments. Additional sources of rural air quality data could include the USDA Forest Service, EPRI (SURE), EPA (NCLAN), as well as permit monitoring programs (e.g., PSD applications). This analysis is to include:*

    1) *Search the data base for locations where either co-occurrence or sequential exposures occur. This search would include separate listings at several threshold concentrations (e.g., 0.05, 0.04, 0.03, and 0.02 ppm).*
    2) *Once locations are identified, the monitoring data bases at the locations should be presented as joint frequency distributions and as diurnal time series. The utility of spectral analysis (Fourier series) and the Box Jenkins model should be explored.*
    3) *The results of this process should be disseminated to research groups to guide experimental exposures used in interaction experiments.*

- *potential data displays for individual pollutants could include: 1) three-dimensional plots of concentration, duration, and frequency; 2) diurnal plots for individual pollutants in terms of mean levels and frequency above particular levels. These analyses would utilize data from the growing season (or some relevant time period) and could also serve to identify potential anomalies (in terms of data values or sites).*

The air quality analyses would provide information that could be used to identify general patterns in terms of geographic region or source configuration. It may be necessary to supplement the air quality data for point source pollutants by considering the use of dispersion models to provide information on levels, diurnal patterns, and time between episodic events.

As a result of the analysis, it is anticipated that the information would provide researchers with relevant exposure patterns that have a known probability of occurrence. In addition, the information that describes the rural site exposures might also be compared to urban results so that researchers can establish possible exposure relationships.

BIOLOGICAL EFFECTS

Introduction

The identified research needs are divided into two areas which have not been assigned a specific funding. The panel believes the activities are complementary and fill gaps in the information base that describe the effects of pollutant mixtures on vegetation. The work group members stated that

- *research efforts should be directed toward major crop species, cultivated plants (including commercial crops and plants utilized for garden and home use), and native herbaceous and tree species.*
- *to assist in the design of future research on the effects of pollutant mixtures on vegetation, available air quality data should be re-evaluated to help identify appropriate response data needed from plant experiments.*

The panel feels that knowledge gaps can only be filled by an integrated research effort with growth chambers, greenhouses, and field plots.

Realistic Exposure Regimes

The panel believes it essential to understand the response of plants to various exposure regimes.

- *Effects must be associated with air pollutant peaks, means, length of exposure, and time between exposures. Research using realistic ambient pollutant exposures should evaluate the vegetation effects associated with sequential exposures of pollutant mixtures that mimic ambient conditions.*

Development of Minimum Recommendations for Research Protocols

The panel recommends that

- *a* *minimum* *set of standardized procedures, to ensure the quality assurance of plant response studies, should be established. Generalized guidelines should be proposed for 1) plant growth conditions, 2) environmental and plant monitoring, 3) pollutant exposures, and 4) uniform terminology (describing plant response characteristics). The most efficient experimental designs and analysis (relevant to a specific experimental goal) should be implemented (e.g., covariant analysis, analysis of variance, and rotatable design). It is proposed that these minimum guidelines be developed through a series of workshops involving scientists experienced in designing and implementing research on the impacts of air pollution on vegetation.*

- *as part of a generalized protocol that researchers should clearly define the meaning of agreed upon concepts (e.g., less than additive, synergism, and antagonism).*

Predictive Capabilities

The purpose of the pollutant interaction research is to develop predictive capabilities for assessing vegetation effects when experimental data are insufficient. Predictive capabilities allow for the extrapolation of results to ambient exposure conditions that have previously not been tested. To properly develop the information necessary to predict vegetation effects associated with pollutant mixtures, it is necessary to implement a research program involving studies that elucidate 1) the modes of action and 2) the sources of biological variation. From these experimental results will come the modeling required to develop the predictive capabilities that are necessary to quantify possible effects on vegetation.

As part of the development of predictive capabilites, the panel recommends that the following research activities be implemented:

- *Modes of Action: The objectives of this research activity is to understand how air contaminants influence biological processes. Studies need to address modes of action of pollutants singly and in combination. The research effort should include both sequential and co-occurrence exposures and should be conducted with an appreciation of realistic exposure regimes. The biological level of organization should focus on processes at all levels of plant organization (i.e., the cell, whole-plant, population, and ecosystem). The panel believes that there should be two major areas of interest*

    a) *The relationship between the different mechanisms of pollutant response.*

    b) *The varying biological responses attributed to different levels of air pollutant exposure.*

- *Sources of Variation: The plant response to a given exposure regime varies significantly with specific environments, and stage of plant development. The panel recommends research that focuses on each of the following:*

  a) *genotype-the significance of intra- (e.g., cultivar, population) and interspecific genotypes. This includes phenology as a source of variation.*

  b) *environment-the significance of edaphic (e.g., soil water availability, soil nutrients), climatic (e.g., temperature, light, relative humidity, elevated carbon dioxide, etc.) and biotic factors (e.g., pathogens, symbionts, competition, etc.).*

- *Modeling-The development of data that describe the process and mechanistic activities associated with air pollutant mixture vegetation effects should allow for the development of conceptual and quantitative models of biological response.*

CONCLUSION

It is the opinion of the workshop participants that the position paper (which is presented in Chapter 2) focused on ambient exposures and represented an initial attempt to identify realistic exposure regimes that exist in the ambient air. The panel believes that

- *additional efforts should be made to supplement the existing analysis.*

- *the efforts should proceed simultaneously as the biological vegetation effects research is implemented.*

- *the results of the air quality characterization should be used in developing the design of the pollutant mixture experimental protocols.*

The first two stages of the process and mechanistic research should include

- *a biological effects screening exercise to prioritize which air pollutant mixture exposures are most likely to be significant. This effort is suitable in controlled exposure facilities, and*

- *a more detailed investigation performed under field and laboratory situations for the purpose of quantifying the significance of the major factors affecting plant response.*

In regard to conceptual models, it was the conclusion of the panel members that

- *they should combine existing models of joint action with the data that describe the modes of biological action. The quantitative models should be capable of providing accurate and precise estimates of plant response. In addition, the models should be compatible with the conceptual interpretation of the modes of action.*

REFERENCES

Amundson, R.G., and L.H. Weinstein. 1980. The effects of SO_2 and NO_2 alone and in combination on the yield of soybean. Plant Physiol. 65:152 supplement.

Amundson, R.G., and L.H. Weinstein. 1981. Joint action of sulfur dioxide and nitrogen dioxide on foliar injury and stomatal behaviour in soybean. J. Environ. Quality 10:204-206.

Amundson, R.G., L.H. Weinstein, P. Van Leuken, and L.J. Colavito. 1982. Joint action of HF and NO_2 on growth, fluorine accumulation, and leaf resistance in Marcross sweet corn. Environ. Exp. Bot. 22:49-55.

Ashenden, T.W. 1978. Extreme pollution sensitivity of grasses when SO_2 and NO_2 are present in the atmosphere together. Nature, 273:142-143.

Ashenden, T.W. 1979a. The effects of long-term exposures to SO_2 and NO_2 pollution on the growth of Dactylis glomerata L. and Poa pratensis L. Environ. Pollut. 18:249-258.

Ashenden, T.W. 1979b. Effects of SO_2 and NO_2 pollution on transpiration in Phaseolus vulgaris L. Environ. Pollut. 18:45-50.

Ashenden, T.W., and T.A. Mansfield. 1978. Extreme pollution sensitivity of grasses when SO_2 and NO_2 are present in the atmosphere together. Nature 273:142-143.

Ashenden, T.W., and I.A.D. Williams. 1980. Growth reductions in Lolium multiforum and Phleum Pratense as a result of SO_2 and NO_2 pollution. Environ. Pollut. 21:131-139.

Ashenden, T.W., P.W. Tabner, P. Williams, M.E. Whitmore, and T.A. Mansfield. 1982. A large scale system for fumigating plants with SO_2 and NO_2. Environ. Pollut. (Ser. B.) 3:21-26.

Bachmann, J., and L.J. Zaragoza. 1983. Protecting public welfare: the role of environmental effects information in air standards development. Presented at the 76th Air Pollution Control Association National Meeting, Atlanta, GA. June 19-24, 1983. 83-3.1.

Beckerson, D.W., and G. Hofstra. 1979a. Response of leaf diffusive resistance of radish, cucumber and soybean to O_3 and SO_2 singly or in combination. Atmos. Environ. 13:1263-1268.

Beckerson, D.W., and G. Hofstra. 1979b. Stomatal responses of white bean to O_3 and SO_2 singly or in combination. Atmos. Environ. 13:533-535.

Beckerson, D.W., and G. Hofstra. 1980. Effects of sulphur dioxide and ozone, singly or in combination, on membrane permeability. Can. J. Bot. 58:451-457.

Beckerson, D.W., G. Hofstra, and R. Wukasch. 1979. The relative sensitivities of 33 bean cultivars to ozone and sulfur dioxide singly or in combination in controlled exposures and to oxidants in the field. Plant Dis. Rep. 63:478:482.

Bennett, J.P., H.M. Resh, and V.C. Runneckles. 1974. Apparent stimulation of plant growth by air pollutants. Can. J. Bot. 52:35-41.

Bennett, J.P., K. Barnes, and J.H. Shinn. 1980. Interactive effects of H_2S and O_3 on the yield of snap beans (Phaseolus vulgaris L.) Environ. Exp. Bot. 20:107-114.

Black, V.J., D.P. Ormrod, and M.H. Unsworth. 1982. Effects of low concentrations of ozone, singly and in combination with sulphur dioxide on net photosynthesis rates of Vicia faba L. J. Exp. Bot. 33:1302-1311.

Black, V.J. 1982. Effects of sulfur dioxide on physiological processes in plants. In: M.H. Unsworth and D.P. Ormrod (eds.) Effects of Gaseous Air Pollution in Agriculture and Horticulture. 11:225-246. London/Boston: Butterworth Scientific. 532 pp.

Bull, J.N., and T.A. Mansfield. 1974. Photosynthesis in leaves exposed to SO_2 and NO_2. Nature 250:433-444.

Carlson, R.W. 1979. Reduction in the photosynthetic rate of Acer, Quercus and Fraxinus species caused by sulphur dioxide and ozone. Environ. Pollut. 18:159-170.

Costonis, A.C. 1973. Injury to eastern white pine by sulfur dioxide and ozone alone and in mixtures. Eur. J. Forest Pathol. 3:50-55.

Cowling, D.W., and M.J. Koziol. 1982. Mineral nutrition and plant responses to air pollutants.pp. 349-375. In: M.H. Unsworth and D.P. Ormrod (eds.) Effects of Gaseous Air Pollution in Agriculture and Horticulture. London/Boston: Butterworth Scientific. 532 pp.

Coyne, P.I., and G.E. Bingham. 1978. Photosynthesis and stomatal light responses in snap beans exposed to hydrogen sulfide and ozone. J. Air Pollut. Control Assoc. 28:1119-1128.

Czuba, M., and D.P. Ormrod. 1974. Effects of cadmium and zinc on ozone-induced phytotoxicity in cress and lettuce. Can. J. Bot. 52:645-649.

Czuba, M., and D.P. Ormrod. 1981. Cadmium concentrations in cress shoots in relation to cadmium-enhanced ozone phytotoxicity. Environ. Pollut. (Ser. A) 25:67-76.

Decormis, L., and M. Luttringer. 1977. Influence of sulfur dioxide and nitrogen dioxide synergism. (Effects sur les vegetaux de la synergie dioxxyde de soufre-oxides d. azote). Pollut. Atmos. 75:245-247.

Department of Health, Education, and Welfare (DHEW). 1970. Air quality criteria for sulfur oxides. National Air Pollution Control Administration, Washington, DC. pp. 61-66, 160-161.

Dochinger, L.S., F.W. Bender, F.O. Box, and W.W. Heck. 1970. Chlorotic dwarf of eastern white pine caused by an ozone and sulphur dioxide interaction. Nature 255:476.

Electric Power Research Institute. 1982. Eastern Regional Air Quality Measurements. Vol. 1. Prepared by Environmental Research & Technology, Inc. EPRI EA-1914, Palo Alto, CA.

Elkiey, T., and D.P. Ormrod. 1979a. Leaf diffusion resistance responses of three petunia cultivars to ozone and/or sulfur dioxide. J. Air Pollut. Control Assoc. 29:622-625.

Elkiey, T., and D.P. Ormrod. 1979b. Ozone and/or sulphur dioxide effects on tissue permeability in petunia leaves. Atmos. Environ. 13:1165-1168.

Elkiey, T., and D.P. Ormrod. 1979c. Ozone and sulphur dioxide effects on leaf water potential of _Petunia_. Z. Pfansenphysiologie 91:177-181.

Elkiey, T., and D.P. Ormrod. 1980. Response of turfgrass cultivars to ozone, sulfur dioxide, nitrogen dioxide, and their mixture. J. Amer. Soc. Hort. Sci. 105:664-668.

Elkiey T., and D.P. Ormrod. 1981a. Sulphite, total sulphur and total nitrogen accumulation by petunia leaves exposed to ozone, sulphur dioxide and nitrogen dioxide. Environ. Pollut. (Ser. A). 24:233-241.

Elkiey, T., and D.P. Ormrod. 1981b. Sorption of O_3, SO_2, NO_2 or their mixtures by nine _Poa pratensis_ cultivars of differing pollutant sensitivity. Atmos. Environ. 15:1739-1743.

Elkiey, T., and D.P. Ormrod. 1981c. Absorption of ozone, sulfur-dioxide, and nitrogen dioxide by petunia plants. Environ. Exp. Bot. 21:63-70.

Elkiey, T., D.P. Ormrod, and B. Marie. 1982. Foliar sorption of sulfur dioxide, nitrogen dioxide, and ozone by ornamental woody plants. HortScience 17:358-360.

Environmental Protection Agency. 1976. AEROS Manual Series Volume V: AEROS Manual of Codes. Office of Air and Waste Management, Office of Air Quality Planning and Standards, Research Triangle Park, NC. EPA-450/2-76-005.

Environmental Protection Agency. 1978. Air Quality Criteria for Ozone and Other Photochemical Oxidants. Environmental Criteria and Assessment Office, Research Triangle Park, NC. EPA-600/8-78-004.

Environmental Protection Agency. 1981a. Preliminary assessment of health and welfare effects associated with nitrogen oxides for standard-setting purposes. Prepared by the Division of Strategies and Air Standards. United States Environmental Protection Agency, Research Triangle Park, North Carolina.

Environmental Protection Agency. 1981b. 1980 ambient assessment--air portion. Office of Air Quality Planning and Standards, Research Triangle Park, North Carolina.

Environmental Protection Agency. 1981c. Air Quality Criteria for Oxides of Nitrogen. Prepared by the Environmental Criteria and Assessment Office. Research Triangle Park, North Carolina. EPA-600/8-82-026.

Environmental Protection Agency. 1982a. Review of the National Ambient Air Quality Standards for Nitrogen Oxides: Assessment of Scientific and Technical Information: OAQPS Staff Paper. Prepared by the Office of Air Quality Planning and Standards, EPA, Research Triangle Park, NC. EPA-450/5-82-002.

Environmental Protection Agency. 1982b. Review of the National Ambient Air Quality Standards for Sulfur Oxides; Assessment of Scientific and Technical Information. Prepared by the Division of Strategies and Air Standards. United States Environmental Protection Agency, Research Triangle Park, North Carolina.

Environmental Protection Agency. 1982c. Air Quality Data - 1981 Annual Statistics Including Summaries with Reference to Standards. Office of Air Planning and Standards, Research Triangle Park, North Carolina. EPA-450/4-82-007.

Evans, L.S., and P.R. Miller. 1975. Histological comparison of single and additive O_3 and SO_2 injuries to elongating Ponderosa pine needles. Amer. J. Bot. 62:416-421.

Foster, K.W., H. Timm, C.K. Labanauskas and R.J. Oshima. 1983. Effects of ozone and sulfur dioxide on tuber yield and quality of potatoes. J. Environ. Qual. 12: In press.

Fujiwara, T. 1973. Damage to plants caused by complex pollution of atmosphere. Snokubutsu Boeki. (Plant Protection) 27:233-236.

Fujiwara, T., and H. Ishikawa. 1976. Actual effects of combination of sulfur dioxide, nitrogen dioxide and ozone on some plants (in Japanese with English summary). Bull. Bio-Environ. Lab., No. 476001. 1019 pp.

Furukawa, A., and T. Totsuka. 1979. Effects of NO_2, SO_2 and O_3 alone and in combination on net photosynthesis in sunflower. Environ. Control Biol. 17:161-166.

Gardner, J.O., and D.P. Ormrod. 1977. Response of Reiger begonia to ozone and sulphur dioxide. Sci. Hort. 5:171-181.

Glass, G.E., and T.G. Brydges. 1981. Problem complexity in predicting impacts from altered precipitation chemistry. Presented at Int. Conf. on Acid Precipitation Impacts, Cornell University, Ithaca, NY. August 1981.

Grosso, J.J., H.A. Menser, G.H. Hodges, and H.H. McKinney. 1971. Effects of air pollutants on Nicotiana cultivars and species used for virus studies. Phytopathology 61:945-950.

Halbwachs, G., and W. Kronberger. 1979. Das Verhalten F- and SO$_2$- resistenter fichtenklone verschledener herkunft in pinem rauchschadens gebiet. Zb. Inst. Gozdno. Lesn. Gospos. Ljubljana, pp. 259-268.

Heagle, A.S., and J.W. Johnston. 1979. Variable responses of soybeans to mixtures of ozone and sulfur dioxide. J. Air Pollut. Control Assoc. 29:729-732.

Heagle, A.S., D.E. Body, and G.E. Neely. 1974. Injury and yield responses of soybean to chronic doses of ozone and sulfur dioxide in the field. Phytopathology 64:132-136.

Heagle, A.S., W.W. Heck, J.O. Rawlings, and R.B. Philbeck. 1983. Effects of chronic doses of ozone and sulfur dioxide on injury and yield of soybeans in open-top chambers. Crop Sci. In press.

Heck, W.W., S.V. Krupa, and S.N. Linzon. 1979. Methodology for the Assessment of Air Pollution Effects on Vegetation. Air Pollution Control Association, Pittsburgh.

Heck, W.W., W.W. Cure, D.S. Shriner, R.J. Olson, and A.S. Heagle. 1982. Ozone impacts on the productivity of selected crops. In: J.S. Jacobson and A.A. Millen (eds.) Symposium on the Effects of Air Pollution on Farm Commodities. Isaak Walton League of America, Arlington, VA.

Heggestad, H.E., and J.H. Bennett. 1981. Photochemical oxidants potentiate yield losses in snap beans attributable to sulfur dioxide. Science 213:1008-1010.

Hill, A.C., S. Hill, C. Lamb, and T.W. Barrett. 1974. Sensitivity of native desert vegetation to SO$_2$ and to SO$_2$ and NO$_2$ combined. J. Air Pollut. Control Assoc. 24:153-157.

Hodges, G.H., H.A. Menser, and W.B. Ogden. 1971. Susceptibility of Wisconsin Havanna tobacco cultivars to air pollutants. Argon. J. 63:107-111.

Hofstra, G., and D.W. Beckerson, 1981. Foliar responses of five plant species to ozone and a sulphur dioxide/ozone mixture after a sulphur dioxide pre-exposure. Atmos. Environ. 15:383-389.

Hofstra, G., and D.P. Ormrod. 1977. Ozone and sulphur dioxide interaction in white bean and soybean. Can. J. Plant Sci. 57:1193-1198.

Horsman, D.C., and A.R. Wellburn. 1975. Synergistic effect of SO_2 and NO_2 polluted air upon enzyme activity in pea seedlings. Environ. Pollut. 8:123-133.

Horsman, D.C., and A.R. Wellburn. 1976. Guide to the metabolic and biochemcial effects of air pollutants on higher plants. In: T.A. Mansfield (ed.) Effects of Air Pollutants on Plants. pp. 185-189.

Hou, L.Y., A.C. Hill, and A. Soleimani. 1977. Influence of CO_2 on the effects of SO_2 and NO_2 on alfalfa. Environ. Pollut. 12:7-16.

Houston, D.B. 1974. Response of selected Pinus strobus L. clones to fumigation with sulfur dioxide and ozone. Can. J. For. Res. 4:65:68.

Irving, P.M., and J.E. Miller, 1981. Productivity of field-grown soybeans exposed to acid rain and sulfur dioxide alone and in combination J. Environ. Quality 10:473-478.

Irving, P., J.E. Miller, and P.B. Xerikos. 1982. The effect of NO_2 and SO_2 alone and in combination on the productivity of field-grown soybeans. In: T. Schneider and L. Grand (eds.) Air Pollution by Nitrogen Oxides. pp. 521-531. Amsterdam, The Netherlands: Elsevier Scientific.

Ishikawa, H. 1976. Sensitivity of cultivated plants to air pollutants. Report from Bio Enviroment Laboratory, Central Research Institute of Electric Power Industry, 1646 Abiko Chiba, Japan.

Jacobson, J.S., and L.J. Colavito. 1976. The combined effect of sulfur dioxide and ozone on bean and tobacco plants. Environ. Exp. Bot. 16:277-285.

Jaeger, J., and W. Banfield. 1970. Response of eastern white pine to prolonged exposure to atmospheric levels of ozone, sulfur dioxide, or mixtures of these pollutants. Phytopathology 60:577.

Karnosky, D.F. 1976. Threshold levels for foliar injury to Populus tremuloides by sulfur dioxide and ozone. Can. J. For. Res. 6:166-169.

Kender, W.J., and F.H.F.G. Spierings. 1975. Effects of sulfur dioxide, ozone, and their interactions on 'Golden Delicious' apple trees. Neth. J. Plant Pathol. 81:149-151.

Klarer, C.I. 1982. Effects of sulfur dioxide and nitrogen dioxide, singly and in combination over time on ribulose bisphosphate carboxylase activity and vegetative growth of soybean. M.S. Thesis, North Carolina State Univ., Raleigh, NC. 76 pp.

Kohut, R.J., and D.D. Davis. 1978. Response of pinto bean to simultaneous exposure to ozone and PAN. Phytopathology 68:567-569.

Krause, C.R., and K.F. Jensen. 1978. Microtopographical changes in hybrid poplar leaves associated with air pollution exposure. Scan. Elec. Micros. 2:755-758.

Krause, C.R., and K.F. Jensen. 1979. Surface changes on hybrid poplar leaves exposed to ozone and sulfur dioxide. Scan. Elec. Micros. 3:77-80.

Krause, G.H.M., and Kaiser, H. 1977. Plant response to heavy metal and sulphur dioxide. Environ. Pollut. 12:63-71.

Kress, L.W., and J.M. Skelly. 1982. Response of several eastern forest tree species to chronic doses of ozone and nitrogen dioxide. Plant Dis. 66:1149-1152.

Kress, L.W., J.M. Skelly, and K.H. Hinkelmann. 1982a. Growth impact of O_3, NO_2 and/or SO_2 on Pinus taeda. Environ. Monit. Assess. 1:229-239.

Kress, L.W., J.M. Skelly, and K.H. Hinkelmann. 1982b. Growth impact of O_3, NO_2 and/or SO_2 on Plantanus occidentalis. Agric. Environ. 7:265-274.

Lamoreaux, R.J., and W.R. Chaney. 1978. Photosynthesis and transpiration of excised silver maple leaves exposed to cadmium and sulphur dioxide. Environ. Pollut. 17:259-268.

Le Sueur-Brymer, N.M., and D.P. Ormrod. 1983. Net photosynthesis rates of fruiting soybean plants exposed to ozone and sulphur dioxide singly or in combination. Can. J. Plant Sci. (submitted for publication).

Lewis, E., and E. Brennan. 1978. Ozone and sulfur dioxide mixtures cause a PAN-type injury to petunia. Phytopathology 68:1011-1014.

Macdowall, F.D.H., and A.F.W. Cole. 1971. Threshold and synergistic damage to tobacco by ozone and sulfur dioxide. Atmos. Environ. 5:553-559.

Malhotra, S.S., and D. Hocking. 1976. Biochemical and cytological effects of sulphur dioxide on plant metabolism. New. Phytol. 76:227-237.

Mandl, R.H., L.H. Weinstein, and M. Keveny. 1975. Effects of hydrogen fluoride and sulphur dioxide alone and in combination on several species of plants. Environ. Pollut. 9:133-143.

Mandl, R.H., L.H. Weinstein, M. Dean, and M. Wheeler. 1980. The response of sweet corn to HF and SO_2 under field conditions. Environ. Exp. Bot. 20:359-365.

Mansfield, T.A., and T.W. Ashenden. 1978. Extreme pollution sensitivity of grasses when SO_2 and NO_2 are present in the atmosphere together. Nature 273:142-143.

Masaru, N., F. Syozo, and K. Saburo. 1976. Effects of exposure to various injurious gases on germination of lily pollen. Environ. Pollut. 11:181-187.

Matsushima, J., and R.F. Brewer. 1972. Influence of sulfur dioxide and hydrogen fluoride as a mix or reciprocal exposure on citrus growth and development. J. Air Pollut. Control Assoc. 22:710-713.

McCune, D.C. 1983. Interaction of fluorides with air pollutants. Intern. Fluoride Symp. Utah State Univ., Logan, UT, May 24-27, 1982.

McLaughlin, S.B., D.S. Shriner, R.K. M'Conathy, and L.K. Mann. 1979. The effects of SO_2 dosage kinetics and exposure frequency on photosynthesis and transpiration of kidney beans (Phaseolus vulgaris, L.). Environ. Exp. Bot. 19:179-191.

Menser, H.A., and H.E. Heggestad. 1966. Ozone and sulfur dioxide synergism: injury to tobacco plants. Science 153:424-425.

Menser, H.A., and G.H. Hodges. 1970. Effects of air pollutants on burley tobacco cultivars. Agronomy J. 63:265-269.

Miller, C.A., and D.D. Davis. 1981a. Response of pinto bean plants exposed to O_3, SO_2, or mixtures at varying temperatures. HortScience 16:548-550.

Miller, C.A., and D.D. Davis. 1981b. Effect of temperature on stomatal conductance and ozone injury of pinto bean leaves. Plant Dis. 65:750:751.

Miller, V.C., R.K. Howell, and B.E. Caldwell. 1974. Relative sensitivity of soybean genotypes to ozone and sulfur dioxide. J. Environ. Qual. 3:35-37.

Mueller, P.K., G. M. Hidy, K. Warren, T.F. Lavery, and R.L. Baskett. 1980. The occurrence of atmospheric aerosols in the northeastern United States. In: Aerosols: Anthropogenic and Natural, Sources and Transport. New York Academy of Sciences, New York, N.Y., pp. 463-482.

Murray, J.J., R.K. Howell, and A.C. Wilton. 1979. Differential response of seventeen Poa protansis cultivars to ozone and sulfur dioxide. Plant Dis. Rep. 59:852-854.

Noble, R.D., and K.F. Jensen. 1980. Effects of sulfur dioxide and ozone on growth of hybrid poplar leaves. Amer. J. Bot. 67:1005-1009.

Noggle, J.C., and H.C. Jones. 1981. Regional effects of multiple air pollutants on plants. Presented at the 1981 Annual Meeting of the Air Pollution Control Association, Philadelphia, PA. Paper #42.5.

O'Connor, J.R. 1980. Overview of the criteria review and standard setting process. Presented at the 1980 Air Pollution Control Association Specialty Conference on the Technical Basis for a Size Specific Particulate Standard. Danvers, Massachusetts. pp. 15-25.

Olszyk, D.M., and T.W. Tibbitts. 1981. Stomatal response and leaf injury of Pisum sativum L. with SO_2 and O_3 exposures. Plant Physiol. 67:539-544.

Ormrod, D.P. 1977. Cadmium and nickel effects on growth and ozone sensitivity of pea. Water, Air, Soil Pollut. 8:263-270.

Ormrod, D.P. 1978. Pollution in Horticulture. Elsevier, Amsterdam.

Ormrod, D.P. 1982. Air pollutant interactions in mixtures. Pages 307-331. In: M.H. Unsworth and D.P. Ormrod (eds.). Effects of Air Pollution in Agriculture and Horticulture. Butterworths, London.

Ormrod, D.P., V.J. Black, and M.H. Unsworth. 1981. Depression of net photosynthesis in Vicia faba L. exposed to sulphur dioxide and ozone. Nature 291:585-586.

Ormrod, D.P., D.T. Tingey, and M.L. Gumpertz, and D.M. Olszyk. 1983a. Utilization of a response surface technique in the study of physiological responses to ozone and sulfur dioxide mixtures. Ms. in preparation.

Ormrod, D.P., D.T. Tingey, and M.L. Gumpertz. 1983b. Covariate measurements for increasing the precision of plant response to O_3 and SO_2. HortScience (submitted for publication).

Oshima, R.J. 1978. The impact of sulfur dioxide on vegetation: A sulfur dioxide-ozone response model. California Air Resources Board, Agreement No. A6-162-30.

Padgett, J., and H. Richmond. 1983. The process of establishing and revising National Ambient Air Quality Standards. J. of Air Pollut. Control Assoc. 33:13-16.

Pell, E.J. 1976. Influence of benomyl soil treatment on pinto bean plants exposed to peroxyacetyl nitrate and ozone. Phytopathology, 66:731-733.

Proctor, J.T.A., and D.P. Ormrod. 1981. Sensitivity of ginseng to ozone and sulfur dioxide. HortScience 16:647:648.

Rajput, C.B.S., D.P. Ormrod, and W.D. Evans. 1977. The resistance of strawberry to ozone and sulfur dioxide. Plant Dis. Rep. 61:222-225.

Reich, P.B., R.G. Amundson, and J.P. Lassoie. 1982. Reduction i soybean yield often exposure to ozone and sulfur dioxide using a Linear Gradient Exposure Technique. Water, Air, Soil Pollut. 17:29-36.

Reinert, R.A., and T.N. Gray. 1981. The response of radish to nitrogen dioxide, sulfur dioxide, and ozone, alone and in combination. J. Environ. Quality 10:240-243.

Reinert, R.A., and W.W. Heck. 1982. Effects of nitrogen dioxide in combination with sulfur dioxide and ozone on selected crops. In: (T. Schneider and L. Grant (eds.) Air Pollution by Nitrogen Oxides. pp. 533-546. Amsterdam, The Netherlands: Elsevier Scientific.

Reinert, R.A., and P.V. Nelson. 1980. Sensitivity and growth of five Elatior Begonia cultivars to SO_2 and O_3 alone and in combination. J. Amer. Soc. Hort. Sci. 105:721-723.

Reinert, R.A., and J.S. Sanders. 1982. Growth of radish and marigold following repeated exposure to nitrogen dioxide, sulfur dioxide, and ozone. Plant. Dis. 66:122-124.

Reinert, R.A., and D.E. Weber. 1980. Ozone and sulfur dioxide-induced changes in soybean growth. Phytopathology 70:914-916.

Reinert, R.A., A.S. Heagle, and W.W. Heck. 1975. Plant response to pollutant combinations. In: J.B. Mudd, and T.T. Koslowski (eds.) Responses of Plants to Air Pollution. pp. 159-177. Academic Press, New York.

Reinert, R.A., D.S. Shriner, and J.O. Rawlings. 1982. Responses of radish to all combinations of three concentrations of nitrogen dioxide, sulfur dioxide, and ozone. J. Environ. Quality. 11:52-57.

Roques, A., M. Kerjean, and D. Auclair. 1980. Effets de la pollution atmospherique par le fluor et le dioxide de soufre sur l'appareil reproducteur fermelle de Pinus sylverstris en foret de roumare (Seine Maritime, France). Environ. Pollut. (Ser. A). 21:191-201.

Sanders, J.A., and R.A. Reinert. 1982a. Screening azalea cultivars for sensitivity to nitrogen dioxide, sulfur dioxide and ozone alone and in mixtures. J. Amer. Soc. Hort. Sci. 107:87-90.

Sanders, J.S., and R.A. Reinert. 1982b. Weight changes of radish and marigold exposed at three ages to NO_2, SO_2 and O_3 alone and in mixture. J. Amer. Soc. Hort. Sci. 107:726-730.

Shertz, R.D., W.J. Kender, and R.C. Musselman. 1980a. Foliar response and growth of apple trees following exposure to ozone and sulfur dioxide. J. Amer. Soc. Hort. Sci. 105:594-598.

Shertz, R.D., W.J. Kender, and R.C. Musselman. 1980b. Effects of ozone and sulfur dioxide on grapevines. Sci. Hort. 13:37-45.

Shew, B.B., R.A. Reinert and R.R. Barker. 1982. Response of tomatoes to ozone, sulfur dioxide, and infection by Pratylenchus penetrans. Phytopathology 72:822-826.

Singh, S.N. 1980. Synergistic action of particulate and gaseous pollutants on the growth of Triticum aestivum L. J. Exp. Bot. 31:1701-1705.

Skelly, J.M., L.D. Moore, and L.L. Stone. 1972. Symptom expression of eastern white pine located near a source of oxide-nitrogen and sulfur dioxide. Plant Dis. Rep. 56:3-6.

Solberg, R.A., and R.F. Adams. 1956. Histological responses of some plant leaves to hydrogen fluoride and sulfur dioxide. Amer. J. Bot. 43-755-760.

Thompson, C.R., G. Kats, and R.W. Lennox. 1980. Effects of SO_2 and/or NO_2 on native plants of the Mohave Desert and eastern Mohave-Colorado Desert. J. Air Pollut. Contr. Assoc. 30:1304-1309.

Tingey, D.T., and R.A. Reinert. 1975. The effects of ozone and sulphur dioxide singly and in combination on plant growth. Environ. Pollut. 9:117-125.

Tingey, D.T., and G.E. Taylor, Jr. 1982. Variation in plant response to ozone: a conceptual model of physiological events. Pages 113-138. In: M.H. Unsworth and D.P. Ormrod (eds.). Effects of Gaseous Air Pollution in Agriculture and Horticulture. Butterworths, London.

Tingey, D.T., R.A. Reinert, J.A. Dunning, and W.W. Heck. 1971a. Vegetation injury from the interaction of nitrogen dioxide and sulfur dioxide. Phytopathology 61:1506-1511.

Tingey, D.T., W.W. Heck, and R.A. Reinert. 1971b. Effect of low concentrations of ozone and sulfur dioxide of foliage, growth and yield of radish. J. Amer. Soc. Hort. Sci. 96:369-371.

Tingey, D.T., R.A. Reinert, J.A. Dunning, and W.W. Heck. 1973a. Foliar injury responses of eleven plant species to ozone/sulfur dioxide mixtures. Atmos. Environ. 7:201-208.

Tingey, D.T., R.A. Reinert, C. Wickliff, and W.W. Heck. 1973b. Chronic ozone or sulfur dioxide exposures, or both, affect the early vegetative growth of soybean. Can. J. Plant Sci. 53:875-879.

Toivonen, P.M.A., and G. Hofstra. 1979. The interaction of copper and sulphur dioxide in plant injury. Can. J. Plant Sci. 59:475-479.

Troiano, J., L. Colavito, T. Heller, and D.C. McCune. 1981. Effect of simulated acid rain and photochemical oxidant on seed development in soybean. Phytopathology 71:565 (abst.).

Troiano, J., L. Colavito, T. Heller, and D.C. McCune. 1982. Viability, vigor, and maturity of seed harvested from two soybean cultivars exposed to simulated acidic rain and photochemical oxidants. Agric. Environ. 7:275-283.

Unsworth, M.H., and D.P. Ormrod. 1982. Effects of Gaseous Air Pollution in Agriculture and Horticulture. London/Boston: Butterworth Scientific. 532 pp.

Weber, D.E., R.A. Reinert, and K.R. Barker. 1979. Ozone and sulfur dioxide effects on reproduction and host-parasite relationships of nematodes. Phytopathology 69:624-628.

Wellburn, A.R. 1982. Effects of SO_2 and NO_2 on metabolic function. In: M.H. Unsworth and D.P. Ormord (eds.) Effects of Gaseous Air Pollution in Agriculture and Horticulture. 8:169-188. London/Boston: Butterworth Scientific. 532 pp.

Wellburn, A.R., T.M. Capron, H.S. Chan, and D.C. Horsman. 1976. Biochemical effects of atmospheric pollutants on plants. Pp. 105-114. In: T.A. Mansfield (ed.). Effects of Air Pollution on Plants. Cambridge University Press, Cambridge.

Wellburn, A.R., C. Higginson, D. Robinson, and C. Walmsley. 1981. Biochemical explanations of more than additive inhibitory effects of low atmospheric levels of sulfur dioxide plus nitrogen dioxide upon plants. New Phytologist 88:223-237.

White, K.L., A.C. Hill, and J.H. Bennett. 1974. Synergistic inhibition of apparent photosynthesis rate of alfalfa by combinations of sulfur dioxide and nitrogen dioxide. Environ. Sci. Technol. 8:574-576.

Whitmore, M.E., P.H. Freer-Smith, and T. Davis. 1982. Some effects of low concentrations of SO_2 and/or NO_2 on the growth of grasses and poplar. pp. 483-485. In: M.H. Unsworth and D.P. Ormrod (eds.) Effects of Gaseous Air Pollution in Agriculture and Horticulture. London/Boston: Butterworth Scientific. 532 pp.

Williams, R.J.H., and G.R. Ricks. 1975. Effects of combinations of atmospheric pollutants upon vegetation. In: M.J. Chadwick and G.T. Goodman (eds.) 15th Symposium of the British Ecological Society. London: Blackwells Science Publication. pp. 127-137.

Williams, R.J.H., M.M. Lloyd, and G.R. Ricks. 1971. Effects of atmospheric pollution on deciduous woodland. I. Some effects on leaves of Quercus petraea (Mattuschka). Leibl. Environ. Pollut. 2:57-68.

Yamazoe, F., and H. Mayumi. 1977. Vegetation injury from interaction of mixed air pollutants. Proc. Inter. Clean Air. Congr. Tokyo, Japan.

Zaragoza, L.J. 1982. The Use of Biological Information in the Development of National Ambient Air Quality Standards. Available through University Microfilms International. Ann Arbor, MI.

☆USGPO: 1984 — 759-102/0875