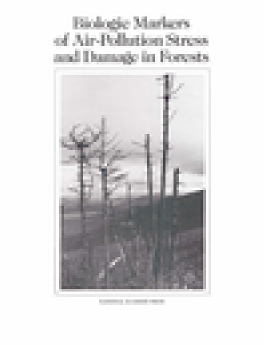
http://www.nap.edu/catalog/1414.html

We ship printed books within 1 business day; personal PDFs are available immediately.



Biologic Markers of Air-Pollution Stress and Damage in Forests

Committee on Biologic Markers of Air-Pollution Damage in Trees, National Research Council

ISBN: 0-309-55549-3, 380 pages, , (1989)

This PDF is available from the National Academies Press at: http://www.nap.edu/catalog/1414.html

Visit the National Academies Press online, the authoritative source for all books from the National Academy of Sciences, the National Academy of Engineering, the Institute of Medicine, and the National Research Council:

- Download hundreds of free books in PDF
- Read thousands of books online for free
- Explore our innovative research tools – try the "Research Dashboard" now!
- Sign up to be notified when new books are published
- Purchase printed books and selected PDF files

Thank you for downloading this PDF. If you have comments, questions or just want more information about the books published by the National Academies Press, you may contact our customer service department toll-free at 888-624-8373, visit us online, or send an email to feedback@nap.edu.

This book plus thousands more are available at http://www.nap.edu.

Copyright © National Academy of Sciences. All rights reserved.
Unless otherwise indicated, all materials in this PDF File are copyrighted by the National Academy of Sciences. Distribution, posting, or copying is strictly prohibited without written permission of the National Academies Press. Request reprint permission for this book.

THE NATIONAL ACADEMIES
Advisers to the Nation on Science, Engineering, and Medicine

Biologic Markers of Air-Pollution Stress and Damage in Forests

Committee on Biologic Markers of Air-Pollution Damage in Trees
Board on Environmental Studies and Toxicology
Commission on Life Sciences
National Research Council

NATIONAL ACADEMY PRESS
Washington, D.C. 1989

NATIONAL ACADEMY PRESS 2101 Constitution Avenue, NW Washington, DC 20418

NOTICE: The project that is the subject of this report was approved by the Governing Board of the National Research Council, whose members are drawn from the councils of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine. The members of the committee responsible for the report were chosen for their special competences and with regard for appropriate balance.

This report has been reviewed by a group other than the authors according to procedures approved by a Report Review Committee consisting of members of the National Academy of Sciences, the National Academy of Engineering, and the Institute of Medicine.

The National Academy of Sciences is a private, nonprofit, self-perpetuating society of distinguished scholars engaged in scientific and engineering research, dedicated to the furtherance of science and technology and to their use for the general welfare. Upon the authority of the charter granted to it by the Congress in 1863, the Academy has a mandate that requires it to advise the federal government of scientific and technical matters. Dr. Frank Press is president of the National Academy of Sciences.

The National Academy of Engineering was established in 1964, under the charter of the National Academy of Sciences, as a parallel organization of outstanding engineers. It is autonomous in its administration and in the selection of its members, sharing with the National Academy of Sciences the responsibility for advising the federal government. The National Academy of Engineering also sponsors engineering programs aimed at meeting national needs, encourages education and research, and recognizes the superior achievements of engineers. Dr. Robert M. White is president of the National Academy of Engineering.

The Institute of Medicine was established in 1970 by the National Academy of Sciences to secure the services of eminent members of appropriate professions in the examination of policy matters pertaining to the health of the public. The Institute acts under the responsibility given to the National Academy of Sciences by its congressional charter to be an adviser to the federal government and, upon its own initiative, to identify issues of medical care, research, and education. Dr. Samuel O. Thier is president of the Institute of Medicine.

The National Research Council was organized by the National Academy of Sciences in 1916 to associate the broad community of science and technology with the Academy's purposes of furthering knowledge and advising the federal government. Functioning in accordance with general policies determined by the Academy, the Council has become the principal operating agency of both the National Academy of Sciences and the National Academy of Engineering in providing services to the government, the public, and the scientific and engineering communities. The Council is administered jointly by both Academies and the Institute of Medicine. Dr. Frank Press and Dr. Robert M. White are chairman and vice chairman, respectively, of the National Research Council.

The project was supported by the Environmental Protection Agency through cooperative agreement No. CR-814248-01.

Library of Congress Number 89-62584
ISBN 0-309-04078-7

Printed in the United States of America

Cover photograph by Y. Yee, USDA Forest Service

Committee on Biologic Markers of Air-Pollution Damage in Trees

George M. Woodwell, *Chairman*, Woods Hole Research Center, Massachusetts
Edward R. Cook, Tree Ring Research Laboratory, Palisades, New York
Ellis B. Cowling, North Carolina State University, Raleigh
Arthur H. Johnson, University of Pennsylvania, Philadelphia
Thomas W. Kimmerer, University of Kentucky, Lexington
Pamela A. Matson, NASA/Ames Research Center, Moffitt Field, California
Samuel S. McLaughlin, Oak Ridge National Laboratory, Tennessee
Dudley J. Raynal, State University of New York, Syracuse
Wayne T. Swank, Coweeta Hydrologic Laboratory, Otto, North Carolina
Richard H. Waring, Oregon State University, Corvallis
William E. Winner, Oregon State University, Corvallis
James N. Woodman, North Carolina State University, Raleigh

Staff

David Policansky, Program Officer
Robert Smythe, Senior Staff Officer
Dave Johnston, Senior Staff Officer
Norman Grossblatt, Editor
Sylvia Tognetti, Research Assistant
Bernidean Williams, Information Specialist
Melanie Knight, Project Assistant
Leah Gales, Project Assistant
Sandi Fitzpatrick, Project Assistant

About this PDF file: This new digital representation of the original work has been recomposed from XML files created from the original paper book, not from the original typesetting files. Page breaks are true to the original; line lengths, word breaks, heading styles, and other typesetting-specific formatting, however, cannot be retained, and some typographic errors may have been accidentally inserted. Please use the print version of this publication as the authoritative version for attribution.

Board on Environmental Studies and Toxicology

Gilbert S. Omenn, *Chairman*, University of Washington, Seattle, Washington
Frederick R. Anderson, Washington School of Law, American University, Washington, D.C.
John Bailar, McGill University School of Medicine, Montreal, Quebec
David Bates, University of British Columbia Health Science Center Hospital, Vancouver, British Columbia
Joanna Burger, Nelson Laboratory, Rutgers University, Piscataway, New Jersey
Richard A. Conway, Union Carbide Corporation, South Charleston, West Virginia
William E. Cooper, Michigan State University, East Lansing, Michigan
Sheldon K. Friedlander, University of California, Los Angeles, California
Bernard Goldstein, UMDNJ-Robert Wood Johnson Medical School, Piscataway, New Jersey
Donald Mattison, National Center for Toxicological Research and University of Arkansas for Medical Sciences, Little Rock, Arkansas
Duncan T. Patten, Arizona State University, Tempe, Arizona
Emil Pfitzer, Hoffmann-La Roche, Inc., Nutley, New Jersey
William H. Rodgers, University of Washington, Seattle, Washington
F. Sherwood Rowland, University of California, Irvine, California
Liane B. Russell, Oak Ridge National Laboratory
Milton Russell, Oak Ridge National Laboratory
Ellen K. Silbergeld, Environmental Defense Fund, Washington, D.C.
I. Glenn Sipes, University of Arizona, Tuscon, Arizona
Bruce M. Alberts, *Ex Officio*, University of California, San Francisco

Staff

Devra L. Davis, Director
James J. Reisa, Associate Director
Karen L. Hulebak, Exposure Assessment and Risk Reduction Program Director
David J. Policansky, Natural Resources and Applied Ecology Program Director
Richard D. Thomas, Human Toxicology and Risk Assessment Program Director
Lee R. Paulson, Manager, Toxicology Information Center

Copyright © National Academy of Sciences. All rights reserved.

About this PDF file: This new digital representation of the original work has been recomposed from XML files created from the original paper book, not from the original typesetting files. Page breaks are true to the original; line lengths, word breaks, heading styles, and other typesetting-specific formatting, however, cannot be retained, and some typographic errors may have been accidentally inserted. Please use the print version of this publication as the authoritative version for attribution.

Commission on Life Sciences

Bruce M. Alberts, *Chairman*, University of California, San Francisco
Perry L. Adkisson, Texas A & M University, College Station
Francisco J. Ayala, University of California, Irvine
J. Michael Bishop, University of California Medical Center, San Francisco
Freeman J. Dyson, Princeton University, New Jersey
Nina V. Fedoroff, Carnegie Institution of Washington, Baltimore
Ralph W.F. Hardy, Cornell University, Ithaca
Richard J. Havel, University of California, San Francisco
Leroy E. Hood, California Institute of Technology, Pasadena
Donald F. Hornig, Harvard School of Public Health, Boston
Ernest G. Jaworski, Monsanto Company, St. Louis
Simon A. Levin, Cornell University, Ithaca
Harold A. Mooney, Stanford University, California
Steven P. Pakes, University of Texas, Dallas
Joseph E. Rall, National Institutes of Health, Bethesda
Richard D. Remington, University of Iowa
Paul G. Risser, University of New Mexico, Albuquerque
Richard B. Setlow, Brookhaven National Laboratory, Upton, New York
Torsten N. Wiesel, Rockefeller University, New York

Staff

John E. Burris, *Executive Director*

Copyright © National Academy of Sciences. All rights reserved.

PREFACE

There is not much question about the sensitivity of plants to air pollution, nor is there doubt that air pollution is affecting forests and agriculture in Europe, North America, Brazil, and elsewhere. The effects on forests are well known—they follow patterns of impoverishment long recognized as the result of chronic disturbance. Depending on the severity of the disturbance, the results are reduced growth of plants, morbidity of trees, shifts in species, and ultimately, replacement of forests by shrubland, grassland, or barren land that supports little or no vegetation, including agriculture. Once the process starts, it can be difficult to reverse. Nutrients are lost from plants and soils; nutrient ratios change; the character of the soils changes; and, as the vegetation changes, seed sources are lost as well.

Experience is rich, and the causes of damage are known; and so are the cures. But the cures seem expensive; they require specific action aimed at specific pollutants. The costs of cures are well focused and great, while the damage is diffuse and its increments seem small. The familiar cry is for better resolution of the relationships between cause and effect before investing in a cure that might be only marginally effective. Meanwhile, the damage accumulates and forests move inexorably down the scale of impoverishment.

The difficulties in addressing the effects of air pollution are made worse by the fact that effects of many types of disturbances are similar and that the responses of plants are responses to general stress and are not easily used to diagnose specific insults. Stress has many causes and collateral effects; such as diseases and pests that become important when plants are otherwise weakened. Sorting out cause and effect has frustrated pathologists and ecologists for years.

In a new effort to address this classical challenge that touches sensitive economic interests and equally sensitive nerves among scientists, the Environmental Protection Agency sought help from the National Research Council to review whether recent progress in science might be opening new doors that would lead to more specific diagnoses and narrow the issues. The Committee on Biologic Markers of Air-Pollutant Damage in Trees borrowed from experience in diagnosing human disease to search for specific criteria to diagnosis the effects of air pollution on trees and forests. The committee sought the aid of a group of distinguished scholars selected for their recent technical contributions to this difficult topic. This group met with the committee in

Little Switzerland, North Carolina, to explore the potential of new approaches. This book is their answer:. progress lags the need, but progress is accumulating. There will be, however, no simple diagnosis. Diagnosis will be a "most probable cause" derived from many lines of evidence, each gathered over time and used with other evidence as the basis of analysis. The potential for progress, however, is real, as shown in this book. It depends on intensified research, but as in medicine, refinements are available now, and others clearly are possible.

The completion of this report required not only the tireless efforts of the committee, but also the patience and persistence of a diverse group of other scholars who contributed to the symposium, prepared papers for the report, and responded to the flow of questions that emerged as the report progressed. Dr. David Policansky and Dr. Robert Smythe of the NRC staff had the awkward duty of reconciling often opposing views of earnest scholars unaccustomed to compromise with an equally adamant chairman. They carried the burden masterfully and graciously, aided throughout by the skill of Norman Grossblatt, editor for the Commission on Life Sciences. To all I offer thanks.

GEORGE M. WOODWELL, CHAIRMAN
COMMITTEE ON BIOLOGIC MARKERS OF AIR-POLLUTION DAMAGE IN FORESTS

CONTENTS

Part I: The Committee's Report

Part II: The Workshop Papers Introductory Session

Forest Applications of Biologic Markers: Regional Session

Tree-Stand/Ecosystem Session

Biochemical/Cell-Tissue Session

PART I

THE COMMITTEE'S REPORT

Executive Summary

Forests are subject to stress from natural causes and human activities. The natural causes include competition, predation, pathogens, extremes of weather, and climatic cycles. The human activities include physical disturbance and air pollution, including acids, oxidants, toxic organic compounds, and trace metals. Stresses often occur in combination, and effects of particular stresses on trees and forests are difficult to recognize.

Biologic markers (i.e., indicators) associated with specific air pollutant stress and damage in forests have been sought to improve discrimination among the many anthropogenic and natural causes of stress. To summarize the current state of knowledge and research and to identify and evaluate promising techniques that might lead to the development of new markers, the Environmental Protection Agency (EPA) asked the National Research Council (NRC) to arrange a workshop to bring together researchers in forest science and complementary disciplines. The Board on Environmental Studies and Toxicology of the NRC's Commission on Life Sciences appointed the Committee on Biologic Markers of Air-Pollution Damage in Trees, which organized a workshop held in Little Switzerland, North Carolina, in April 1988. The workshop brought together over 40 experts in forestry, ecology, plant pathology, physiology and biochemistry, and a broad range of other scientific and engineering disciplines. The group reviewed the current and potential uses of biologic markers at a variety of organizational levels, from the subcellular to the ecosystem level.

This publication is in two parts. Part I, the committee's report, provides a summary of the workshop, a review of criteria for the establishment of cause and effect in complex relationships, a discussion of the use of biologic markers to identify stress and damage in trees and forests, and the committee's conclusions and recommendations for further development and application of biologic markers. The workshop on which the committee based its report produced 32 papers by individual participants; those papers are included as Part II of this publication.

One task of the committee was to describe the status of work on markers of forest responses to air pollutants. A consensus emerged from the workshop that, although the currently available array of biologic markers is insufficient for resolving complex issues of forest decline, when used in combination these markers can facilitate evaluation of the mechanisms and consequences of environmental stress and damage from atmospheric pollutants. The use of biologic markers should be expanded as basic knowledge of forest ecology and physiology increases to allow more discriminating measurements of responses to natural and anthropogenic stresses. The committee's general conclusions follow.

CONCLUSIONS

1. No readily detectable, pollutant-specific single marker for identifying the effects of air pollution on forests or trees has been identified. Other stresses can produce symptoms in plants that mimic or conceal damage caused by air pollution.
2. Plants differ within and between species in their genetic capacities to absorb, assimilate, and respond to air pollutants; therefore, they exhibit different sensitivities to air pollutants via markers of pollution-caused damage. Dose-response relationships derived from air pollution exposure experiments can also vary within a single species in response to environmental conditions. Such variations add to the complexity of marker analysis.
3. Most current biologic markers of responses of trees to stress measure changes in plant canopies; additional markers are needed to measure effects on roots and shoots and to provide greater specificity in relating effects to causes of stress and damage.
4. A better understanding of spatial and temporal variations in natural processes that affect forests is needed to establish baselines against which to measure effects of pollutants.
5. Markers of forest-level effects can best identify sites of possible air-pollution damage when analyzed in conjunction with spatial and temporal patterns of air-pollution distribution.
6. The most useful analyses of the effects of air pollutants on forests combine surveys with controlled-exposure studies of potential cause-and-effect relationships.

A second task of the committee was to formulate recommendations on the basis of the workshop presentations and the committee's own deliberations for the further development and use of markers of tree and forest damage from air pollution. The committee believes that several current efforts to develop and apply markers of air-pollutant effects are promising and should be expanded. Those efforts should include the use of new technologies in an integrated analytic strategy, and they also should include the development of a protocol for interpreting the relative effects of various air pollutants on the function and composition of forest ecosystems. The committee's recommendations follow.

RECOMMENDATIONS

1. Increased emphasis should be placed on identifying suites of biologic markers for detecting forest responses to pollutants at various levels of biologic organization. The approach should include the application of statistical techniques that allow inferences to be stated in terms of probability.
2. Further development of markers of forest-level responses to air pollutants is especially needed. Examples of promising forest-level marker techniques include stream chemistry analysis and remote sensing from aircraft and satellites. Such techniques will be particularly useful if applied along known gradients of air-pollution intensity.

3. Because markers currently used to survey forest and tree responses to ambient concentrations of air pollutants cannot by themselves delineate cause-and-effect relationships, they must be used in concert with controlled-exposure techniques that involve monitoring and experimentation.
4. Ongoing regional surveys of forest responses to air pollution should be used to help identify specific areas for detailed studies of air-pollution effects.
5. Government and industry should continue supporting the development and use of biologic markers of air-pollutant stress and damage in forests; this work should be coordinated with continuing efforts to identify and model air-pollution distribution patterns.

Biologic Markers of Air-Pollution Stress and Damage in Forests

Introduction

The effects of air pollution on forests have long been the object of study and public concern. For example, smelters in Copper Hill, Tennessee, in Sudbury, Ontario, and in Palmerton, Pennsylvania (reviewed by Kozlowski, 1985) have devastated forests over large areas. In contrast, the effects of New York City's air pollution on the growth of corticolous lichen populations on Long Island (Brodo, 1966) have been subtle: the changes documented were so obscure that even an experienced botanist observing a segment of the gradient might overlook the changes and dismiss the differences as being well within the normal range of variation. Similarly, patchiness in the distribution, vigor, reproductive success, and other attributes of trees in forests often is accepted as normal. But the extent of normal variation is not well known, so understanding normal variability has taken on new importance with the accumulation of evidence of declines over the last 25 years in the vitality of many forests in the United States and in Europe (e.g., Johnson and Siccama, 1983; Andersson, 1984; Schütt and Cowling, 1985; McLaughlin et al., 1987; Sheffield and Cost, 1987; Woodman and Cowling, 1987; Pitelka and Raynal, 1989).

Even if forests are distant from sources of pollutants, ambient concentrations of airborne chemicals can be sufficient to produce visible injury, alter biochemical and physiologic processes that control metabolism and carbon allocation, reduce resistance to disease, reduce resistance to abiotic stress, and lead to the death of individual trees (Berry and Riperton, 1963; Linzon, 1966; Dochinger, 1968; Cobb and Stark, 1970; Miller and McBride, 1975; Cowling, 1985; Kozlowski, 1985; McBride et al., 1985; McLaughlin, 1985). However, the cause-and-effect relationships in such examples often are difficult to discern.

The pollutants most often suspected in cases of forest stress and damage are combinations of sulfuric acid and sulfur oxides, nitric acid and nitrogen oxides, and ozone. As research progresses, the list might grow to include an array of other organic and inorganic substances. The effects of a wide variety of stresses on trees and forests often are similar, and it remains to be seen whether a particular set of symptoms can be associated unequivocally with one pollutant, given that several pollutants are commonly present.

The NRC's Committee on Biologic Markers of Air-Pollution Damage in Trees was convened by the Board on Environmental Studies and Toxicology of the Commission on Life Sciences at the request of the EPA. This committee was asked to conduct a workshop to review, summarize, and evaluate current and potential uses of a broad range of biologic markers of stress and damage in trees and forests that might be caused by air pollutants. In a related effort, the NRC's Committee on Biologic Markers has been examining the use of markers in environmental (human) health research. That committee has defined biologic markers as "indicators of variation in cellular or biochemical components or processes, structure, or function that are measurable in biologic systems or samples" (NRC, 1989) and is considering biologic markers of exposure, effect, and susceptibility. A marker of effect is defined as "a measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease."

This committee has used similar definitions. However, to deal with forests and trees, it concentrated on biologic markers of effect and considered all measurable variations in structure or function to be potential markers. Stresses can often cause effects for which trees have compensatory mechanisms that prevent measurable damage or dysfunction; the term "effects," as used in this publication, is intended to include measurable compensatory responses as well as damage.

Using Markers in Combination

The application of biologic markers of air-pollution effects in forests reflects two related objectives: documenting that changes have occurred in natural systems exposed to high levels of stress and demonstrating under controlled conditions that a given pollutant dose can elicit a measurable response in some specific biologic or chemical process. The ultimate aim of developing markers is to integrate the two objectives to provide a set of markers that will point toward primary mechanisms and likely consequences of stress.

Biologic markers can be used in forests at several organizational levels, including watersheds, forest stands, individual trees, and metabolic processes. The workshop emphasized responses that indicate dysfunctions in single trees and at lower organizational levels, but useful indicators of ecosystem dysfunction were also reviewed. Such indicators include chemical attributes of soils and streams, hydrologic budgets (including evapotranspiration and water yield), and nutrient budgets (based on fluxes and pool sizes) that describe the net movement of water and nutrients through forest ecosystems. For example, despite the difficulties of documenting the specific process alterations and assigning them to specific causes, the integration of system function reflected in stream chemistry could turn out to provide a useful index of cumulative effects of some air pollutants on forest systems (see D.W. Johnson et al. in Part II).

Time—a fundamental variable—often is inadequately addressed. Biologic processes are strongly influenced by daily and seasonal cycles and by changes in surrounding plant communities that take place annually or over longer periods. The consideration of time is particularly important in selecting markers to evaluate forest responses to chronic low-level pollution, which can take months or years to be expressed. For example, a change in net photosynthesis might be a useful marker of short-term high-concentration exposure to pollutant gases, but be less useful than a change in carbohydrate storage reserves or loss of membrane integrity as a marker of chronic exposure. The permanent-plot system used by Forest Inventory and Assessment personnel of the U. S. Forest Service and tree-ring analysis are particularly useful for integrating long-term effects of pollutant stresses on trees and forests.

The Workshop

The committee's workshop was held on April 25-27, 1988, at Little Switzerland, North Carolina. A fundamental question discussed at the workshop was whether there are any unequivocal markers of the effects of air pollution on trees. Can changes in the structure, color, metabolism, or reproductive success of trees and associated plants or changes in whole forest stands or systems unequivocally indicate effects of specific air pollutants not caused by any other factors? Or are the effects of individual pollutants so confounded with the effects of other stresses that they are impossible to identify without extensive experimentation?

INTRODUCTORY PAPERS

Several papers at the workshop provided overviews of current programs for monitoring forest conditions and air-pollutant concentrations. Husar described a large-scale program for monitoring chemical air pollutants over North America and Europe. He provided, in effect, a distributional atlas of atmospheric pollutants, such as sulfur and nitrogen oxides, including their emissions, deposition, and concentrations. This atlas, which can be examined with microcomputers, is intended to reveal patterns of pollutant occurrence and of forest change. On a smaller scale, Mohnen examined elevational gradients of air pollutants based on data accumulated by the Mountain Cloud Chemistry Program (MCCP) in the eastern United States. At MCCP sites, chemical and physical measurement of selected acidic compounds and associated oxidants are being taken annually; the data will be useful for detecting altitudinal and latitudinal variations. Barnard reviewed the Forest Response Program of the National Vegetation Survey (NVS), a program begun in 1985 to determine the extent and magnitude of forest conditions in the United States. Several NVS projects, such as one on dendrochronology, give promise of a broad-scale monitoring program for the identification of visible symptoms in forest stands. In the southern United States, for example, a geographic information network will provide data on forest properties and on atmospheric deposition of pollutants. According to Cape, monitoring European forest conditions using routine visual assessments of leaf loss and discoloration has presented problems of interpretation. He suggested the use of alternative methods, such as comparison of visibly damaged versus undamaged trees, comparison of trees at different sites exposed to different pollution regimes, and controlled-exposure experiments intended to improve the validity of large-scale field surveys.

Tingey examined general applications and constraints in the use of biologic markers for air pollution research. Because markers usually are relatively nonspecific indicators of a problem, they must be used with caution in drawing conclusions about cause-and-effect relationships. Ideally, markers are easily measurable, are responses to specific pollutants, and produce distinctive symptoms that are not confused with those caused by other environmental stresses. Sharpe and Spence examined several new and emerging technologies for potential use in the detection of responses to stress, including short-lived radioisotopes, nuclear magnetic resonance, infrared reflectance, fiber optics, and semiconductors. Successful implementation of these tools would not be easy or inexpensive, but preliminary information suggests that these new and emerging methods would be remarkably cost-effective, compared with traditional experimental protocols, for detecting some types of stress.

REGIONAL APPLICATIONS OF BIOLOGIC MARKERS

The next group of papers covered the use of biologic markers at forest sites in different regions. A. H. Johnson reported on abiotic stresses and air pollution in the decline of northern Appalachian red spruce forests. Multiple stress factors in winter, coupled with pathogen and insect injuries, have been associated with declines in these forests. Air-pollution stress (e.g., related to ozone) might increase the effects of winter injury or affect energy balance as trees deplete resources in response to pathogens and insect attacks. Anderson reviewed surveys showing relationships between airborne pollutants and symptoms in several sensitive plants in southeastern U.S. forests: eastern white pine, milkweed, and lichens. The use of those plants as markers has provided information on extent of injury, type of associated pollutant, and temporal variations. However, limitations in their use were found to be related to mimicked symptoms and genetic, as well as climatic, variations. For western coniferous forests, Miller considered the relation of biologic markers at tissue and whole-tree levels to air-pollution exposure. An array of markers (foliage injury and changes in needle tissues and elemental contents of leaves) was found to be more effective than a single marker in suggesting the cause of observed effects. The evaluation of air-pollution effects is improved by measuring the concentration of suspected pollutants along gradients of decreasing pollutant deposition in combination with controlled exposures of seedlings or small trees in enclosures that compare ambient air with carbon-filtered air. More specific markers are needed to distinguish air-pollution effects from those of other abiotic stress factors. Schütt discussed some problems in understanding forest decline in Europe, such as the presence of multiple air pollutants and possible synergistic effects. The detection of reliable specific symptoms is complicated by the large number of tree species and wide-ranging variations in climate, soil, ecology, and altitude. The existence of all those variations and gaps in knowledge makes it difficult to determine whether different groups of scientists in different locations are working on similar or different sets of forest decline problems.

PHYSIOLOGIC, MORPHOLOGIC, AND ECOLOGIC MARKERS

The authors of this group of papers examined physiologic processes and structural features as potentially useful markers of air pollution in trees. Waring focused on resource allocation in various parts of a tree as an aid to interpreting signals of environmental change. For example, nutritional imbalances brought about by environmental changes can alter the ratios of essential minerals to one another in foliage and roots. Comparing changes in selected structural ratios (e.g., the leaf-to-bole ratio) might make it possible to distinguish specific causes of stress and to correlate them with changes in pollution loads on forests. D. W. Johnson, Van Miegroet, and Swank noted how air pollution might affect cycles of forest nutrients, particularly sulfates and nitrates in natural waters. Although nutrient pools might be affected by air pollution, their relatively large size makes them rather insensitive markers. Even so, well-documented long-term elemental budgets should be useful for determining changes in ecosystem nutrient pools caused by air pollution and other factors. Fry looked at the degree to which stable isotopes (in tree rings, lake sediments, and the atmosphere) can serve as historical tracers of anthropogenic pollutants. Large isotopic changes in sulfur were found in recent lake sediments in response to relatively large changes in sulfate loading. Historical elemental records were compared with records of regional and national emission patterns.

Cook and Innes described the use of tree-ring analysis to assess the impact of air pollutants on forests and pointed out that anomalous tree-ring behavior could be important in understanding pollution effects on forests. However, inferring a causal link between air pollution and forest declines from tree-ring analyses alone requires a better understanding of air-pollution effects on tree growth under natural conditions. Richards evaluated root growth and function after pollution-caused reduction in carbon allocation. Four techniques showed particular promise for assessing root-system damage caused by air pollution. As pointed out in several other papers given at the workshop, the use of multiple measurements will yield more reliable information from biologic markers than will attempts to relate air pollution to tree damage on the basis of a single measurement.

Rock, Vogelmann, and Defeo discussed the use of remote-sensing techniques for monitoring air-pollution effects in several forests. Remote sensing has shown changes in the health of portions of spruce-fir forests in the eastern United States that are too large to attribute to typical trends and natural variability. Some spectral signatures are characteristic of forest damage and can be used on a large scale to identify various specific symptoms of damage. Weinstein and Laurence evaluated the use of indigenous and cultivated plants as biologic indicators of air pollution. Carefully selected indicator species not only can identify some pollutants but also can provide estimates of pollutant distribution and a rough approximation of source strength. Of the many plant species that are sensitive to air pollution, a large number are low in cultivation cost, require little maintenance, and can be grown over wide geographic areas, including remote sites where the absence of electric power precludes many on-site measurements.

Scott and Hutchinson explored the suitability of using epiphytic lichens at high altitudes as early indicators of forest decline. Preliminary work indicated that growth abnormalities and metal accumulation can be demonstrated in some lichens growing on healthy conifers. Although epiphytic lichens appear to have potential as biologic markers of forest decline, their use as early indicators requires the identification of specific structural and chemical predictors of severe tree dieback. Little-investigated microbial markers were the subjects of papers by Marx and Shafer, who used fungal and bacterial symbiosis, and by Antibus and Linkens, who examined rhizosphere activities. In the absence of baseline data on and adequate techniques for assessing symbiosis in forests, it is doubtful whether microbial associations have potential as biologic markers of air-pollution effects in forests. Similarly, data are too sparse to suggest that any specific rhizosphere organisms or functions can serve as simple biologic markers of air-pollution effects. However, measurements of root and rhizosphere enzyme activities do offer a simple approach to the study of air-pollution effects on rhizosphere physiology.

BIOCHEMICAL, CELLULAR, AND TISSUE-LEVEL MARKERS

The final group of workshop papers focused on potential biologic markers at the biochemical, cell, and tissue levels. Norby concentrated on foliar nitrate reductase, an enzyme that responds to gaseous nitrogen pollutants, can be measured in the field, and thus might be a useful marker of the effects of nitrogen pollution on trees. Richardson, DiGiulio, and Tandy looked at the potential use of free-radical-mediated processes as early biologic markers of stress in trees. The authors established dose-response relationships between ozone and acid-rain exposures and growth, photosynthesis, and other biochemical activities in loblolly pines. Mechanisms of action and interspecific variations are poorly understood, but it appears that antioxidants can be used as early markers of oxidative stress in trees. Jones and Coleman also investigated the use of biochemical indicators to predict changes in secondary metabolites and nitrogen in leaves; their models, supported by preliminary data, indicate that two or more of these characteristics will help to identify air-pollution stress or damage in trees.

Shortle was concerned with changing concentrations of metals in various tree tissues. Some soil-related effects of acidic deposition might be linked to specific adverse plant conditions (e.g., suppression of cambial growth). The mechanism is believed to involve the release of aluminum cations into solution in acidic soils (pH 3-4.5) followed by the gradual replacement of essential metal cations, such as calcium and magnesium, with aluminum in the rooting zone of spruce-fir stands. Bondietti, Baes, and McLaughlin focused on changes in aluminum mobilization. The trend in Al:Ca cation ratios in a tree growing on a relatively undisturbed site is a sensitive marker of aluminum mobilization caused by atmospheric acid deposition. The use of correlations between Al:Ca ratios and radial growth rates is a promising approach to evaluating the linkage between aluminum mobilization (from atmospheric deposition) and growth-rate declines.

McLaughlin examined the allocation and partitioning of carbon among various physiologic processes as indicators of pollutant impacts on forest trees. The use of carbon allocation pathways to identify and diagnose responses of trees to air pollutants can include (1) identification of stress-induced shifts in patterns of carbon production, storage, and use; (2) quantification of changes in the amount, timing, and distribution of growth; and (3) evaluation of alterations in susceptibility to other stresses. A whole-tree perspective is recommended as the best approach for detection of changes in carbon allocation.

Winner dealt with the use of photosynthesis and transpiration changes as biologic indicators in forests. Although those processes are affected by pollutant exposures, nonpollution environmental factors, as well as seasonal variations, can produce similar effects or confounding problems. Luxmoore was concerned with nutrient-use efficiency (NUE) as a biologic indicator of air-pollution stress in trees. A review of reported experiments led Luxmoore to suggest that NUE can be an insensitive indicator of stress. He concluded that the use of NUE as an indicator of stress is impractical on a whole-plant basis and is not definitive on a tissue basis. The possibility of using changes in leaf cuticle structure as a marker of air pollution was discussed by Berg, who used field and laboratory studies to demonstrate pollution-caused changes in ultrastructure and physiologic properties.

Alscher, Cumming, and Fincher focused on ozone effects in red spruce during different seasons. Results from a dose-response experiment on red spruce seedlings suggest that ozone exposure during the summer and fall leads to changes in carbohydrate metabolism associated with winter hardening and to cell damage during the late fall and early winter. Heath examined the use of chlorophyll fluorescence as an early indicator of leaf stress; the loss of chlorophyll from plants has long been used to assess injury induced by air pollutants. Changes in chlorophyll fluorescence kinetics, although indicative of pollutant injury, might not be specific to air-pollutant injury. Therefore, several markers should be correlated before air pollutants alone can be identified as causing stresses observed in plants. Tyree's paper discussed drought as a factor that might interact with or be confused with stresses from atmospheric pollutants. After reviewing the relevant literature on drought and related stress mechanisms, Tyree pointed out forest decline symptoms caused by drought that could be confused with symptoms caused by air pollutants.

Establishing Cause-and-Effect Relationships

Where should researchers look to detect early stages of the effects of air pollution on forests and trees, and how might they address the classical problem of establishing relationships between causes and effects? The answers are complicated and somewhat unsatisfactory, but, in view of the importance of this issue for understanding and controlling air pollution in the United States and elsewhere, are worthy of review.

For biologic markers to be accepted as indicators of the effects of specific air pollutants, a relationship must be shown to exist between the pollutants (causes) and the responses (effects) measured by the markers. In approaching its task of evaluating the potential usefulness of various markers of air-pollution effects, the committee found it necessary to consider several fundamental criteria for the establishment of cause and effect in complex environmental relationships. This section reviews those criteria and suggests guidelines for their application to biologic markers of air-pollutant stress and damage in forests.

Air pollution affects forests and trees in many ways. Smith (1981) classified interactions between air pollutants and forests into three categories. When pollutant concentrations are low (Class I), forest vegetation and soil serve both as sinks and sources of pollutants. At intermediate concentrations (Class II), vegetation is subtly and adversely affected. Acute effects on forests, including morbidity and mortality, occur at high pollutant concentrations (Class III). Gradations between classes result in a continuum of effects on forests, occurring from the subcellular to the ecosystem levels.

Chronic stresses can induce a series of changes (including species impoverishment) that are as systematic as plant succession, although less well recognized. Of the many examples of the effects of chronic stress, some of the best defined were shown in experimental studies of the ecologic effects of ionizing radiation (McCormick, 1963; Woodwell, 1970; Fraley, 1971; Woodwell and Houghton, in press), in which the primary effect was well defined and measurable, whereas secondary effects, such as insect damage, were clearly recognized as secondary.

In forest stands affected by regional pollution, where effects of several stresses might be integrated, cause-and-effect relationships are not as clear as in the case of ionizing radiation. The challenge of relating specific causes to specific effects is complicated further by the similarity of patterns of forest changes caused by a wide variety of toxicants to changes caused by other stresses.

Experimental studies have been designed to investigate the effects of specific pollutants or combinations of pollutants on forests or trees and to test whether a particular pollutant might be a factor in damage observed in an existing stand or single tree. However, in a forested region subjected to a variety of air pollutants and natural stresses, the effects of each stress are not necessarily unique and identifiable. Furthermore, simple field studies of affected forest stands cannot yield clear information about the specific causes of damage—only the general conclusion that the observed pollutants appear to be associated with the observed damage. The damage might eventually be noticed as changes in the distribution of species, rather than immediately as specific physiologic symptoms in individual plants. If an affected species were an ephemeral or an occasional participant in the community, such changes would be particularly difficult to recognize and attribute to a cause.

Knowledge of the structure and physiology of forests and trees is now sufficient to develop a basis for detecting disruption or disturbance from a variety of causes. Although the task is complicated and efforts are incomplete, the committee's review suggested some answers to the questions posed during the workshop. The workshop papers confirmed that many biologic markers of stress are known for trees and forests, but that few (if any) such markers are unequivocal indicators of single causal agents. Because many natural and anthropogenic stress factors (summarized in Table 1) can combine to cause changes in the trends of forest metabolism, growth, and mortality (summarized in Table 2), it is difficult to link causes and effects with certainty. To help to clarify the links, the committee suggests a set of guidelines for inferring cause-and-effect relationships in cases of local and regional changes in the health and growth of trees and forests. As in much of science, the relationships will be expressed as probabilities based on current information and experience, not as certainties.

A related problem was addressed by bacteriologist Robert Koch in the nineteenth century, when the germ theory of disease was gaining wide acceptance (Koch, 1876). Koch pointed to the need for systematic and rigorous proof that a disease was caused by a particular organism.

To establish the cause-and-effect relationship, Koch advanced a set of postulates that now seem obvious. His postulates, summarized by Yerushalmy and Palmer (1959), were as follows:

1. The [causative] organism must be found in every case of the disease.
2. [The causative organism] must be isolated from patients and grown in pure culture.
3. When the pure culture is introduced into a susceptible subject, it must produce the disease.

These postulates were formulated to deal with bacterial diseases and are widely accepted in the biomedical sciences. They clearly are inadequate to deal with diseases that have several etiologic components or to assess cause-and-effect relationships between tree damage and environmental pollutants. But they can be used to focus thinking about important questions and can help to rule out particular pathogens as causes.

Hill (1965), Mosteller and Tukey (1977), Cochran (1983), and Holland (1986) have described various criteria for establishing cause-and-effect relationships. As Holland pointed out, it is necessary that, "for causal inference, each unit [e.g., each tree, each stand, etc.] be *potentially exposable* to any one of the causes."

This committee sought criteria that might help to define the most probable causes of stress symptoms seen in nature. On the basis of a few common principles, the criteria for establishing causality that appeared most useful for assessing pollutant effects on forests are the following:

- **Strong correlation.** Is there a consistent relationship between the measured effect and the suspected cause(s)? This criterion includes the concept of generality of association or **consistency** (Koch's first postulate) and the concept of **strength** of correlation. Mosteller and Tukey (1977) wrote about "a clear and consistent association."
- **Plausibility or mechanism.** Is there a biologic explanation of the mechanism of the observed association that is reasonable, coherent, or analogous to another case that is understood? Does it appear to contradict other, known mechanisms? Hill (1965) cautioned that "what is biologically plausible depends on the biological knowledge of the day."

Table 1. Environmental Stress Factors Known to Affect Forests and Trees

- Competition for resources:
- Growing space
- Nutrients
- Sunlight
- Water
- Weather and climate:
- Temperature extremes
- Drought
- High winds
- Low humidity
- High altitude with high ultraviolet radiation
- Heavy loads of ice or snow
- Wild fire
- Biologic agents:
- Fungi
- Insects
- Nematodes
- Bacteria
- Viruses and related forms
- Parasitic plants
- Predators
- Lack of essential symbionts
- Chemical factors:
- Deficiencies or imbalances in essential nutrients
- Toxic elements
- Allelopathic chemicals
- Herbicides
- Air pollutants:
  - Toxic gases
  - Toxic aerosols
  - Growth-altering chemicals
  - Acid deposition leading to direct injury
  - Atmospheric deposition of toxic particulates
- Human disturbances:
- Mechanical injury to individual trees
- Clearing of forests
- Burning of forests
- Physical disturbance of soil induced by:
  - Excessive drainage or flooding
  - Compaction
  - Erosion by wind or water
  - Removal or destruction of organic matter
  - Mechanical disturbance of soil

Table 2. Effects or Symptoms of Stress and Damage in Forests and Trees

Effects on Individual Trees
Visible symptoms of injury:
    Change in shape, size, color, and number of leaves
    Change in normal patterns of growth and development
    Change in normal patterns of foliage flushing or senescence
    Decreased annual height growth or radial increments
Alteration of physiologic processes:
    Photosynthesis
    Respiration and metabolism
    Transpiration
    Mineral nutrition
    Transport and allocation of photosynthate
    Hormonal control of growth
    Symbiotic relationships with other organisms
Changes in susceptibility to other stress factors
Life-history changes:
    Decreased longevity; early onset of dieback
    Changes in reproductive behavior
Effects on Forests
Decreased productivity of stands
Changes in age-class distribution
Changes in normal patterns of competition and mortality
Changes in normal patterns of community succession
Changes in species composition
Changes in nutrient cycling
Changes in hydrologic behavior, watershed functions, or wildlife habitat
Changes in genetic structure of populations

- **Responsiveness or experimental replication.** Can the effect be duplicated by experiment? Can the effect be stopped or prevented by removing the putative causal agent? Such a result is "strong causal evidence when you can find it" (Holland, 1986). This criterion represents Koch's third postulate and includes Hill's (1965) idea of **biologic gradient**—i.e., dose-response relationship.
- **Temporality.** A cause must precede its effect or at least be present at an appropriate time. Testing this criterion requires adequate histories of exposure and trends in a forest's growth and composition. It might be difficult to isolate subtle shifts in the genetic makeup of a tree population that increase the susceptibility of the population to other stresses.
- **Weight of evidence.** The individual components of establishing cause-and-effect relationships—correlation, plausibility, responsiveness, and temporality—do not by themselves provide sufficient evidence. However, studies that provide information on

> those categories contribute to the formation of a view that helps to put air-pollution stress in perspective with other stresses, provide a view of the current status of forests, and contribute to evidence that air pollution is affecting forests. For example, in 1986, an NRC committee reviewing acid deposition used the criteria of consistency, responsiveness, and mechanism to infer causal relationships among sulfur emissions, sulfate aerosol concentrations in the atmosphere, decrease in visibility, chemical makeup of rain, and acidification of surface waters in sensitive regions (NRC, 1986).

The more nearly these criteria are met, the stronger the inference becomes. The criteria suggested are reasonably simple and comprehensive, and they are similar to those often used by epidemiologists to separate causes from chance associations in studying human diseases. In the use of biologic markers, documentation of change often is a critical first step in determining that a problem might exist. A marker might be specific enough to serve as a reliable indicator of system dysfunction, but not specific enough to support the inference of a causal relationship by itself. In that case, it can be used with appropriate information to test for more specific relationships with potential causal agents. The distinctions between general, integrative biologic markers of stress and markers that might be more suitable for discriminating among potential causes are discussed in the following sections.

Using Markers in Surveys and Experimental Studies

Three types of studies are being used in forest air-pollution research, as described by the papers in Part II:

- Surveys of damage coupled with measurements of pollutants.
- Controlled-exposure tests to determine dose-response relationships.
- Experiments to determine mechanisms linking air pollution to specific effects.

No approach alone is sufficient to establish cause-and-effect relationships for instances of forest damage observed in the field. Results of the three types of studies can be used together for assessing cause-and-effect relationships in the analysis of regional-scale changes in forests.

SURVEYS OF STRESS AND DAMAGE

In addition to reporting the number and species of trees showing visible injury, decreased growth, or mortality, damage surveys or monitoring programs also provide information on spatial or temporal trends in forest-canopy variables. Properly designed surveys can be used to determine the strength of an association between suspected causal agents and degree of damage in forests, determine the degree of spatial and temporal consistency, and define biologic and environmental gradients. Causality is supported if, for example, specific airborne chemicals (or other stress factors) are present consistently when or shortly before a change in condition or a measure of dysfunction in forests occurs. If a spatial or temporal gradient in the amount of injury or damage is observed along a corresponding gradient in exposure, causation is further supported. Surveys are strictly correlative and can be misleading if the suspected causal factor is not well measured and placed in temporal perspective. The strength of the inference is decreased if—as is often true—the suspected cause is not specific to the observed effect. Because numerous natural and anthropogenic stresses occur together, it is rare for forest damage or dysfunction to correlate well with only one potential agent or cause.

Among the biologic markers reviewed in the workshop papers, the committee found the following to be especially appropriate for survey and monitoring work: **visible symptoms** (as discussed by Anderson, Barnard, Kape, Miller, and Schütt), **changes in leaf morphology and histology** (Miller and Schütt), **tree-ring changes** (Cook and Innes and Bondietti, et al.), **nutrient balances** (D.W. Johnson et al., Tingey, and Waring), **stream chemistry** (D.W. Johnson et al.), and **indicator plants**, including lichens (Weinstein and Laurence and Scott and Hutchinson). Some methods or techniques reviewed in the workshop appear to have consideral potential for use in survey and monitoring and deserve further research support: **remote sensing** of leaf morphology and biochemical changes (Rock et al.), **study of resource allocation** of carbon and other elements (Bondietti et al., Jones and Coleman, McLaughlin, Shortle, and Waring), and **use of some metabolic characteristics** (Norby, Richards, and Winner).

CONTROLLED-EXPOSURE STUDIES

Controlled-exposure tests range from simple comparisons of effects on otherwise healthy plants exposed to one suspected causal factor at a time to complex experiments in which plants are exposed to various combinations and amounts of suspected causal factors. Most controlled-exposure tests are performed with seedlings or small saplings of a single species in growth chambers, greenhouses, or open-top chambers. Experimental tests in which branches of mature trees are protected from exposure to pollutants or are exposed to known amounts of pollutants are becoming more common. The results of controlled-exposure tests are usually expressed as dose-response curves or as the minimal (threshold) exposure needed to induce symptoms of injury or dysfunction in the plant species under study.

To satisfy the criterion of responsiveness, a quantitative relationship must be found between degree of exposure and extent of injury or dysfunction. Dose-response curves can be difficult to interpret, because the response of a plant to various air pollutant exposures can be influenced by other biotic and abiotic environmental factors, including other kinds of stress. Controlled-exposure studies should be used to examine the interactions between air pollutants and other stresses. The more differences there are between the controlled environment and the field situation of interest, the more difficult it is to extrapolate from the experiment to the field.

Most of the biologic markers mentioned above as appropriate for survey and monitoring studies should also provide valuable information if used in controlled-exposure studies; the results of the latter studies should improve understanding of the linkage between the effects measured by such markers and their causes. Additional markers reviewed in the workshop papers that the committee considered to be particularly appropriate for use in controlled-exposure studies are: **lichens and other plant indicators** (discussed by Scott and Hutchinson and Weinstein and Laurence) and certain **secondary metabolites** (Jones and Coleman). Other markers discussed during the workshop that showed promise for use in research to determine cause-and-effect relationships include **aluminum mobilization** (Bondietti et al. and Shortle) and **leaf pigments** (Heath and Richardson et al.).

EXPERIMENTS TO DETERMINE MECHANISM

In establishing cause and effect, it is important to determine whether an observed change or dysfunction resulted from one or more suspected causal factors through a specific physical or biologic process. Experiments are most valuable if the mechanisms can be demonstrated both in field studies and in carefully controlled experiments. Demonstration of a physical or biologic mechanism of action is an important criterion of causality, as discussed above. Experiments to determine mechanisms go beyond the measurement of given characteristics of markers to the determination of the specific processes by which pollutants or other stress factors might be linked to the effects measured by those markers. As is discussed below, studies of the physiology, pathology, and biochemistry of individual trees, tissues, and cells are more likely to be useful in elucidating mechanisms of action than are studies of changes in natural systems at higher organizational levels. Nevertheless, coordinated measurements of higher-level effects are needed to determine the ultimate scope of expression and impact of the mechanisms in question.

DEVELOPING A DIAGNOSTIC APPROACH

Anyone attempting to use biologic markers in forest ecosystems should recognize that the current array of atmospheric pollutants includes agents that might affect forests at many physiologic and biogeochemical loci. It is therefore essential to select an array, or suite, of markers of effects on several metabolic pathways and structural features to aid in the cause-and-effect analysis described above. In developing a diagnostic approach, it is important to test various hypotheses that are formulated to explain the changes observed. If the hypotheses are organized around known or suspected changes in uptake and use of carbon, water, and nutrients, the analysis of forest responses becomes relevant to a broader range of natural and anthropogenic stresses that are known to affect resource availability.

Critical points and mechanisms that determine pollutant effects on forest systems often are biochemical. Therefore, markers related to changes in growth and structure alone are insufficient; they must be used in combination with biochemical markers related to metabolic processes that reflect responses to stress, such as compensation and dysfunction. The next section presents a framework for the integrated application of biologic markers to analyze the effects of air pollutants and other stresses in forest systems.

A Strategy for Using Biologic Markers of Stress in Forests

A strategy for the effective use of markers of forest damage should involve classifying markers into functional groups and then using the groups in a systematic, diagnostic way. One approach to the integration and interpretation of biologic markers of forest responses to air pollutants is presented below. Other approaches are needed and can be developed.

Foresters and resource managers need biologic markers that are specific for evaluating changes in forest health due to air pollutants. Air pollution is one of the few environmental features that, if stressful, could be corrected by human intervention. Thus, simply using biologic markers to locate unhealthy forests is not particularly useful, whereas locating and diagnosing forests damaged by air pollution could provide the basis for constructive regulatory and management responses.

Suites of markers can be developed to help foresters and resource managers to detect effects specific to air pollutants. The committee suggests classifying markers into the categories described below and using them in a sequential diagnostic pattern to identify forest regions suffering from air-pollution stress. The committee then suggests an outline for the application of a suite of markers to detect stresses in forest systems.

1. **Biologic Markers of Response to Environmental Change.** The responses of plants to environmental change are continuous and are evident from the use of an array of biologic markers (see Table 3). An example of biologic markers of environmental change at the ecosystem level is a dramatic shift in nutrient leakage; this can sometimes be determined by measurements of stream chemistry. Biologic markers of environmental change at the tree level might include sudden changes in tree-ring size or abnormal rates of photosynthesis and other metabolic processes.
2. **Biologic Markers of Compensation to Stress.** A useful way to evaluate the importance of environmental stresses for trees is to determine the degree to which the trees have developed compensatory responses to them. Compensation maximizes productivity and the likelihood of survival. It also has potential diagnostic value; compensation mechanisms can be specific to particular environmental stresses. Table 4 lists some potentially useful markers of compensation that are discussed in the workshop papers.

   Compensation by plants in response to environmental stresses can take place at the biochemical, physiologic, and ecosystem levels. For example, trees can compensate for drought by closing stomata to conserve water and by shifting resources to foster greater root growth to enhance acquisition of water from soils. Those forms of compensation differ in important ways from simpler responses of trees to drought; they specifically enhance the acquisition of resources that are most limiting. The growth, survival, and perhaps fitness of compensating plants are depressed, compared with those of unstressed plants, but greater than those of stressed plants that did not compensate.

   If distinct compensation responses of trees to various air pollutants can be described, and biologic markers of them defined, it should be possible to identify trees that have compensated in response to specific air-pollution stresses.
3. **Biologic Markers of Toxicity.** Toxic effects of air pollutants occur in plants when absorption of toxic chemicals exceeds the capacity to compensate. In such cases, plants are unable to maintain themselves in a healthy state. There are important biologic markers of such events (see Table 5). Air pollutants, like other types of stresses, can damage or even kill individual cells. Pollutants can also damage membranes, rendering them less able to select against toxic substances and allowing concentrations of heavy metals or other pollutants to increase in tissues. Cellular damage can become so widespread that the tree is compromised and dead or dying cells become visible. Such visible injury is known to be associated with gaseous pollutants such as ozone and sulfur and nitrogen oxides. Visible injury typical of these pollutants includes foliar chlorosis, necrosis, stippling, and needle banding. Those visible symptoms can be specific for key pollutants, but some nonpollution stresses can induce similar symptoms.

The loss of leaf area due to air-pollution toxicity can occur via mechanisms other than cellular death. For example, air pollutants are known to cause premature senescence and casting of deciduous and evergreen foliage. That process can give the canopy of affected trees the appearance of being transparent—a symptom widely used in air-pollution damage surveys.

Direct absorption of gaseous pollutants—such as ozone and sulfur dioxide—might eventually overcome the repair capacity of a plant and initiate injury that not only kills individual cells, but also induces production of plant resins and phenols that fill dead cells or spread and wall off surrounding tissue. Damage can also result in formation of visibly thickened cells.

Table 3. Some important biologic markers of environmental change that can be determined by assessing trees, as presented in Part II of this publication.

MARKER	WORKSHOP PAPER
Tree—Stand level:	
Nutrient cycling	Johnson et al.
Stable isotopes of carbon, nitrogen, and sulfur	Fry
Tree ring analysis	Cook and Innes; Johnson
Canopy spectral analysis	Rock et al.
Shifts in phenology	Rock et al., Barnard; Schutt; Anderson, Miller; Cape
Root growth	Richards
Symbiotic rhizosphere fungi	Marx and Shafer
Symbiotic rhizosphere bacteria	Antibus and Linkens
Biochemical—Tissue level:	
Foliar nitrate reductase	Norby
Free-radical processes	Richardson et al.
Photosynthesis and transpiration	Winner
Nutrient-use efficiency	Luxmoore
Carbon partitioning	McLaughlin
Cuticular competence	Berg
Secondary metabolites	Jones and Coleman
Chlorophyll content	Heath

Table 4. Some important biologic markers of compensation to air-pollution stress, as presented in Part II of this publication.

MARKER	WORKSHOP PAPER
Tree-Stand Level:	
Allocation of internal resources	Waring
Leaf retention and crown density	Miller; Cape; Schutt; Barnard; Anderson
Bud damage	Johnson
Biochemical-Tissue Level:	
Carbon partitioning	McLaughlin

Table 5. Some important biologic markers of air-pollution-caused toxicity and absorption, as presented in Part II of this publication.

MARKER	WORKSHOP PAPER
Tree-Stand Level:	
Indigenous and cultivated plants	Weinstein and Laurence
Epiphytic cryptogams	Scott and Hutchinson
Foliar damage	Miller
Biochemical-Tissue Level:	
Loss of membrane integrity and selectivity	Alscher
Pollutant content in tissues	Shortle; Bondietti et al.
Foliage histology	Rock; Miller
Phloem damage	Sharpe and Spence

Foliar discoloration, canopy thinning, and tissue isolation can be caused by air pollution as well as other stresses. Consequently, analysis using biologic markers of air-pollution absorption can help to link putative air-pollution toxicity to a specific chemical agent. Such markers might include the presence of sulfur compounds in leaves exposed to sulfur dioxide and abnormal concentrations of heavy metals in plant tissues.

The three types of biologic markers discussed above—of response to environmental change, of compensation to stress, and of toxicity—can be used in a systematic, phased manner to elucidate air-pollution-caused changes in forest health. For any given research location, the process requires evaluation of air-quality data and assembly of an appropriate suite of biologic markers that is based on an understanding of air-pollution deposition and site-specific biologic processes. The process should not only reveal the nature of air-pollution-caused changes in forest health, but also identify other key stress factors that influence trees and forests.

To identify changes in forest health caused by air pollution, biologic markers in the three categories described can be evaluated in a systematic fashion. If the biologic markers of response to environmental change suggest a stable environment, air-pollution effects have probably not occurred recently. If, however, they indicate significant shifts in environmental factors, recent changes in air quality could be important. Analysis of air-quality data can be useful in showing whether air-pollution deposition at the site has taken place over a long period or has changed sharply recently.

Once analysis of biologic markers of response to environmental change and air-quality data suggest that air pollutants are important, markers of compensation and acute toxicity that are specific for air pollution can be evaluated. Because compensation for air-pollutant damage takes time, detection of compensation with biologic markers implies that air pollutants with chronic impacts are present. Such impacts might include a reduction in capacity to compensate for nonpollution stresses; the result might be decreases in growth, productivity, and reproduction. Detection of compensation markers also justifies analysis with markers of pollution toxicity and absorption. In this case, foliar injury and other such indicators of pollution damage can be interpreted with more certainty.

The sequence of analysis can be reversed. If biologic markers of pollution toxicity and absorption at a site become apparent, air quality can be evaluated to determine whether ozone, sulfur dioxide, acid deposition, or other pollutants are likely causes of observed symptoms. If so, analysis of markers of compensation and environmental change can help to put air-pollution stress in perspective with other stresses.

Because the nature of compensation and toxicity responses to each air pollutant can differ, monitoring pollution concentration and deposition is essential for interpreting the effects of pollutants on forest health. Without monitoring, interpretation of information from any set of biologic markers is unreliable.

Use of biologic markers will never completely answer questions of cause and effect, nor will markers provide all the information needed by foresters, resource managers, and regulators about the role of air pollutants in forests. That requires supplementing the use of markers with studies of air-pollution response mechanisms of trees and an analysis of ecosystem processes. Only the combination of those approaches can yield all the information necessary to manage air quality and forest resources.

Conclusions and Recommendations

Used individually, the available biologic markers are insufficient for resolving complex issues of forest decline, but used in selected combinations, they can help to simplify the evaluation of the mechanisms and consequences of environmental stress due to atmospheric pollutants. Markers currently in use constitute a starting point for diagnosis that should be expanded as knowledge of forest ecology, physiology, and responses to natural and anthropogenic stresses increase.

One task of the committee was to describe the status of research on markers of forest responses to air pollutants. The committee reviewed the workshop presentations and papers and drew general conclusions intended to synthesize and summarize applications of biologic markers to trees and forests.

CONCLUSIONS

1. No readily detectable, pollutant-specific single marker for identifying the effects of air pollution on forests or trees has been identified. Other stresses can produce symptoms in plants that mimic or conceal damage caused by air pollution.

Current markers of forest change typically fail to distinguish the effects of different pollutants. Examples of nonpollution stresses are those caused by drought, insufficiency of nitrogen, pathogens, and insects. In addition, a single air pollutant can cause an array of symptoms in plants. For example, ozone is known to cause chlorosis, stippling, bronzing, flecking, and blackening of broadleaf plants and tip burn, mottling, and banding on conifer needles, as well as reductions in growth and changes in susceptibility to other stresses.

2. Plants differ within and between species in their genetic capacities to absorb, assimilate, and respond to air pollutants; therefore, they exhibit different sensitivities to air pollutants via markers of pollution-caused damage. Dose-response relationships derived from air-pollution exposure experiments can also vary within a single species in response to environmental conditions. Such variations add to the complexity of marker analysis.

Differences among species in sensitivity to air pollution can be exploited by focusing analysis on the most sensitive species. For example, lichens are known to be sensitive to sulfur dioxide. Milkweed, some strains of white pine, and tobacco foliage are known to be sensitive to ozone. A given ambient air-pollution concentration can have different effects on a single species, because of variations in environment, genotype, symbionts and plant age, if such variations alter absorption or assimilation of air pollutants. Environmental factors that increase stomatal conductance are likely to enhance uptake of gaseous pollutants and therefore increase their effect. Genotypic differences between populations that confer contrasting physiologic adaptations to environmental stresses can result in a physiologic basis for population differences in air-pollution sensitivity. Such genetically based variation is well known in white pine, loblolly pine, and poplar. In addition, symbiotic microorganisms growing in the rhizosphere and on foliage, as well as macroscopic symbionts and changes in age, can alter the functioning of stomata and the metabolic capacity of an entire tree. Such changes are likely to alter sensitivity of a plant to air pollution. Thus, a single air-pollution concentration might or might not elicit a recognizable response.

3. Most current biologic markers of responses of trees to stress measure changes in plant canopies; additional markers are needed to measure effects on roots and shoots and to provide greater specificity in relating effects to causes of stress and damage.

Most current markers of forest responses to air pollutants measure particular characteristics of plant canopies. These markers cannot be used to characterize pollution-caused changes in the growth of roots, the growth of whole trees, forest productivity, or other forest characteristics. Further research is needed to develop markers of such changes, particularly at the forest-stand and ecosystem levels.

4. A better understanding of spatial and temporal variations in natural processes that affect forests is needed to establish baselines against which to measure effects of pollutants.

Much of the ambiguity associated with interpreting data from biologic markers of stress in forests is due to the potential for interactions between natural and anthropogenic changes in the forest environment. More detailed and precise knowledge of forest responses to drought, low temperatures, and long-term environmental variables is needed to help researchers separate responses to these variables from responses to air pollutants.

5. Markers of forest-level effects can best identify sites of possible air-pollution damage when analyzed in conjunction with spatial and temporal patterns of air-pollution distribution.

Markers of change in forests—such as visible symptoms, gaseous emissions, shifts in nutrient allocation, and other general markers of stress—can be effectively used when they are related to patterns of air-pollution distribution. Sites of greatest interest are those where forest status either is beginning to change or is changing rapidly and where air pollutants are present at concentrations known to affect plants. Rates of change in forest status and correlations with air-pollution concentrations can be assessed only through repeated surveys and analyses.

6. The most useful analyses of the effects of air pollutants on forests combine surveys with controlled-exposure studies of potential cause-and-effect relationships.

Controlled experimental exposures of forest species to air pollution are necessary to confirm a conclusion that forest changes observed in survey studies are due to ambient exposures. Such experiments can be done on potted plants in greenhouses and other laboratory facilities, or in the field with chambers for seedlings or branches, or in both places simultaneously. Without such experiments, it is difficult to link observed changes in trees, stands, or forests with air pollutants in a cause-and-effect relationship.

RECOMMENDATIONS

A second task of the committee was to formulate recommendations—on the basis of the workshop presentations and its own conclusions—regarding the use of markers of forest responses to air pollutants. The committee believes that several current efforts to develop and apply markers of air-pollutant effects are promising and should be expanded. The efforts should include the use of newly available technologies in an integrated analytical strategy, along with the development of a protocol for interpreting the relative effects of various air pollutants on the functions and composition of forest ecosystems.

The committee's review yielded recommendations for several other activities that should advance the identification and measurement of specific types of air-pollutant damage to trees and forests. The committee believes that such advancement will continue to present challenges, but that the potential results justify the effort.

1. **Increased emphasis should be placed on identifying suites of biologic markers for detecting forest responses to pollutants at various levels of biological organization. This approach should include the application of statistical techniques that allow inferences to be stated in terms of probability.**
2. **Further development of markers of forest-level responses to air pollutants is especially needed. Examples of promising forest-level marker techniques include stream chemistry analysis and remote sensing from aircraft and satellites. Such techniques will be particularly useful if developed along known gradients of air-pollution intensity.**
3. **Because markers currently used to survey forest and tree responses to ambient concentrations of air pollutants cannot by themselves delineate cause-and-effect relationships, they must be used in concert with controlled-exposure techniques that involve monitoring and experimentation.**
4. **Ongoing regional surveys of forest responses to air pollution should be used to help identify specific areas for detailed studies of air-pollution effects.**
5. **Government and industry should continue supporting the development and use of biologic markers of air-pollutant stress and damage in forests; this work should be coordinated with continuing efforts to identify and model air-pollution distribution patterns.**

REFERENCES

Andersson, F. 1984. Forest Ecosystem Research: Past and Present Trends. In State and Change of Forest Ecosystems: Indicators in Current Research, G. I. Agren, ed. Uppsala: Swedish University of Agricultural Research, Department of Ecology Environmental Research.

Berry, C. R., and L. A. Riperton. 1963. Ozone: A possible cause of white pine evergreen tipburn. Phytopathology 53:552-557.

Brodo, I. M. 1966. Lichen growth and cities: A study on Long Island, New York. Bryologist 69:427-449.

Cobb, F. W., and R. W. Stark. 1970. Decline and mortality of smog-injured ponderosa pine. J. For. 68:147-149.

Cochran, W. G. 1983. Planning and Analysis of Observational Studies. New York: John Wiley & Sons.

Cowling, E. B. 1985. Critical review discussion papers: Effects of air pollution on forests. J. Air Pollut. Control Assoc. 35:916-919.

Dochinger, L. S. 1968. The impact of air pollution on eastern white pine: The chlorotic dwarf disease. J. Air Pollut. Control Assoc. 18:814-816.

Fraley, L., Jr. 1971. The Response of Shortgrass Plains Vegetation to Chronic and Seasonally Administered Gamma Radiation. Ph.D. Dissertation, Colorado State University, Fort Collins.

Hill, A. B. 1965. The environment and disease: Association or causality. Proc. R. Soc. Med. 58:295-300.

Holland, P. W. 1986. Statistics and causal inference. J. Am. Stat. Assoc. 81:945-960.

Johnson, A. H., and T. G. Siccama. 1983. Acid deposition and forest decline. Environ. Sci. Technol. 17:294a-305a.

Koch, R. 1876. Die aetiologie der Milzbrand-Krankheit, begundet auf die Entwick-lungschiachte des Baiollus Antracis. Beitr. Biol. Pflanz. 2:277.

Kozlowski, T. T. 1985. Sulfur dioxide effects on plant community structure. Pp. 431-453 in Sulfur Dioxide and Vegetation, W. E. Winner, R. A. Mooney, and R. A. Goldstein, eds. Stanford, Calif.: Stanford University Press.

Linzon, S. N. 1966. Damage to eastern white pine by sulfur dioxide, semimature-tissue needle blight and ozone. J. Air Pollut. Control Assoc. 16:140-144.

McBride, J. R., P. R. Miller, and R. B. Laven. 1985. Effects of oxidant air pollutants on forest succession in the mixed conifer forest type of Southern California. In Proceedings of Air Pollutants Effects on Forest Ecosystems. St. Paul, Minn.: Acid Rain Foundation.

McCormick, F. 1963. Changes in a herbaceous plant community during a three-year period following exposure to ionizing radiation gradients. In Radioecology: Proceedings of the National Symposium on Radioecology held September 10-15, 1961, Colorado State University, V. Schultz and A. W. Klement, Jr., eds. New York: Reinhold.

McLaughlin, S. B. 1985. Effects of air pollution on forests: A critical review. J. Air Pollut. Control Assoc. 35:512-534.

McLaughlin, S. B., D. J. Downing, T. J. Blasing, E. R. Cook, and H. S. Adams. 1987. An analysis of climate and competition as contributors to the decline of red spruce in the high elevation Appalachian forests of the eastern United States. Oecologia 72:487-501.

Miller, P. R., and J. R. McBride. 1975. Effects of air pollutants on forests. Pp. 196-236 in Responses of Plants to Air Pollutants, J. B. Mudd and T. T. Kozlowski, eds. New York: Academic Press.

Mosteller, F., and J. W. Tukey. 1977. Data Analysis and Regression: A Second Course in Statistics. Reading, Mass.: Addison-Wesley.

NRC (National Research Council). 1986. Acid Deposition: Long-Term Trends. Washington, D.C.: National Academy Press.

NRC (National Research Council). 1989. Biologic Markers in Pulmonary Toxicology. Washington, D.C.: National Academy Press.

Pitelka, L. F., and D. J. Raynal. 1989. Forest decline and acidic deposition. Ecology 70:2-10.

Schütt, P., and E. B. Cowling. 1985. Waldsterben, a general decline of forests in central Europe: Symptoms, development, and possible causes. Plant Dis. 69:548-558.

Sheffield, R. M., and N. D. Cost. 1987. Behind the decline. J. For. 85:29-33.

Smith, W. H. 1981. Air Pollution and Forests. New York: Springer-Verlag.

Woodman, J. N., and E. B. Cowling. 1987. Airborne chemicals and forest health. Environ. Sci. Technol. 21:120-126.

Woodwell, G. M. 1970. Effects of pollution on the structure and physiology of ecosystems. Science 168:429-433.

Woodwell, G. M., and R. A. Houghton. In press. The experimental impoverishment of natural communities: Effects of ionizing radiation on plant communities. In Patterns and Processes of Biotic Impoverishment, G. M. Woodwell, ed. New York: Cambridge University Press.

Yerushalmy, J., and C. E. Palmer. 1959. On the methodology of investigation of etiologic factors in chronic diseases. J. Chronic Dis. 10:27-40.

PART II

THE WORKSHOP PAPERS INTRODUCTORY SESSION

Air Pollutant Distribution and Trends

Rudolf B. Husar
Center for Air Pollution Impact and Trend Analysis (CAPITA), Washington University, Box 1124, St. Louis, MO 63130

ABSTRACT
Forest health is influenced by the soil, physical climate, and its chemical climate. Historically, among the environmental factors, the role of chemical climate has received the least attention. This paper constitutes an annotated chemical atlas of the atmosphere over North America and to a lesser extent Europe. It covers the emissions for sulfur and nitrogen oxides, wet deposition of sulfate, nitrate, and ammonia, airborne concentrations of ozone and sulfate, and the spatial temporal pattern of atmospheric haziness.

INTRODUCTION

Forest growth is determined by the environmental conditions that include soil and physical and chemical climate of the atmosphere. The role of soil and physical climate has been dealt with extensively in the literature for the past several centuries. Much less is known about the long-term effect of atmospheric chemicals, i.e., chemical climate of the forests. Recognizing the severe uncertainties as to what specific atmospheric chemicals may be responsible for potential forest damage, it seems beneficial to assemble an annotated atlas of atmospheric chemicals that may be relevant to forests. An examination of such a chemical-climatic atlas and trends in conjunction with physical climate and observed forest change may reveal the chemical that could be significant to the damage.

MAN-MADE SOX AND NOX EMISSION PATTERN AND TRENDS

The North American emission densities for SO_x and NO_x for 1977-78 are displayed in Fig. 1. The contour lines have units of grams of sulfur or nitrogen per square meter per year. Currently, the sulfur emissions in the subcontinent are estimated to range between 11 and 15 million tons/year (22-30 million tons SO_2/year). It is evident that the highest sulfur emission density occurs over the Ohio River region. The highest NO_x emission density is over the states north of the Ohio and east of the Mississippi rivers.

There are substantial differences in the emission trends of smaller regions within eastern North America. Comparisons of the sulfur emissions north and south of Ohio River are given in Fig. 2, expressed as emission per unit area (g $S/m^2/yr$). The emissions north of the Ohio River have increased about 25% since the 1920s. In contrast, the emissions south of the Ohio River show a threefold increase since the 1930s. Currently, the sulfur emissions density is comparable for the regions north and south of the Ohio River.

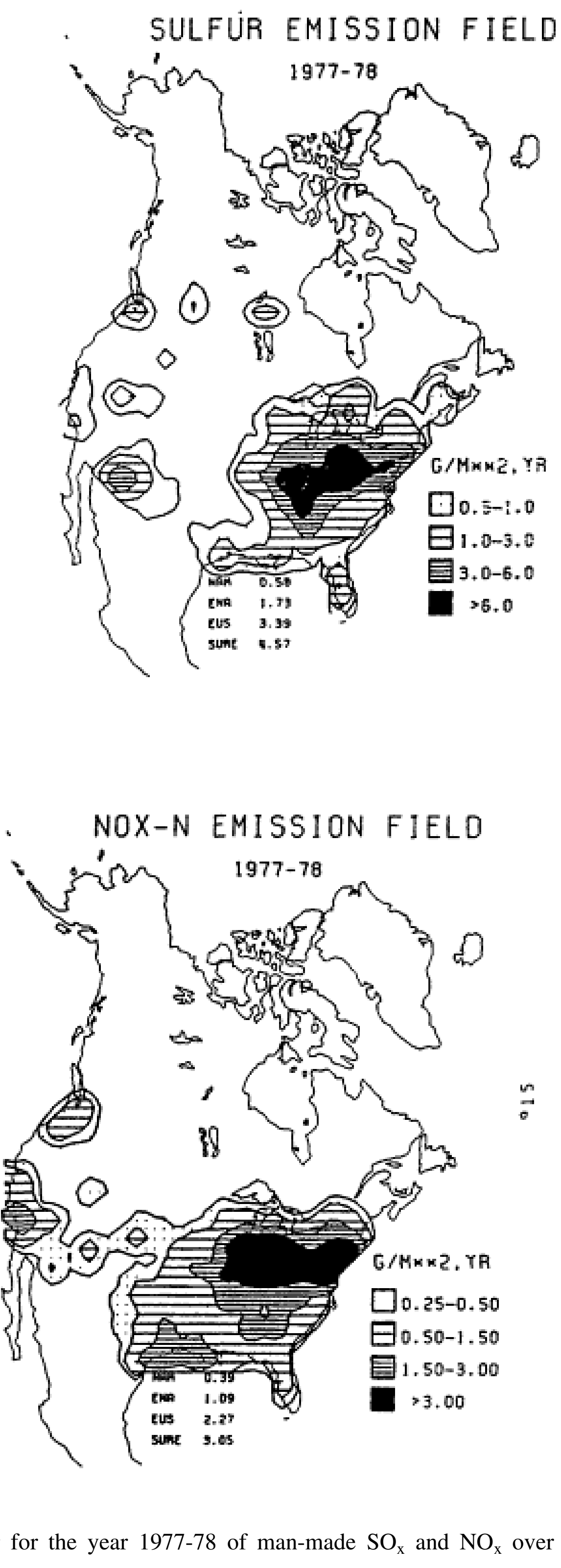


Fig. 1a, 1b.

Emission densities, g/m^2/yr for the year 1977-78 of man-made SO_x and NO_x over North America (Husar and Holloway, 1983).

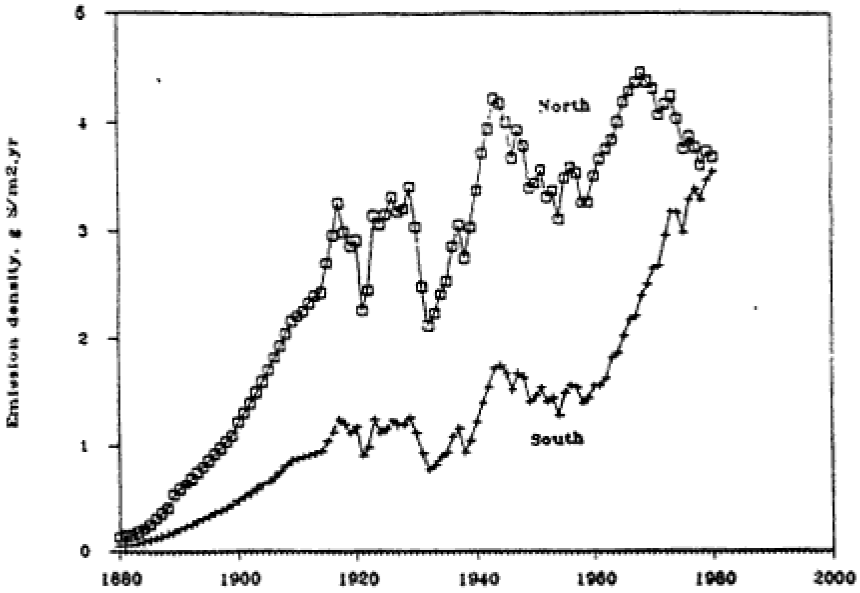


Fig. 2.
Sulfur emission density, g/m^2/yr for regions north and south of the Ohio River (Husar, 1985).

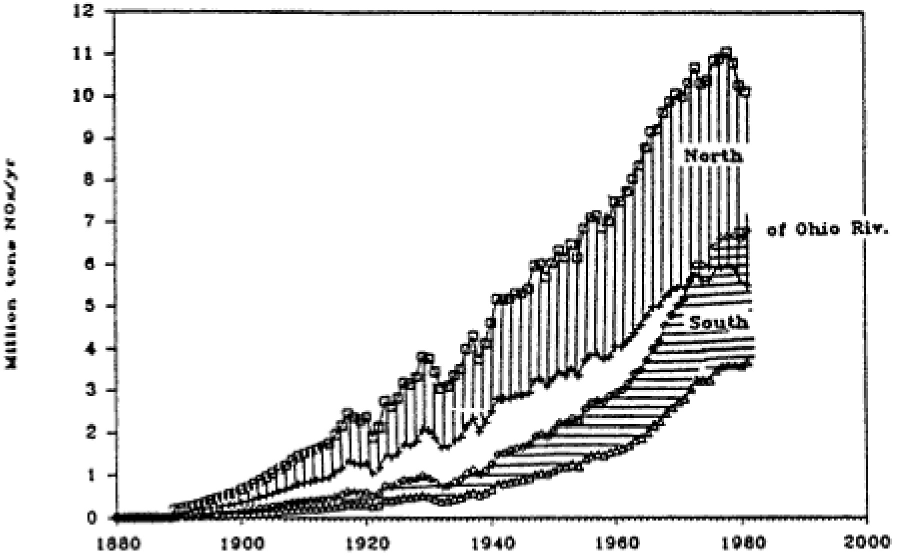


Fig. 3.
NO_x emission trend for regions north and south of the Ohio River (Husar, 1985).

The overwhelming fraction of NO_x emissions arises from the combustion of fossil fuels (coal, oil, and gas). Fuel consumption data constitute the most important input for the estimation of NO_x emissions. However, the estimation of the current and historical emission trends is more difficult than for sulfur compounds. Since most of the nitrogen oxides are formed by the fixation of air nitrogen at high temperatures rather than oxidation of fuel nitrogen. Thus, NO_x, emission depends primarily on the combustion process and to a lesser degree on the fuel properties. The fuel combustion processes in internal combustion engines and boilers have changed since the turn of the century. It is thus likely that the NO_x emission per fuel consumption has also changed historically. Hence, the following NO_x emission trends have substantially higher uncertainty than those for sulfur oxides.

The NO_x emissions since 1940 (Fig. 3) have been estimated to have increased by threefold by the 1970s. The sharp rise in the 1960s is attributed to the rise of the emissions from industrial and electric utility sources. Evidently, the NO_x increase was more pronounced north of the Ohio River compared to the south.

WET DEPOSITION DATA FOR SULFATE, NITRATE, AND AMMONIA

Motivated by increasing concern about "acid rain," at least five major precipitation chemistry networks have operated over North America since 1978. In Europe, most of the monitoring is conducted in the framework of the Economic Community of Europe (ECE), Co-operative Programme for Monitoring and Evaluation of the Long Range Transport (EMEP) network.

Sulfate

The yearly average sulfur deposition and the precipitation-weighted concentration fields arising from the five network data sets for the time period 1977-1980 are given in Fig. 4. Some of the sulfur in precipitation is due to sea salt and its contribution may be estimated either from Na or Cl data. Taking the sea salt sulfate to be 0.047 × Cl, the marine sulfate deposition over the continent was calculated to be only about 6% of the sulfur wet deposition over North America. For the sites more than 100 km from the coastline the marine sulfur deposition was insignificant. In the following discussion S refers to the excess beyond the sea salt sulfur.

For the North American continent (NAM), excluding Mexico the average sulfur wet deposition is 0.34 g $S/m^2/yr$. The area considered is 18 × 1012 m^2 and the total wet S deposition is thus 6.2 Tg S/yr. The highest sulfate deposition rate and concentration in precipitation occurs in the region surrounding the eastern Great Lakes. The sulfate wet deposition there exceeds 1 g $S/m^2/yr$. For the region east of the Mississippi River and south of the James Bay (defining here eastern North America ENA area 5.8 × 1012 m^2) the sulfate wet deposition is about 0.63 g $S/m^2/yr$. The lowest sulfate deposition rate of about 0.1 $g/m^2/yr$ is measured in northwestern Canada and southwestern U.S. Both of these regions have less than 0.5 m/yr of rainfall.

The weighted average sulfate concentration in precipitation (Fig. 4) ranges between 15 μeq/l in remote U.S. and Canadian regions to about 70 μeq/l in the vicinity of the Great Lakes. Hence, while the average deposition rate varies tenfold over the continent, the average precipitation sulfate concentration increases only fivefold from remote regions to industrial hot spots.

There are few areas of North America for which it can be safely assumed that the sulfate deposition values represent the "natural background." Considering the wind pattern and the anthropogenic S emission fields over the continent, a possible area uninfluenced by man may be northwestern Canada, inland from the Pacific Coast. The

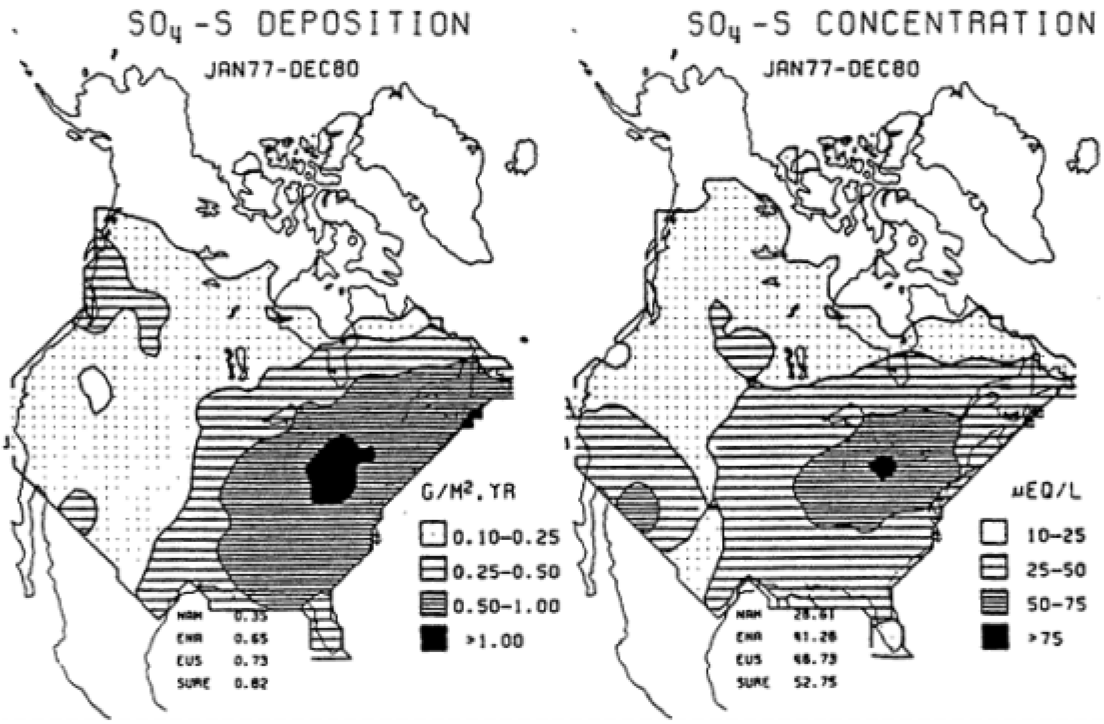


Fig. 4.
Maps of sulfate wet deposition rate, (g/m^2/yr) and precipitation-weighted average concentration (µeq/yr) over North America (Husar and Holloway, 1983).

measured sulfur wet deposition rate in that region is 0.1 g S/m^2/yr, compared to the North American average of 0.34 g S/m^2/yr. In estimating the natural global sulfur cycle from rain chemistry data, Granat et al. (1976) have chosen 0.15 g S/m^2/yr as the representative background deposition over nondesert land areas. Considering the severe uncertainties of the deposition estimates, 0.07 g S/m^2/yr would also seem reasonable as a North American average background. Scaled to the nondesert land area of the world (120 × 1012 m^2), the global S wet deposition from the above "background" sources would be 9-18 Tg S/yr, which is below the estimate of 18 Tg S/yr by Granat et al. (1976). An increasing data base of precipitation sulfur values obtained in the southern hemisphere, e.g., Stallard and Edmond (1981), from the Amazon region, and data from the polar ice caps-dictates that continental average background deposition outside the 0.07 to 0.15 g S/m^2/yr range is unlikely. A documentation of this statement is best available in Granat et al. (1976), but will require continuous reevaluation as the global data base expands.

The lower (0.07 g S/m^2/yr; 1.3 Tg/yr) and the upper bound (0.15 g S/m^2/yr; 2.5 Tg/yr) of the estimated "natural background" sulfur deposition constitutes 20 to 40% of the measured total sulfur deposition (0.34 g S/m^2/yr; 6.2 Tg/yr) over North America. For eastern North America the average excess sulfur deposition rate is about 0.63 g S/m^2/yr (3.7 Tg/yr) and the "background" (0.5-1.0 Tg S/yr) deposition would account for 12-25% of the total ENA wet deposition. This is a substantial upward revision of previous estimates of "natural contribution" for North America (Galloway and Whelpdale 1980).

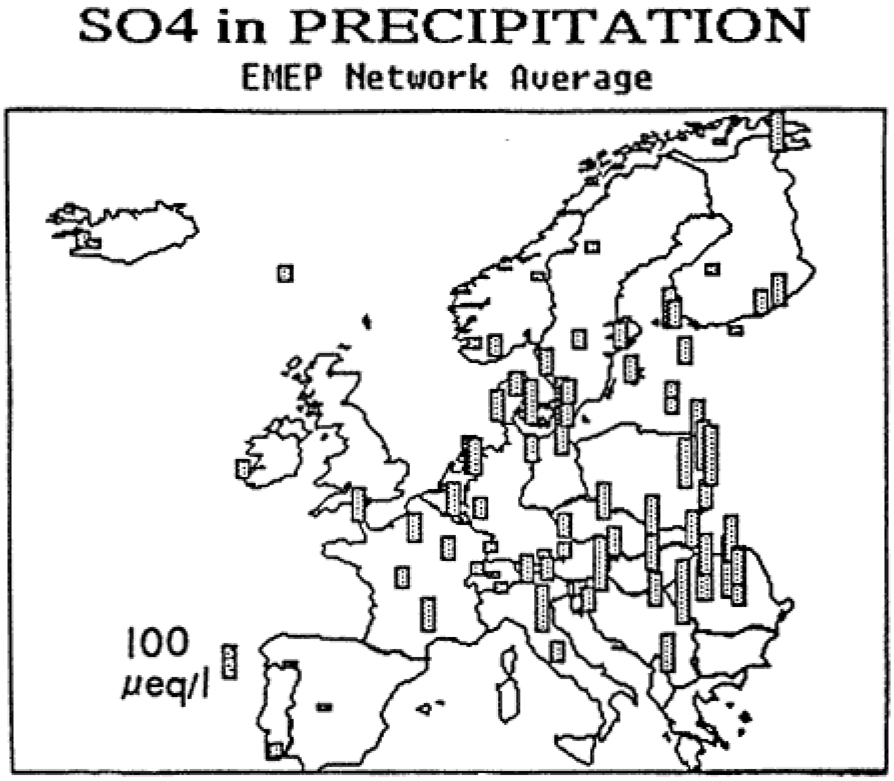
It is also instructive to compare the measured wet deposition pattern and rates to the emission field of man-made sulfur over North America (Fig. 1). The average emission density over eastern North America is 1.9 g S/m^2/yr while the average wet deposition over the same region is 0.63 g S/m^2/yr, i.e., about 30% of the known man-made emissions. If we further assume that the natural sources contribute on the average 0.07 g/m^2/yr, the measured wet deposition of sulfur amounts to only 25-30% of the man-made sulfur. The remaining 70-75% of the man-made sulfur is then either dry deposited as SO_2 or SO_4, or exported to the Atlantic by the prevailing winds.

The sulfate concentration in precipitation over Europe (Fig. 5) shows the highest yearly average values over eastern Europe (GDR, Poland, Hungary, Romania). In those areas it exceeds 100 μeq/l which is more than double the highest concentrations over eastern North America.

Nitrate

The average deposition and concentration pattern of nitrate over NAM is given in Fig. 6. The average NAM wet deposition rate is 0.13 g N/m^2/yr corresponding to 2.4 Tg/yr. The highest deposition rates (0.4g N/m^2/yr) are observed in the area surrounding the eastern Great Lakes. The average over ENA is 0.23 g N/m^2/yr. Using again the deposition data for remote western Canada (0.03-0.06 g N/m^2/yr), approximately an order of magnitude increase may be observed from remote to high emission regions. Similarly as for the sulfate, the nitrate concentration in rain has only a fivefold increase from remote (3-6 μeq/l) to industrialized regions (25 μeq/l).

If we take the "background" nitrate wet deposition rate to range between 0.03-0.06 g N/m^2/yr over the entire continent, the "background" wet removal will contribute 0.5-1.0 Tg N/yr. As for sulfate wet deposition, the "background" nitrate deposition would account for 20-40% of the measured nitrate deposition in precipitation. Over eastern North America, the corresponding background contribution would be 10-25% of the total measured wet deposition. Taking 0.03-0.06 g N/m^2/yr as representative over the nondesert land areas (1.2 × 1014 m^2) of the world, the global nitrate wet deposition would range between 3 and 7 Tg N/yr. This is an order of magnitude less than the total



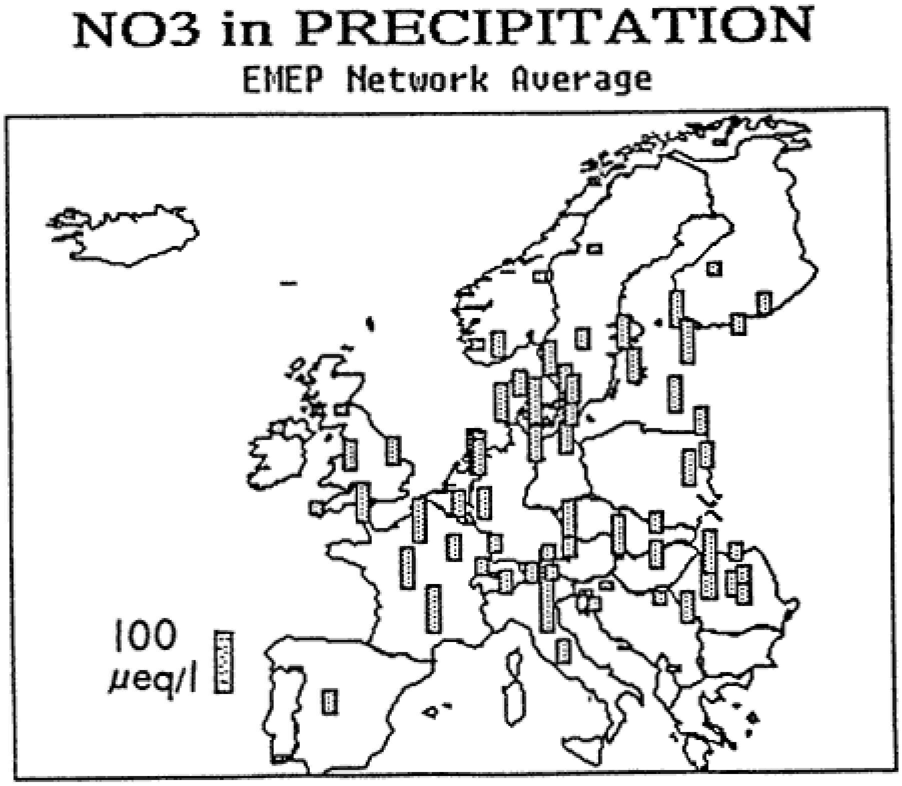


Fig. 5.
Sulfate and nitrate concentration, (μeq/l) in Europe (EMEP network average).

(dry and wet removed, natural and man-made) nitrate deposition (32-83 Tg N/yr) estimated by Soderland and Svennson (1976). Using the average measured NAM nitrate wet deposition of 0.13 g N/m^2/yr and applying it to the global nondesert land area, yields 16 Tg N/yr which is still a factor of two to five less than the nitrate deposition (dry and wet) estimated by Soderland and Svennson (1976).

An emission map (Fig. 1b) for man-made NO_x over eastern North America shows that the highest emission density is in excess of 3 g N/m^2/yr in the Great Lakes region and it roughly coincides with the location of the highest deposition density (Fig. 6). The comparison with the deposition data also reveals that wet deposition accounts for only 20% of the known man-made NO_x emissions over eastern North America. The remaining 80% is thus either dry deposited or exported from the continent.

The nitrate concentration in precipitation over Europe (Fig. 5) shows the highest values over western Europe including northern Italy and France. In those areas it exceeds 80 μeq/l which is more than double the highest nitrate concentrations over eastern North America.

Ammonia

The yearly average ammonium ion deposition field is shown in Fig. 7. The North American average deposition is 0.11 g N/m^2/yr (2.0 Tg N/yr) and for eastern North America 0.16 g N/m^2/yr (1.0 Tg N/yr). The highest deposition rates, ranging 0.25-0.50 g N/m^2/yr, occur from the Great Lakes to the Rocky Mountains, an area generally known as the corn belt region. West from the Rockies and in northern Canada the deposition rate is below 0.1 g N/m^2/yr. Conspicuously, the industrialized northeastern part of the U.S. does not show high ammonia deposition values. Here again, it is useful to compare the measured NAM ammonium wet deposition rates (2 Tg N/yr), scaled to the globe (13 Tg N/yr), to the global dry and wet ammonium/ammonia deposition rates (91-186 Tg N/yr), estimated by Soderland and Svennson (1976).

Possible sources of ammonia. Unlike SO_x and NO_x, an emission inventory of man-made X. X or "known" ammonia sources over North America currently does not exist. As an aid to interpret the measured ammonia wet deposition field, we have constructed a tentative inventory for the U.S. The emission factors for domestic animals were taken from Bottger et al. (1978). For nitrogen fertilizer it was assumed that 10% N is volatilized to the atmosphere. Minor contributions from known industrial sources were also included. The resulting ammonia emission density map is shown in Fig. 8. The total U.S. ammonia emission from the above "known" sources is estimated at 3.4 Tg N/yr with the highest emission density in the corn belt region exceeding 1 g N/m^2/yr.

There is a rough coincidence of the area of high measured deposition rate and estimated emission density, both extending through the corn belt region. The estimated U.S. ammonia emission rate from "known" U.S. sources is 3.4 Tg N/yr, which is comparable to the wet deposition integral of 2 Tg N/m^2/yr for NAM. However, considering the severe uncertainties of the "known" source estimates, little significance is attached to the emission values beyond suggesting that the inventory is within the right order of magnitude.

The ammonia concentration in precipitation over Europe (Fig. 9) shows the highest yearly average values over central and eastern Europe stretching from Great Britain through Poland and Romania. In many areas the concentration exceeds 100 μeq/l which is more than three times the highest concentrations over eastern North America.

In spite of the uncertainties associated with the spatial-temporal coverage, sampling, and analytical procedures, and the interpretation of the wet deposition data, it is most gratifying that such continental-scale data bases currently exists for North

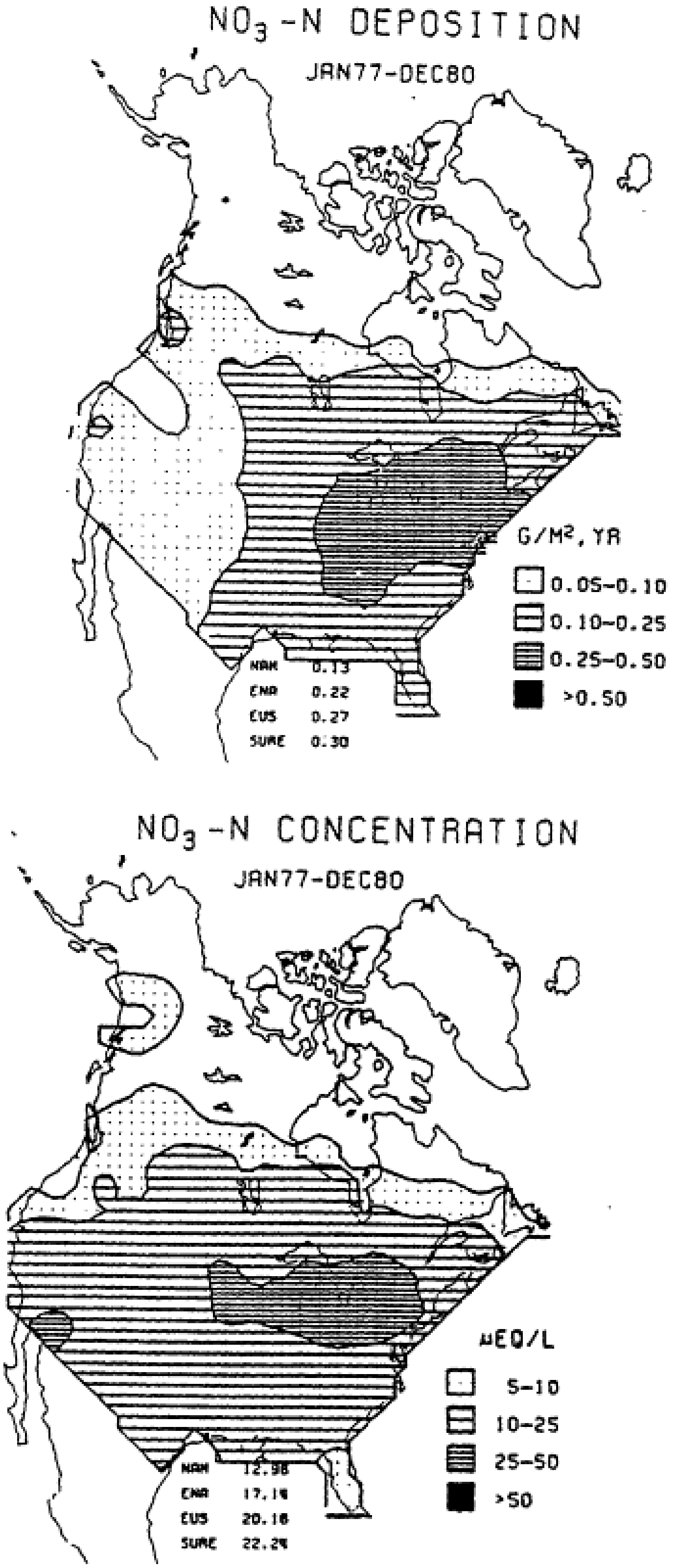


Fig. 6.
Maps of nitrate wet deposition rate (g/m^2/yr) and Precipitation-weighted average concentration (μeq/l) (Husar and Holloway, 1983).

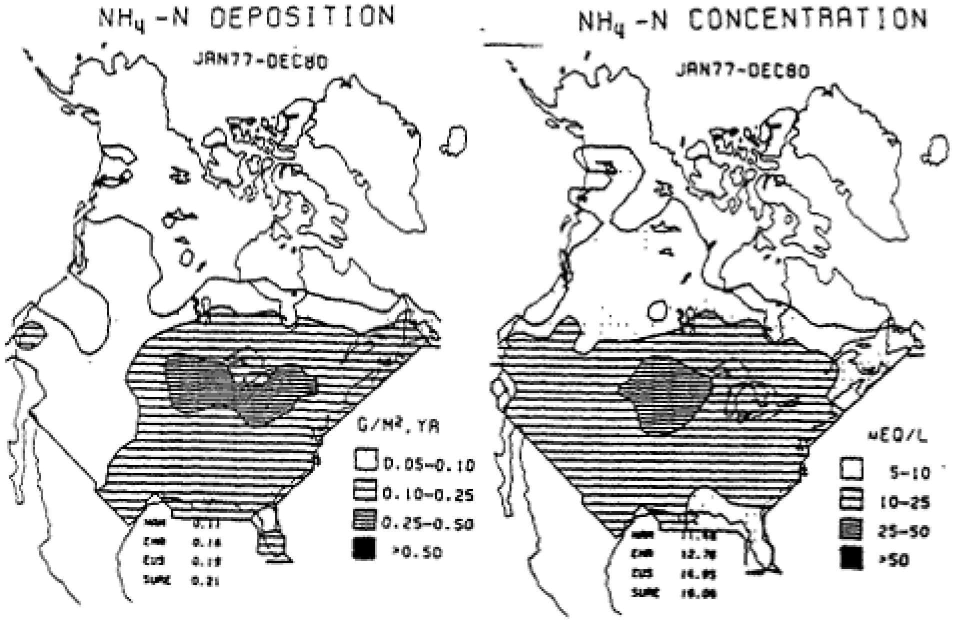


Fig. 7.
Maps of ammonia wet deposition rate, (g/m^2/yr) and precipitation-weighted average concentration, (μeq/l) (Husar and Holloway, 1983).

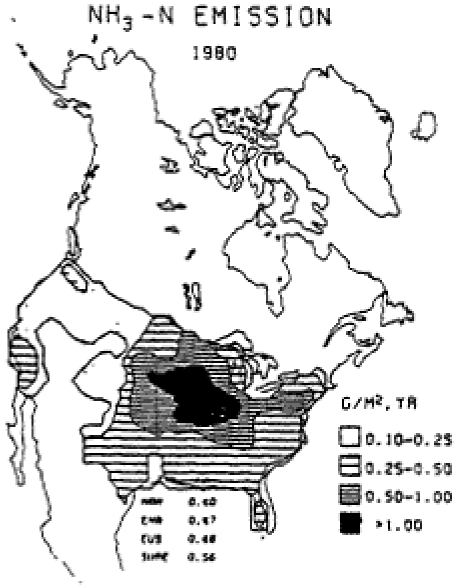
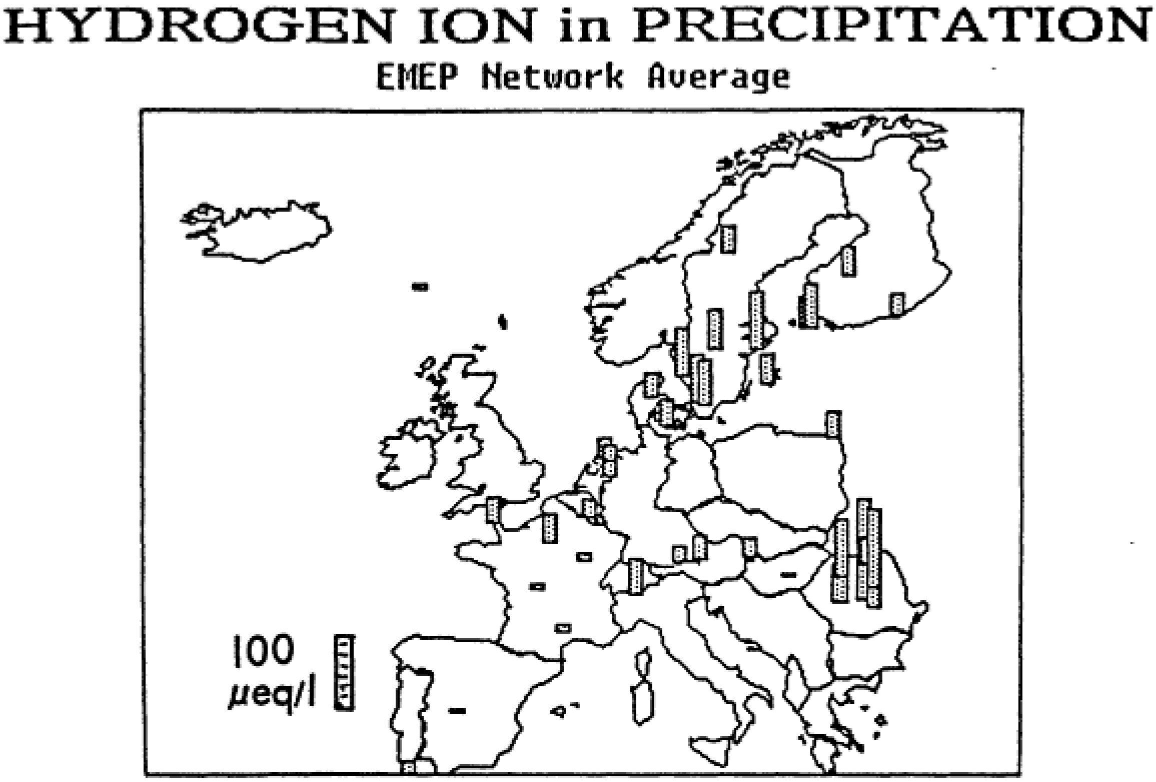


Fig. 8.
Estimated emission density of ammonia emissions for the U.S. (Husar and Holloway, 1983).



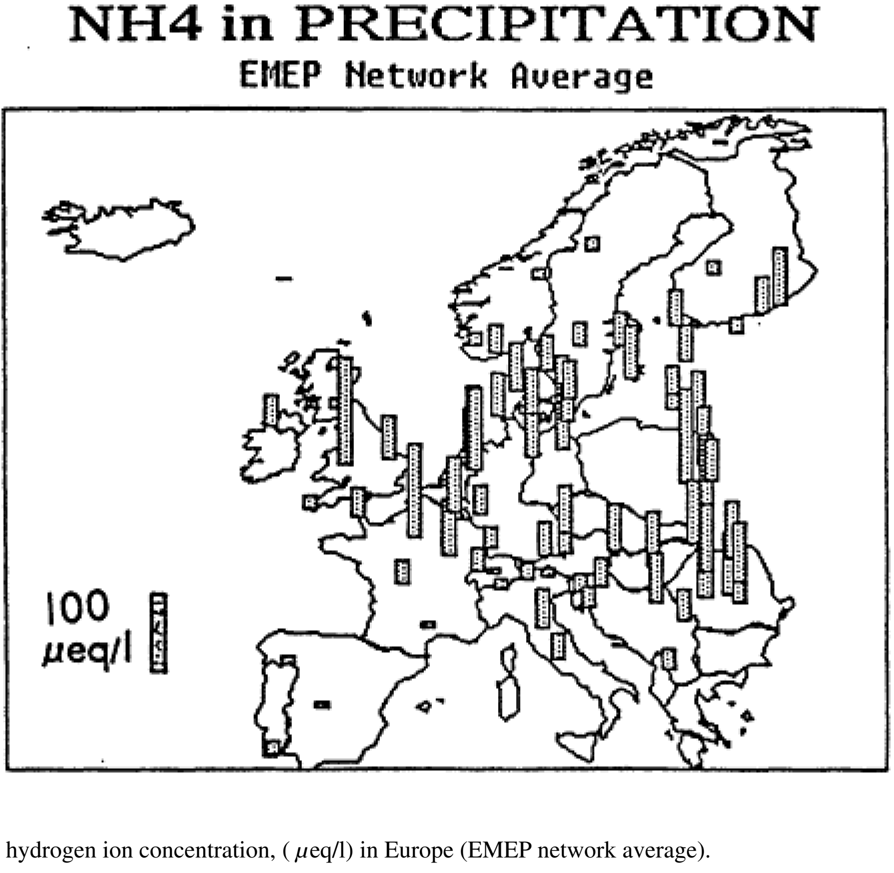


Fig. 9.
Ammonia and hydrogen ion concentration, (μeq/l) in Europe (EMEP network average).

America and Europe. Prudent use of such data bases will undoubtedly provide us with a much improved perspective on both scientific and other aspects of the "acid rain" problem.

AIRBORNE OZONE CONCENTRATION

It is well established that high airborne ozone concentrations can damage forests. Regrettably, national climatic maps for ozone are not available. One of the most difficult problems pertains to the calculation of "representative" concentrations. The concentration at a given location has a diurnal, synoptic scale (3 to 5 days), seasonal and long-term trends that result from the interacting NO_x, hydrocarbon sources, physical and chemical removal processes, and atmospheric transport. Nevertheless, attempts have been made to compile the ozone distribution pattern. Vukovich et al. (1985) have explored the daytime ozone concentrations for nonurban locations over the eastern U.S. for August 1978-1981 as displayed in Fig. 10. The daytime ozone concentrations exceeding 60 ppb cover the area of the Ohio River valley and the mid-Atlantic states, New Jersey-North Carolina. It is worth noting, however, that the concentrations are about 40 ppb in the upper Midwest. Hence, the regional variability of the summer daytime ozone is only about a factor of two. It is likely that the spatial gradients in the winter time are more pronounced.

Singh et al. (1978) have proposed a semi-quantitative picture of the seasonal variation of the ozone in the lower layers of the atmosphere (Fig. 11). The natural ozone pattern is indicated by the shaded area marked A. Superimposed on this natural background is a man-induced perturbation marked with B and C. At remote sites the natural ozone concentration reaches the maximum in the early spring. In areas influenced by man-made sources, a summer peak may arise. Here again, we emphasize that on the average the man-made ozone is a mere perturbation over a substantial natural background.

VISIBILITY

Atmospheric optical data are much more abundant on a continental scale than chemical composition data, with the exception of the water vapor content. Hence, if a reasonably well defined relationship can be established between, for example, the visual range or turbidity and the fine particle content of the atmosphere, then the extensive meteorological observations by human observers inherent in the interpretation of the visibility data obtained by routine meteorological observation networks. These include the subjectivity of the human observer, the lack of suitable visual targets, and the numerous natural phenomena that perturb the visual environment (rain, fog, blowing dust, natural haze, etc.). The spatial and trend analysis of the visibility data, therefore, needs to be conducted with utmost caution. The data presented below arise from the analysis of 147 U.S. and 177 Canadian stations from 1948 to 1980.

The quarterly average extinction coefficient (3.9/visual range, km) from noon observations in the absence of precipitation and fog is shown in Fig. 12. At midlatitudes (40-60° N) there are three hazy regions: surrounding the Great Lakes, the Mississippi Delta and southern California including the San Joaquin Valley. A conspicuously hazy region also exists north of the arctic circle, the cause of which is unknown and will be ignored here. The lowest mean extinction coefficient (<0.1 l/km) occurs in the U.S. Rocky Mountain Region. The long-term availability of visibility data also permits the examination of secular trends of continental haze from about 1950 to 1980. The five-year average map for 1950-1954 shows substantially lower haziness than the 1976-1980 period. The important changes in the three decades occurred in the southeastern U.S. (mostly in the summer months) as well as over the northwestern U.S. and adjacent southwestern Canada (mostly in the winter months).

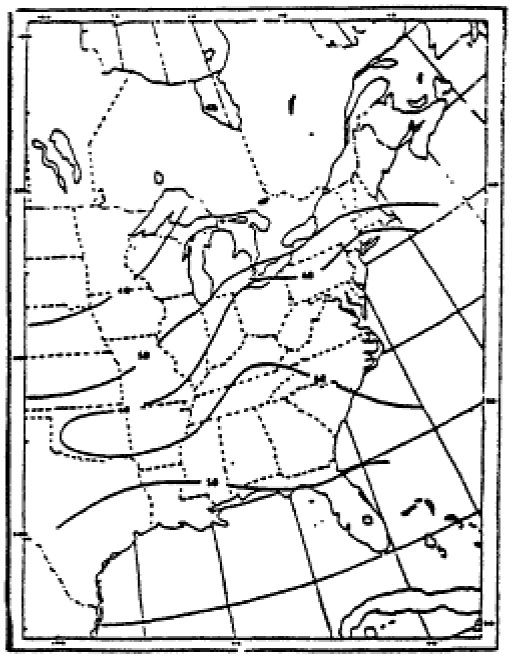


Fig. 10.
Mean diurnal maximum ozone concentration isopleths for August 1978-1981 (Vukovich et al., 1985).

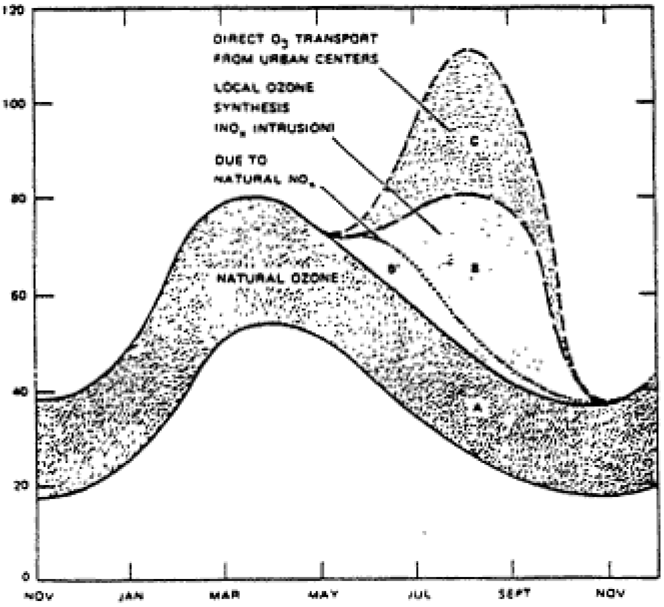


Fig. 11.
Idealized ozone variations at remote locations (Singh et al., 1978).

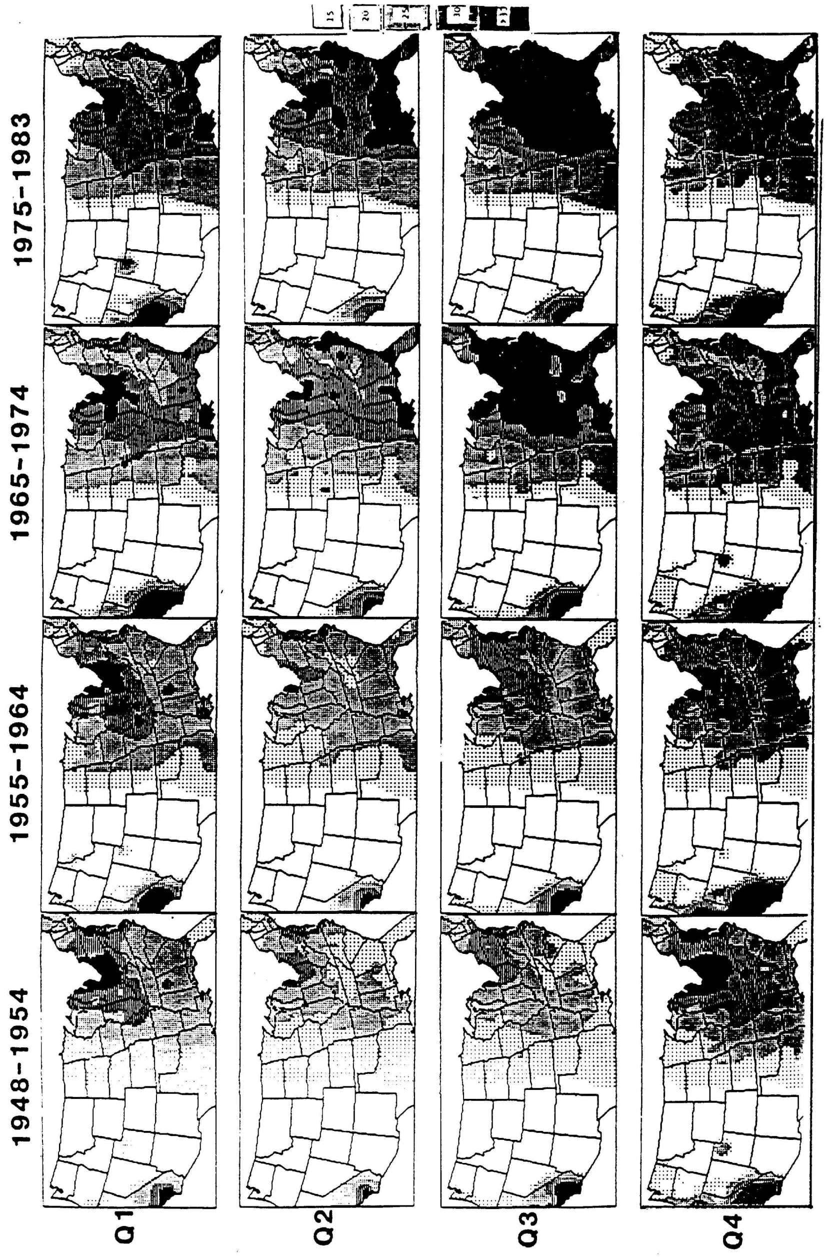


Fig. 12.
The quarterly (Q1 Jan-March, Q2 April-June, Q3 July-Sept, Q4 Oct-Dec) average extinction coefficient from noon observations in the absence of precipitation and fog (Husar and Patterson, 1987).

A more detailed trend pattern for eastern U.S. haze is shown in Fig. 13. It is apparent that in some areas, such as in New England, the haze decreased for the first quarter of the year. Other regions, such as surrounding the Smoky Mountains, exhibit a strong increase, particularly during the third quarter (July, August, September). In the New England and New York area the SO_x emissions have declined since the 1950s primarily because of the shift from coal to oil. The Smoky Mountain region on the other hand, experienced a substantial increase of SO_x emission primarily due to increasing electric utility coal consumption (see Emission section above).

Beyond demonstrating that substantial changes have occurred in the optical environment, the visibility data demonstrate that within the eastern U.S. some subregions may exhibit a decline, others an increase of haziness. This implies that in spite of "long range transport" subregions covering several U.S. states will exhibit trends in fine particle concentration consistent with their own emission trends. In other words, the long-term haze data base confirms the most revealing conclusion of the European OECD project, namely that every source region impacts on itself more than on any other region.

ACKNOWLEDGMENTS

This research was partially supported by the U.S. Environmental Protection Agency cooperative agreement #CR 810351-02, the National Academy of Sciences, and Washington University. Special thanks to Dr. Ellis Cowling for his encouragement to pursue and refine our concepts of Chemical Climatology.

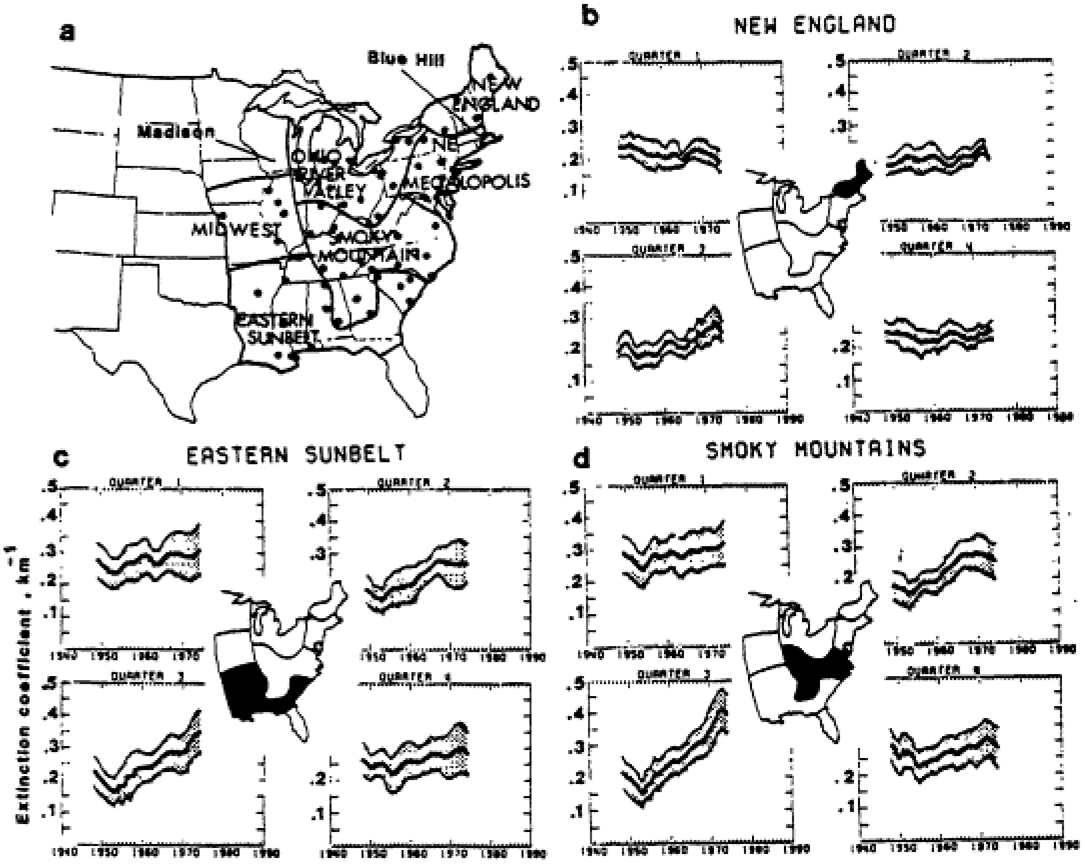


Fig. 13
Location of 70 eastern U.S. sites where detailed trend analysis was performed. The trend lines indicate mean and arithmetic standard deviation among the stations within each region; b.-d. Trends of extinction coefficient by yearly quarters for New England, southeastern sun belt, and the Smoky Mountain region (Husar et al., 1979).

References

Bottger, A., Ehhalt, D.H., and Gravenhorst, G. 1978. Atmospherische Kreislaufe von Stickstoffen und Ammoniakum. Berichte der Kernforschunsanlage Julich, 1558.

Galloway, J.N. and Whelpdale, D.M. 1980. An atmospheric sulfur budget for eastern North America. Atmospheric Environ. 14, 409.

Granat, L., Rodhe, H., and Hallberg, R.O. 1976. The global sulfur cycle. In Nitrogen, Phosphorus, and Sulfur-Global Cycles. Svensson, B.H. and Soderland, R., eds., SCOPE, Report 7. Ecol. Bull. (Stockholm) 22, 23.

Husar, R.B. and Patterson, D.E. 1987. Project Summary: Haze Climate of the United States. U.S. Environmental Protection Agency, EPA/600/SB-86/071.

Husar, R.B., Patterson, D.E., Holloway, J.M., Wilson, Jr., W.E., and Ellerstad, T.G. 1979. Trends of Eastern U.S. Haziness Since 1948. Proceedings of the Fourth Symposium on Turbulence, Diffusion, and Air Pollution, Jan. 15-18, 1979.

Husar, R.B. and Holloway, J.M. 1983. Sulfur and nitrogen over North America. In Ecological Effects of Acid Deposition, National Swedish Environment Protection Board-Report PM 1636, Stockholm, Sweden.

Husar, R.B. 1985. Manmade SO_x and NO_x emission and trends of Eastern North America. Background paper for the National Academy of Sciences Committee on Monitoring and Assessment of Trends in Acid Deposition.

Singh, H.B., Ludwig, F.L., and Johnson, W.B. 1978. Tropospheric ozone: concentrations and variations in clean remote atmospheres. Atmospheric Environ., 12, 2185-2196.

Soderland, R., Svensson, B.H. 1976. The global nitrogen cycle. In Sevensson, B.H. and Soderland, R., eds., Nitrogen, Phosphorous, and Sulphur-global cycles. SCOPE, Report 7. Ecol. Bull. (Stockholm) 22:23-73.

Stallard, R.F. and Edmond, J.M. 1981. Geochemistry of the Amazon. 1. Precipitation chemistry and the marine contribution to the dissolved load at the time of peak discharge. J. Geophys. Res. 86,9844.

Vukovich, F.M., Fishman J. and Browell E.V. (1985). The reservoir of ozone in the boundary layer of the Eastern United States and its potential impact on the global tropospheric ozone budget. J. Geophysical Res. , 90-03, 5687-5698.

List of Figures

Elevational Gradients/Local Chemistry

Volker A. Mohnen

Professor, Department of Atmospheric Sciences State University of New York at Albany Albany, New York 12222

ABSTRACT

Since some air pollutant concentrations are known to vary systematically with latitude, mountain sites were located at several latitudes, reflecting different transport distances and times from major air pollution source regions. MCCP's (Mountain Clouds Chemistry Project) specific locations are: Mt. Mitchell, NC; Whitetop Mt., VA; Shenandoah National Park, VA; Whiteface Mt., NY; Mt. Moosilauke, NY; and Howland Forest, ME. The sites were established during the late 1985-early 1986 time frame and all are currently functioning. Three of the sites are operating monitoring stations at several elevations. The Atmospheric Environment Service of Canada is collaborating with the United States EPA in this project by operating similar sites at Mt. Tremblant and Roundtop Mountain in Quebec Province.

Experimental design at the EPA-funded Mountain Cloud Chemistry Project (MCCP) includes the selection of sites, establishment of protocols, coordination, data base oversight, and preparation of interpretive reports. The MCCP is designed to define the concentration of selected acidic compounds and associated oxidants in the gaseous and liquid phases for above-cloud base forests.

To achieve the program objectives, the following measurements are made at one or all MCCP sites:

(A) Gaseous chemical measurements: ozone-continuous (hourly average), sulfur dioxide-continuous (hourly average), oxides of nitrogen-continuous (hourly average), hydrogen peroxide (hourly average), ammonia/ammonium-filterpack (weekly), fine particulate sulfate aerosol-filterpack (weekly), sulfur dioxide-filterpack (weekly).
(B) Aqueous chemical measurements: cloud water chemistry (hourly), cloud water hydrogen peroxide (hourly), rain chemistry from precipitating clouds (hourly), precipitation chemistry, NTN (weekly), throughfall/stemflow chemistry (weekly).
(C) Physical measurements: cloud liquid water content, presence of cloud-continuous (hourly), precipitation amount (hourly).
(D) Climatic measurements (forest stress and model evaluation parameters): air temperatures (hourly), wind speed and direction (hourly), relative humidity (hourly), insolation (hourly), barometric pressure (hourly).

Measurement data collected at the MCCP sites are transferred to the MCCP Central Data Center. At the Data Center the data are loaded into a VAX/ORACLE database. These data are subsequently validated and certified in accordance with the procedures defined in the MCCP QA Plan. This database is exercised by a series of report generation software programs to yield the desired frequency distribution graphic reports and other statistical reports required by MCCP scientists or by the forest research community.

The following segments summarize the 1986-87 results.

CLOUD EXPOSURE FREQUENCY

Various techniques for monitoring the presence of clouds have been studied and tested during the 1986-87 field seasons and have been summarized in a MCCP interim technical report. For the 1988 field season, a uniform cloud detection technique has been implemented network-wide using an optical cloud detection instrument developed originally by the Netherlands Research Foundation.

Table 1 summarizes cloud exposure statistics by site for the June-October 1987 period:

Table 1. Cloud Exposure Statistics

				Diurnal Cloud Frequency (%)			
Site	Cloud Frequency % time	No. of Cloud Events	Average Event Duration (hrs)	1-6 AM	7-Noon	1-6 PM	7-Mdnt
Whiteface	40	80	15	53	41	25	45
Whitetop	28	142	7	39	33	12	27
Mitchell	29	143	7	46	24	10	35
Moosilauke	21	52	13	26	21	15	23
Shenandoah	7	29	8	10	6	3	9

CLOUD CHEMISTRY EXPOSURE

Table 2. Cloud Chemistry Exposure (Non-precipitating Clouds) for 1986 and 1987 All concentrations are in micromoles per liter.

	Site/Year	Ion	Max	Mean	Std Dev Samples	Number of Hourly
WHITEFACE	1986	H^+	1585	254	254	306
	1987	H^+	1738	188	212	196
	1986	NH_4^+	776	110	136	308
	1987	NH_4^+	493	123	108	198
	1986	SO_4^{--}	747	130	132	308
	1987	SO_4^{--}	940	120	129	198
	1986	NO_3^-	804	82	95	308
	1987	NO_3^-	547	95	97	198
SHENANDOAH						
	1986	H^+	589	237	157	25
	1987	H^+	1202	212	210	57
	1986	NH_4^+	263	123	75	25
	1987	NH_4^+	602	96	118	57
	1986	SO_4^{--}	267	101	76	25
	1987	SO_4^{--}	619	104	115	57
	1986	NO_3^-	388	157	110	23
	1987	NO_3^-	541	104	110	57
WHITETOP						
	1986	H^+	3020	274	395	153
	1987	H^+	1413	308	240	153
	1986	NH_4^+	834	136	159	154
	1987	NH_4^+	1070	199	182	153
	1986	SO_4^{--}	943	132	159	154
	1987	SO_4^{--}	1070	199	156	152
	1986	NO_3^-	1744	141	236	155
	1987	NO_3^-	648	150	117	152
MT. MITCHELL						
	1986	H^+	1096	346	254	111
	1987	H^+	724	374	140	38
	1986	NH_4^+	670	191	143	114
	1987	NH_4^+	430	154	89	38
	1986	SO_4^{--}	670	191	143	114
	1987	SO_4^{--}	435	198	83	38
	1986	NO_3^-	373	121	80	115
	1987	NO_3^-	265	138	55	38
MOOSILAUKE						
	1987	H^+	1202	284	324	54
	1987	NH_4^+	488	110	130	54
	1987	SO_4^{--}	768	142	182	54
	1987	NO_3^-	548	114	123	54

OZONE

Table 3. Frequency Distribution of Ozone (Summer) All values are given in parts per billion (ppb).

Site	Min	Max	Mean	Standard Deviation
Whiteface Mtn 1986	15.3	98.5	44.1	13.8
Whiteface Mtn 1987	6.0	105.0	46.0	14.3
Whitetop 1986	1.0	120.0	59.0	15.0
Mount Mitchell 1986	12.0	112.9	47.2	15.3

(Other sites had less extensive data records for 1986 and thus are omitted here.)

HYDROGEN PEROXIDE

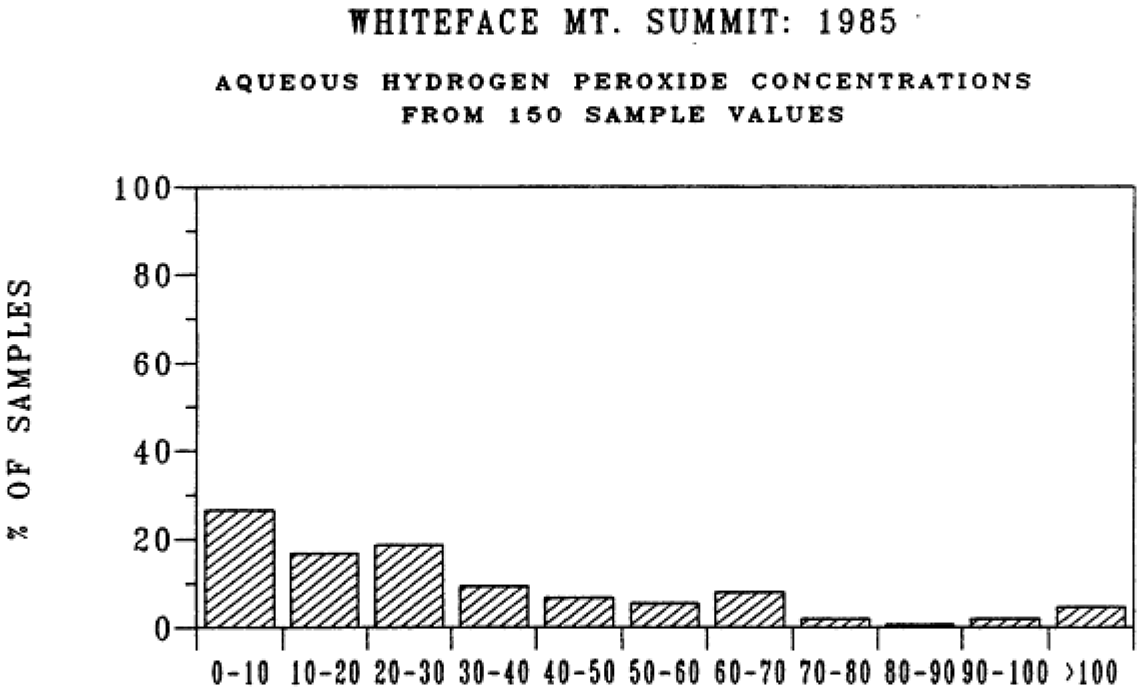


Fig. 1a.
Frequency Distribution of Hydrogen Peroxide, 1985

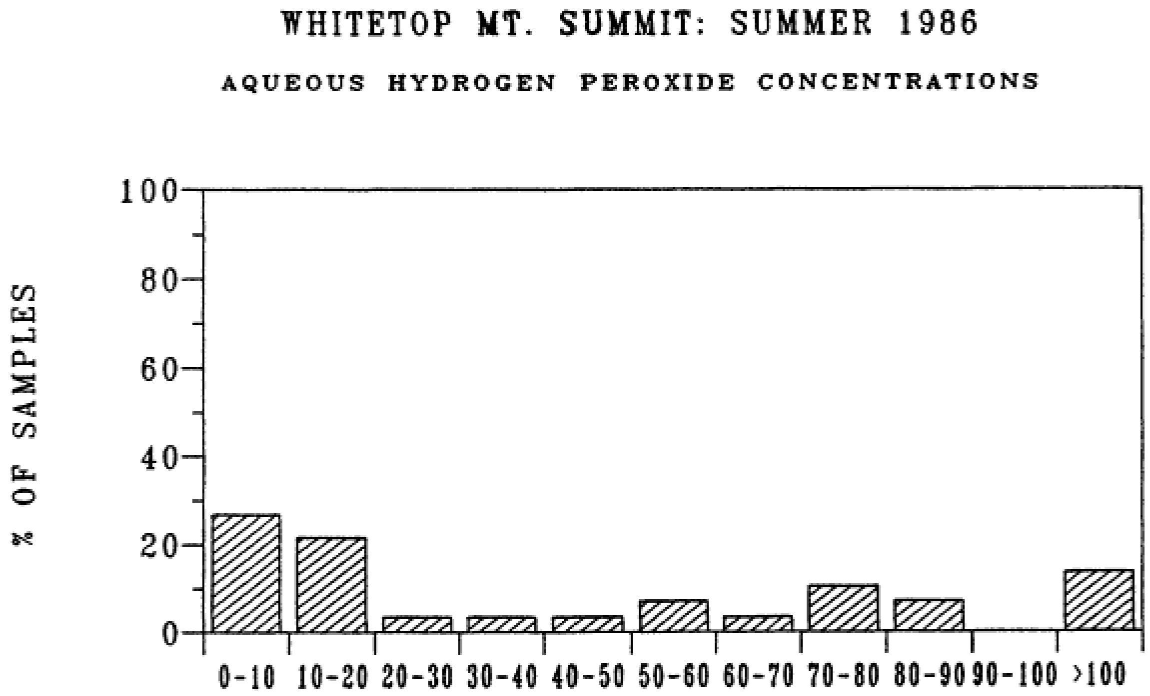


Fig. 1b.
Frequency Distribution of Hydrogen Peroxide, 1986

A few cloud samples from all other sites have been shipped to the above sites for H_2O_2. analysis.

Gas phase H_2O_2: because of the complexity in measuring the gas phase concentration of H_2O_2, only two sites are operating on a continuous basis.

Table 4. Hydrogen Peroxide, 1986

Site	Min	Max	Mean	Standard Deviation	No. of Hourly Samples
Whiteface Mtn 1986	BDL	2.82	0.72	0.55	424
Fall 1986	BDL	0.75	0.18	0.15	76
Whitetop 1986	BDL	2.60	0.80	0.50	183
Fall 1986	BDL	0.57	0.15	0.11	96

CLOUD LIQUID WATER CONTENT

Cloud liquid water content (LWC) measurement is necessary to convert cloud water chemical concentrations into pollutant inputs. A filter sampler designed by TVA collects cloud water isokinetically on a filter which is weighed manually during cloud events to obtain hourly integrated LWC values. The frequency distribution of 1987 cloud LWC values was determined for all sites for both precipitating and non-precipitating cloud (see sample figure). Whiteface exhibits a broad, bimodal range (0.1-1.1 g/m^3) compared with Mitchell and Moosilauke where most values are less than 0.5. Values measured at Shenandoah cover a broad range similar to that for Whiteface, but the sample size is significantly smaller.

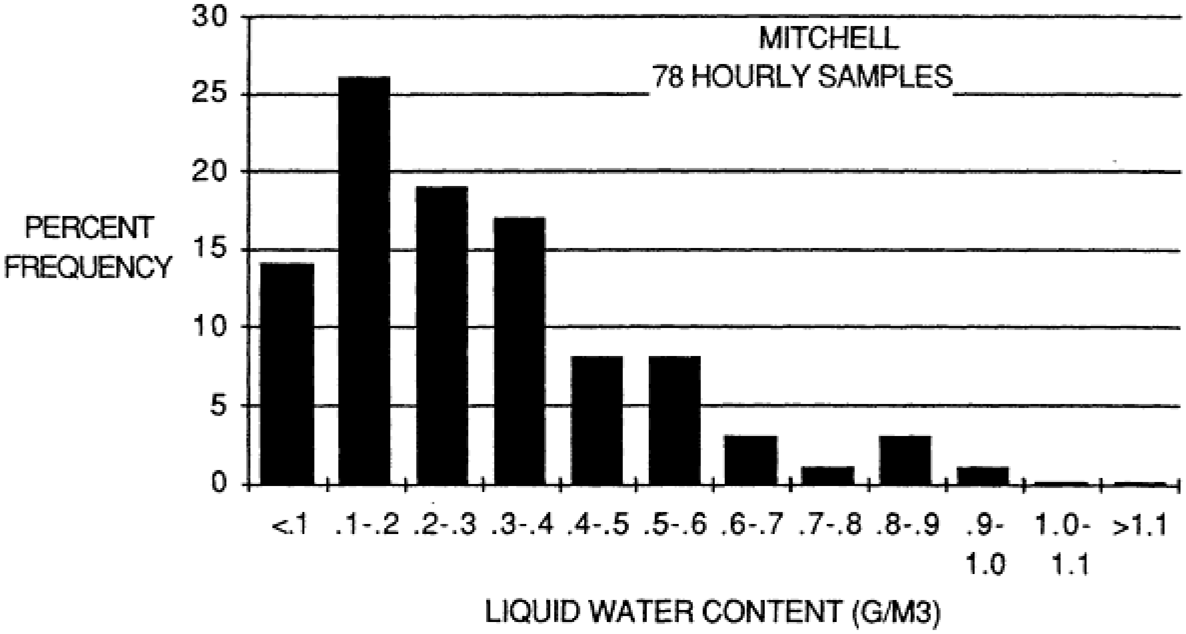


Fig. 2.
Frequency Distribution of Cloud Liquid Water Content For Non-Precipitating Cloud-1987 Field Season

DISCUSSION

Concentrations of pollution related ions in cloud water (non-precipitating clouds) are significantly higher than in rain, typically by a factor of 5 to 10.

There is a considerable interannual and intersite variation in cloud water ion concentration, reflecting the different weather patterns and meteorological conditions that give rise to clouds at the MCCP sites. In order to define "typical" cloud exposure, we need to monitor clouds for several years as was originally envisioned and as provided for in the project plan.

The mean hydrogen ion concentration of cloud water samples (non-precipitating clouds) are in the range of 174 to 374 micromoles per liter for all MCCP sites.

Cloud exposure frequency over the first two field seasons ranged from about 10% to 45% for the MCCP sites (see fig. 3). There is no clear pattern evident of cloud frequency as a function of latitude or elevation. Current studies are focusing on the monitoring of cloud exposure at several mountain sub-sites to determine relationships with elevation, slope and aspect.

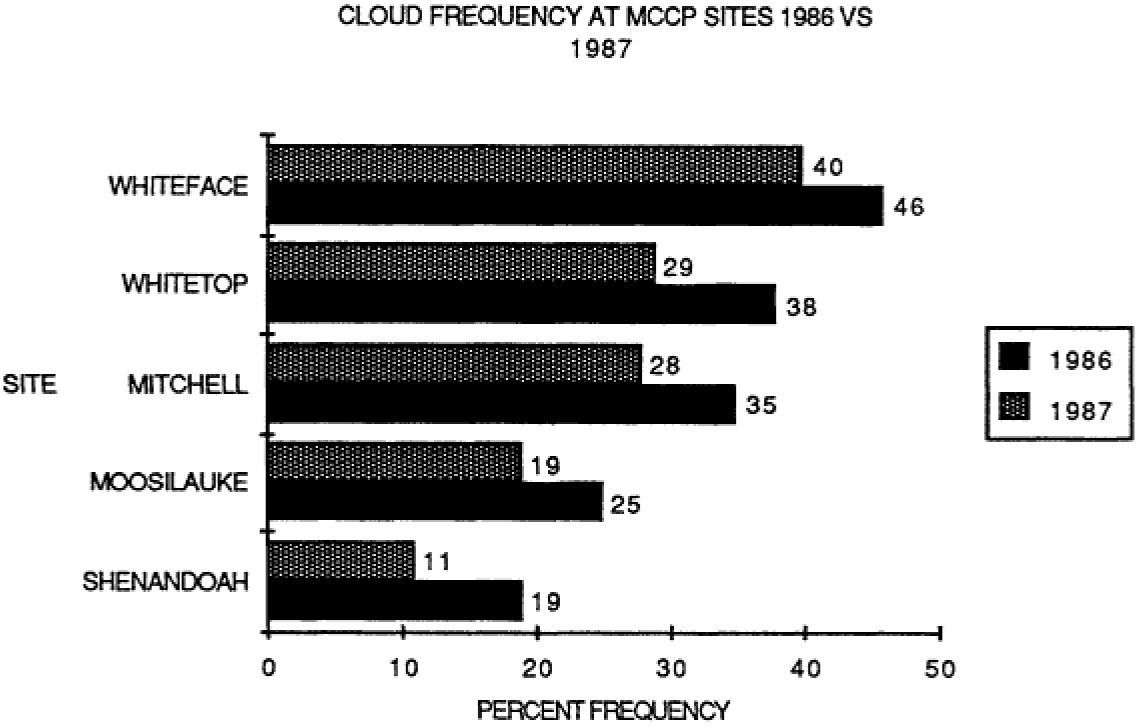


Fig. 3.
Cloud Frequency at MCCP sites 1986 vs 1987

The observed differences in liquid water content values among sites emphasizes the need to distinguish between cloud types (e.g., cap cloud vs. regional cloud) and cloud formation mechanisms.

Sulfur dioxide concentrations (not shown here) are very low at all sites except Shenandoah. At Whiteface Mountain for example, the mean SO_2 concentration in 1987 was 1.3 ppb and 65 percent of the time the values were below the detection level of the instrument, i.e., 0.5 ppb.

Ozone, hydrogen peroxide and sulfur dioxide show almost no diurnal variation in concentration at high elevation sites. Hydrogen peroxide is a typical summertime problem, being very low in spring and fall and non-detectable in winter (gas and aqueous phase).

Total deposition fluxes computed for each 1986 Whitetop intensive study were converted to a monthly basis (i.e., kg ha^{-1} mo^{-1}) for ease of comparison between studies of different lengths. Figure 4a illustrates the computed precipitation (wet) deposition fluxes. The length of a horizontal bar represents the computational uncertainty for each combination of chemical species and season. Dry deposition flux estimates for Whitetop are illustrated in Figure 4b. "Total SO_{-4}"deposition is the sum of particle sulfate deposition and the sulfate equivalent of the deposited SO_2 gas. Figure 4c illustrates cloud deposition flux estimates for Whitetop. Extrapolations to entire seasons yield the seasonal deposition totals listed in Table 5. In this extrapolation exercise, the seasons are defined as: spring=April-June, summer=June-September, fall=October-December. Caution must be taken in extrapolating these values further to annual values.

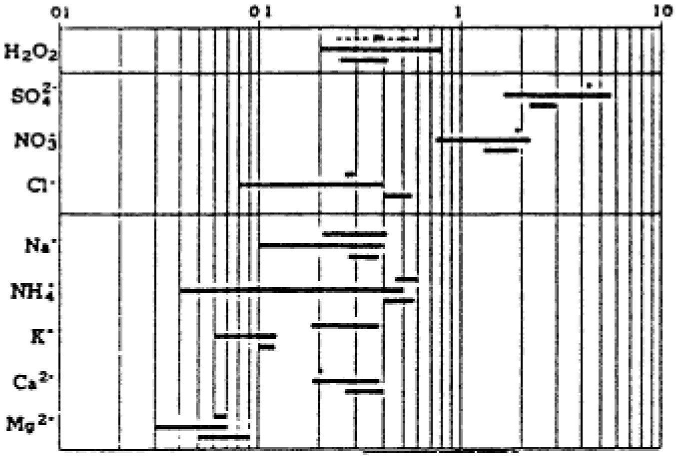


Fig. 4a.
Plots of Whitetop Mountain Wet Deposition Flux (kg ha^{-1} $^{mo-1}$) Estimates during the Spring, Summer, and Fall intensive Field

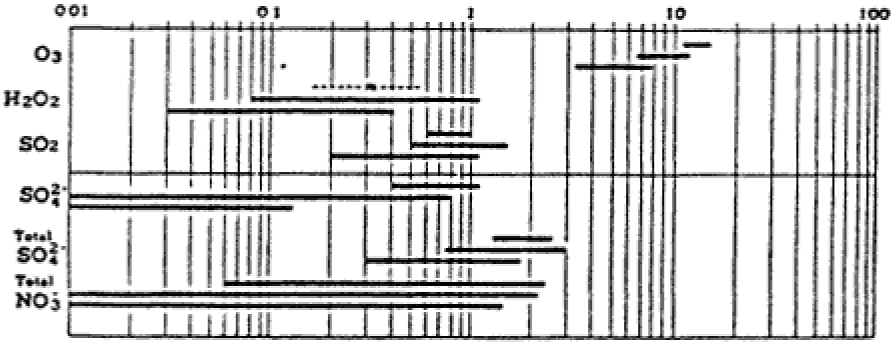


Fig. 4b.
Plots of Whitetop Mountain Dry Deposition Flux (kg ha^{-1} $^{mo-1}$) Estimates During the Spring, Summer, and Fall Intensive Field Studies of 1989

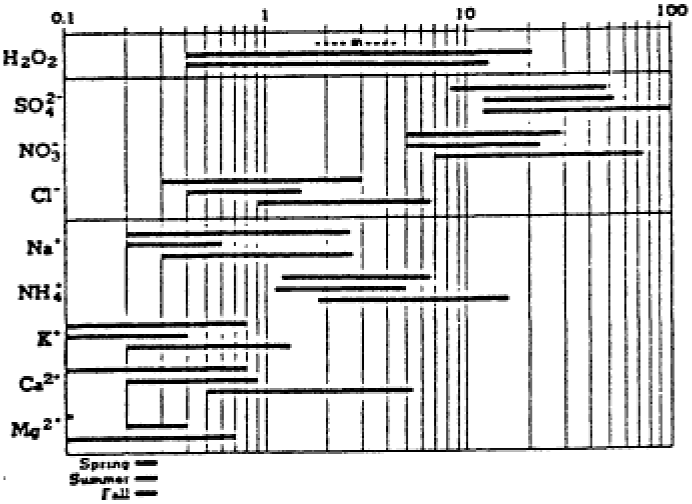


Fig. 4c.
Plots of Whitetop Mountain Cloud Deposition Flux (kg ha^{-1} $^{mo-1}$) Estimates During the Spring, Summer, and Fall Intensive Field Studies of 1986.

Table 5. 1986 Estimates Seasonal Deposition Budgets for Whitetop Mountain

Chemical Species	Spring	Total Deposition Summer	(kg ha^{-1}) Fall	All 3 Seasons
O_3	34-41	22-34	<20[a]	<95
H_2O_2	-	9-18	3-6	-
SO_2	2-3	2-5	-	-
SO_4^{2-}	56-123[b]	86-167[b]	64-131[b]	206-421[b]
Total SO_4^{2-} $(SO_2 + SO_4^{2-})$	59-128	88-174	-	-
Total NO_3^- $(HNO_3 + NO_3^-)$	59-128	38-78	38-79	106-229
$C1^-$	1.5-3.2[c]	1.3-2.3[c]	1.6-3.1[c]	4.4-8.6[c]
Na^+	1.2-2.5[c]	0.7-1.2[c]	0.6-1.1[c]	2.5-4.8[c]
NH_4^+	7-16[d]	7-15[d]	8-15[d]	22-46[d]
K^+	0.6-1.0[c]	0.4-0.6[c]	0.4-0.8[c]	1.4-2.4[c]
Ca^{2+}	0.6-1.0[c]	1.4-2.0[c]	1.2-2.4[c]	3.2-5.4[c]
Mg^{2+}	0.2-0.3[c]	0.3-0.6[c]	0.1-0.3[c]	0.6-1.2[c]

a. Upper limit based on fall average O_3 concentrations and decline in daylight hours.

b. Neglects the dry deposition of SO_4^{2-} which was probably <1% of the total deposition.

c. Neglects dry deposition which was computed to be typically <1% of the total deposition.

d. Neglects the dry deposition of NH_4^+ and NH_3.

Large-Scale Monitoring

Joseph E. Barnard, Program Manager

NATIONAL VEGETATION SURVEY Forestry Sciences Laboratory Southeastern Forest Experiment Station P.O. Box 12254 Research Triangle Park, NC 27709

ABSTRACT

The need to begin monitoring and visible symptom identification on forest trees is evident. Monitoring of forests can be defined as the long-term, periodic measurement of selected physical and biological parameters for establishing baselines, that is, for determining the extent and magnitude of current conditions for specified forest values, and for detecting and quantifying change over time. Within the Forest Response Program, the National Vegetation Survey (NVS) is responsible for survey and monitoring of U.S. forests. Four general categories of research are underway in the NVS: (1) Dendrochronology, (2) Mensuration and Statistics, (3) Mapping and Geographic Information Systems, and (4) Monitoring. Results of several NVS projects are discussed.

Research is underway to develop and implement appropriate inventory techniques to assess the current health of forest trees and stands. This includes evaluations of procedures to identify and rate specific kinds of air pollution damage on forest trees, field plot measurement procedures and techniques, remote sensing applications, soil and moisture rating procedures, and the assessment of current impact of air pollutants on species with known visible symptoms. As an aid for identifying locations where there may be air pollution impacts, a Geographic Information System has been developed for the South to provide information on forest resources, climate, soils, and atmospheric deposition.

Existing inventory data and procedures, while providing important insights, have not met present analytical needs. Now we have an opportunity to design and perhaps implement a monitoring program as a legacy of research on the effects of acidic deposition on forests. This opportunity will involve an effort to identify and coordinate all aspects of forest condition monitoring.

There is continuing interest in the assessment of the condition and trends of the Nation's natural resources (Barnard et al., 1985). Monitoring of forests can be defined as the long-term, periodic measurement of selected physical and biological parameters for establishing baselines and to detect and quantify change over time. With monitoring, we can determine the extent and magnitude of current conditions for specified forest values in relation to natural and anthropogenic stresses, generate plausible cause-effect hypotheses to explain these observations, and suggest management or regulatory actions where appropriate.

When the National Vegetation Survey (NVS) began in 1985, those involved in the planning of the research effort recognized:

a. The limitations imposed by time and funding.
b. The extent of the forest area in question. Probably more than 300 million acres needed to be considered.
c. The European literature and personal communications indicated that radial growth declines had preceded the appearance of visible symptoms of foliage loss and/or discoloration in the crowns of most of the species being studied.
d. The lack of a clearly defined or recognized visible symptomatology for atmospheric pollutant damage to most eastern United States forests or individual trees.
e. The availability of a number of extensive remeasured plot or increment core data sets for much of the forested area of concern.

They also were aware that scientists had observed foliar damage, unexplained decline, and death of trees of various species in the eastern United States and were raising the concern that atmospheric pollutants were involved. Thus, it seemed appropriate that the initial research should focus on the evaluation of existing data and be conducted in such a manner that early results would guide the future direction of the studies (Barnard, 1986). Four general categories of research were followed : (1) Dendrochronology, (2) Mapping and Geographic Information Systems, (3) Mensuration and Statistics, and (4) Monitoring. Progress in each of these areas of research is such that we are now revisiting the issue of additional field work to describe more fully the current health (especially the visible component) of the eastern U.S. forest resource. The availability of the manual, for Diagnosing Injury To Eastern Forest Trees (Skelly et al., 1987), as well as further experience by inventory experts in the United States and Europe were important factors in this decision.

RESULTS OF STUDIES IN THE NATIONAL VEGETATION SURVEY

A brief discussion of the results of several studies conducted by investigators with NVS funding is appropriate to this discussion. Since monitoring is the specific topic of interest, I will limit the discussion to information related to that subject.

Dendrochronology

Researchers in the NVS have been using the techniques of dendrochronology (and developing more powerful statistical applications) to enable the evaluation of evidence for change in the growth patterns of a number of eastern tree species. The effort has concentrated on New York and the New England region with some additional studies of spruce and loblolly pine in the South.

- Research studies conducted by Hornbeck, Smith and Federer (1986), Federer and Hornbeck (1987) and Van Deusen (1987) have identified a definite decline since 1960 in the basal area growth rate of second-growth stands (age 60-75 years) of red spruce and balsam fir growing at elevations less than 700 m throughout New England and New York. In-depth analysis of the data and comparison to published findings in the mid-1920s has identified stand dynamics as the major factor in the growth rate change. Although these analyses have not completely eliminated the possible role of atmospheric pollutants, THEY HAVE DEMONSTRATED THAT THE DECREASE IN BASAL AREA INCREMENT OVER THE PAST 20 TO 25 YEARS IS EXPLICABLE AT LEAST IN PART BY NATURAL GROWTH PROCESSES.

Research using increment cores collected throughout New England has demonstrated that ALL MAJOR FOREST TREE SPECIES EXCEPT BALSAM FIR AND RED SPRUCE ARE CURRENTLY GROWING AT RATES THAT EQUAL OR EXCEED THE RATES PRIOR TO 1960 (Hornbeck, Smith and Federer, 1986).

A more rigorous statistical procedure (the Kalman filter) has been successfully applied to the analysis of tree ring data (Van Deusen, 1987).

Mapping and Geographic Information Systems

Scientists in the NVS are using this capability to store and display potentially interrelated data on a common base.

- The Southern Atlas, consisting of four specific data bases, is in place and is being used to address issues related to the research needs of the Southern Commercial Forest Research Cooperative. The system is designed to be responsive to direction by researchers with specific information needs related to vegetation, climate, soils, and atmospheric deposition in particular regions. Currently the data base is complete for Georgia and North and South Carolina. Data for the remaining southern states are being input as rapidly as possible.

The location and condition of all the spruce/fir stands in the southern Appalachian Mountains are stored in the GIS. Maps and appropriate data summaries are prepared and made available on a regular basis.

The GIS contains the location and local land-use description of all atmospheric monitoring stations in the South. These data have been used to define procedures and to address other questions specific to the use of air pollution and deposition data for evaluation of forest conditions in the South.

Mensuration and Statistics

Researchers in the NVS have been analyzing data collected according to standard forest mensurational procedures and techniques. Using statistical procedures to assist in the identification of the distribution patterns of these biological data, they apply the rules of probability to the evaluation of the data. Specific applications have included the deposition gradient studies in Pennsylvania and the North Central region, the Southeastern Station's Forest Inventory and Analysis (FIA) data base, and the southwide softwood remeasurement data base. Each of the above data bases is in place and currently undergoing specific statistical evaluation. There have been some findings of a preliminary nature, but major results are anticipated later this year.

- Statistical analysis and modeling of the FIA data set has assisted in the clarification of hypotheses regarding the stand development factors and associated

> changes in stand growth patterns for loblolly pine (Sheffield et al., 1985 and Knight, 1987). Recent, more rigorous statistical analyses have verified the earlier finding of a significant difference in the growth of natural loblolly pine in the Piedmont region of Georgia and North and South Carolina between the last two decades. A study nearing completion will identify the significant factors, either natural or anthropogenic, associated with the change.

Analyses of data from the two gradient studies are moving toward completion and several manuscripts have been prepared. One manuscript describes a sulphur gradient in the forest soils that corresponds to the deposition gradient for that region.

Monitoring

Since the NVS began, scientists have been keenly aware of the need to monitor the forest resource with respect to its condition or health status. Although existing inventory activities enabled some level of comparison analysis, it was not possible to make the detailed statistical evaluations needed to define and identify probable causes. While defining and identifying what a monitoring effort should include, researchers also identified other specific procedural and measurement needs which have been addressed. The most significant of these was the documentation of atmospheric deposition symptoms on trees.

- A manual with over 100 color plates showing recognized air pollution damage symptoms on trees and indications of other nonpollutant stresses that may produce similar symptoms has been prepared by a panel of experts and is now available for use by scientists, foresters, pathologists, and other interested individuals. A German translation of the text and figures has been prepared and is in the printing process.

Remotely-sensed data from a satellite thematic mapper is being evaluated for its potential as an early indicator of stress that may be induced by atmospheric pollutants in forest trees. This summer, photographic representations of selected bands from the TM scenes are being evaluated at the intensive research sites of the Spruce/Fir Research Cooperative by field crews familiar with the forest conditions at each site. Evaluation of results is anticipated early this fall.

A bioindicator survey for ozone injury in natural loblolly pine stands in the Piedmont of North Carolina, South Carolina and Georgia was conducted (Chappelka et al., 1986). The study identified and clarified the plants (both trees and shrubs) most likely to be of value as indicators in future survey or monitoring work in the Piedmont. However, as Jones and Heck have noted in Worf (1980), plants used as bioindicators do not serve as monitors as I have defined monitoring in this paper. They are indicators of pollution presence above some threshold level.

A series of plots has been established in the spruce/fir forests of New England and New York and repeated visits by trained pathologists and entomologists are ongoing to identify and describe the development of damage symptoms on individual trees (Miller-Weeks et al., 1987). Whenever possible, the cause of such damage is being identified and documented. The European and an American visual damage assessment procedure are being used as part of this study. They provide a baseline and some data for comparison to survey data being distributed by the UNECE annual forest damage survey. The third series of measurements is underway this field season. No specific air pollution related damage symptoms have been identified thus far in the study.

DISCUSSION

Research is underway to develop and implement appropriate inventory techniques to assess the current health of forest trees and stands. This includes evaluations of procedures to identify and rate specific kinds of air pollution damage on forest trees, field plot measurement procedures and techniques, remote sensing applications, soil and moisture rating procedures, and the assessment of current impact of air pollutants on species with known visible symptoms. The manual for diagnosing visible symptoms is published. The Spruce Symptomatology study has established baseline information on visual symptoms occurring in these forest stands. Some contrasts have been noted between red spruce in the U.S. and spruce data from Europe.

Atmospheric deposition monitoring has identified regional gradients in the deposition of a number of species of air pollutants. Several such gradients are being studied to identify possible impacts of such deposition on forest growth and soil chemical properties. There are two studies sponsored by the NVS, one across western and central Pennsylvania and the other across Minnesota and Michigan, to evaluate the impact of sulphur deposition on growth of trees in the forests of those areas. Data collection and laboratory analysis is completed and analysis are underway. Publications presenting initial results of the analysis of these data are prepared. One manuscript discusses the verification of a sulphur gradient in the forest soil that corresponds to the atmospheric sulphur deposition gradient for the region.

With the implementation of the GIS Atlas for the South, researchers can make use of the system on an as needed basis. They can develop maps that describe the vegetation, climate, soils, and atmospheric deposition patterns for specific areas of interest. In this way it is possible to identify locations where the potential for air pollution impacts on forest vegetation is greatest. This will guide the development of monitoring activities as well as focusing research on damage appraisal and growth changes.

The need to begin monitoring and visible symptom identification on forest trees is evident. The study of incremental growth in trees has not identified a measurable pollutant-related response. Inventory data and procedures, while providing important insights, have not met present analytical needs of researchers in the Forest Response Program. We realize that part of this problem was due to the original design of the inventories involved. Now we have an opportunity to design and perhaps implement a monitoring program as a legacy of research on the effects of acidic deposition on forests. A proposal for a substantial research effort to identify and correlate all aspects of forest condition monitoring into an implementable package has been developed and will be reviewed later this summer prior to its circulation as a request for research proposals.

References

Barnard, J.E. 1986. National Vegetation Survey/Forest Response Program. In Proceedings, Fourth regional technical conference, Appalachian Society of American Foresters: Atmospheric deposition and forest productivity. Raleigh, NC, January 29-31, 1986.

Barnard, J., Myers, W., Pearce, J., Ramsey, F., Siisenwine, M. and Smith, W. 1985. Surveys for monitoring changes and trends in renewable resources: forests and marine fisheries. American Statistician 39(4): 363-373.

Chappelka, A.H., Chevone, B.I., Brown, H.D. and Anderson, R.L. 1986. Bioindicator survey for ozone injury in Georgia, North Carolina, and South Carolina [Abstract] Phytopathology 76(10) No. 222:1085.

Federer, C. Anthony and Hornbeck, James W. 1987. Expected decrease in diameter growth of even-aged red spruce. Canadian Journal Forest Research 17:266-269.

Hornbeck, James W., Smith, Robert B, and Federer, C. Anthony. 1986. Growth decline in red spruce and balsam fir relative to natural processes. Water, Air, and Soil Pollution 31:425-430.

Miller-Weeks, M., Millers, I., and Cooke, R. 1987. Description of crown symptoms on red spruce (*Picea rubens* Sarg.) and balsam fir (*Abies balsamea* (L.) Mill.) in the Northeastern United States-A Progress Report. In Proceedings of the effects of atmospheric pollution on spruce and fir forests in the eastern United States and the Federal Republic of Germany; Burlington, VT. October 19-23, 1987. (in press).

Knight, Herbert A. 1987. The pine decline. Journal of Forestry 85(1):25-28.

Sheffield, R.M., Cost, N.D., Bechtold, W.A., and McClure, J.P. 1985. Pine Growth reductions in the Southeast. USDA For. Serv. Resource Bull. SE-83. U.S. Department of Agriculture, Forest Service, Southeastern Forest Experiment Station. Asheville, NC. 112 pp.

Skelly, J. M., Davis, D. D., Merrill, W., Cameron, E. A., Brown, H. D., Drummond, D. B., and Dochinger, L. S. 1987. Diagnosing injury to eastern forest trees: A manual for identifying damage caused by air pollution, pathogens, insects, and abiotic stresses. The Pennsylvania State University, College of Agriculture, University Park, PA. 120 pp.

Van Deusen, Paul C. 1987. Testing for stand dynamics effects on red spruce growth trends. Canadian Journal Forest Research 17:1487-1495.

Worf, Douglas L. 1980. Biological Monitoring for Environmental Effects. Lexington Books, D.C. Heath and Company. Lexington, MA. 227 pp.

The Use of Biomarkers to Monitor Forest Damage in Europe

J. N. Cape
Institute of Terrestrial Ecology Bush Estate, Penicuik EH26 OQB Midlothian, Scotland

ABSTRACT

Forest health is now routinely monitored in most Western European countries, using a common protocol based upon visual assessment of leaf loss, crown structure and discoloration. Problems of observer bias have been recognized, and training is now more thorough. There are also problems of interpretation; it is not clear what are "normal" patterns, and how they vary with latitude, maritime/continental climates and altitude. Many hypotheses have been formulated to explain observed patterns of damage.

Attention is now being turned to alternative methods of assessment, aimed at providing objective quantitative measurements, and early diagnosis (i.e., pre-visible symptoms.) These techniques, still in the exploratory stages, rely on three approaches: (i) comparison of visibly damaged and undamaged trees at the same sites, (ii) comparison of trees at different sites exposed to different pollution climates, and (iii) direct controlled experiments (e.g., whole trees in open-top chambers). Specific biochemical/physiological tests have been applied using some or all of these three approaches. Collaborative research and further development are required before such tests can be applied in large-scale field surveys.

INTRODUCTION

The possibility that the long-range transport of air pollutants derived from fossil fuel combustion could cause damage to forest health was raised by Scandinavian scientists some 16 years ago (Anon., 1972). However, it was not until this decade that the possibility became a reality in the public mind, following a widespread visible decline in forest health in West Germany. The scientific community remains to be convinced, perhaps not that air pollutants are involved, but that air pollutants are primarily responsible for the observed effects.

MONITORING FOREST HEALTH

One of the difficulties encountered has been the quantification of this 'new' type of forest decline, both in space and time. Systematic surveys of forest tree health started in W. Germany in the early 1980s and are now routinely undertaken in most European countries, under the auspices of the United Nations Economic Commission for Europe (UNECE) (Anon., 1987a). Within the European Economic Community, such surveys, based on a uniform 16 x 16 km grid with protocols drawn up by UNECE, are now mandatory (Anon., 1986a). In addition, several countries conduct more detailed studies using their own protocols. In Britain, for example, the grid-based survey is complemented by a stratified sampling system for economically important tree species in 12 regions (Innes & Boswell, 1987). Damage is scored visually in late summer each year by comparison with "standard" illustrations for each of the major tree species (e.g., Bosshard, 1986). The scoring system is based upon crown structure, leaf loss and foliar discoloration (Table 1).

Table 1. Criteria used under the UNECE protocol for visual assessment of damage.

• Grid 16 km × 16 km intersecting with woodland area > 0.5 ha.		
• Results reported for 5 most important broadleaved species and 5 most important conifer species.		
• Crown density (leaf loss) expressed in 5 categories:		
0	0-10%	no damage
1	11-25%	indicative
2	26-60%	moderate damage
3+4	> 60%	severe damage and death
• Discoloration expressed in 4 categories		
0	0-10%	none
1	11-25%	indicative
2	26-60%	moderate
3	> 60%	severe

It has become apparent that such survey techniques require thorough training of assessors. The documented biases in the earlier W. German surveys (Krause et al., 1986) have also been observed in Britain (Innes et al., 1986). Training is now an important component of international cooperation. Since efficient methods have been established, the apparent vitality of W. German conifer forests has changed little, but broadleaved species such as beech (*Fagus sylvatica*) and oak (*Quercus spp*.) are showing a clear, if slow, deterioration (Anon., 1986b). It is too early yet to identify time trends from other national surveys.

The debate on the efficacy of such surveys continues, despite the agreed protocols. Some simple sources of error, such as sampling with or against the light, are amenable to assessor training. The most serious point of contention is the use of "standard" reference photographs over a wide range of latitude, altitude and ecotype. It has been suggested (Kilz & Hanisch, 1988) that separate standards should be set up for different types of ecosystems, became crown form and crown density are known to be influenced strongly by environmental factors other than air pollution. This suggestion may overcome difficulties associated with crown form, but in the absence of good historical data it is impossible to define "normal" crown density at sites which are now subject to air pollution stress, and the interpretation of the visible "damage" remains subjective. Other refinements have been suggested, such as surveying forests twice yearly. More frequent observations would enable the crown structure of deciduous trees to be evaluated better, and pathogenic influences to be identified. However, resources are unlikely to be available to progress from a simple inventory toward the identification of the possible causes of the observed symptoms.

The visible "damage" symptoms (leaf loss and discoloration) used in such monitoring surveys of this type are non-specific, and may be produced by biotic, climatic or edaphic factors in the absence of air pollution. Visible symptoms alone give no information on the role of air pollutants. In general, data on the levels of air pollution are unavailable for the forest sites where symptoms appear, so it is not even possible to look for spatial or temporal correlation between air pollution damage and symptoms. More specific markers are required to link forest decline with air pollution.

THE USE OF BIOMARKERS

A biomarker is any biological measure which may be used to monitor the effects of stress on plants, at every level from the plant cell to the ecosystem. The range of possible biomarkers may be subdivided into visible biomarkers (usually giving qualitative information) and non-visible, which may give qualitative and quantitative information.

The objective of the study must be defined before an appropriate biomarker is selected. If one wishes to use plants as biological indicators of air pollution exposure, lichens, herbaceous and woody plants may be chosen to indicate critical levels of exposure to specific air pollutants (Arndt et al., 1987). If one wishes to study subtle effects of air pollutants on forest ecosystems, then it may be more appropriate to use sensitive herbaceous species on the forest floor as bioindicators, or to study changes in species composition. There have been large changes in the species composition and production of fruiting bodies of ectomycorrhizal fungi over the past two decades in the Netherlands (Anon., 1987b), which are thought the result from excess deposition of nitrogen. The above are examples of the use of visible biomarkers, or bioindicators.

In studying forest decline, however, most attention has been given to detailed investigations of trees, and non-visible biomarkers of tree health have been developed to extend observations beyond the visible symptoms of crown thinning and discoloration. Recent investigations of the vitality of forest trees in Europe have been reviewed by Cape (1988), and the methods used are summarized in Table 2.

Table 2. Methods for investigating the effects of air pollutants on trees (after Cape, 1988).

microscopic-	external surfaces
	cell ultrastructure
'whole leaf'-	electrolyte leakage
	element composition
external surfaces-	leaf wettability
	amount and composition of surface wax
	Härtel turbidity test
physiological-	photosynthesis/respiration
	water relations
	biochemical

(nondestructive)-	reflected light (remote sensing)
	induced fluorescence
	electron paramagnetic resonance
	nuclear magnetic resonance
	emission of organic compounds
	biochemical
(destructive)-	enzymes and substrates/precursors
	pigments
	other metabolites
	buffer capacity
roots-	elemental analysis
	mycorrhizal associations

The ways in which such methods are used may be classified under three headings:

i) comparison of visibly damaged and non-damaged trees at the same site.
ii) comparison of trees (usually showing some damage symptoms) at different sites.
iii) experimental manipulation of whole trees or parts of trees, to determine the types of measurement which give clear indications of specific stresses, and which may be useful for diagnosis under field conditions.

In the first case, it may be assumed that climate and exposure to pollutants are similar across the site, and, more dubiously, that soils are homogeneous. Measurements may identify the limiting factors or impaired functions in individual trees showing visible symptoms, and may indicate possible causes. Markers of tolerance may then be identified as part of the genetic variability expressed in the visible symptoms. If a good correlation can be found between a biomarker (or biomarkers) and visible symptoms, then the biomarker may be used objectively to evaluate the degree of "risk" of the forest populations at that site, and in the selection of resistant genotypes for future breeding. However, as the precise cause of the differential response is not known, there are no guarantees that the same biomarker will be useful elsewhere. An example of such an approach may be found in the work of Villanueva et al. (1988); they measured a wide range of biochemical constituents of individual trees showing different degrees of damage but growing at the same site.

In the second case, there are many possible sources of variability when comparing different sites. To be useful, a biomarker should be less variable within sites than between sites, so that significant differences may be observed between sites. If biomarkers that show good spatial and temporal correlation with visible damage symptoms can be identified, then there is a basis for a more objective assessment of forest health, possibly even before the appearance of visible symptoms. One recent example is a pilot study, supported financially by the Commission of the European Communities, which involved four institutions in Britain and Germany (Cape et al., 1988). In this study, samples were taken from three tree species growing at 12 sites in a transect from southwest Germany to northeast Scotland. This material was subjected to a wide range of potential diagnostic tests (Table 3), and significant differences were sought between "damaged" and "undamaged" trees within sites, and between sites. A unique feature of this study was the wide range of tests applied to the same plant material. Some interesting correlations occurred between apparently unrelated properties; for example, the emission of ethylene from spruce needles was strongly correlated with their buffering capacity after maceration. Although some of the tests were too variable between trees to be useful as biomarkers, others allowed discrimination between sites. Of the 42 measurements made on Norway spruce, 14 were shown to be significant in terms of site discrimination. The straightforward determination of foliar nutrients ranked as the most important discriminating factor. The range of pollution climates (i.e. different mixtures of pollutants and climate) at the different sites also permitted the correlation of specific effects with exposure to different pollutants, and pointed to possible underlying mechanisms. Those tests which appeared to be useful are now being applied to experimentally-treated trees in an attempt to understand the genetic, climatic and dynamic factors affecting the response.

Table 3. The range of tests of tree vitality applied to samples of foliage collected from 12 sites in Europe as part of a study on the early diagnosis of forest decline (Cape et al., 1988).

Visible symptoms
Histology of leaf sections
Leaf wettability
Amounts of surface wax and dust
Härtel turbidity test
Hydrocarbon emission
Pigment analysis (chlorophylls + carotenoids)
Anti-oxidants (alpha-tocopherol)
Buffering capacity
Modulated fluorescence
Water relations
Nutrient analysis (S, N, K, Ca, Mg)

In the third case, experimental approaches have been of three types: the exclusion of supposed causes (for example, by growing trees in filtered or unfiltered air), the removal of symptoms (by adding fertilizer to nutrient deficient trees), and by the addition of supposed causal factors (fumigation or spraying with acid rain). Each of these approaches addresses a different aspect of the cause-and-effect relationship. In all cases a response is sought that is specific for a given treatment, and which may be used in the field to indicate the presence or absence of a particular pollutant stress.

Exclusion of supposed causes may narrow the range of possible causal factors if an effect is observed, but it may not give sufficiently detailed information to indicate the appropriate strategy for pollution control. On the other hand, such experiments show the likely benefit of a given reduction in air pollutant exposure. The use of open-top chambers in studying effects of air filtration on mature trees has formed part of the CEC initiative for studying effects of air pollutants on crops and trees (CEC, 1987). Work on mature trees is expensive, and the experimental design must permit optimum use of the facility. For example, at the open-top chamber site set up by Arndt and colleagues (Arndt, 1987) at Edelmannshof in the Swabian-Frankonian Forest of West Germany, research workers from other institutions make measurements and take samples of plant material. Not all of the studies are relevant to the development of biomarkers, but the facility provides an opportunity to test potential biomarkers under conditions similar to those of a mature forest stand.

In the absence of a meaningful decrease in pollutant emissions, the use of ameliorative experiments may give little or no information on the causes of observed symptoms, but points to the feasibility of practical approaches in alleviating damage, at least in the short term. Zöttl and co-workers (Zöttl & Hüttl, 1986) have shown, for example, that discoloration caused by magnesium or potassium deficiency can be rapidly reversed by applying fertilizer, but the underlying cause of the nutrient deficiency is still unknown. Such studies may again be used to test the response of potential biomarkers.

The direct testing of supposed causal agents, following the ideas enshrined in Koch's Postulates (e.g., Last et al., 1984), has been employed primarily under well-controlled conditions in the laboratory. The aim is to reproduce symptoms typical of field conditions, and thereby establish a direct cause-and-effect relationship. Such experiments have been used both to identify potential biomarkers, and to test biomarkers against a known stress. Although this appears to be a straightforward approach, a number of problems may arise. First, if the field symptoms or biomarker responses are non-specific, reproduction of symptoms or responses experimentally in the laboratory only indicates that an applied treatment *can* produce the effect, but it does not necessarily indicate that the treatment *does* produce the effect under field conditions. Second, there are subtleties of interaction between different pollutants, and between pollutants and other stresses (climatic or pathogenic), which may be untestable in the laboratory. Under these circumstances, Koch's Postulates may be unworkable. Third, at the most basic level, such studies may experience difficulties in reproducing appropriate experimental treatments. Nevertheless, the value of such experiments is that they (i) isolate the effects of individual pollutants at all levels in the plant, from biochemical processes through to the overall effect on growth, and (ii) suggest potential biomarkers for study in the field. Microscopic examination, for example, can now distinguish between the effects of ozone exposure, winter desiccation and magnesium deficiency, based on results obtained from laboratory experiments (Fink, 1988). The best example of this experimental approach is the study based around the environmental chambers of the GSF near Munich, where complex control over pollutant exposure and climate can be achieved. A large number of measurements are being made on the exposed trees in a major collaborative experiment (see Payer et al., 1986). However, such experiments cover only a minute fraction of the combinations and permutations available in the field.

The widespread application of biomarkers will be determined by the practical constraints of logistics and finance. The benefits derived from the use of specific biomarkers (for example, a biochemical assay) will need to be seen as cost-effective relative to other techniques (for example, an increased frequency of scoring visible damage). However, biomarkers that measure the biochemical and physiological responses of trees to their environment provide the best means of quantifying non-visible injury and identifying causes. The attention given to tree health in relation to air pollution stress has highlighted the deficiencies in our knowledge of the fundamental biochemical and physiological mechanisms that operate in forest trees.

CURRENT DEVELOPMENTS

A recent CEC workshop on Forest Decline Symptomatology (CEC, 1988) concluded that the problem of forest decline cannot be solved by foresters or biochemists working in isolation, and that an intensive multi-disciplinary approach should be adopted at a few key sites. Specific biomarkers need to be identified which can be applied on a large scale in forests, and several research groups in Europe are working toward this end. The scale of the problem is encouraging a multi-disciplinary approach, but too often the research effort is fragmented, with intensive measurement of air pollutants at one site, and intensive research into tree physiology and biochemistry at another. The major funding agencies have attempted to bring together the different approaches, and publish regular reports. The French "DEFORPA" (Dépérissement des Forêts Attribué à la Pollution Atmosphérique) programme (Landmann, 1988) and the West German "PEF"

(Projekt Europäisches Forschungszentrum für Massnahmen zur Luftreinhaltung) project publish regular reports in conjunction with the CEC, which also publishes its own series of "Air Pollution Research Reports" (in English) as the proceedings of workshops held under its auspices.

SUMMARY

In Europe, national and international surveys of forest health have been developed in response to widespread visible symptoms of decline in both coniferous and broadleaved trees. These surveys cannot of themselves be used to establish cause-and-effect relationships, and many studies are now in progress on the use of tree biomarkers in detecting and quantifying damage attributable to air pollutants. There are, however, a number of questions that arise, and a number of statements that can be made.

1) In the visual assessment of tree vitality, can or should allowance be made for the influence of known factors such as climate or genotype? Does a "normal" tree exist, against which comparisons can be made?
2) Within sites, a range of damage symptoms may occur. Detailed studies of individual trees at the same site may give quantitative information, and may be used to identify markers of damage before visible symptoms appear.
3) Between sites, a biomarker used as a diagnostic test must show a correlation with visible symptoms.
4) It is unlikely that cause-and-effect relationships can ever be established from field data. Plausible mechanisms of action of air pollutants or other factors may be derived from well-designed and controlled manipulative experiments on trees. Such experiments are more easily performed on small or young trees, and extrapolation to older trees in forests is unlikely to be straightforward.
5) In field based studies there is a need to use as much information as possible in attempting to understand ecosystem responses to air pollution stress. To this end, research efforts should be concentrated at key sites to ensure that the different disciplines (atmospheric chemistry to plant population ecology) act in coordination.
6) The dynamics of forest responses to air pollutants are poorly understood, both on short time scales (minutes) and long (decades). The sequence of events in the development of damage is also not well known, and may indicate mechanisms of cause-and-effect.

These issues were discussed at a recent workshop on forest decline symptomatology sponsored by the Commission of the European Communities (CEC, 1988). The conclusions will be used as the basis for the development of research programmes on forest decline in Europe.

References

Anon. 1972. Sweden's Case Study for the UN Conference on the Human Environment. The Impact on the Environment of Sulfur in Air and Precipitation, Stockholm.

Anon. 1986a. Council Regulation (EEC) No. 3528/86 on the protection of the Community's forests against atmospheric pollution. Official Journal of the European Communities, L326/2-4.

Anon. 1986b. 1986 Forest Damage Survey. Federal Ministry of Food, Agriculture and Forestry, Bonn.

Anon. 1987a. Manual on methodologies and criteria for harmonized sampling, assessment, monitoring and analysis of the effects of air pollution on forests. Convention on Long-range Transboundary Air Pollution, International Cooperative Programme on Assessment and Monitoring of Air Pollution Effects on Forests. UNECE, Geneva.

Anon. 1987b. Qualitative and quantitative research on the relation between ectomycorrhiza of Pseudotsuga menziesii, vitality of the host and acid rain. Report 25-01 Agricultural University, Wageningen.

Arndt, U. 1987. Open-top chamber projects in Hohenheim and Edelmannshof. Pp. 299-317 in Microclimate and plant growth in open-top chambers. CEC Air Pollution Research Report 5 (EUR 11257). CEC, Brussels.

Arndt, U., Nobel, W., & Schweizer, B. 1987. Bioindikatoren: M⊃glichkeiten Grenzen und neue Erkenntnisse. Ulmer, Stuttgart.

Bosshard, W. (ed). 1986. Kronenbilder. Eidgenössische Anstalt für das Forstliche Versuchswesen, Birmensdorf, Switzerland.

Cape, J.N. 1988. Recent developments in the diagnosis and quantification of forest decline. Pp. 262-305 in Air Pollution and Ecosystems (P. Mathy, ed.). D. Reidel, Dordrecht.

Cape, J.N., Paterson, I.S., Wellburn, A.R., Wolfenden, J., Mehlhorn, H., Freer-Smith, P.H., & Fink, S. 1988. Early Diagnosis of Forest Decline. Institute of Terrestrial Ecology, Grange-over-Sands, UK.

CEC. 1987. Microclimate and plant growth in open-top chambers. Air Pollution Research Report 5. (EUR 11257), CEC, Brussels.

CEC. 1988. Forest Decline Symptomatology. Air Pollution Research Report (in press) CEC, Brussels.

Fink, S. 1988. Histological and cytological changes caused by air pollutants and other abiotic factors. Pp. 36-54 in Int. Symp. Air Pollution and Plant Metabolism, S. Schulte-Hostede, N.M. Darrall, L. Blank, and A.R. Wellburn (eds), Elsevier, London.

Innes, J.L., Boswell, R., Binns, W.O. & Redfern, D.B. 1986. Forest health and air pollution: 1986 survey. Forestry Commission Research and Development Paper 150. Forestry Commission, Edinburgh.

Innes, J.L., & Boswell, R.C. 1987. Forest Health Surveys 1987, Part 1: Results. Forestry Commission Bulletin 74, HMSO, London.

Kilz, E., & Hanisch, B. 1988. Characterization of visible symptoms. In Forest Decline Symptomatology, CEC Air Pollution Research Report, Brussels (in press).

Krause, G.H.M., Arndt, U., Brandt, C.J., Bucher, J., Kink, G., & Matzner, E. 1986. Forest decline in Europe: development and possible causes. Water, Air, and Soil Polln. 31, 647-668.

Landmann, G. 1988. Les recherches sur le dépérissement des forêts en France: structure et principaux resultats du programme DEFORPA. Pp. 261-281 in Air Pollution & Ecosystems (P. Mathy, ed.). D. Reidel, Dordrecht.

Last, F.T., Fowler, D., & Freer-Smith, P.H. 1984. Die Postulate von Koch und die Luftverschmutzung. Forst. Centralblatt, 103, 28-48.

Payer, H.D., Bosch, C., Blank, L.W, Eisenmann, T., & Runkel, K.H. 1986. Beschreibung der Expositionskammern und der Versuchsbedingungen bei der Belastung von Pflanzen mit Luftschadstoffen und Klimastress. Forstw. Cbl. 105, 207-218 (see also other papers in this volume).

Villanueva, V.R., Mardon, M., Moncelon, F., & Santerre, A. 1988. Biochemical markers in polluted Picea trees. Pp. 88-00 in 2nd Int. Syrup. Air Pollution and Plant Metabolism, GSF-Bericht 9/87, GSF München.

Zöttl, H.W., & Hüttl, R.F. 1986. Nutrient supply and forest decline in southwest Germany. Water, Air, and Soil Poll., 31, 449-462.

Bioindicators in Air Pollution Research — Applications and Constraints

David T. Tingey
U.S. Environmental Protection Agency 200 SW 35th Street Corvallis, OR 97333

ABSTRACT

Physical and chemical measurements of air pollutants provide a precise measure of pollutant exposure which is frequently used to estimate probable biological impacts. In contrast, a biological response to the exposure indicates if the exposure had a biological effect. The biological response integrates the pollutant exposure and the modifying factors of genotype, climate and edaphic conditions. There is a growing interest in developing and using bioindicators to detect early changes in plant performance. Bioindicators may be classified as either accumulators of the pollutant or reactors to the pollutant. The ultimate selection of a bioindicator(s) depends on the pollutant and the ultimate use of the data. A bioindicator should: (1) provide a readily detectable response to the pollutant; (2) be easy and efficient to use; (3) be readily related to the response(s) of interest; and (4) have a distinctive syndrome not readily confused with other causes. Bioindicators clearly have value in assessing environmental problems, but, at most, they are only indicators of a problem. Corrective or mitigative actions require additional data (e.g., air monitoring).

INTRODUCTION

When attempting to assess the impact of an air pollutant(s) on individual organisms/species or whole ecosystems, the following questions are frequently posed: (1) 'What is the current status of the species or ecosystem?" (2) "What is the current trend in the status of the species or ecosystem?" These questions imply that there is a spatial or temporal change in the status of the system that has been or can be measured. The implied hope is that the data are currently available to conduct the assessment and that no additional studies are required to provide a timely and precise answer. However, if the data are not available, the issue becomes, "What physical or biological methods are readily available to conduct the assessment?"

CLASSIFICATION OF ASSESSMENT METHODS

Physical/Chemical Methods: Physical/chemical methods (Guderian et al., 1985) can accurately describe, in "real time," the ambient exposure at a given site or series of sites. These methods are sensitive, specific and reproducible but require electrical power making it difficult to situate monitors in remote locations. Also, monitors are generally expensive to purchase and operate, requiring trained personnel. If resources and funds are available, monitors can be located throughout the study area permitting a more detailed characterization of exposure. Despite providing an accurate description of the exposure, these methods fail to consider the influence of other important factors that control biological responses. Although physical/chemical methods can establish the potential for biological impacts, they do not provide sufficient information to establish the risk of an exposure.

Biological Methods: A biological method is any response to a stress by an organism or community of organisms whose presence or other easily measured attribute can be assessed either qualitatively or quantitatively. Biological methods that include bioindicators show the presence or absence of the stress and biomonitors which attempt to provide additional information about the intensity of the stress (Arndt et al., 1987; Guderian et al., 1985, Knabe, 1982). In contrast to physical/chemical methods, a biological response cannot provide a precise measure of exposure. The biological response is influenced, not only by the concentration and duration of exposure to air pollutant(s) but also by the relative sensitivity of the organism. The relative sensitivity is a function of the stage of plant development, the previous and current edaphic and climatic conditions and the genetic composition of the plant. The unique advantage of biological response is that it integrates the influence of the various factors that control biological response; consequently they permit the direct assessment of risk from an exposure (i.e., they show if a given exposure(s) was harmful). Biological methods are frequently inexpensive and data can be obtained by periodic (e.g., monthly or yearly) visits to the sites.

The above comparisons of the various types of methods available for risk assessment lead to the obvious conclusion: there is no better indicator of the status of a species or a system than the species or system itself (i.e., biological methods). Biological methods integrate the effects of pollutant exposure and modifying factors. Consequently, biological methods should play a significant role in assessing the status of a species or ecosystem over temporal or spatial gradients, provided suitable measures of response can be found and properly applied (Guderian et al., 1985; Knabe, 1982).

CLASSIFICATION OF BIOLOGICAL METHODS

Biological methods may be classified (Arndt et al., 1987; Guderian et al., 1985) as either passive or active (Fig. 1). Passive methods use plants growing naturally in the study area, where they are ecologically adapted. However, with *in situ* vegetation it is difficult to partition the variation in response among individuals between that caused by different exposures and that caused by genotypic variation. Also, the specific responses of many species to specific pollutants have not been established, rendering a definitive diagnosis difficult. Despite these limitations, visible injury to vegetation has repeatedly provided the first indication of pollutant impacts. In contrast, active biological methods use standardized plants (known genotype and response) placed at specific locations to detect the presence of air pollutants. Bel W3 tobacco has been used extensively in North America and Europe as an active indicator to detect and confirm the widespread occurrence of photochemical oxidant air pollutants (Guderian et al., 1985).

An organism may be classified as either a reactor to or an accumulator of the pollutant (Fig. 1). A reactor displays a typical symptom or a measurable response to the pollutant. Any measurable response of an organism can be used; however, foliar injury is probably used most frequently. To be a suitable bioindicator, the response of an organism must be specific for a particular pollutant and not readily confused with other similar symptoms with different causes. In contrast to a reactor, an accumulator will not necessarily display an overt symptom but will accumulate the pollutant causing a significant tissue enrichment. Accumulators are only suitable for pollutants (e.g., fluorides or heavy metals) that have a long residence time in the tissue or for which the metabolic products are known.

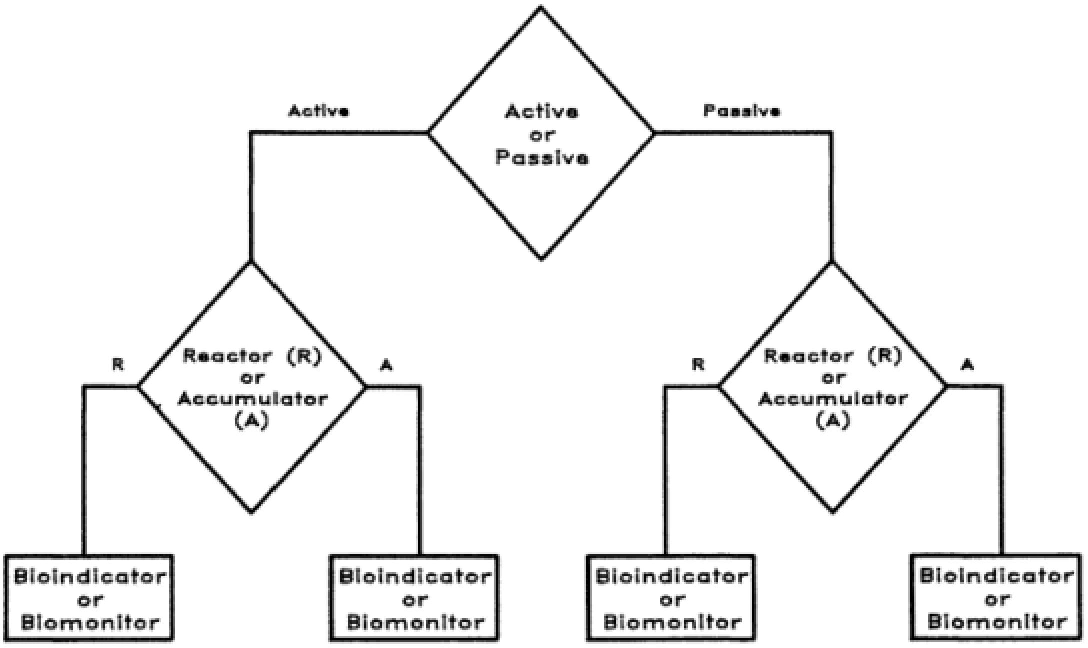


Figure 1.
A classification of biological methods used in air pollution studies.

A distinction is frequently made between using organisms as bioindicators or biomonitors (Fig. 1). A bioindicator is an organism or biological response that reveals the presence or absence of an air pollutant by the occurrence of typical symptoms or measurable responses. A biomonitor provides information on the presence of the pollutant and attempts to provide additional information about the amount or intensity of the exposure. The bioaccumulation of pollutants has been proposed as a biomonitor. However, it is difficult to relate tissue concentrations of a pollutant to a specific concentration and/or duration of exposure because the tissue levels are influenced by the temporal nature of the pollutant occurrence as well as edaphic, climatic and biological factors. In general, plants exposed to low concentrations absorb higher tissue levels than plants exposed to high concentrations that readily injure and kill the tissue. At this time, bioindicators are clearly important and have a history of use. However, biomonitors are not sufficiently defined and calibrated with respect to exposure to be reliable.

BIOINDICATORS

In various forms, bioindicators have been used almost as long as we have been aware of air pollution problems, and there appears to be a renewed interest in using bioindicators to solve environmental problems. The primary goal of environmental protection is to protect humans, animals, plants and materials from adverse effects, not to reduce emissions or ambient concentrations. Bioindicators have a clear advantage over physical/chemical monitors because they indicate the status of the organism. A broad range of possible plant responses could potentially be used as bioindicators (Table 1).

Table 1. Examples of possible plant responses that could be used as bioindicators.

Plant Biomass	Tissue Analysis
Growth/yield	Metabolite pools
Tree-ring analysis	Pollutant concentrations
Morphological Responses	Plant Water Relations
Foliar symptoms	Transpiration
Cellular/ultrastructural changes	Water potential and its components
	Membrane permeability
Physiological Responses	
Photosynthesis	Enzyme Activity
Respiration	
Stable isotope ratios	Plant Pigments
Bark Turbidity	

Because plants and ecosystems function across a wide range of scales of time and space, a diverse array of responses ranging from cellular processes (i.e., photosynthesis) to ecosystem responses (i.e., community changes) can be used to assess the state of the system.

The selection of a bioindicator(s) should consider several factors:

- be easily measured and describe responses of concern within the ecosystem.
- have a distinct response which is capable of predicting how the species/ecosystem will respond to the stress.
- measure the response with acceptable accuracy and precision.
- be based on a knowledge of the pollutant and its characteristics.
- consider the final use of the data.

Although not a requirement, bioindicators are frequently selected to characterize responses of concern to humans. The bioindicator may be the response itself or related to the response of interest. For example, people are frequently interested in forest productivity but its measurement is a difficult process that is time consuming and can be expensive and uncertain. Consequently, a bioindicator or a surrogate for productivity, such as mid-summer leaf starch or leaf duration may be used rapidly to assess the status of the system. The hope is that these indicators will have sufficient accuracy and can rapidly and more easily be measured than productivity.

The selection of a bioindicator must consider the intended use of the data (Knabe, 1982). Will the data be used to determine the status of a species or the status of a community or ecosystem? A single bioindicator is not suitable for providing information at all levels of biological organization. The bioindicator must be appropriate for the different scales of time and space at which plants and ecosystems operate. Is the bioindicator being used to determine if there is an environmental problem in an area or to establish the bounds of a known problem? What level of accuracy and precision is needed? There is no reason to use a method that yields greater precision and accuracy than needed for the intended use of the data. Will the bioindicator be used to assess the effects of air pollutants in the field or a laboratory study?

Knowledge of the particular air pollutant(s) is a principal consideration in the choice of a bioindicator(s). Not all bioindicators are appropriate for assessing the impacts of all air pollutants (Knabe, 1982).

EXAMPLES OF BIOINDICATORS AND THEIR LIMITATIONS:

Foliar symptoms are probably the most widely used bioindicator. There are numerous publications documenting the symptoms caused by specific air pollutants on a wide variety of plant species (e.g., Jacobson and Hill, 1970; van Haut and Strattman, 1970; Manning and Feder, 1980). The presence or absence of foliar injury has been used to establish zones of impact, while the type of foliar injury has been used to discriminate among various possible air pollutants. For example, foliar symptoms of ozone injury have been used extensively in field studies to establish the presence of ozone and the extent of its impact (Guderian et al., 1985). These applications are based on the assumption that ozone produces a unique set of foliar symptoms which are not produced by other air pollutants or stressors. This assumption does not hold; for example, mixtures of ozone and sulfur dioxide yield symptoms that are indistinguishable from those produced by ozone alone (Tingey et al., 1973). This problem is highlighted by the fact that publications describing symptoms of air pollutant injury also describe mimicking symptoms (i.e., symptoms induced by other stress factors which are readily confused with known air pollutant symptoms). Another limitation of foliar symptoms is that not all species have been exposed to all known air pollutants to establish their symptom expression. Consequently, it is still an art to diagnose the probable air pollutant on an untested species or genotype in the field. Additional data, such as symptoms on adjacent (previously documented) species/genotypes or the availability of air monitoring data displaying elevated concentrations of the suspected air pollutant in the proper temporal sequence, are required.

Elevated tissue concentrations of various elements has been extensively used to establish the presence of various air pollutants (Arndt et al., 1987). When chemical analysis is used, it is especially important to have some knowledge about the identity of the suspected air pollutant and its possible source(s). For example, if one has no knowledge about the probable air pollutant, it is very difficult to analyze plant tissue to determine if there are abnormal concentrations or accumulations of any inorganic element or organic compound in the tissue. These broad ranging analyses are very expensive and require large amounts of tissue and numerous extraction and analytical techniques in the hope of finding elevated levels of an element or compound that can be judged to be a pollutant. Even if this broad spectrum analysis fails to identify a single compound, one cannot dismiss the cumulative impact of several elements/compounds as a possible problem. However, when the suspected source of the pollutant or the likely chemical contaminant is known, it is much easier to pose focused questions, e.g., "Are fluoride levels elevated in the tissue?" "Is the tissue enriched in cadmium?" When the probable source is known, the optimum sampling design and analytical techniques can be chosen to determine the possible degree of contamination. Tissue concentrations of various materials have been used as both passive and active bioindicators to map the concentrations of fluoride and various metals around pollution sources (e.g., Arndt et al., 1987; Tingey et al., 1979). Once the concentration zones are mapped, then other biological responses, such as increased peroxidase activity, can be associated with various levels of the toxicant (Keller and Schwager, 1971). Tissue concentrations integrate the total exposure at the site, but it difficult to use this method as a biomonitor. Although tissue concentrations are dependent on the pollutant of exposure, the extent of tissue enrichment is influenced by several other factors, such as the location of the tissue on the plant, the plant developmental stage, environmental conditions and metabolic vigor of the organism. Plants absorb higher tissue levels when exposed to low concentrations than to high concentrations that injure the tissue. Enriched tissue concentrations alone is not a sufficient proof that a pollution problem exists. Additional analyses must establish that the tissue enrichment resulted from contamination by an anthropogenic source.

The identification of a proper control is critical when bioindicators are used to establish changes in plant performance over gradients in time or space in the field. This problem is highlighted by several examples. Air pollutants and other chemical stresses, as well as various abiotic and biotic stresses stimulate increased ethylene evolution by vegetation (Tingey, 1980). This observation has been used to develop plant bioassays for a broad range of chemicals (Tingey 1980). However, it is doubtful if this approach can be used in the field because of the difficulty in establishing normal and abnormal ethylene emission rates. Also, this elevated evolution of ethylene is a transient response. Similar difficulties exist when using plant pigment concentrations or a broad range of physiological or metabolic responses. Even the measure of plant growth suffers from the problem of establishing the appropriate control.

Care must be used in the interpretation of bioindicator data in air pollution studies. In most cases cause-and-effect and specificity of the response have not been established. For example, ozone has been shown to induce the formation of hydroxyl radical adducts in chloroplast DNA (Floyd, personal communication). However, the stability of the adducts has not been determined and it is not known if the adducts are formed by other stresses. Consequently, the use of this response as a bioindicator is very limited. Similar types of problems limit the application of most bioindicators.

Bioindicators provide at most correlative responses that are not sufficient to instigate mitigative actions. A bioindicator response is analogous to having an elevated fever and going to the doctor. The fever is an indication of system dysfunction; it does not tell you what the disease is. Additional information and tests are required before the doctor can diagnose and propose a cure. So it is with bioindicator responses; a response is indicative of a potential problem but additional studies are required to establish the problem and its magnitude. Only after the cause of the problem is established can specific mitigative or restorative actions be taken. Both physical/chemical and bioindicator methods have unique attributes and a combination of both approaches is necessary to conduct an adequate environmental assessment.

CONCLUSIONS

Because bioindicators integrate the influence of environmental factors and provide direct measures of phytotoxicity of exposure(s) (i.e., risk), they clearly provide desirable information for environmental assessment. However, before they are used several factors must be established.

A bioindicator should provide readily detectable responses to the pollutant in the organism or the system of interest.
A bioindicator should be easy and efficient to use.
It is not necessary that there be a cause-and-effect association but the bioindicator must be a reliable predictor of the status of the organism or system.
A bioindicator should produce distinctive symptoms that are not readily confused with mimicking symptoms produced by other environmental stresses.
Both biological response and physical/chemical monitoring data are required for an environmental assessment program.

References

Arndt, U., W. Nobel, and B. Nobel. 1987. Bioindikatoren. Möglichkeiten, Grenzen und neue Erkenntisse. Eugen Ulmer Verlag, Stuttgart, Federal Republic of Germany.
Guderian, R., D.T. Tingey, and R. Rabe. 1985. Part 2. Effects of photochemical oxidants on plants. In Air Pollution by Photochemical Oxidants, (ed) R. Guderian. Springer Verlag. Berlin.
Jacobson, J.S., and A.C. Hill. 1970. Recognition of Air Pollution Injury to Vegetation: A Pictorial Atlas. Air Pollution Control Association, Pittsburgh, PA.
Keller, Th., and H. Schwager. 1971. Der Nachweiss unsichtbar ("physiologisher") Flour-Immionsschädigungen an Waldbäumen durch eine einfache Kolorismetrische Bestimmung der Peroxidase-Activität. European Journal Forest Pathology 1:6-18.
Knabe, W. 1982. Monitoring of pollutants by wild life plants and plant exposure: Suitable bioindicators of different immisions types. Pp. 59-72 in Monitoring of Air Pollutants by Plants Methods and Problems, (ed) L. Steubing and H-J Jäger. Dr. W. Junk Publishers, The Hague.
Manning, W.J., and W.A. Feder. 1980. Biomonitoring Air Pollutants with Plants. Applied Science Publishers, London.
Tingey, D.T. 1980. Stress ethylene production-a measure of plant response to stress. HortScience 15:630-633.
Tingey, D.T., R.A. Reinert, J.A. Dunning, and W.W. Heck. 1973. Foliar injury responses of eleven plant species to ozone/sulfur dioxide mixtures. Atmospheric Environment 7:201-208.

Tingey, D.T., R.G. Wilhour, and O.C. Taylor. 1979. The measurement of plant responses . Pp. 7-1 to 7-35 in Handbook of Metholodology for the Assessement of Air Pollution Effects on Vegetation. (eds) W.W. Heck, S.V. Krupa, and S.N. Linzon. Air Pollution Control Association, Pittsburgh, PA.

van Haut, H., and H. Stratmann. 1970. Farbtafelatlas über Schwefeldioxid-Wirkungen an Pflanzen. W. Girardet Verlag, Essen, Federal Republic of Germany.

Biological Markers: New and Emerging Technologies

Peter J. H. Sharpe
Biosystems Research Group Department of Industrial Engineering Texas A & M University College Station, TX 77843
Richard D. Spence
Biosystems Research Group Department of Industrial Engineering Texas A & M University College Station, TX 77843

ABSTRACT

Widespread, chronic, low concentrations of atmospheric pollutants lead to circumstances where visible impact upon forests is generally not evident. For this reason an array of reliable markers is needed to assess physiological damage to vegetation long before it becomes visible. Several new and emerging technologies offer promising techniques for use as markers. Short-lived radioisotopes (SLR) of metabolizable compounds can now be introduced to plants and their assimilation, translocation and allocation patterns characterized *in vivo*, nondestructively, and in real time as functions of pollutant impact. Nuclear magnetic resonance (NMR) has recently developed into a powerful tool for quantitative analysis of metabolic processes in living tissues, identifying chemical groups and their concentrations. Infrared reflectance (IR) techniques have been used extensively in analytical laboratories and are now a viable technique for physiological measurements in intact plants. Finally, the development of fiber optics, semiconductor and membrane technologies has set the stage for the near-future development of a variety of microsensors capable of making physiological and biochemical measurements localized to the level of single cells or tissues. These latter techniques provide even more opportunities for markers yet to be identified. The implementation of short-lived radioisotopes to measure biological markers in field environments is discussed, together with the cost-effectiveness of emerging technologies.

INTRODUCTION

Air pollutant effects on plants, especially impacts at low concentrations where visible injury is not evident, have been difficult to assess. Measurements of biochemical process rates, such as photosynthesis, are confounded by environmental factors as well as the cryptic nature of plant physiology. The effects of pollutants are offset to some degree by compensatory mechanisms employed by the plant to overcome limitations in individual resources (Bloom et al., 1985). If air pollutants interfere with the acquisition and/or assimilation of one or more resources, and the plant as a consequence initiates compensatory shifts in metabolism, cause-and-effect relationships become difficult to establish.

Compensatory mechanisms, therefore, can hide many direct effects of air pollutants until the stresses exceed the compensatory limits. At present, there is no method for determining how near a plant community or even an individual plant is to its compensatory limit. In addition, the compensatory limit is much more than just a function of air pollution exposure. It is influenced by the availability of resources for growth, climatic variables and the structure of biotic interaction within the ecosystem or controlled experiment. The correlation of damage to dose is appropriate only at higher levels of exposure above the injury threshold. Below this threshold, the relationship between dose and response is not evident to an external observer.

NEED FOR BIOLOGICAL MARKERS

If compensatory mechanisms hide the response of plants to low doses of air pollutants, then the traditional statistically based experimental design approaches are ineffective. Alternatively, a method of measurement is required to identify and quantify the degree to which compensatory changes are occurring within the plant. Although these homeostatic shifts may not result in a reduction in overall yield, they serve as an estimate of internal stress imposed upon the plant by air pollutants. Further investigation of internal stress responses can lead to an understanding of how close the compensatory shifts are to the breakdown point, beyond which further stress leads to yield reduction or physiological disruption of normal metabolism.

A marker can therefore be defined as an indicator of the physiological strain experienced by a plant subjected to a range of interacting stresses. A marker may not be indicative of any external change in plant growth or yield, especially where anthropogenic stresses mimic the action of natural stresses. Where this occurs, the plant has an built-in compensatory response partially to overcome limitations imposed in this manner. For example, if ozone interferes with carbon dioxide assimilation, the plant might respond by treating ozone stress as a reduction in CO_2 concentration and allocating a greater proportion of photosynthate to synthesizing Calvin cycle enzymes and new leaves, until the perceived CO_2 limitation was compensated for and equilibrated with other limitations for water, nutrients and light. If this is accomplished easily, then no reduction in total yield becomes obvious. If the physiological strain cannot be compensated then reductions in yield will become evident. Even where yield reductions can be compensated, the compensation response may leave the plant vulnerable to drought, insect, disease or other natural catastrophes (Sharpe and Wu, 1985; Sharpe et al., 1985; Sharpe and Scheld, 1986).

An approach using markers contrasts with the traditional dose-response approach because it emphasizes the need for recognizing how basic processess affect plant performance. The physiological and morphological systems of the plant must be monitored to reveal how, and to what extent, the plant is affected by air pollution stresses even when no yield response is evident. The physiological and morphological measures chosen for monitoring internal changes, then, must be interpreted by physiologically based metabolic models.

DESIRED CHARACTERISTICS OF MARKERS

Ideally, a biological marker should be easily measured with a minimum of equipment and skill. In both medical and plant physiology applications, however, the greatest breakthroughs in monitoring the internal status of living systems have been accomplished using rather sophisticated biotechnology. In other words, the important markers in medicine and biology require a considerable investment in technology and expertise. The second desirable characteristic of a marker is that its interpretation should be unambiguous. There should be no uncertainty in its level of significance. It should be obvious when it occurs. Third, a marker should be manifested in such a way that it can be explained in physiological terms. To have confidence in its predictive capability as an indicator of stress compensation, a marker should form a component of the scientific framework of concepts and knowledge relating to internal biochemical and biophysical processes. Fourth, physiological markers should foreshadow morphological changes. Fifth, a marker should allow measurements that are non-destructive, *in vivo* and observable in real time. With the exception of the high cost of the monitoring technology, all of the above desirable characteristics for applying markers can be met in air pollution research using a range of new and emerging technologies.

NEW AND EMERGING TECHNOLOGIES

Although many of the new and emerging technologies have become associated with medicine, these techniques arose in the biological and bioengineering sciences. Their use has been most obvious in the medical sciences largely because of the larger funding base and the need for innovative methods for studying internal processes leading to cancer, heart disease and other intractable medical problems. There has been no reason why the plant sciences could not have maintained a lead in many of these technologies, especially for diagnosing the internal effects of air stresses on plants.

The power of personal computers attached to measurement instruments is providing new techniques to analyze and display quantitative data. Probably the most sophisticated general application of computers in emerging technologies involves Fourier transform (FT) spectroscopy. Each application of FT techniques has led to the development of whole new fields of instruments (Hirschfeld, 1985). Three examples of FT-based quantitative analysis are gas chromatography-mass spectroscopy, FT-infrared (FT-IR) and FT nuclear magnetic resonance (FT-NMR).

The power of the current approaches to "intelligent" instrumentation is demonstrated by near infrared reflectance analysis (FT-NIRA). This technique can now be used for biological analysis (Hirschfeld and Stark, 1984). The spectral signatures of various constituents are inaccurately known and heavily overlapped. Instead of conventional data reduction, NIRA uses a combination of spectral correlation and a "self-learning" algorithm. This operates by measuring a set of preanalyzed samples, cross-correlating the spectra to composition by multilinear regression, and using an optimization algorithm to select a set of measurement wavelengths and calibration coefficients for the analysis. NIRA is verified with a second, independent, preanalyzed sample set. This approach provides approximately 0.1 percent repeatable analysis using data in which a 10 percent compositional variation is barely perceptible to the eye (Hirschfeld, 1985).

In addition to IR spectral methods, Sharpe and Scheld (1986) identified three other emerging technologies for *in vivo* studies of air pollution effects on plants. These are (1) short-lived, high-energy radioisotopes (SLR) of metabolizable compounds, (2) NMR and (3) microsensors using combinations of fiber optics, semiconductors and membrane techniques. This list is not complete, as new techniques are being developed and adapted continuously. We focus in this paper on the complementary technologies offered by SLR and NMR methods.

SHORT-LIVED RADIOISOTOPE TECHNOLOGIES

The role played by isotopes, stable and radioactive, in plant physiology studies is well established. Long-lived radioisotopes (e.g.,^{14}C and ^{35}S) have been used for some years (Fares et al., 1983). The use of SLR is comparatively more recent, but the development of small accelerators, e.g., 3-5 MeV Van de Graaff generators and 14-35 MeV cyclotrons (Fares et al., 1978; McKinney et al., 1988), and the advent of minicomputers and microprocessors (Fares et al., 1978) for data acquisition and analysis have made the production and use of SLR in dynamic plant studies a viable technique. SLR have many advantages for use as physiological markers: (1) Many SLR are positron (β^+) emitters, e.g., ^{11}C, ^{13}N, ^{15}O, ^{30}P, which emit two δ-rays at 180° to each other with sufficient energy (0.511 MeV) to penetrate several centimeters of tissue, allowing for *in vivo* coincident counting highly localized within the tissue. (2) The short half life of the isotope makes it possible to conduct several experiments simultaneously or in rapid succession on the same plant under the same or contrasting sets of environmental conditions. (3) The half life of the isotope is often comparable to the turnover times of metabolic pools. (4) The nondestructive nature of the isotope means that the plant can be used as its own control. (5) The potentially high sensitivities of the experiments often reveal unsuspected phenomena. Thus, the use of SLR allows for the highly localized characterization of plant physiological processes which is non-invasive, nondestructive, *in vivo* and in real time.

In the last decade 11C (half life = 20.4 minutes), in particular, has been effective in characterizing many plant physiological processes and responses to environmental stresses. Phloem loading, unloading, concentration and transport speeds have been characterized to elucidate controlling mechanisms (Minchin and Thorpe, 1982; Goeschl et al., 1984, Magnuson et al., 1986). Phloem transport has been shown to be severely affected by temperature, especially cold temperature (Potvin et al., 1984; 1985; Goeschl et al., 1984) and by plant exposure to ozone (Sharpe et al., 1988). These last studies have all confirmed that 11C makes an excellent marker in studies of plant response to stress because it documents the severity of physiological strains long before visible damage occurs.

NUCLEAR MAGNETIC RESONANCE TECHNOLOGIES

NMR began as a technique of analytical chemistry but evolved into a methodology that has been widely used in molecular biology and studies of biosynthetics of natural products (Scott, 1985; Bax and Lerner, 1986). Roberts (1984) reviewed over 20 NMR studies of *in vivo* plant metabolism. NMR is one of the most powerful methods for quantitative analysis of metabolic processes in living tissues. Over the past 10 years, the sensitivity of commercially available NMR spectrometers has increased dramatically. The technique is based on placing the living sample in a strong, homogeneous magnetic field which aligns the nuclear magnets of certain chemical isotopes, such as ^{1}H, ^{13}C, ^{15}N and ^{31}P. These elements possess a permanent magnetic moment from their nuclear spin. Irradiation of a live plant with a pulse of the appropriate frequency range excites the nuclear magnets aligned against the external magnetic field, providing the following physiological information: (1) The positions of absorption peaks are indicative of chemical groups, their ionization states, and bindings to other chemical species of the nuclei. (2) The intensity of the spectral line is linearly proportional to the number of nuclei in a particular chemical group. This enables tissue concentrations of metabolites to be determined by NMR. (3) Where magnetic nuclei are close to other magnetic nuclei, spectral lines can be assigned to particular chemical groups if two-dimensional NMR techniques are used (Bax and Lerner, 1986). In the past decade ^{31}P NMR spectroscopy studies on *in vivo* plant and animal cell cultures have been used to measure the pH of cell cytoplasm and vacuoles (Roberts et al., 1980; Foyer et al., 1982), phosphate, ATPase and MgATP activity in root (Roberts et al., 1984) and photosynthetic tissue (Foyer et al., 1982), and the effects of ammonium (NH_4^+) vs. nitrate (NO_3^-) nutrition (Andrade and Anderson, 1986). To date, these NMR studies have focused on *in vivo* tissue cultures, but there is the promise of live whole-plant analysis. In anticipation, Roberts (1984) outlines numerous studies that could be modified to monitor changes in plant metabolism as a result of air pollutant exposure. Of particular interest are changes in carbon, nitrogen and phosphate metabolites in intact leaves, stems, and roots.

OTHER EMERGING MARKER TECHNOLOGIES

The development of fiber optics, semiconductor and membrane technologies has set the stage for the development of a variety of microsensors that are capable of taking physiological/biochemical measurements localized to the level of single cells or implanted to measure localized tissue parameters (Hirschfeld, 1985). The driver for this instrumentation is primarily biomedical research, but there is considerable potential for use in plant research. IR techniques (Hirschfeld, 1985) have been used quite extensively in analytical laboratories, and now, as a result of the space program, a method for physiological measurements has been developed for intact plants. It is claimed that measurements of such components as chlorophyll, lignin, and protein have developed to the level of accuracy obtainable by wet chemical analysis.

DISCUSSION

The successful implementation of these new and emerging biological marker technologies, unfortunately, is usually neither easy nor inexpensive. Sophisticated instrumentation and computers are required for most of these new technologies, plus well-trained researchers and technicians to operate them. In the case of SLR, for example, there is the need for (1) a dedicated accelerator near an environmentally controlled plant growth facility, (2) sophisticated electronics for tracer profile measurement, (3) an on-line computing facility for data acquisition and for the analysis of large data blocks in a relatively short time, (4) advanced and novel mathematical methods of data analysis and dynamic modeling, and (5) a multidisciplinary approach coupled with a multidisciplinary team of scientists (Fares et al., 1983). An NMR facility requires a similar large investment in facilities, instrumentation and personnel.

The major practical disadvantage to the use of many of these technologies as biological markers is that, at present, they are of limited use in field studies. So far, all studies of plant physiology or metabolism using these technologies have been laboratory studies. The difficulties in implementing these techniques in the field are obvious, revolving largely around the bulk and delicacy of the instrumentation and the need for a considerable source of power. Many of these limitations are not insurmountable, however, and limited field studies using some of these technologies could conceivably be implemented in the very near future.

The feasibility of bringing ^{11}C technology to the field has been enhanced by recent developments in storing high-activity $^{11}CO_2$ immediately after production (J.D. Goeschl and C.J. McKinney, pers. comm.). A small, portable, lead-lined pressurized storage container (whimsically called a "pig") has been charged with enough $^{11}CO_2$ so that even after several hours enough activity remains to conduct a series of viable experiments at another location. The pig is currently being tested at remote laboratory locations at Duke University. The next step entails loading detector and gas-monitoring instrumentation onto a truck with the pig and driving to an experimental field site. The only remaining requirement is electrical power, which is supplied to most research field sites anyway. At the field site the gas-delivery cuvettes could be attached to a leaf on a tree as easily as on one in the laboratory. Thus, the physiological responses of a plant to air pollutants or other environmental factors could be characterized for field conditions.

The benefit that balances the cost of establishing and maintaining sophisticated facilities, either for laboratory or future field studies, is the enormous amount of invaluable data which can be collected simultaneously over short time intervals. The ^{11}C technique, for example, can make simultaneous, nondestructive, *in vivo* measurements of transpiration, stomatal conductance, net photosynthesis both by ^{12}C exchange and ^{11}C assimilation, net photosynthate storage in and export from the leaf, turnover time and pool size of exportable products, axial speed of photosynthate translocation, activity level of translocated photosynthates, and unloading rate into any given sink tissue. The significance of these measurements is readily apparent, especially if and when they can be made in the field. The systems behavior of the overall physiological response may be characterized. Dynamic carbon allocation patterns may be observed. Genetically and environmentally induced changes in plant physiology may be evaluated. Genetic and environmental effects of physiology or growth may be measured. Many of these parameters cannot be measured through conventional laboratory or field experimental techniques and those that can usually require months of painstaking replication. When the amount of information that can be obtained in such a short period from those new technologies is compared to traditional experimental protocols, these new and emerging technologies are remarkably cost-effective.

ACKNOWLEDGMENTS

Our carbon-ll studies were made possible by grant No. 86-11 from the Air Quality/Forest Health Program, National Council of the Paper Industry for Air and Stream Improvement. For scientific collaboration and support, we thank Alan A. Lucier of NCASI; John D. Goeschl, Robert L. Musser, Charles E. Magnuson and Collin J. McKinney, Phytokinetics Division of PhytoResource Research, Inc.; Boyd R. Strain and the staff of the Duke University Phytotron;. Walter W. Heck of the Air Pollution Lab, USDA-ARS, North Carolina State University; and William E. Winner and Richard H. Waring, Oregon State University. For assistance in outlining the role of NMR, we thank A. Ian Scott and Howard J. Williams, Center for Biological NMR, Department of Chemistry, and Ray W. Flumerfelt and Bruce Dale, Department of Chemical Engineering, Texas A & M University.

References

Andrade, F.H., and I.C. Anderson. 1986. Physiological effects of the form of nitrogen on corn root tips: A. ^{13}P nuclear magnetic resonance study. Crop Science 26:293-296.

Bax, A., and L. Lerner. 1986. Two-dimensional nuclear magnetic resonance spectroscopy. Science 232:960-967.

Bloom, A.J., F.S. Chapin, and H.A. Mooney. 1985. Resource limitations in plants: An economic analogy. Ann. Rev. Ecol. Syst. 16:363-392.

Fares, Y., D. W. DeMichele, J.D. Goeschl., and D.A. Baltuskonis. 1978. Continuously produced, high specific activity ^{11}C for studies of photosynthesis, transport, and metabolism. Internat. I. Appl. Radiation and Isotopes 29:431-441.

Fares, Y., J.D. Goeschl, C.E. Magnuson, B.R. Strain, C.E. Nelson, and H.M.Sadek. 1983. Use of short-lived isotopes in the study of xenobiotic transport. Pp. 364-369 in IUPAC Pesticide Chemistry, Human Welfare and the Environment (J. Muyamoto et al., eds.). Pergamon Press, New York.

Foyer, C., D. Walker, C. Spenser, and B. Mann. 1982. Observations on the phosphate status and intracellular pH of intact cells, protoplasts and chloroplasts from photosynthetic tissue using phosphorus-31 nuclear magnetic resonance. Biochem. J. 202:429-434.

Goeschl, J.D., C.E. Magnuson, Y. Fares, C.H. Jaeger, C.E. Nelson, and B.R. Strain. 1984. Spontaneous and induced blocking and unblocking of phloem transport. Plant, Cell and Environment 7:607-613.

Hirschfeld, T., and E.W. Stark. 1984. Near-infrared reflectance analysis of foodstuffs. Pp. 505-551 in Analysis of Food and Beverages (G. Charalambous, ed.). Academic Press, Orlando, Fl.

Hirschfeld, T. 1985. Instrumentation in the next decade. Science 230:286-291.

Magnuson, C. E., J.D. Goeschl, and Y. Fares. 1986. Experimental tests of the Munch-Horwitz theory of phloem transport: effects of loading rates. Plant, Cell and Environment 9:103-109.

McKinney, C.J., Y. Fares, C.E. Magnuson,, C.H. Jaeger, J.D. Goeschl, and J.L. Need. 1988. Automatic system for the control of batch-produced $^{11}CO_2$ for continuous labeling experiments. Review of Scientific Instruments 59:467-469.

Minchin, P.E.H, and M.R. Thorpe. 1982. Evidence for a flow of water into sieve tubes' associated with phloem loading . J. Exp Botany 33:233-240.

Potvin, C., J.D. Goeschl, and B.R. Strain. 1984. Effects of temperature and CO_2 enrichment on carbon translocation of plants of the C_4 grass species Echinochloa crus-galli (L.) Beauv. from cool to warm environments. Plant Physiol. 75:1054-1057.

Potvin, C., B.R. Strain, and J.D. Goeschl. 1985. Low night temperature effect on C_4 grass species: II. Effect on photosynthate translocation. Oecologia 67:305-309.

Roberts, J.K.M., P.M. Ray, N. Wade-Jardetzky, and O. Jardetzky. 1980. Estimation of cytoplasmic and vacuolar pH in higher plant cells by ^{31}P NMR. Nature 283:870-872.

Roberts, J.K.M., D. Wemmer, and O. Jardetzky. 1984. Measurement of mitochondrial ATPase activity in maize root tips by saturation transfer of ^{31}P nuclear magnetic resonance. Plant Physiol. 74:632-639.

Roberts, J.K.M. 1984. Study of plant metabolism *in vivo* using NMR spectroscopy. Ann. Rev. Plant Physiol. 35:375-386.

Scott, A. I. 1985. Applications of C-13 NMR in the study of biosynthetic mechanism. J. Natural Products 48:689-707.

Sharpe, P.J.H., and H. Wu. 1985. A preliminary model of host susceptibility to bark beetle attack. Pp. 108-127 in Proceedings, International Union of Forest Research Organizations (L. Safranyik and A.A. Berryman, eds.). Host Insect Work Group, Banff, Alberta, September, 1983.

Sharpe, P.J.H., H. Wu, R.G. Cates, and J.D. Goeschl. 1985. Energetics of pine defense systems to bark beetle attack. Pp. 206-223 in Proceedings Integrated Pest Management Research Symposium, (S.J. Branham and R.C. Thatcher, eds.) Southern Forest Experiment Station General Technical Report 50-56. New Orleans, Louisiana.

Sharpe, P.J.H., and H.W. Scheld, 1986. Role of mechanistic modeling in estimating long-term pollution effects upon natural and man-influenced forest ecosystems. NCASI Technical Bulletin No. 500: Proceedings of Workshop on Controlled Exposure Techniques and Evaluation of Tree Responses to Airborne Chemicals, pp. 72-82.

Sharpe, P.J.H., R.D. Spence, and E.J. Rykiel. 1988. Diagnosis of sequential ozone effects on carbon assimilation, translocation and allocation in cottonwood and loblolly pine. NCASI Technical Bulletin (in press).

THE WORKSHOP PAPERS FOREST APPLICATIONS OF BIOLOGIC MARKERS: REGIONAL SESSION

Decline of Red Spruce in the Northern Appalachians: Determining if Air Pollution is an Important Factor

A.H. Johnson
Department of Geology University of Pennsylvania Philadelphia, PA 19104

ABSTRACT

Declines are multiple stress diseases in which combinations of abiotic and biotic stresses weaken trees, eventually causing death. Airborne chemicals could play a role in decline diseases by altering normal functions and enhancing natural stresses which initiate a decline, and by causing injury which competes for carbon with naturally-induced injuries, ultimately causing available carbon to drop below a level critical for maintaining vital functions. The decline of red spruce in mountain forests of the northern Appalachians appears to have been initiated by repeated winter damage to needles and buds from freezing or desiccation. The spatial pattern of decline severity is associated with age, site conditions, and the nature of the pathogens and insects which serve as contributing stresses. Emerging research results suggest that air pollution at ambient levels is capable of altering resistance to winter stress in high-elevation spruce. Additional research is needed to determine if ambient levels of airborne chemicals serve to reduce the carbon available for defense against and repair of the injuries caused by pathogens, drought and winter weather.

INTRODUCTION

Recently, the unusual mortality of red spruce in high-elevation forests of New York and New England has raised the possibility that air pollution is responsible, at least in part, for the decline of that species. Similarly, a recent episode of unusual mortality and symptoms in several species in central Europe has intensified the effort to determine if airborne chemicals are important stresses contributing to "forest decline." Some current forest problems in eastern North America and Europe are occurring in areas subject to relatively high doses of air pollutants, but many other declines (diseases characterized by a combination of biotic and abiotic stresses) have occurred in places where, and at times when, air pollution is almost certainly not a factor (e.g., Mueller-Dombois 1983).

One useful conceptual framework divides the stresses involved in declines into predisposing, inciting, and contributing (or secondary) stresses (Manion 1981). Figure 1 puts this concept in a simple model showing how the resources a tree has available to repair injury and resist pathogens might change through a period of decline. During a "decline" some trees in a population will be depleted in usable reserves to the point that death, although possibly several years away, is inevitable (e.g., Waring 1987, McLaughlin 1987). Other individuals may be stressed by the inciting factors, but recover due to lesser effects of the predisposing and secondary stresses.

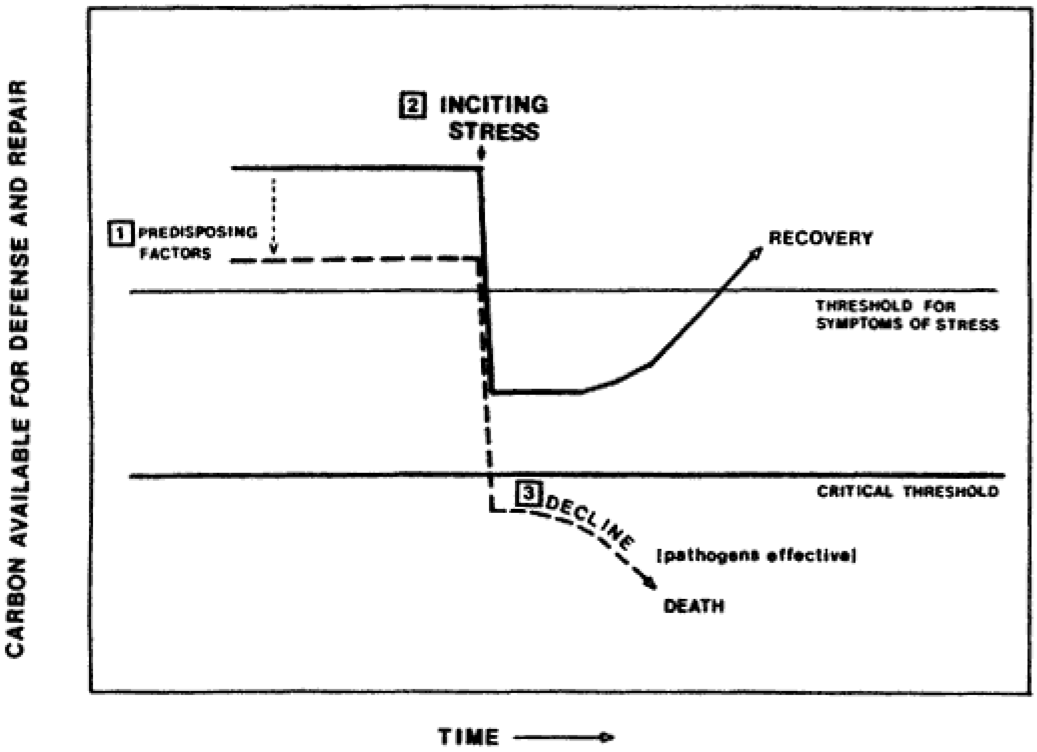


Figure 1.
Schematic representation of energy available for defense and repair during various phases of a decline disease. Represented are, (1) predisposing factors that influence the vigor of an individual prior to injury, (2) a drop in reserves as repair begins after an initiating stress, and a period of recovery or (3) decline when pathogens or other stresses eventually become lethal.

Using Figure 1 as a guide, I have summarized the natural factors that appear to be involved in the decline of high-elevation red spruce, suggested how air pollution might fit into the disease complex, and reviewed recent evidence suggesting that air pollution at ambient levels may alter red spruce to a degree sufficient to promote a decline.

FACTORS RELATED TO THE OCCURRENCE AND SEVERITY OF SPRUCE DECLINE

The gradients in vegetation, soil and climate on the Adirondack, Green and White Mountains have been summarized by many authors, and reviewed in the context of the current decline by Johnson and McLaughlin (1986) and Johnson and Siccama (1983). Exposure of trees to airborne chemicals in those mountain forests is now being characterized with considerable precision (e.g., Mohnen, this volume). Earlier estimates have all suggested relatively high deposition of acids and heavy metals, and prolonged exposure to acidic cloud water and elevated levels of ozone (e.g., Lovett et al. 1982, Burgess et al. 1984, J. Panek, unpublished data on hourly ozone measurements at 1026 m at Whiteface Mt., NY). In particular, the frequent occurrence of clouds above 1000 m and the lack of diurnal fluctuations in ozone concentrations above 1000 m make for prolonged exposure to airborne chemicals.

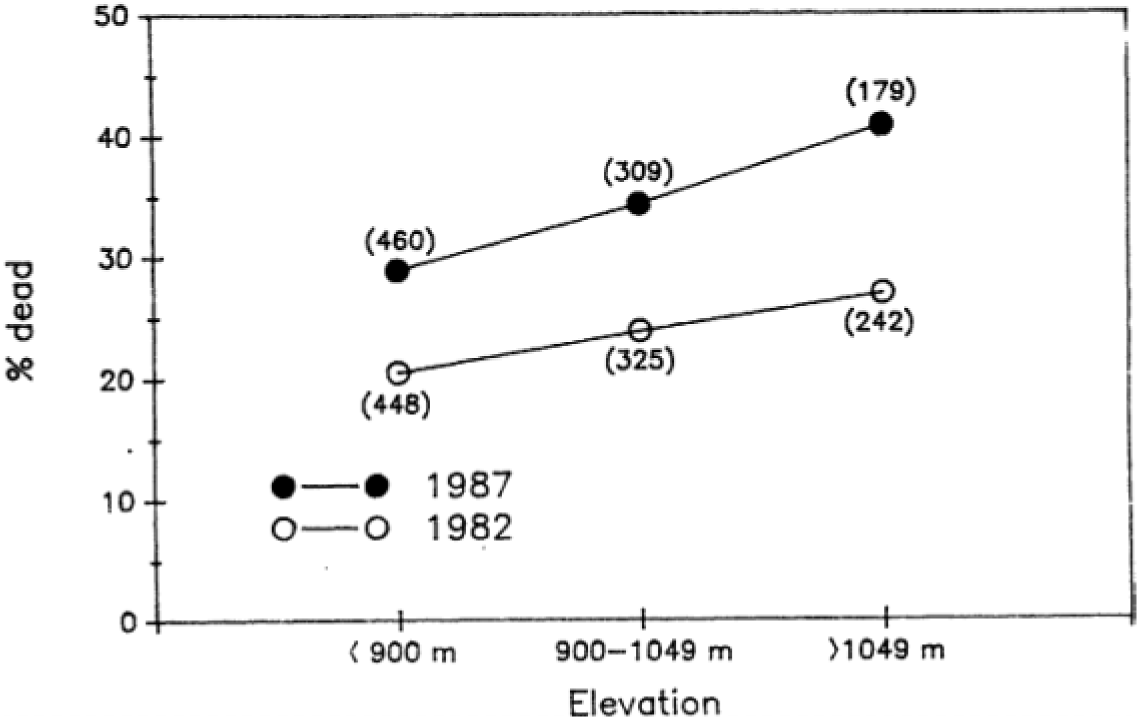


Figure 2.
Changes in percent dead red spruce over 5 years on 56 100-m transects on Mt. Washington, NH, Mt. Mansfield, VT, and Whiteface Mt., NY. Differences are significant in all elevational bands at P<.05 (W.L. Silver, A.H. Johnson and T.G. Siccama, unpublished data).

Johnson and McLaughlin (1986) summarized the data from several studies which showed that red spruce density and basal area decreased dramatically (40-80%) on five mountains in New York and New England between the mid-1960s and early 1980s. Surveys of mature spruce on Whiteface Mt. (NY), Mr. Mansfield (VT) and Mt. Washington (NH) showed unusually rapid mortality between 1982 and 1987, particularly at high elevation (Fig. 2). The most prominent symptom in declining high-elevation spruce has been a deterioration of the crown due to death of twigs and branches, and recent detailed studies have shown many specific symptoms associated with locally important insects and diseases.

In a study of 331 permanent plots on Whiteface Mr. (NY), Battles et al. (1987) showed the following with respect to spatial associations between forest characteristics and the density of dead spruce:

1. More than half of the red spruce in all size classes are standing-dead in the three 100-m elevational bands above 1000 m, while about 25% are dead in all size classes in the three 100-m elevational bands below 1000 m.
2. The percent dead spruce is slightly greater in larger size classes than in smaller size classes.

3. The percent dead spruce is greater in all size classes above 1000 m on the northwest exposure which experiences the most severe winter winds capable of defoliating conifers.

Thus, it appears that age, elevation (representing poorer site conditions and shorter growing seasons) and unfavorable exposure at high elevation can be considered as conditions which predisposed the trees to greater damage from other environmental stresses.

Friedland et al. (1984) suggested that damage to foliage and buds which became visible in early spring was probably an important type of injury promoting the decline of red spruce. Johnson et al. (1986, 1988) suggested that several consecutive years of winter damage to the previous year's foliage and buds probably initiated and synchronized the decline across New York and New England. The winters of the 1960s were unusually cold (Namias 1970). Figures 3-6 show that the several years of winter damage were accompanied and followed by regionwide reports of dead and dying spruce, a synchronized regional-scale decrease in ring width, and an abrupt change in the long-term relationship between climate and ring width. Substantial and/or repeated loss of new foliage from spruce growing near the top of their altitudinal range is likely to be significant because it reduces photosynthetic biomass, removes storage tissue and energy reserves, and judging by the production of adventitious shoots (P. Wargo, U.S. Forest Service, and personal observations), shifts carbon allocation to the production of new shoots and needles. A systematic study in the Adirondacks by Curry and Church (1952) and repeated observations in western Maine (Stark, 1962-1970) and in patches of dead and dying spruce in the White Mountains of New Hampshire (Tegethoff 1964, Wheeler 1965, Kelso 1965) indicate that severe episodes of winter injury can kill red spruce. Thus, the repeated and severe episodes of injury to foliage and buds two decades ago represent a likely stress which initiated and synchronized the spruce decline, and later episodes of winter injury may have served to keep the decline going.

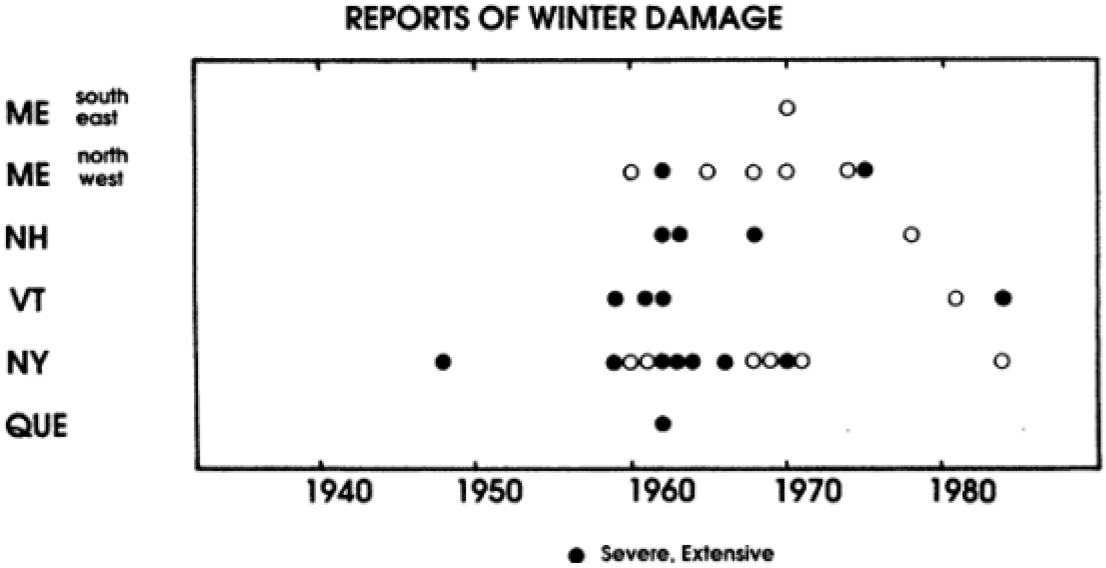


Figure 3.
Reports of "winter drying," "winter burn," "winterkill," etc. where red foliage was observed in spring. The tabulated incidents were generally widespread within the geographic area. Source: Reprinted with permission of Kluwer Academic Publishers. Copyright c 1986 by D. Reidel Publishing Company.

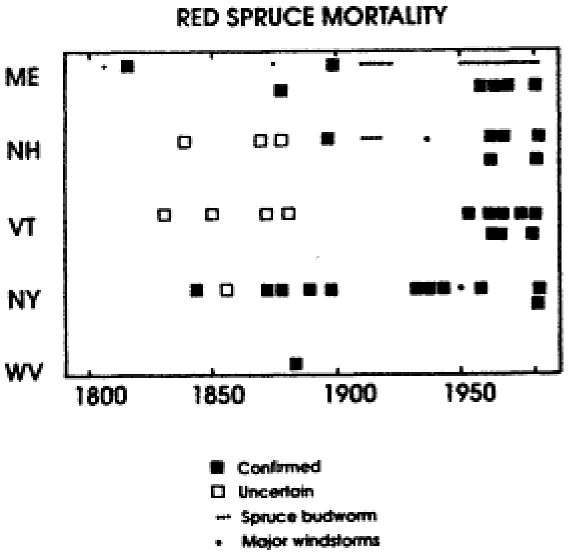


Figure 4.

Periods when extensive or severe red spruce mortality were recorded.

Source: Reprinted with permission of Kluwer Academic Publishers. Copyright c 1986 by D. Reidel Publishing Company.

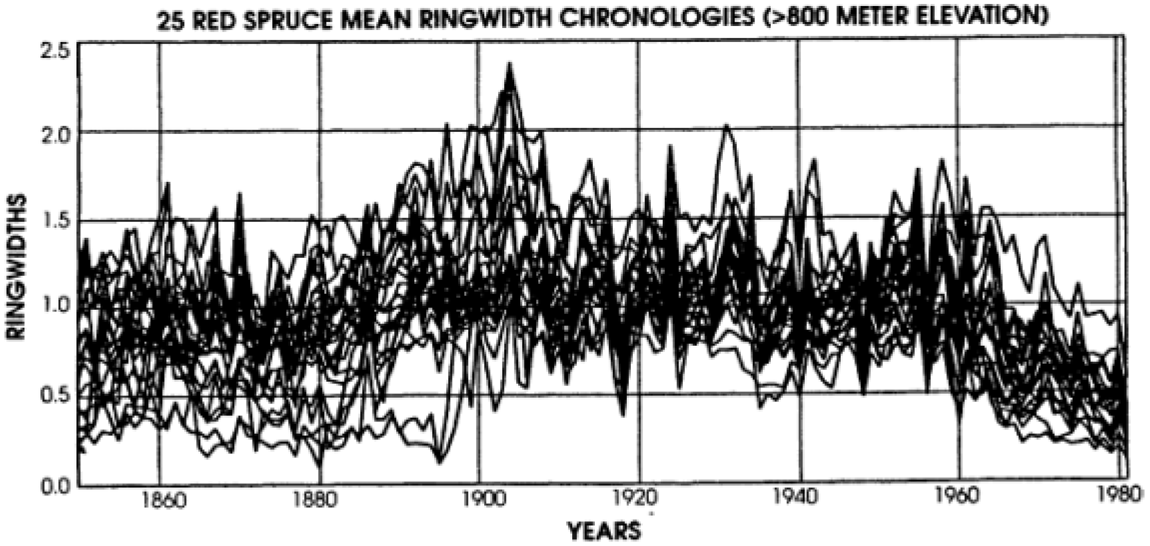


Figure 5.

Red spruce tree ring chronologies at 25 high-elevation sites in the Catskill, Adirondack, Green and White Mountains. Each site is represented by two cores from 12-20 trees >10 cm dbh. Values are raw ring widths for each year averaged for each site, then normalized to the 131-year mean ring width. (After Johnson et al. 1988).

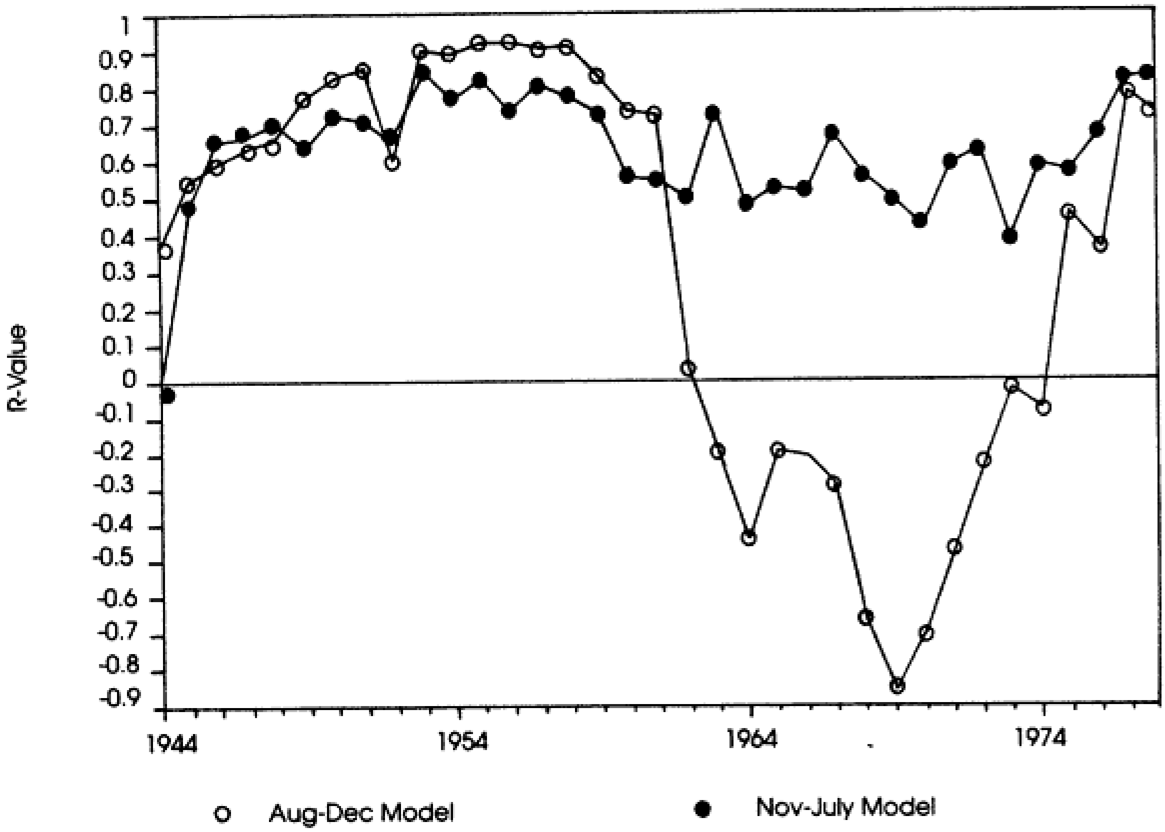


Figure 6.
Seven-year running correlation coefficients for actual vs. climate-predicted standardized ring widths. Open circles reflect the predictive ability of a regression model which uses average monthly temperatures of the prior August and prior December. This combination of temperatures was significantly correlated with ring width from 1856-1960. Prior August ceases to be related to ring width from 1960 to 1981. Instead, a model using prior November and current July is related to ring width. Methods are explained by Johnson et al. (1988).

Johnson et al. (1988), McLaughlin et al. (1987) and Cook et al. (1987) used tree-ring analysis to show that radial growth in high-elevation red spruce across New York and New England responded unfavorably to warm Augusts (year prior to ring formation) and cold Decembers and Januarys (winter prior to ring formation) for the 100 years prior to 1960 when the relationship between climate and ring width changed abruptly. While the physiological bases for those relationships are unknown, it is interesting to note that the most severe recorded periods of red spruce mortality occurred during periods of unfavorable summer and winter temperatures (Johnson et al. 1988, Fig. 7).

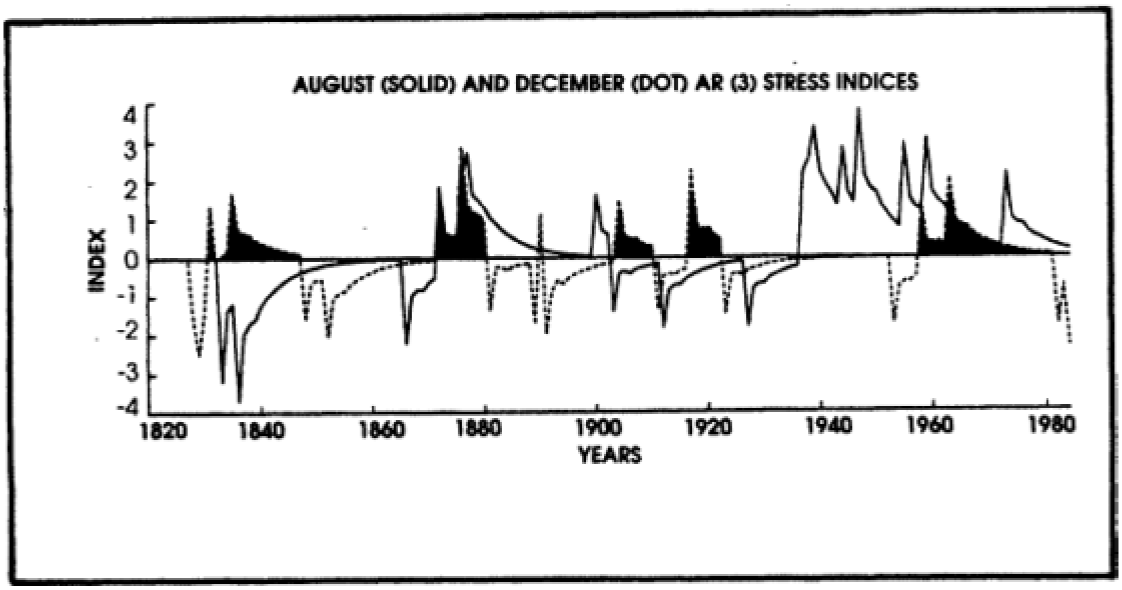


Figure 7.
Years when unusually warm Augusts and/or unusually cold Decembers occurred. Only monthly average temperatures that have a probability of <.05 are included. Positive index values represent periods of stress, negative values represent favorable periods. Solid areas are unusually cold Decembers. Methods are explained by Johnson et al. (1988).

Particularly notable was the spruce decline of 1871-1885 (Hopkins 1901) which killed an estimated 50% of the spruce in the Adirondacks, and coincided well with the very unusual years 1871 and 1876 which had both especially warm Augusts and especially cold December temperatures. The most recent period of spruce decline apparently started at the end of a period of warm summers in the 1950s and at the start of a period of unusually cold winters (see Fig. 7 and Namias 1970). From those findings, it seems plausible that warm summer temperatures may have served as a predisposing factor.

Several researchers have noted the occurrence of severe drought in the mid-1960s which could serve as an initiating stress. Johnson and McLaughlin (1986) and Johnson et al. (1988) discussed the conflicting evidence related to the possible importance of the mid-1960s drought. Although regionwide red spruce mortality was noted prior to severe drought, large negative residuals from the temperature-predicted ring widths during 1965-67 suggest at least a contributing and, possibly in some areas, an initiating role.

Many of the insects and diseases which have affected red spruce historically are present in dead and dying trees, but their occurrence varies spatially. The spruce beetle (Dendroctonus rufipennis Kirby) has been observed in many areas (McCreery et al. 1987) but is rare in others, such as at Whiteface Mt. (T.C. Weidensaul, personal communication). Armillaria mellea, the shoestring root rot fungus is present in declining spruce at lower elevation, but rare at higher elevations (Carey et al. 1984). Other fungi (Fomes pini and Cytospora canker) have been identified in several places over the last two decades (e.g., Hadfield 1968). At lower elevations, dwarf mistletoe (Arceuthobium pusillum Peck) is associated with growth loss and mortality (McCreery et al. 1987). The presence of many more pests and disease organisms is expected to be confirmed by ongoing work.

Early observers of spruce mortality at high elevation in New Hampshire and western Maine (Kelso 1965, Wheeler 1965, Tegethoff 1964, Stark 1962) looked for, but found no evidence of insects or fungal diseases as primary causes of the spruce mortality they studied through repeated visits in 1962-64. They attributed the mortality and visible decline of spruce to severe winter conditions. As in most declines, insects and diseases appear to be serving as contributing or secondary stresses in the recent episode of spruce decline.

To date, many of the naturally-occurring factors that have been implicated in other declines have been shown to be temporally or spatially associated with the red spruce decline. These are summarized in table 1.

TABLE 1. Stresses temporally or spatially associated with the occurrence or severity of the recent red spruce decline in the northern Appalachians and Adirondacks.

PREDISPOSING	INCITING	CONTRIBUTING
age	winter damage to foliage and buds	insects
elevation (site)		fungal diseases
severe exposure to winter winds	(drought?)	winter injury
(warm summers?)		

THE POSSIBILITY OF AIR POLLUTION INVOLVEMENT

The presence of the factors listed in Table 1 does not rule out air pollution as a contributor to spruce decline. Figure 8 shows how air pollution could serve as a predisposing or contributing factor by reducing reserves available to repair injuries caused by the winter damage, drought etc. Possibly, air pollution could make trees more susceptible to the conditions that cause the winter injury to foliage and buds. Emerging findings (e.g., Alscher, this volume) suggest that those topics deserve attention.

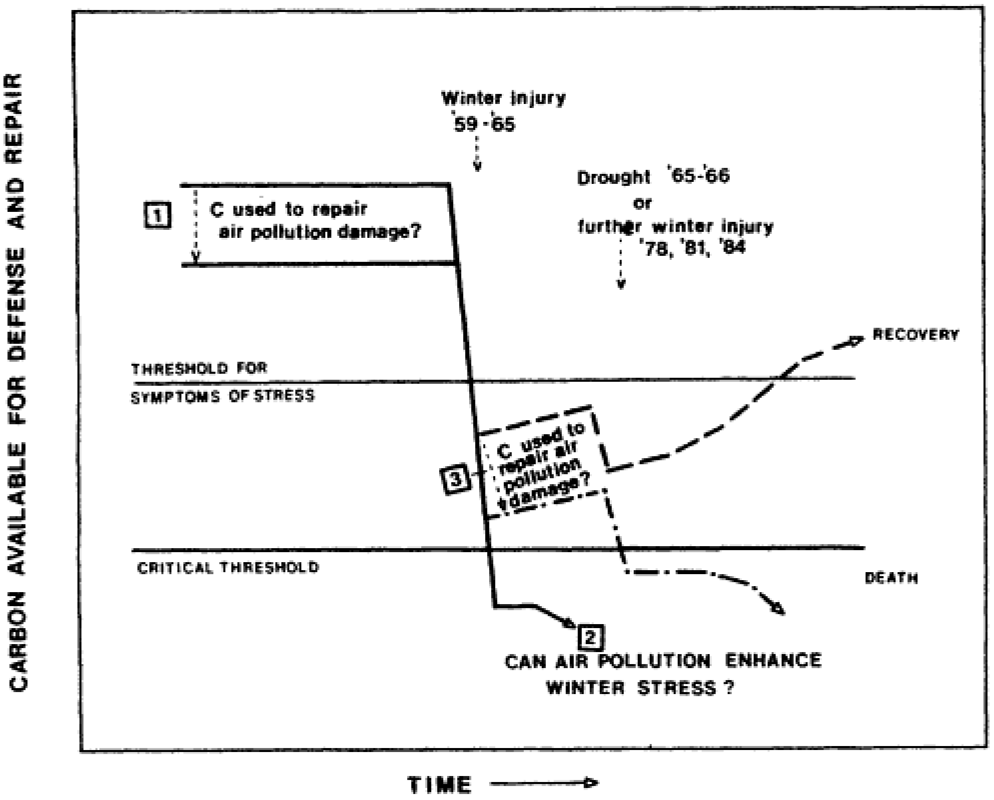


Figure 8.
Events that contributed to the recent spruce decline in the northern Appalachians and possible ways in which air pollution might contribute.

Many factors contribute to a tree's ability to resist damage in winter (e.g., Weiser 1970). Davison et al. (1987) and Alscher et al. (this volume) reviewed the types of damage plants are subject to in winter, and the ways in which air pollution might alter resistance to those types of stress. In winter, high elevation conifers must contend with photo-oxidation of chlorophyll (resulting in bleaching of the needles), desiccation (thought to be caused by water loss through cuticles on bright, sunny days when water in conducting tissues is frozen), and freezing injury (usually called "frost damage"). The latter types of injury cause the death of needles which turn red-brown in the spring. The exact mechanism that caused the winter damage observed in red spruce over the past two decades is unknown, and the climatic conditions under which the injuries occurred are, likewise, unknown. Freezing damage early in winter, desiccation on appropriate days in mid-winter, mid-winter thaws followed by very low temperatures, and spring frosts all have the potential to cause the damage observed.

There is evidence that air pollution might affect resistance to winter stresses. Electron microscopy studies carried out in Finland along a gradient of air pollution exposure showed greater alterations of cells and greater winter damage in areas of higher pollutant exposure (Davison et al. 1987), but mechanisms or the causal agents are not known. Since several pollutants attack constituents of cell membranes, and since changes in cell membranes occur during the fall hardening period, Davison et al. (1987) suggested that there is a sound theoretical basis for suspecting that air pollution might interfere with the development of freezing resistance. Their experiments with ozone fumigations (120 ppb ozone, 6 h per day for 70 days) indicated that ozone at that dose rate was associated with increased cuticular water loss from previous year's needles in one of eight clones of Norway spruce tested. Four clones showed an increase in freezing-related damage when exposed to the ozone treatments.

Weinstein et al. (1987) and Cumming et al. (1988) and Alscher et al. (this volume) exposed red spruce seedlings in open-top chambers to filtered air and to ozone at ambient and twice ambient levels at Ithaca, NY. Ozone at ambient and twice ambient levels was associated with several factors that indicated a delay in hardening. Ozone at ambient levels was associated with increased respiration, decreased pigment concentrations, delayed starch export or conversion, a delay in tannin accumulation, and unusually high photosynthesis rates during the hardening period.

While there are obvious problems in extrapolating from seedlings grown under the climatic conditions in Ithaca to mature trees growing on the mountains, it is interesting to note that ozone exposure in the spruce-fir forest at Whiteface Mt. in the summer of 1987 was in the ambient to twice ambient range used in the experiments noted above. Hourly ozone concentrations averaged 47 ppb, day and night from June 1 through August 31. The maximum reading was 129 ppb, and 22% of the time, ozone concentrations exceeded 60 ppb (J. Panek, Atmospheric Sciences Research Center, SUNY-Albany, unpublished data). Long-term data from the summit of Whiteface (Burgess et al. 1984) indicate that those values are representative of normal conditions.

As indicated in Figure 8, it is also important to try to determine if ambient levels of airborne chemicals cause a decrease in the carbon available for defense and repair. A clear record has been established with seedlings and saplings of several species that suggests ozone at ambient levels can reduce net photosynthesis and growth without visible symptoms (e.g., Wang et al. 1986, Skelly et al. 1983, Reich and Amundson 1985). Thus, there is reason to suspect that air pollution might be capable of inhibiting repair of the winter damage sustained by red spruce during the decline. It would be useful to have experimental evidence from mature trees in the field for the best possible assessment.

In this regard, Vann and Johnson (1988) have begun experiments using branch chambers for the exclusion of airborne chemicals from branches of mature red spruce. Their preliminary results suggest improved foliage with filtered air (Fig. 9), but the results are not sufficient at present to make judgments about productivity and carbon reserves.

SUMMARY

The recent mortality of red spruce has components which have been commonly associated with declines of other forest species in the northeastern U.S. Natural factors such as age, poor sites, exposure to winter winds, and possibly climate warming may act as predisposing factors. Repeated and severe winter damage to foliage and buds, and possibly drought, may act as inciting factors, and insects and pathogens appear to serve as contributing or secondary stresses. Air pollution stress might be a contributor by enhancing the effects of the conditions leading to winter injury, or by consuming energy reserves which might otherwise have been used to defend against pathogens or to repair damage from winter injury or drought. The information available at the present time suggests that ozone is the most likely pollutant for further study. The climatic conditions and mechanisms leading to winter injury in red spruce need to be understood in detail, and the effects of airborne chemicals on those mechanisms need to be determined before a confident assessment can be made of the role played by air pollution.

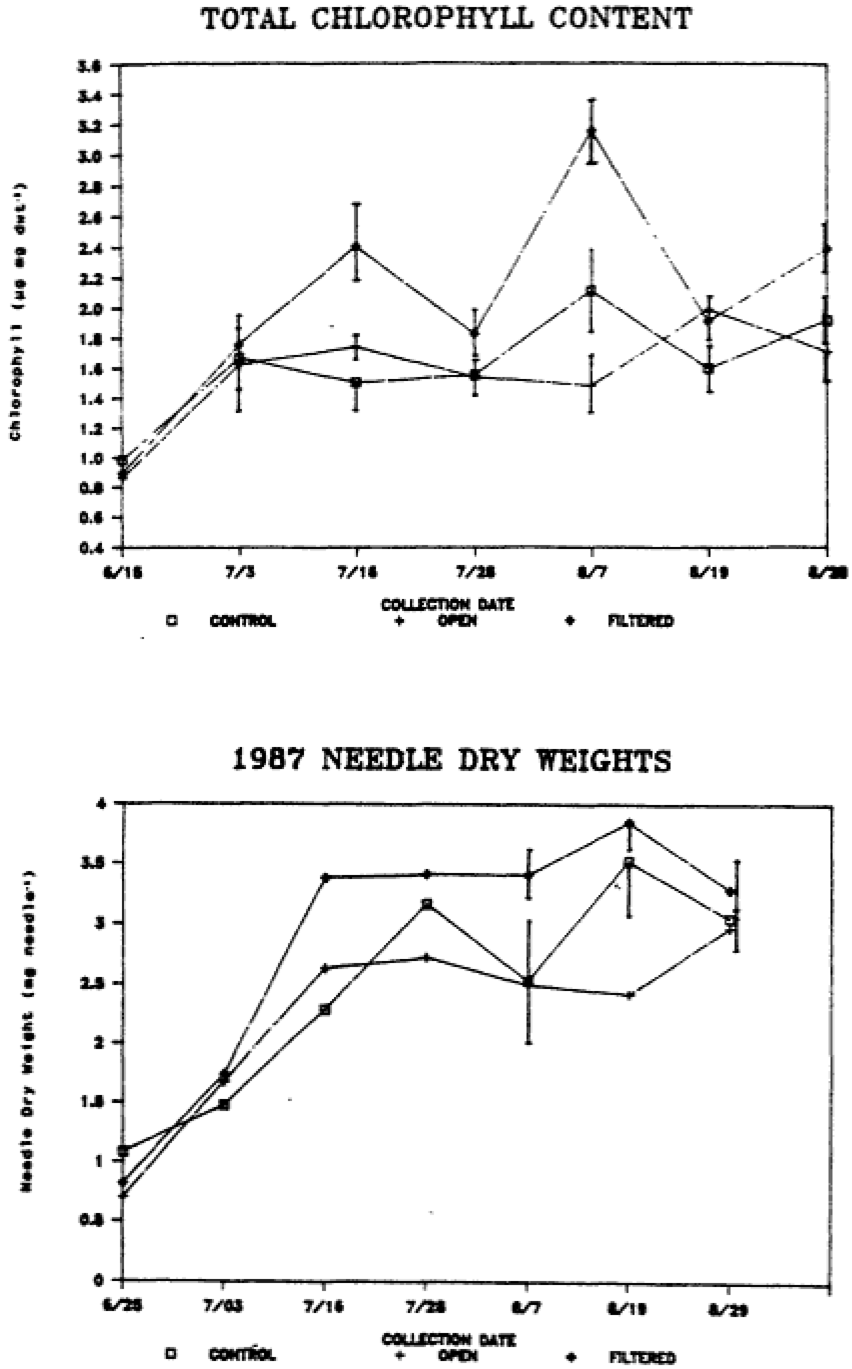


Figure 9.
Chlorophyll content and dry weight of red spruce foliage from a control chamber, a chamber receiving charcoal-filtered air, and an open branch. The experimental tree was approximately 125 years old (at dbh), and growing at 1170 m on Whiteface Mt., NY. Chambers were installed on July 3. Standard errors are calculated from replicate twigs from one branch. Methods and operating conditions in the chambers (temperature, PAR, relative humidity) are given by Vann and Johnson (1988).

References

Battles, J., A.H. Johnson, T.G. Siccama, and W.L. Silver. 1987. Recent changes in spruce-fir forests of the New York, Vermont and New Hampshire. In: proc. of a U.S.-German Conference on forest decline. Burlington, VT. October 19-24, 1987. U.S. Forest Service, Broomall, PA.

Burgess, R.L., M.B. David, P.D. Manion, M.J. Mitchell, V.A. Mohnen, D.J. Raynal, M. Schaedle, and E.H. White. 1984. Effects of acidic deposition on forest ecosystems in the northeastern United States: an evaluation of current evidence. New York State College of Environmental Science and Forestry, Syracuse, NY.

Carey, A.C., E.A. Miller, G.T. Geballe, P.M. Wargo, W.H. Smith, and T.G. Siccama. 1984. Armillaria mellea and decline of red spruce. Plant Dis. 68:794-795.

Cook, E.R., A.H. Johnson, and T.J. Blasing. 1987. Modelling the climate effect in tree rings for forest decline studies. Tree Phys. (3) 27-40.

Cumming, J.R., R.G. Alscher, J. Chabot, and L.H. Weinstein. 1988. Effects of ozone on the physiology of red spruce seedlings. In: proc. of a U.S.-FRG conference on forest secline. Burlington, VT. Oct. 1987. U.S. Forest Service, Broomall, PA (in press).

Curry, J.R., and T.W. Church. 1952. Winter drying of conifers in the Adirondacks. J. Forestry (50) 114-116.

Davison, A.W., J.D. Barnes, and C.J. Renner. 1987. Interactions between air pollution and cold stress. Proc. 2nd Internat. Symposium on Air Pollution and Plant Metabolism. Apr. 6-9, 1987. Neuhenburg, Fed. Repub. of Germany.

Friedland, A.J., R.A. Gregory, L. Karenlampi, and A.H. Johnson. 1984. Winter damage to foliage as a factor in red spruce decline. Can. J. Forest Res. 14:963-965.

Hadfield, J.S. 1968. Evaluation of diseases of red spruce on the Chamberlain Hill sale, Rochester Ranger District, Green Mt. Nat. Forest. File Report A-68-8 5230. Amherst, MA: USDA-Forest Service Northeastern Area, State and Private Forestry, Amherst FPC Field Office.

Hopkins, A.D. 1901. Insect enemies of the spruce in the Northeast. U.S. Dept. of Agriculture, Div. of Entomology Bull. No.28, new series. Pp. 15-29.

Johnson, A.H., and T.G. Siccama. 1983. Acid deposition and forest decline. Environ. Sci. Technology. 17:294a-305a.

Johnson, A.H., and S.B. McLaughlin. 1986. The nature and timing of the deterioration of red spruce in the northern Appalachians. Report of the Committee on Monitoring and Trends in Acidic Deposition. National Research Council, National Academy Press, Washington D.C. Pp. 200-230.

Johnson, A.H., A.J. Friedland, and J. Dushoff. 1986. Recent and historic red spruce mortality: evidence of climatic influence. Water Air Soil Pollut. 30:319-330.

Johnson, A.H., E.R. Cook, and T.G. Siccama. 1988. Relationships between climate and red spruce growth and decline. Proc. Nat. Academy Sci. (in press).

Kelso, E.G. 1965. Memorandum 5220,2480, July 23, 1965. Amherst, Mass. U.S. Forest Service Northern FPC Zone.

Lovett, G.M., W.A. Reiners, and R.K. Olson. 1982. Cloud droplet deposition in subalpine balsam fir forests: hydrological and chemical inputs. Science 218:1303-1304.

Manion, P.D. 1981. Tree Disease Concepts. Englewood Cliffs, N.J. Prentice Hall.

McCreery, L.R., M.M. Weeks, M.J. Weiss and I. Millers. 1987. Cooperative survey of red spruce and balsam fir decline and mortality in New York, Vermont and New Hampshire: a progress report. In: proc. Integrated Pest Management symposium for northern forests March 24-27, 1986. Cooperative Extension Service, University of Wisconsin, Madison.

McLaughlin, S.B. 1987. Carbon allocation as an indicator of pollutant impact on forest trees. In Proc. of a IUFRO Conference on Woody Plant Growth In a Changing Chemical and Physical Environment. M. Cannell and D.L. Lavander (eds.). Vancouver, B.C July 1987. (in press).

McLaughlin, S.B., D.J. Downing, T.J. Biasing, E.R. Cook, and H.S. Adams. 1987. An analysis of climate and competition as contributors to the decline of red spruce in the high elevation Appalachian forests. Oecologia 72:487-501.

Mueller-Dombois, D. 1983. Tree-group deaths in North American and Hawaiian forests: a pathological problem or a new problem for vegetation ecology? Phytocoenologia 11:117-137.

Namias, J. 1970. Climatic anomaly over the United States during the 1960's. Science 170:741-743.

Reich, P.B., and R.G. Amundson. 1985. Ambient levels of ozone reduce net photosynthesis in tree and crop species. Science 230:566-570.

Skelly, J.M., Y. Yang, B.I. Chevone, S.J. Long, J.E. Nellessen, and W.E. Winner. 1983. Ozone concentrations and their influence on forest species in the Blue Ridge Mountains of Virginia. In: Air Pollution and Productivity of the Forest. Pp. 143-59. Izaak Walton League, Washington, D.C.

Stark, D. Unpublished notes on red spruce disease, mortality and winter injury 1957-1977. State of Maine Department of Conservation, Entomology Laboratory. Augusta.

Tegethoff, A.C. 1964. High Elevation Spruce Mortality. Memorandum 5220, September 25, 1964. Amherst Mass.: U.S. Forest Service Northern FPC Zone.

Vann, D.R., and A.H. Johnson. 1988. Design and testing of branch chambers for the exclusion of atmospheric pollutants. In Proc. of a U.S.-FRG Conference on Forest Decline. Burlington, VT, Oct. 1987. U.S. Forest Service, Broomall, PA (in press).

Wang, D., D.F. Karnosky, and F.H. Bormann. 1986. Effects of ambient ozone on productivity of Populus tremuloides Michx. grown under field conditions. Can. J. Forest Research. 16:47-55.

Waring, R.H. 1987. Characteristics of trees predisposed to die. BioScience 37: 569-575.

Weinstein, L., R.J. Kohut, and Jay S. Jacobson. 1987. Research at Boyce Thompson Institute on the effects of ozone and acidic precipitation on red spruce. Proc. 80th Ann. Meeting, Air Pollut. Control Assn. June 21-26, 1987, New York, NY.

Weiser, C.J. 1970. Cold resistance and injury in woody plants. Science 169:1269-1278.

Wheeler, G.S. 1965. Memorandum 2400, 5100 July 1, 1965. Laconia, NH U.S. Forest Service Northern FPC Zone.

Forest Applications of Biomarkers in Southeastern Forests

Robert L. Anderson
Plant Pathologist USDA Forest Service, R-8 Asheville, NC 28806

ABSTRACT

Air pollution symptoms on sensitive tree species have long been recognized as a way to assess air pollution damage. In 1974, the USDA Forest Service reported that forests in the southeastern United States were being affected near point sources and may be affected over large areas. Eastern white pine was viewed as a classic biomarker. Clones of different sensitivity were propagated and planted out as survey tools. Many of the more sensitive clones have died. The National Park Service has used biomarkers such as milkweed and eastern white pine to survey for air pollution injury in southeastern National Parks since 1983. In 1986, a survey was made of the Piedmont of Georgia, South Carolina, and North Carolina, using existing biomarkers such as cherry. It showed that sensitive plants were widely distributed, but were more common around sources of pollution. In 1986, eastern white pines were used as biomarkers for a survey of portions of Virginia, North Carolina, South Carolina, Georgia, Tennessee, and Kentucky. Surveys of wilderness areas in South Carolina and Virginia have also been made using biomarkers. Lichens have also been used as biomarkers. The European system of crown density classification has been evaluated and modified for use on loblolly and shortleaf pines. Surveys using the crown vigor system are being developed.

Injury to vegetation by phytotoxic levels of ozone, fluoride, sulfur dioxide, and other pollutants has been reported in the southeastern United States for a number of years. Much of the reported injury has been associated with industrial sources. In some cases, the injury has produced severe symptoms on sensitive vegetation miles from the source. The symptoms are more common around point sources and in areas where meteorological or topographical conditions inhibit pollutant dispersal (Davis, 1970).

Ozone is a pollutant that is a product of photochemical reactions of a number of pollutants originating from a number of sources, such as automobiles. Whereas pollution-caused symptoms from most point sources usually occur in a limited area ozone occurs over a wide area. In 1974, the USDA Forest Service (Loomis, 1974) reported that forests in the southeastern United States were being affected near point sources and may be affected over large areas. We now know that ozone does cause injurious effects on forest trees at concentrations that occur on a regional scale in the Southeast (Woodman, 1986).

A number of forest tree species or plants commonly found in association with the plant species are more sensitive to pollutants such as ozone (Skelly et al., 1988). Several of these more sensitive species, such as eastern white pine, blackberry, black cherry, sweetgum, ragweed, white ash, milkweed, and poison ivy, show symptoms of ozone while located next to more resistant species, such as oak, that do not show any visible symptoms. These more sensitive plants are referred to as bioindicators or biomarkers. These plant species offer an opportunity to assess the relative incidence of pollutant injury in a given area. By looking at the biomarkers in a geographic area and comparing them to the same species in another area, one can assess whether the damage is more severe in one area as compared to another, and from year to year. However, estimates on impact or the level of pollutants required to cause the symptoms cannot be easily made. Another use is to select biomarkers that are sensitive to certain levels of pollutants and plant them in the field. These biomarkers can then be roughly correlated with the amount of pollutants required to cause the noted injury.

A number of factors must be considered when using biomarkers. Injury to leaves from air pollutants is easily confused with mimicking symptoms caused by factors such as temperature and soil moisture extremes, mites, certain insects and fungi, viruses, salt application, herbicides, fertilizers, and the list continues. Also, the use of biomarkers requires that the plants be examined at a point when symptom expression is at its maximum, but fall colors have not started. This normally means that the surveys have to be conducted from mid-July until the start of fall colors. In spite of the limitations, biomarkers have been used for a number of years in the southeastern United States to assess air pollution incidence.

Eastern white pine has been viewed as a biomarker since 1964 (Berry, 1964). Berry selected clones of eastern white pine that would detect low levels of oxidants, sulfur dioxide, or fluorides. By using the sensitive clones, the pollutant could be identified, as well as a rough estimate made of the concentration. Of the 1,428 seedlings Berry screened, 53 were sensitive only to oxidants, 8 to sulfur dioxide, and 14 to fluorides. Only 4.5% were tolerant to all three types of pollutants. Selections from these clones were planted in portions of Tennessee, Kentucky, Virginia, and North Carolina in the 1960s. Many of the plantings have been lost for a number of reasons. The few that do remain have a story to tell. The clones more sensitive to ozone are for the most part dead. The intermediate clones are still alive but show substantial growth reductions when compared to resistant selections. The USDA Forest Service at Asheville, N.C., has continued to propagate the eastern white pine biomarkers selected by Berry. Recently, clonal plants were provided to the National Park Service, Virginia Polytechnic Institute, and Penn State. Additional material is being propagated at this time for use in controlled pollutant studies.

Other biomarkers, such as milkweed and tulip poplar, occur over a very large geographic area and lend themselves to use in surveys. In 1983-84, the National Park Service used milkweed to conduct biomarker surveys on a number of parks in the southeastern United States (Bennett and Stalte, 1985). They found the milkweed surveys were useful to identify areas with higher levels of ozone. For a number of years they have used eastern white pine as a biomarker. The Park Service has established permanent plots in a number of parks and from 1984-87 recorded the amount of chlorotic mottling on eastern white pine. This helps identify "hot spots" and can be used to compare incidence from year to year. In general, the percentage of trees with some damage has been high, but the severity has been low. They have also used chlorotic mottling to survey for damage on slash pine in the Everglades National Park. Hardwood biomarkers, such as tulip poplar and cherry, were used to survey several Park Service battlefields in northern Virginia (Davis and Bennett, 1985). They found the incidence of ozone damage on the biomarkers to be very common, with the degree of injury varying by location. The Park Service has also used the presence or absence of lichen and biochemical analysis of the lichen to assess air pollution injury (personal communication, J. Bennett).

In 1986, the USDA Forest Service used eastern white pine as a biomarker to survey for ozone symptoms in the natural range of white pine in Georgia, South Carolina, North Carolina, Virginia, Tennessee, and Kentucky (Anderson et al., 1988). They found the ozone symptoms of chlorotic mottling and tip burn to occur throughout the range, but the symptomatic trees were more common around areas of urban development. About 23% of the stands surveyed had some symptomatic trees. Trees with ozone symptoms had 49% less volume than symptom-free trees.

In 1985, the Southeastern Forest Experiment Station reported a growth loss in loblolly pine in the Piedmont of Georgia, North Carolina, and South Carolina. Chevone, Chappelka, and Brown (1986) used biomarkers to survey for ozone injury in the area showing growth loss. Plots were established in the vicinity of pine growth plots so correlations could be made. They found that typical ozone injury was common on the biomarkers throughout the survey area, varied geographically, and was more pronounced around urban areas. They theorized that the damage on the biomarkers may be severe enough to cause a growth loss in loblolly pine.

In 1986-87, the USDA Forest Service made a survey of two class-one wilderness areas: Bull Island in South Carolina and the James River Face Wilderness Area in Virginia. Both of these biomarker surveys found the percent of plants with ozone symptoms to be low and the severity on the symptomatic plants to be slight. The data show that ozone damage was low in these years, but should be used with caution because ozone is highly variable from year to year.

All of these surveys used plants that are sensitive to pollutants. There are several benefits to these kinds of surveys:

1. The damage is relatively easy to see and evaluate.
2. The surveys use native plants that are part of the area's ecology.
3. The data permit comparisons of injury over time and geographic areas.
4. Data collection is relatively inexpensive and can be done in a short time.

However, there are several disadvantages:

1. There are a number of mimicking symptoms that can confuse the observations.
2. The biomarkers may vary in their genetic sensitivity to the pollutant.
3. Some pollutants are highly variable from year to year.
4. Normally, the surveys have to be done in a 2-to 4-week period.
5. The concentration of pollutants cannot be determined.
6. Normally, impact cannot be determined from the data.

In addition to the biomarkers, a European system rates the crowns of trees for foliage loss. The USDA Forest Service (Anderson and Belanger, 1986) has evaluated this system on shortleaf and loblolly pine in the South. They found that by estimating the foliage loss and measuring crown width, the relative growth rate of a tree could be accurately estimated.

SUMMARY

Biomarkers can provide valuable information on presence or absence of injury, relative severity of injury, type of pollutant, geographic variation, and seasonal variation, yearly variation. However, there are problems with mimicking symptoms, plant genetics variation, climatic variations, long-term exposure in a given area, etc. As any tool, they should be used with their limitations in mind.

References

Anderson, R.L., and R.P. Belanger. 1986. A crown rating method for assessing tree vigor of loblolly and shortleaf pines. In: Proceedings of the Fourth Biennial Southern Silvicultural Research Conference, Atlanta, Ga., Nov. 4-6. Pp. 538-543. Published by USDA Forest Service, Southwestern Forest Experiment Station, General Technical Report SE-42, Asheville, NC.

Anderson, R.L., and others. 1988. Occurrence of air pollution symptoms (needle tip necrosis and chlorotic mottling) on eastern white pines in the southern Appalachian Mountains. Plant Disease Reporter 72(2):130-132.

Bennett, J.P., and K.W. Stalte. 1985. Using vegetation biomonitors to assess air pollution injury in National Parks: milkweed survey. National Park Service, Air Quality Division, Denver, CO, Natural Resources Report Series No. 85-1.

Berry, C.R., and G.H. Hepting. 1964. Injury to eastern white pine by unidentified constituents. For. Sci. 10(1):1-13.

Chevone, B.I., A.H. Chappelka, and H.D. Brown. 1986. Survey for ozone injury on sensitive plant species on or near natural loblolly pine stands in Georgia, South Carolina, and North Carolina. Cooperative Research Agreement #29-206, USDA Forest Service, National Vegetation Survey. Raleigh, NC. p. 26.

Davis, D.D. 1970. Air pollution and southern forests. Forest Farmer 10:6-8.

Davis, D.D., and J.P. Bennett. 1985. Evaluation of air pollution injury to vegetation in four mid-Atlantic National Park Service areas. Center for Air Environment Studies No. 756-85. National Park Service, Air Quality Division, Denver Colorado. Pp. 72.

Loomis, R.C. 1974. Evaluation of air pollution effects in the South. USDA Forest Service, Forest Pest Management, Atlanta, GA. Report #75-1-4. p. 27.

Skelly, J.M., and others. 1988. Diagnosing injury to eastern forest trees. National Acid Precipitation Assessment Program, Forest Responses Program, Vegetation Survey Cooperative. Published by Agric. Information Service, College of Arizona, Pennsylvania State University, State College, PA. p. 122.

Woodman, J.N. 1986. Air pollution and forest productivity: a critical issue for foresters . In: Proceedings of Atmospheric Deposition and Forest Productivity, Fourth Regional Technical Conference, Appalachian Society of American Foresters, Raleigh, NC, Jan. 29-31. Pp. 1-8.

Biomarkers for Defining Air Pollution Effects in Western Coniferous Forests

Paul R. Miller
Pacific Southwest Forest and Range Experiment Station 4955 Canyon Crest Drive Forest Service, U.S.D.A. Riverside, California 92507

ABSTRACT
Biomarkers, as discussed in this paper, are considered to be tissue level or whole plant level changes which can be directly related to air pollution exposure. In western coniferous forests the markers used most frequently include visible injury to foliage, histological changes in needle tissue, elemental contents of leaf tissue (sulfur, fluoride, etc.). The pattern of markers is more effective in indicating the cause of observed effects than a single marker. The evaluation of air pollution effects is improved by measuring the concentration of suspected pollutants, sampling plants along gradients of decreasing pollutant deposition, and controlled exposures of seedlings or small trees in enclosures comparing ambient with carbon-filtered air. Land managers need rapid methods of assessment of air pollution effects because large numbers of trees are involved. At the same time more specific and convenient markers are needed to distinguish the effects of air pollution from other abiotic stress.

INTRODUCTION

Forest land managers need improved diagnostic procedures to differentiate air pollution effects from the influences of other man-caused perturbations, the extremes of weather, and the action of biotic diseases. Biomarkers have traditionally been used in combinations because more than one line of evidence is usually needed to confirm that an air pollutant is the cause of an observed effect (Figure 1). The purpose of this paper is to provide a brief background on some of the methods frequently used in western forest areas to assess air pollutant effects, and to consider some limitations of those techniques involving biomarkers. Several aspects will be addressed: (1) observations derived from inspection of foliage and whole tree crowns, and microscopic observation of leaf tissue, (2) chemical analysis of foliage, and (3) manipulations that clarify the response of biomarkers.

VISUAL DETECTION AND ESTIMATION OF EFFECTS

Visual detection and assessment of injury can be done with respect to individual needles and leaves, or the entire crown. The particular symptoms caused by sulfur dioxide, hydrogen fluoride, and ozone are well established for western tree species (Bega, 1978, Malhotra and Blauel, 1980). Confirmation of the particular pollutant causing injury is often easy because there is a known source nearby. When sources are not nearby and pollutant concentrations are low or near natural background, particularly in the case of ozone, it is much more difficult to find convincing evidence that observed symptoms are caused by an air pollutant.

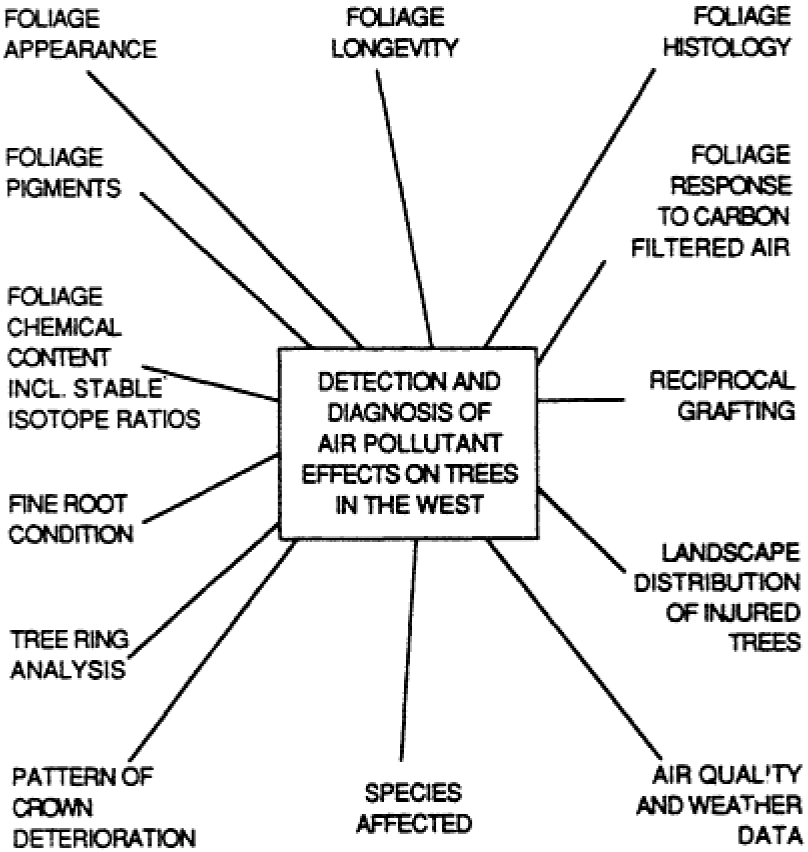


Figure 1.
Biomarkers and supporting information frequently used to assess air pollutant effects in western coniferous forests.

With ozone-injured trees the pattern of crown deterioration is a very important visual clue. The abscission of older foliage is accelerated by ozone so that injured trees have barren branches near the bole, and the lower branches appear to have fewer and shorter needles. The crown deteriorates from the inside-out and the bottom-up. These changes provide the essential markers for estimating change of tree condition over time. Forest management agencies have employed these characteristics in repeated surveys of conifer forests in the Sierra Nevada and other locations in the West (Pronos et al., 1978).

A scoring method developed for ozone-injured pines in the San Bernardino National Forest in southern California uses a combination of characteristics to derive a score for individual trees (Miller, 1973). These characteristics include the average number of needle whorls retained in the upper and lower parts of the crown, a description of the condition and length of needles in each of the remaining whorls in both the upper and lower crown, and an estimate of lower branch mortality. This information is gained by a combination of hands-on and binocular inspection of the foliage and crown condition. The sum derived from the whorl counts and weighted values for foliage condition, foliage length and branch mortality was categorized as: 0, dead; 1-8 very severe; 9-14, severe; 15-21, moderate; 22-28, slight; 29-35, very slight; and 36 or above, no visible injury. This method of evaluation was used successfully to describe condition of ponderosa pine (*Pinus ponderosa* Laws.), and Jeffrey pine (*P. Jeffreyi* Grey. and Balf.) located at plots along a gradient of decreasing ozone dose in the San Bernardino Mountains.

A supplementary method used mainly with sapling trees involved repeated counts of surviving needles in single annual whorls observed for three years in succession, starting during the year the needle whorl was formed. Rates of needle loss were proportional to the ozone dose at each of 5 plot locations (Miller and Van Doren, 1981).

Modified survey methods have been employed in the southern Sierra Nevada mountains but in all cases the presence of the chlorotic mottle symptom was the key variable. Pronos et al. (1978) established 242 ten-tree plots in the Sequoia National Forest at locations where roads and trails intersected the 1220, 1525, 1830, 2135, and 2440-meter contour lines. Chlorotic mottle symptoms were evaluated on branches cut from the lower crown with a pole pruner. The only datum recorded was the youngest annual whorl with any evidence of chlorotic mottle. For example, a tree with symptoms on the youngest (current year whorl of needles) was given a score of 0 and described as very severe injury. At the opposite end of the scale, a tree with symptoms only on the fifth-year whorl or older was given a score of 4 and described as having very slight injury. Only rarely were trees encountered with more than 5 annual whorls in the Sequoia National Forest and these were considered healthy. In 1977, 42 percent of the plots had no injury symptoms, or only very slight injury symptoms, 52 percent had slight injury, and 6 percent had moderate injury.

In a parallel series of observations in the Sequoia National Forest comparisons of ozone-injury to 100 ponderosa pines distributed among four plots were made between 1975 and 1983 (Williams and Williams, 1986). The basis of comparison was the percentage of trees having chlorotic mottle symptoms on each of the needle whorls present. The incidence of chlorotic mottle generally increased between 1975 and 1983. Recent efforts have been made to evaluate the various methods of gathering pine injury data (Muir and Armentano, 1987). The main issues investigated were: 1) the comparison of binocular and spotting scope observations of foliage with "hands-on" evaluations of foliage on branches cut from the crown of the same trees; 2) the difference of foliage condition in the upper and lower crown; 3) the bias introduced by different observers employing the same method; and, 4) the within-tree sample size needed to reach a standard error that was one-third of the specified detection limit (10 percent of the needle surface area occupied by chlorotic mottle).

Observations of needle retention and chlorotic mottle using binoculars and spotting scopes were weakly correlated with "hands-on" assessments of foliage from the upper crown. Optical instruments underestimated both needle retention and chlorotic mottle in the lower crown; however, after adjustment for observer bias, this method did meet the criteria for accuracy and precision for needle retention but not chlorotic mottle. Needle retention and chlorotic mottle did not differ significantly between upper and lower crown but needle length was longer in the upper crown along with a larger amount of injury caused by factors other than ozone. The differences between assessments made by two "hands-on" observers was generally not significant. Observer differences with optical instruments were substantial and varied more for upper crown observations than for lower crown observations. Five branches per tree constituted a sufficient sample size to obtain significant differences at $p = 0.05$ for both "hands-on" assessments and observations with optical instruments.

Questions pertaining to which data types may be best to construct an "index" of tree injury, and what tree sample size may be required have also been investigated (Duriscoe, 1988). There are advantages to the use of an index comprised of several symptoms or characteristics of injured plants (Muir and McCune, 1987). An index allows the simultaneous use of all of the observed variables during the data analysis phase. According to Muir and McCune (1987) the ideal index uses quantitative information which is equally weighted or carefully weighted. The elements should be additive; they should have a minimum at zero and maximum at the highest possible level of injury, and have a nearly linear relationship to dose.

The index proposed by Duriscoe (1988) contains four variables: chlorotic mottle (percent of the surface area of each whorl), needle retention (percent of the fascicles retained in each whorl), needle length (length in centimeters averaged at the tree level), and crown density (live crown ratio representing the tree level). These variables are weighted differently and added together to obtain a number between 0 and 100, where larger numbers mean more injury. It was estimated that 15-20 trees per plot would be required when employing the index, compared with 50 trees per plot if only one variable was recorded per tree, namely, the percent of surface area of the three-year-old needle whorl with chlorotic mottle. Finally, the use of the index decreases the amount of field work and yet provides a more descriptive baseline estimate of tree condition for comparison with future observations.

Rice et al., (1983) used visible symptoms on ponderosa pine foliage as a biomonitor for changes in levels of ambient sulfur dioxide and fluoride at two sites in Montana. Foliage was cut from plot trees annually and brought to the laboratory where assessments of symptoms could be made under a dissecting microscope. The symptoms evaluated were described as: needle tip burn, mottling, basal injury, needle surface area injured, total necrosis, and healthy needles. Tissue content of sulfur and fluoride was measured. These variables were analyzed separately and not combined into an index. Over a five-year period most variables showed significant differences between tree populations located at Colstrip (until recently pristine) and Billings (polluted by several sources for 40 years).

In conclusion, visible injury ratings for foliage and tree crowns will continue to be used on an operational basis to determine the spatial distribution and severity of air pollutant injury to pines in California and other western states. Considerable time and effort is required to make these surveys; therefore, it is important to continue to strive toward a use of the same biomarkers and satisfactory sampling procedures so that data taken by several agencies can be compared from year to year and from place to place. The Air Quality Division of the U.S. National Park Service is providing leadership in this effort.

NEEDLE HISTOLOGY

The kinds of histological effects and their sequence of development depend mainly on whether the injury is acute or chronic. One study of acute injury, typified by needle tip necrosis of conifers, sampled the transition zone between necrotic and healthy tissue to identify individual effects for each of several stress agents (Stewart et al., 1973). They examined the cellular changes in the transition zone tissue caused by natural senescence, sodium chloride, boron, moisture stress, winter injury, suffocation, hydrogen fluoride, sulfur dioxide, ozone and combinations of hydrogen fluoride with sulfur dioxide or ozone. It was concluded that mesophyll collapse, resin duct occlusion, transfusion parenchyma hypertrophy and phloem abnormalities were commonly caused by several of these stresses acting independently. Therefore, the application of needle histology to diagnosis of acute injury has important limitations because cell and tissue responses in the transition zone are the same for several stress agents.

The histological examination of needle tissue with chronic injury (usually no tip necrosis) may offer brighter prospects in the case of ozone. Chronic ozone injury to pine is commonly characterized as a destruction of the contents of the mesophyll or plicate parenchyma cells. Affected cells are typically distributed randomly and are often seen more frequently in the outer layers of the mesophyll but not necessarily in the substomatal region (Evans and Miller, 1972). Phloem abnormalities have not been observed in pine needles with either acute or chronic ozone injury (Stewart et al., 1973, Evans and Miller, 1972).

A recent study of ponderosa pine employing both light and electron microscopy confirmed the typical mesophyll damage by ozone and further characterized the cytological changes associated with both ozone injury and natural senescence (Karenlampi, 1986). Histological examination is definitely useful because the principal macroscopic symptom of ozone injury (chlorotic mottle) may be induced by other stress agents.

In conclusion, histological examination will continue to be useful in the case of ozone to explore for injury to additional tree species in which needle senescence may be hastened without the obvious appearance of specific macroscopic symptoms. Recent reports in the literature regarding tree declines in Europe suggest that histological examination can be developed to finer levels of resolution. Histological changes will continue to be the most effective when used in combination with other biomarkers of air pollutant injury.

CHEMICAL ANALYSIS OF LEAF TISSUE

Tissue analysis for fluoride and sulfur have been an integral part of numerous studies of pollutant effects. At the West Whitecourt study site in Alberta sulfate and total sulfur content of the foliage of several tree species decreased with increasing distance along an emission corridor extending from a gas desulfurization plant. Foliar sulfate concentration was a better indicator of foliar loading than total sulfur (Legge et al., 1981). The stable isotope ratio derived from comparison of foliar sulfur, crustal (soil) sulfur and the fossil sulfur emissions from the gas plant revealed a difference sufficient to conclude that foliar sulfur accumulation was indeed from the fossil source.

Sulfate content was determined for pine needles collected at increasing intervals of distance downwind from Bakersfield, California. There were many sources of sulfur pollution in addition to the local petroleum industry. Therefore, the analysis of stable sulfur isotope ratios of foliar sulfate and the petroleum in the local area did not compare sufficiently to provide conclusive results (Taylor et al., 1986).

The analysis of foliage for fluoride content along 10 sample transects extending away from a fluoride source near Columbia Falls, Montana clearly identified the direction of pollutant transport in terms of a gradient of decreasing foliar fluoride content (Carlson and Dewey, 1971). The measured values of tissue fluoride ranged from slightly more than 1000 ppm at one-half mile from the source to approximately 20 ppm at a distance of 8 miles.

Both total sulfur and fluoride concentrations of ponderosa pine needles were used to monitor the annual accumulations at tree plots near Billings and Colstrip, Montana. The Billings plot had been influenced for 40 years by several pollutant sources. Both sulfur and fluoride levels at Billings remained considerably higher than Colstrip during the 5-year sampling period, even though a new coal-burning power generating plant had recently begun operation at Colstrip. These data provide a valuable background against which to compare future sulfur and fluoride loading at Colstrip (Rice et al., 1983).

The West Whitecourt study discovered an additional marker of sulfur pollution. A dramatic increase in manganese content of foliage was observed with decreasing distance to the source. This result coincided with a similar decrease in soil pH. Greater soil acidity made manganese more available for uptake (Legge et al., 1981).

In conclusion, analysis of tissue for suspected pollutants will continue to be very useful. Recent improvements in the speed and versatility of laboratory analytical equipment may encourage the investigation of the relationships of a whole suite of anions and cations in symptomatic plant tissue.

MANIPULATIONS THAT CLARIFY THE RESPONSE OF BIOMARKERS

Temporary exclusion of foliage from pollutant exposure under natural conditions in the forest provides an opportunity to observe the difference between the new healthy foliage and that which continues to be exposed. Adjacent groups of 10 naturally regenerated ponderosa pine saplings were observed for a 5-year period during which each group received one of the following treatments: ambient air in a greenhouse-like enclosure, carbon-filtered air inside a similar house, and ambient air outside of the enclosures. The treatments were carried out during the May-September "smog" season and greenhouse walls and roofs were removed during the winter. Chlorotic mottle symptoms no longer appeared on the new foliage in the filtered air treatment, foliage retention increased, and both height growth and diameter growth increased sharply compared to both ambient air treatments. Foliage of an outside tree in the path of the air exhausted from the filtered air chamber also ceased to exhibit symptoms while other portions of the tree continued to show symptoms (Miller and Elderman, 1977). These results provided confirming evidence that ozone was the cause of foliage injury and tree decline in the San Bernardino mountains.

The influence of ozone pollution on newly emerged seedlings of Giant Sequoia (*Sequoiadendron giganteum* (Lindl.) Decne.) is currently being studied in an open-top chamber experiment in Sequoia and Kings Canyon National Parks. The treatments include ambient air inside and outside chambers, carbon filtered air, and proportional addition of ozone at the level of 1.5 X ambient. The appearance of foliar symptoms in the ambient and enhanced ozone treatments has provided the information needed to survey nearby groves to determine the natural incidence of these symptoms. The results of one summer show significantly higher shoot and root weights for seedlings in carbon filtered air compared to the 1.5 X ambient treatment (Miller et al., 1988). The particular advantage gained from controlled exposure studies is that plant material is exposed to most of the common abiotic and biotic stresses in the presence and absence of the pollutant. Therefore, it is essential to locate such experiments in the natural habitat of the species in question. In conclusion, the method of long-term exclusion of trees and seedlings from polluted air in their natural environment may be at a threshold of new discovery with the development and application of branch chambers. This technique will assist in making comparative physiological measurements between seedlings and mature trees in different pollution environments.

SUMMARY

Several observations can be made about past uses of biomarkers of pollutant injury to trees that may make their use more effective in the future.

- Combinations of biomarkers must be chosen for each condition to be investigated; it is risky to rely on a single biomarker.
- Sample size must be an important consideration and efforts should be made to construct injury indices from groups of biomarkers.
- The time required for gathering sufficient biomarker data may require months or even years; it is desirable to identify combinations of biomarkers that yield information more rapidly.
- There is sufficient need for discovering and improving manipulations that enhance the utility and specificity of biomarkers.

References

Bega, R. V. 1978. Diseases of Pacific Coast Conifers. U. S. D. A., Forest Service, Agric. Handbk. No. 521. 206 pp.

Carlson, C. E., and J. E. Dewey. 1971. Environmental pollution by fluorides in Flathead National Forest and Glacier National Park. U.S.D.A. Forest Service, Northern Region Headquarters, Missoula, Montana. 55 pp.

Duriscoe, D. M. 1988. Methods for sampling *Pinus ponderosa* and *Pinus jeffreyi* for the evaluation of oxidant-induced foliar injury. Draft Final Report submitted to the National Park Service, Air Quality Division, Denver. Eridanus Research Associates and Holcomb Research Inst., Butler University, Indianapolis, IN. 39 pp.

Evans, L. S., and P. R. Miller. 1972. Ozone damage to ponderosa pine: a histological and histochemical appraisal. Amer. J. Botany. 59:297-304.

Karenlampi, L. 1986. Relationships between macroscopic symptoms of injury and cell structural changes in needles of ponderosa pine exposed to air pollution in California. Ann. Bot. Fennici. 23:225-264.

Legge, A. H., D. R. Jaques, G. W. Harvey, H. R. Krouse, H. M. Brown, E. C. Rhodes, M. Nosal, H. U. Schellhase, J. Mayo, A. P. Hartgerink, P. F. Lester, R. G. Amundson, and R. B Walker. 1981. Sulphur gas emissions in the boreal forest: the West Whitecourt case study. Water, Air, Soil Pollut. 15:77-85.

Malhotra, S. S., and R. A. Blauel. 1980. Diagnosis of Air Pollutant and Natural Stress Symptoms on Forest Vegetation in Western Canada. Info. Rept. NOR-X228, Canadian Forestry Service, Edmonton, 84 pp.

Miller, P. R. 1973. Oxidant-induced community change in a mixed conifer forest. In Air Pollution Damage to Vegetation, Advances in Chem. Ser. No. 122: Amer. Chem. Soc. pp 101-117.

Miller, P. R., and M. J. Elderman (Eds.). 1977. Photochemical oxidant air pollutant effects on a mixed conifer forest ecosystem. U. S. EPA-600/3-77-104. Environmental Research Laboratory, Corvallis, OR. 339 pp.

Miller, P. R., and R. E. Van Doren. 1981. Ponderosa and Jeffrey pine foliage retention indicates ozone dose response. In Proc. Sym. Dynamics and Management of Mediterranean-type Ecosystems, San Diego, CA. U.S.D.A. Forest Service, Berkeley, CA. p. 621.

Miller, P. R., R. D. Wilborn, S. B. Schilling, and A. P. Gomez. 1988. Ozone injury to important tree species of Sequoia and Kings Canyon National Parks. Draft Final Report. Submitted to the National Park Service, Air Quality Division. Denver, CO. 53 pp.

Muir, P. S., and T. V. Armentano. 1987. Evaluating oxidant-induced injury to foliage of Pinus ponderosa (West) and Pinus strobus (East). A comparison of methods. Interim Report to the National Park Service, Air Quality Division, Denver, CO submitted by Holcomb Research Inst., Butler University, Indianapolis, IN. 75pp.

Muir, P. S., and B. McCune. 1987. Index construction for foliar symptoms of air pollution injury. Phytopathology 71:558-565.

Pronos, J., D. R. Vogler, and R. S. Smith, Jr. 1978. An evaluation of ozone injury to pines in the southern Sierra Nevada. U.S.D.A. Forest Service. Report No. 78-1. Forest Insect and Disease Management, Region 5, San Francisco, CA. 17 pp.

Rice, P. M., R. A. Boldi, C. E. Carlson, P. C. Tourangeau, and C. C. Gordon. 1983. Sensitivity of *Pinus ponderosa* foliage to airborne phytotoxins: use in biomonitoring. Can. J. For. Res. 13:1083-1091.

Stewart, D., M. Treshow, and F. M. Harner. 1973. Pathological anatomy of conifer needle necrosis. Can. J. Bot. 51:983-988.

Taylor, O. C., P. R. Miller, A. L. Page, and L. J. Lurid. 1986. Effects of ozone and sulfur dioxide mixtures on forest vegetation of the southern Sierra Nevada. Final Report. Contract AO-135-33. Submitted to the California Air Resources Board. Statewide Air Pollution Research Center, University of California, Riverside, CA. 145 pp.

Williams, W. T., and J. A. Williams. 1986. Effects of oxidant air pollution on needle health and annual-ring width in a ponderosa pine forest. Environ. Con. 13:229-234.

Symptoms as Bioindicators of Decline in European Forests

Peter Schütt
Faculty of Forestry University of Munich West Germany

ABSTRACT

Forest decline in Europe is looked upon by most scientists as a complex disease of forest ecosystems caused or at least predisposed by air pollution. Symptoms of "Waldsterben," however, differ from those of classical air pollution with components like SO_2 or HF and also from damages by O_3, PAN or photochemical smog. This may be due to effects of hundreds of additional phytotoxic compounds in the air or to synergistic effects between them. Pollutants induce higher susceptibility against normal pathological events (abiotic as well as biotic) so that trees seem to die, for instance, from bark beetle-attack, fungi or frost. The consequences for symptomatology of forest decline are embarrassing: the typical, authentic symptom of the syndrome is hard to find, and possibly does not exist at all.

Examples are given, which demonstrate the difficulties *in situ* to give a reliable answer whether or not a given damage belongs to forest decline.

INTRODUCTION

The recent changes in condition of forests in central Europe are remarkable for several reasons:

1) more than 20 species of forest trees and shrubs are affected;
2) the changes in condition of all 20 species first became conspicuous at about the same time — late 1970s and early 1980s;
3) some of these changes are familiar but others have rarely been seen before, or have become evident at different times, places, and stages of forest growth and development;
4) the affected trees are found in an extremely broad range of geographical, climatic, soil, and elevational conditions; and
5) none of the familiar biotic, edaphic, climatic, and air-pollution causes of stress in forests (fungi, insects, poor soil conditions, drought, storms of various types, or airborne sulfur dioxide or fluoride) appear to provide an adequate explanation for the changes observed in the forests (Schütt and Cowling, 1985; Schütt et al., 1986).

In Germany, these remarkable changes in forest condition were given the collective name "Waldsterben" (forest death, forest decline) or "neuartige Waldschaden" (new types of forest damages). Forests have a very special place in the life of European people.

For this reason, forest pathologists, tree physiologists, ecologists, soil scientists, and many other experts in Germany and other countries of central Europe have been called upon to explain why these changes were occurring. So far, none of us have been able to do so with certainty. The reason is that there is no single, well-defined causal factor, or simple combination of one or two or even three causal factors, that can explain all the changes in forest conditions, which have been observed.

A considerable body of evidence points to the probability that airborne pollutant chemicals are involved. But this evidence is almost entirely circumstantial. Thus, most well-informed scientists in central Europe share the view that air pollutants are involved, but we share this view largely because no other set of causal and/or contributing factors has so far been found to explain the diverse symptoms observed on the trees themselves.

THE IMPORTANCE OF SYMPTOMS AS BIOINDICATORS OF STRESS

In human medicine, in the veterinary sciences, and in the study of disease and dysfunction in plants, observations of symptoms are almost invariably the first indicators that something is wrong with the individual organism or the ecosystem in which the individuals live. To put it another way, symptoms (changes in the physical appearance or the physiological function of the host organism itself) are the principal foundation for detection and diagnosis of disease and dysfunction in essentially all living organisms.

In the case of Waldsterben in central Europe, the following three types of symptoms typify the recent changes that have taken place in many species of trees under a wide variety of ecological conditions:

1) transparency of tree crowns (loss of crown density);
2) anomalies of tree growth; and
3) degradation of the fine feeder-root system of trees.

We will discuss the significance of each of three these typical symptoms in turn.

1. Analysis of Crown and Foliage Symptoms.

Crown transparency is a general symptom of decline in many tree species. It occurs under a wide range of edaphic and climatic conditions. Looking closer, however, this general symptom appears to result from three different processes:

(a) premature casting-off of foliage.—This is a common symptom of decline in many broad-leaf trees, especially *Fagus sylvatica* (beech), *Acer pseudoplatanus* (maple), *Betula pendula* (birch), and *Sorbus acuparia* (mountain ash). It is less common in *Quercus* (oak) and *Fraxinus* (ash) species. Premature loss of foliage often is accompanied by discoloration of the leaves. It may occur as early as late July or early August. Frequently, both premature discoloration and casting-off of leaves are interpreted as an early form of senescence. Premature casting-off of needles is also a common symptom of decline in several conifers, including *Abies alba* (silver fir), *Picea abies* (Norway spruce), *Pinus sylvestris* (Scots pine), and *Larix decidua* (European larch). Especially remarkable is the active casting-off of leaves while they are still green. This occurs in both European beech and maple as well as in Norway spruce, Scots pine, and European larch (Schütt and Cowling, 1985).

A special kind of needle casting is observed in *Picea abies* stands at elevations higher than about 700 m above sea level. Here, a pronounced yellowing of needles is observed where needles are frequently exposed to cloud mist. Often this yellowing is accompanied by magnesium deficiency.

In both high and low elevation *Picea abies* and *Abies alba* stands, needles are often cast-off after turning brown. This symptom usually originates with the older needles in the inner parts of the tree crowns. Since these brown needles are sometimes inhabited by fungal endophytes such as *Lophodermium piceae* or *Rhizosphaera kalkhoffii*, a controversy has developed about whether these fungi have a primary or only a secondary role in the etiology of spruce decline (Rehfuess and Rodenkirken, 1984; Schütt, 1985b).

We conclude that crown transparency is a common symptom of decline in many species of trees in central Europe. It often results from premature casting-off of leaves, it may result from the early induction of senescence processes, and it may be triggered by contact with cloud water, elevated concentrations of ozone or other airborne pollutant chemicals, or by fungi.

(b) **changes in leaf size and shape**.—Decreased leaf size, sometimes in combination with a lower leaf frequency and distortion of normal leaf form, especially close to the distal ends of leading shoots, is a common symptom of Waldsterben in *Betula, Fagus*, and *Carpinus* as well as in *Pinus*. It is not observed in *Quercus, Fraxinus, Abies*, or *Picea*, however. This is a second mechanism by which crown transparency can be increased, even in cases in which premature casting of leaves is not common. These changes are also anomalies of tree growth.

(c) **changes in leaf position and branching habit**.—Normal, healthy tree crowns are characterized by a dense and very uniform distribution of leaves over both the periphery and interior parts of the crown. This pattern is based on a well-balanced system of growth of short-shoots and long-shoots. The balance in number and form of these two types of shoots is controlled by growth hormones and usually follows the rules of apical dominance (acrotony). The "rhythm" of development of these two general types of shoots is what gives rise to the characteristic shape of crowns in various species of forest trees.

A common manifestation of disturbance of these hormonal control systems is a reduction in the number of long-shoots and a concomitant increase in the number of short-shoots. These changes are most easily recognized in *Fagus* (European beech), in which, as a consequence, the shape of the whole crown is altered. The crown is turned into a few very long long-shoots that are covered with large numbers of very short short-shoots. Thus, the crown becomes very open and transparent, even though the total number of leaves in the whole crown may not be greatly changed (Roloff, 1986).

In *Quercus*, a similar phenomenon is based on an increased intensity of apical dominance—leaves are formed by only the most distal buds of much foreshortened sets of branch internodes so that the crown consists of some portions that have a very bushy appearance and other portions that are almost devoid of leaves.

In *Abies*, by contrast, almost the opposite changes in branching habit take place. In this case a loss of apical dominance leads to a very flattened appearance of the crown which is often referred to as a "stork's nest." This change in morphology of branching is not uncommon in older *Abies*, but the "stork's nest" form of crowns is now observed on very much younger *Abies* trees (Schütt and Cowling, 1985).

A recent investigation of healthy and declining *Picea abies* in the Bavarian forest also revealed irregularities in branching habit and branching intensity in this species.

During the last 10 years, declining trees formed only 55 percent as many lateral shoots as were formed on nearby healthy trees (Schill, 1988).

We conclude that changes in leaf position due to irregularities in branching habit, or decreased numbers of lateral shoots, provide both a third mechanism leading to increased transparency of tree crowns and a conspicuous illustration of anomalies of tree growth.

2) Anomalies of tree growth.

Anomalies of tree growth also occur under a wide range of ecological conditions. This second general foliage symptom also appears to result from two different processes:

a.) changes in branching habit (part of process 1b (above); and
b.) formation of epicormic shoots.

Formation of epicormic branches.—A common response of trees subject to many different types of stress is the formation of epicormic branches. These abnormal shoots are formed by stimulation of otherwise dormant lateral buds or new buds formed in the cambium. Epicormic branches usually develop on the upper sides of first-order lateral branches. Formation of such shoots usually occurs only during the year immediately following the stress. In the case of Waldsterben-affected trees of *Picea abies* and *Picea rubens*, however, epicormic branches continue to be formed year-after-year so that they gradually replace the normal branches, which tend to lose their foliage by one or more of the mechanisms described above. As a result, trees with many epicormic branches, although seriously injured by loss of normal foliage, are often incorrectly diagnosed as normal because the foliage of epicormic shoots tends to mask the loss of normal foliage.

Thus, formation of epicormic branches year-after-year is a common symptom in many Waldsterben-affected trees. It is a very significant anomaly of growth, but, in extreme cases, tends to mask the loss of normal foliage which leads to increased crown transparency.

3) Analysis of Root Symptoms

Degradation of the fine feeder-root system of trees and decreased abundance of mycorrhizae are common symptoms of decline in all tree species in central Europe. Root systems are much more difficult to study than crown and foliage symptoms and are less well understood as regards timing and causes. Fundamentally, the trees showing above-ground symptoms also show a conspicuous decrease in abundance of fine feeder-roots and mycorrhizae. But very few studies have been made to determine if these changes in root systems precede or follow the occurrence of crown and foliage symptoms (Schütt et al., 1986).

If degradation of feeder-roots or mycorrhizae were to occur first, it might be inferred that failure of the root system is a primary cause of the crown and foliage symptoms. Conversely, if crown and foliage symptoms were to occur first, it might be inferred that failure of the foliage system is a primary cause of degradation of the root system. If both types of symptoms occur simultaneously, or if the sequence of these two events is highly irregular, then possible causal linkages between the two are even more uncertain.

DISCUSSION AND GENERAL CONCLUSIONS

The decline of forests in central Europe is a serious pathological, physiological, and ecological problem. For this reason, forest pathologists, tree physiologists, soil scientists, and ecologists all have a responsibility to contribute our best skills to the challenges of detection, diagnosis, and management of the stress factors involved. Careful study of symptoms with the perspective of discovering clues to the identification of probable causal and contributing factors is one of the special skills of forest pathology. In the case of forest decline in central Europe, the detection of reliable specific symptoms is extremely complicated because of the large number of tree species involved and the very wide range of climatic, edaphic, ecological, and elevational conditions under which the symptoms have been observed.

In practice, crown transparency is used as the most general indicator of forest decline in central Europe. As discussed earlier, however, this general symptom has many different morphological manifestations, which vary from species to species, and involve different physiological mechanisms. Thus, with our present meager state of knowledge, we do not have adequate tools for reliable symptom surveys. For many of the 20 tree and affected shrub species and several different symptom characters (such as branching intensity and bud frequency), we do not even know the border between the appearance of healthy and declining trees. Also, our knowledge about the relationships among the morphology, anatomy, and the physiology of the trees is very sparse (Schütt, 1985a,b).

These gaps in knowledge will not be closed in the immediate future. For this reason, significant efforts should be made to develop herbaceous plants and forest tree seedlings genetically selected for susceptibility to specific airborne chemical and other stress factors. Such plants would be easily movable and manipulatable and thus valuable as a aid to diagnosis.

In addition, the general field of comparative symptomatology of forest trees, which has been severely neglected in recent years, should be reactivated and coordinated on an international level. If this is not done, we will continue to have problems determining whether different groups of scientists in different locations are indeed working on the same or different forest decline problems.

References

Rehfuess, K. E., and H. Rodenkirchen. 1984. Über die nadelröte — Erkrankung der Fichte (*Picea abies* Karst.) in Süddeutschland. Forstw. Cbl. 103:248-262.

Roloff, A. 1986. Morphologische Untersuchungen zum Wachstum und zum Verzweigungssystem der Rotbuche (*Fagus sylvatica* L.). Mitt. d. Dt. Dendr. Ges. 76:5-49.

Schill, H. 1988. Morphologische und ökologische Untersuchungen zum Austriebsund und Verzweigungsverhalten junger Fihten (*Picea abies* (L.) Karst und Lärchen (*Larix decidua* (Mill.)). Diss. Forstwiss., Università München, München, West Germany.

Schütt, P. 1985a. Vernetzte Problemstellung — Vernetzte Forschung — Betrachtungen zur Waldsterbenforschung. Forstarchiv 56:179-181.

Schütt, P. 1985b. Das Waldsterben — eine Pilzkrankheit. Forstw. Cbl. 105:169-177.

Schütt, P., and E. B. Cowling. 1985. Waldsterben, a general decline of forests in central Europe: symptoms, development, and possible causes. Plant Disease 69:548-558.

Schütt, P., W. Koch, H. Blasche, K. J. Lang, H. J. Schunk, and H. Summerer. 1986. So stirbt der Wald. BLV-Verlag, Mnchen. 127 pp.

THE WORKSHOP PAPERS TREE-STAND/ECOSYSTEM SESSION

Resource Allocation in Trees and Ecosystems

R.H. Waring
Forest Science Department Oregon State University Corvallis, Oregon 97331

ABSTRACT

As ecosystems are subjected to various kinds of stresses, the availability of resources required to support life is altered. As a consequence, plants, animals, and microbes alter the way in which resources are expended. When a particular ecosystem is subjected to an unusual combination of stresses, or an unusual intensity of one type of stress, biologically catalyzed reactions are initiated that permeate throughout the system. A record of how the biological components of an ecosystem reacted to various stresses is often encoded in the tissue composition of trees. For example, a nutritional imbalance may alter the normal ratio of essential minerals to one another in foliage or in roots. Other types of stresses affect the amount of carbon stored in various organs, the possibility of seed production, and the form of a tree's bole. Structural indices of leaf/bole, leaf/phloem, and leaf/root allocation may aid in interpreting other signals of environmental change associated with mineral and stable isotope composition.

INTRODUCTION

The allocation of carbon into various components of trees and ecosystems changes depending on the environment (Waring 1983). Trees on an exposed headland exhibit extreme stem taper and an extensive supporting root system. Such trees also contribute a larger proportion of fresh foliage and limbs to annual litterfall than normal. If pollutants affect tree growth and the decomposition process, then deviations from normal patterns in carbon allocation should also be observed. Recognition of deviate responses depends on analysis along environmental gradients, historical reconstructions of changing environment, and experiments. I draw on these sources in suggesting production ratios most likely to change in response to pollutants.

Foresters, as an aid to estimating the value of trees, construct tables and equations that predict the wood volume in stems of specified basal diameters. Depending on the type of tree and the environment in which it grows, the taper of the stem varies. This fact has led foresters to develop local volume tables for many commercially valuable trees. The approach has been extended to estimating the weight of foliage, branches, and large diameter roots from the measurement of stem diameter (Whittaker and Woodwell 1968). Production rates are predicted by assessing the calculated change in weights of various organs in relation to measured changes in stem diameter. Because many of these allometric relationships were determined from trees sampled before pollution was extensive, they serve as a benchmark for comparing any change attributed to pollutants.

In the last decade the basic approach has been refined by recognizing that the cross-sectional area of conducting sapwood is often proportional to the foliage it supports at the time of peak leaf development (Waring 1983). This relationship is more accurate than that determined from diameter alone. Sometimes the historical development of tree crowns can be reconstructed by knowledge of what age sapwood converts to heartwood and from branch scars when limbs die (Margolis et al. 1988). By coring a tree it is possible to compare the amount of wood produced annually from a certain complement of foliage. This "growth efficiency" ratio decreases abruptly when the environment becomes less favorable and more slowly as trees age and the cost of maintenance respiration increases (Fig. 1).

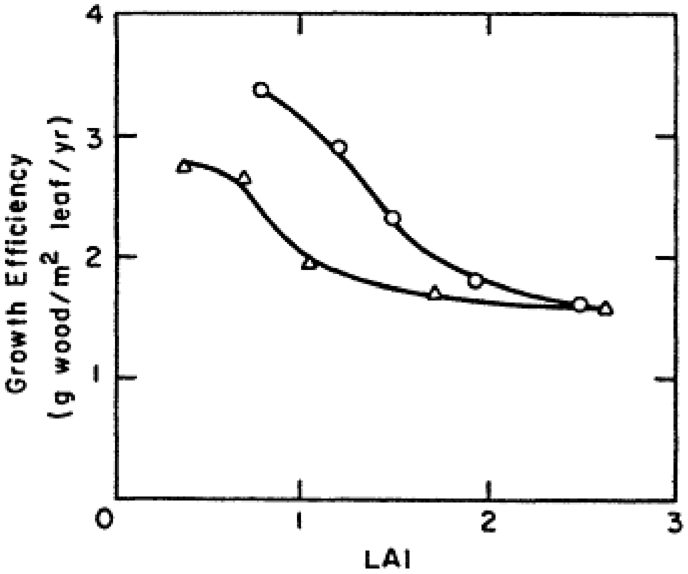


Figure 1.
When shrub cover was removed (○) from beneath an arid zone ponderosa pine forest, the growth efficiency of trees significantly increased over that observed with shrub cover present (△). As the canopies developed, the leaf-area index (LAI) eventually shaded out shrub cover and trees competed with one another to a similar extent. After Waring (1983).

By comparing changes in other selected ratios of structural biomass it may be possible to distinguish more specific causes of stress and to correlate these with parallel changes in the pollution load on forests.

PHOTOSYNTHESIS/BRANCH PRODUCTION

The capture of radiant energy (400-700 nm) in the process of photosynthesis differs depending on the exposure of leaves and their biochemical capacities. Photosynthesis can be predicted for different portions of tree crowns (Caldwell et al. 1986). When branch growth is compared with photosynthesis, a linear relationship results (Fig. 2). The slope of the relationship appears to differ slightly depending on the nutritional balance. Where pollutants are likely to damage phloem and restrict translocation, a higher than normal allocation of photosynthate to branch production would be expected.

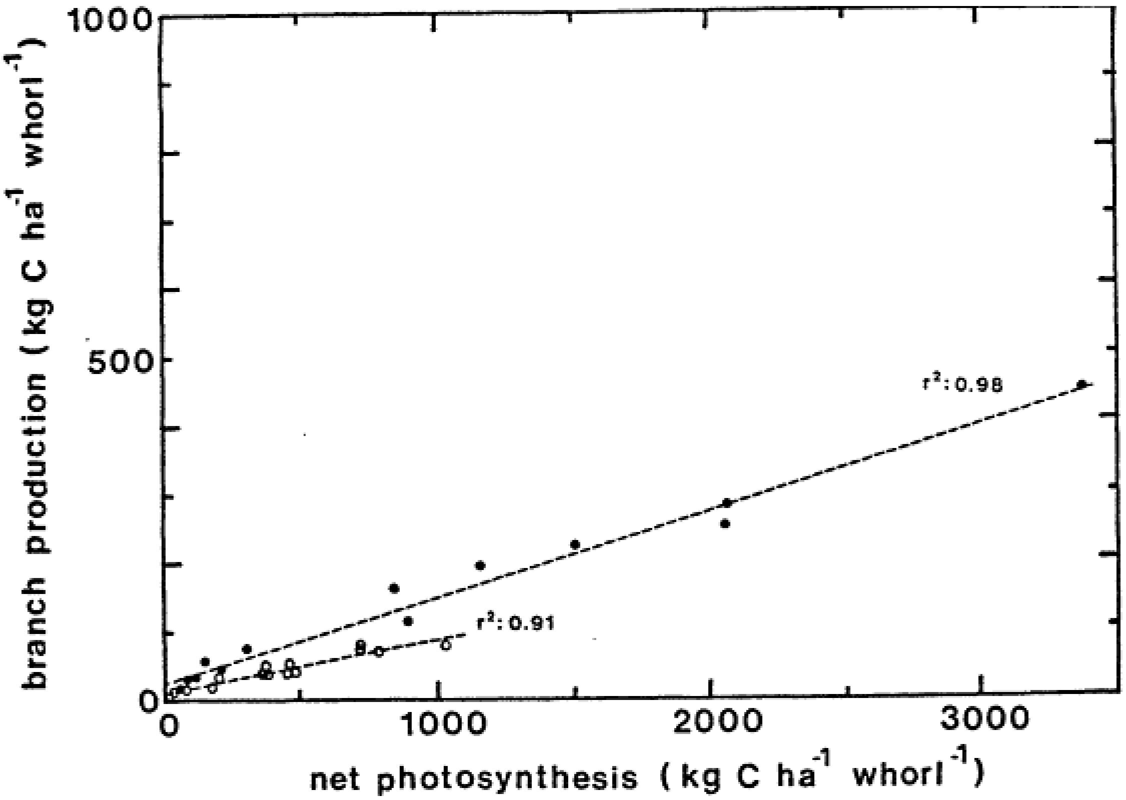


Figure 2.
Annual net photosynthesis by whorls of branches in a Scots pine stand contributes a fixed fraction to branch growth. On fertilized and irrigated plots (upper line) the fraction is higher than on control plots (bottom line). After Linder and Axelsson (1982).

PHLOEM/SAPWOOD AREA

If the transport of photosynthate through phloem is inhibited, then the cross-sectional area of sieve cells should be reduced in relation to leaf area or the surrogate, sapwood cross-sectional area. Scots pine trees provided with optimum water and nutrients exhibit a ratio of phloem/sapwood area in the stem, half that of unfertilized and unirrigated trees (Dr. Erik Mattson-Djos, University of Uppsala, Sweden). This corresponds with a similar reduction in the fraction of photosynthate allocated to fine-root production (Alexsson and Alexsson 1986). If pollutants reduce the functional area of phloem down the bole, annual wood increment should mirror this, resulting in reduced taper as noted by Schütt and Cowling 1985).

LEAF AREA/BOLE MAINTENANCE

As trees grow, the maintenance cost of parenchyma cells in the conducting tissue becomes proportionately larger. These cells make up nearly 30% of the sapwood in oak and 5% or more in the sapwood of other species (Waring and Schlesinger 1985). For conifers with a fairly similar percent of living cells in sapwood, pioneer species usually support fewer leaves with a given amount of sapwood. Species that follow in succession tend to support more leaves with less sapwood and to have lower light-compensation points for photosynthesis. Pollutants that reduce photosynthesis make shaded branches no longer self-sufficient and they die. The original sapwood serving those branches, however, remains alive until a large fraction of foliage is lost (Margolis et al. 1988). Advanced successional species have a slower turnover time of foliage than pioneer species, and thus take much longer to adjust to changing conditions. For these reasons, pioneer trees are likely to be favored in heavily polluted areas.

LEAF/LITTER PRODUCTION

Increased damage to foliage will increase the normal turnover, resulting in an increased fraction of new/total foliage on evergreens, and a temporary increase in litterfall. As the canopy becomes more open, it intercepts less precipitation and radiant energy. This favors a microclimate conducive to improved decomposition. If, however, heavy metals or nutrient imbalances are associated with conditions favoring canopy opening, the rate of carbon breakdown and mineral release may be reduced below that expected. Deviations from expected rates may be indicative of pollutants affecting heavy metal and nutrient balances (O'Neill et al. 1977). Imbalance in N:P:S ratios in foliage and litter also are indicative of nutritional problems affecting tree growth and litter decomposition (Waring 1985, Staaf and Berg 1982).

BARK BEETLE ATTACKS/ GROWTH EFFICIENCY

In many forests, bark beetle attacks follows a reduction in tree vigor. Christiansen et al. (1987) illustrated that any stress that critically reduced the amount of photosynthate being translocated down the bole during the period of insect attack lowers production of defensive compounds. In general, growth efficiency provides a good index to the threshold at which trees are killed by a particular density of attacking beetles (Fig. 3).

CONCLUSION

Changes in carbon allocation must be based on some reference to normal. Local volume tables, stem analyses, and methods that quantify changes in climate and atmospheric deposition can assist in interpreting the significance of observed alterations in allometric relationships. Sometimes it is possible to reconstruct the development of tree canopies by correlation with sapwood cross-sectional area. Shifts in how photosynthate is allocated to branches and bole may be indicative of changes associated with pollution load. Alterations in phloem conducting area may also result. Analysis across pollution gradients may be useful in assessing the value of proposed techniques.

RECOMMENDATIONS

Healthy forests contain trees able to allocate a considerable fraction of photosynthate to wood production. Any environmental stress decreases the fraction of wood produced/unit of foliage. This index of vigor correlates with a tree's ability to withstand a fixed amount of defoliation, bark beetle attacks, pathogenic infection, and dose of air pollutants. Sustained exposure to new stresses will subsequently lower vigor, reduce carbohydrate reserves, and tree resistance to a variety of pests and pathogens.

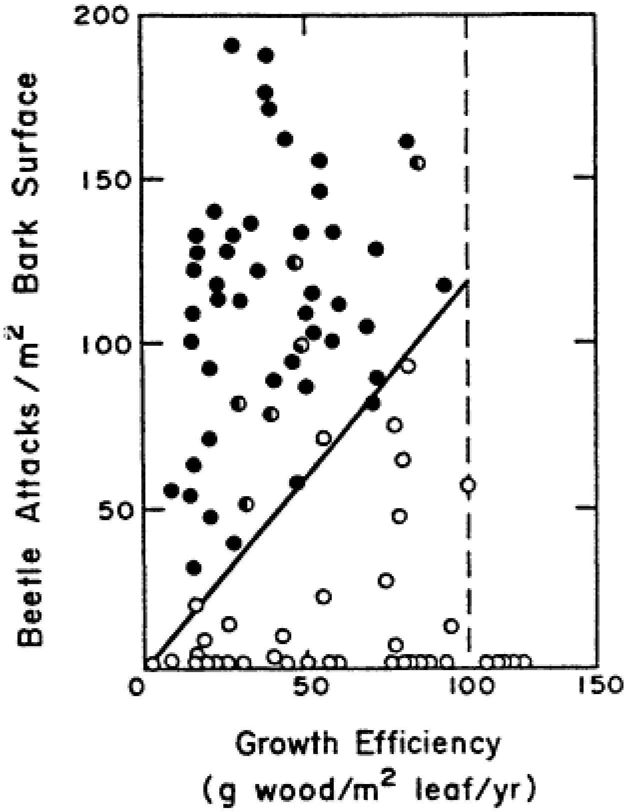


Figure 3.
Growth efficiency provides an index to the density of bark beetle attack required to kill lodgepole pine trees. Filled or partly filled circles represent the proportion of conducting tissue killed on attacked trees. Open circles represent trees able to halt all beetle attacks before any conducting tissue was killed. The dotted vertical line indicates the boundary above which beetle attacks are unlikely to cause tree mortality. After Waring and Pitman (1985).

Tree vigor, defined as grams of wood produced annually per square meter of foliage, can be assessed by extracting wood cores and determining growth, sapwood thickness, and tree diameter. I recommend that all forest studies develop the constants for applying these relationships and use them as a general frame of reference.

In specific cases where low vigor is recorded and air pollution is expected to be a contributing cause, further analyses are warranted. From what we know about ozone, excess nitrate, and sulfur dioxide effects, allocation of wood to branches should increase/ unit of foliage while that to the lower stem and roots should decrease. These expected alterations in branch and bole wood allocation should be sampled because the pattern of response differs from that initiated from most other kinds of environmental stresses.

More detailed analyses of phloem, starch reserves, and nutrient balances could also be made in foliage, twigs, and sapwood but are seasonally dependent variables that are best studies in experimental programs involving stable isotope and remote sensing techniques (see papers by B. Fry and B. Rock).

References

Axelsson, E., and B. Axelsson.1986. Changes in carbon allocation patterns in spruce and pine trees following irrigation and fertilization. Tree Physiol. 2:189-204.

Caldwell, M.M., H.-P. Meister, J.D. Tenhunen, and O.L. Lange. 1986. Canopy structure, light microclimate and leaf gas exchange of *Quercus coccifera* L. in a Portuguese macchia: measurements in different canopy layers and simulation with a canopy model. Trees 1:25-41.

Christiansen, E., R.H. Waring, and A.A. Berryman. 1987. Resistance of conifers to bark beetle attack: searching for general relationships. Forest Ecol. & Management 22:89-106.

O'Neill, R.V., B.S. Ausmus, D.R. Jackson, R.I. Van Hook, P. Van Voris, C. Washburne, and A.P. Watson. 1977. Monitoring terrestrial ecosystems by analysis of nutrient export. Water, Air, Soil Pollut. 8:271-277.

Linder, S., and B. Axelsson. 1982. Changes in carbon uptake and allocation patterns as a result of irrigation and fertilization in a young *Pinus sylvestris* stand. Pp. 38-44 in Carbon uptake and allocation in subalpine ecosystems as a key to management, R.H. Waring (ed.). Proceedings of an I.U.F.R.O. Workshop. Forest Research Lab., Oregon state Univ., Corvallis, OR.

Margolis, H.A., R.R. Gagnon, D. Pothier, and M. Pineau. 1988. The adjustment of growth, sapwood area, heartwood area, and sapwood saturated permeability of balsam fir after different intensities of pruning. Can. J. For. Res. (in press).

Schütt, P., and E.B. Cowling. 1985. Waldsterben, a general decline of forests in central Europe: Symptoms, development, and possible causes. Plant Disease 69:548-558.

Staaf, H., and B. Berg. 1982. Accumulation and release of plant nutrients in decomposing Scots pine needle litter. Long-term decomposition in a Scots pine forest. II. Can. J. Bot. 60:1561-1568.

Waring, R.H. 1983. Estimating forest growth and efficiency in relation to canopy leaf area. Adv. Ecol. Res. 13:327-354.

Waring, R.H. 1985. Imbalanced forest ecosystems: assessment and consequences. Forest Ecol. & Management 12:93-112.

Waring, R.H., and G.B. Pitman. 1985. Modifying lodgepole pine stands to change susceptibility to mountain pine beetle attack. Ecology 66:889-897.

Waring, R.H., and W.H. Schlesinger. 1985. Forest ecosystems: concepts and management. Academic Press, Orlando, Fl.

Whittaker, R.H., and G.M. Woodwell. 1968. Dimension and production relations of trees and shrubs in the Brookhaven forest, New York. J. Ecol. 56:1-25.

Markers of Air Pollution in Forests: Nutrient Cycling

Dale W. Johnson
Environ. Sci. Division Oak Ridge National Lab P.O. Box 2008 Oak Ridge, TN 37831-6038
Helga Van Miegroet
Environ. Sci. Division Oak Ridge National Lab P.O. Box 2008 Oak Ridge, TN 37831-6038
Wayne T. Swank
Coweeta Hydrologic Lab 999 Coweeta Lab Road Otto, NC 28763.

ABSTRACT

Air pollution may affect forest nutrient cycles in a number of ways, but many of these effects are difficult to evaluate because control sites unaffected by air pollution are seldom available for comparison. Air pollution may alter either the nutrient content or the flux rates in forest ecosystems, but, with a few exceptions (e.g., SO_4^{2-}, in foliage, Al^{3+} or Pb in tree rings), flux measurements provide more sensitive measures of air pollution effects. The fact that nutrient pools are typically quite large relative to the flux rates into and out of them accounts for the sensitivity of flux measurements. However, nutrient pools may be significantly affected by air pollution over long periods of time, and the importance of sample archiving as a means of detecting these long-term changes in nutrient pools (e.g., soils) cannot be overemphasized. Acid deposition and/or foliar damage by ozone may cause increased foliar leaching, but the degree to which air pollution affects foliar leaching is very difficult to assess in the absence of control sites for comparison. Decomposition may also be altered by increased acid input, trace metal deposition, or gaseous pollution; but, once again, many of these potential changes are very difficult to evaluate for lack of control sites. Acid deposition nearly always influences soil leaching rates to some degree. In the case of SO_4^{2-}, reasonable assumptions about background concentrations often allow an approximate estimate of the degree to which leaching is affected by sulfate deposition. For NO_3^-, there is considerably less certainty about exactly what background levels should be. It is therefore more difficult to evaluate the degree to which current NO_3^- leaching rates are affected by atmospheric N deposition without a full analysis of the N cycle of the ecosystem in question. The same basic considerations apply to stream concentrations Stream concentrations may be less sensitive to air pollution effects than soil solution concentrations, but the advantages of ease of sampling and integration of whole watershed response make stream concentration monitoring an attractive possibility as a marker of air pollution in forest ecosystems.

INTRODUCTION

In general, nutrient fluxes (particularly solution fluxes such as foliar and soil leaching) respond most readily to air pollution, whereas nutrient pools (e.g., soil, vegetation, and forest floor contents) are often sufficiently large to remain rather insensitive to all but very long-term effects of air pollution inputs. Even in cases where long-term changes in nutrient pools have occurred, they may be difficult to identify and demonstrate if no control sites unaffected (or less affected) by air pollution are available for comparison. Thus, with the exception of certain key nutrients or ions in certain ecosystem components, nutrient fluxes usually represent more sensitive markers of the presence of air pollution than do nutrient pools. The presence of air pollution by no means implies that this pollution is creating any damage to the ecosystem, however. From a nutrient cycling perspective, damage may be defined as either the depletion of a critical nutrient or the elevation of any element to toxicity levels. Thus, while nutrient or element pools are relatively insensitive markers of the presence of air pollution, they may be the most sensitive markers of pollutant damage.

In the following discussion, we review very briefly the literature regarding effects of air pollution on various nutrient fluxes and contents in forest ecosystems, attempt to extract from that literature useful markers of air pollution on forest nutrient cycles, and make some recommendations as to general sampling protocols that may be useful in monitoring pollutant presence and effects on forest nutrient cycles.

DEPOSITION AND CANOPY INTERACTIONS

There has long been a concern over the effects of acid deposition on foliar leaching. Studies involving artificial acid irrigation have variously found either no effect on leaching (e.g., Haines et al. 1985) or an increase in foliar leaching (at very low pH) (e.g., Wood and Bormann 1974). Amthor (1986) argues convincingly that the energy costs of increased uptake to compensate for accelerated foliar leaching are inconsequential. However, there may be significant nutritional consequences if uptake does not compensate for these losses. Rehfuess (1987) and Prinz (1985) advance a foliar leaching hypothesis to explain the decline of high-elevation forests in Germany. They hypothesize that foliage predamaged by exposure to photo-oxidants like ozone is especially susceptible to increased Ca and Mg leaching. In soils low in exchangeable Ca and Mg, uptake apparently does not compensate for this additional foliar leaching, and foliar Ca and Mg concentrations decline, eventually to deficiency levels. These deficiencies, in turn, lead to reduced frost hardiness, reduced chlorophyll content and photosynthesis rates, and reduced root growth and uptake.

Unfortunately, the lack of control sites presents serious problems for testing the foliar leaching hypothesis and for using foliar leaching as a marker of air pollution stress. As noted by Lovett et al. (1986), the disappearance or H^+ in the forest canopy does not necessarily mean that foliar leaching has increased: the H^+ may simply have gone into a neutralization reaction with organic anions that naturally leach from the canopy. This does not imply, however, that acid deposition has no effect on the ecosystem: H^+ in combination with organic acid would still result in throughfall with greater total acidity than that in throughfall in an unpolluted environment. Low foliar Mg could not serve as a reliable marker either because Mg deficiencies could clearly occur in pristine as well as in polluted environments.

Tissue concentration of certain pollutants has obvious potential use as a marker of air pollution. For instance, Bieberdorf et al. (1958) found that SO_4^{2-} in Pinus taeda foliage was a very sensitive index of proximity to an SO_2 source, presumably because of SO_2 absorption by the foliage. Such accumulation of SO_4^{2-} in the foliage will occur only to the extent that trees are not S deficient. Kelly and Lambert (1972) indeed found foliar SO_4^{2-} to be a reliable indicator of S excess (i.e., SO_4^{2-} accumulates only when the S supply is greater than tree S requirements). Foliar SO_4^{2-} may not be a sensitive index of greatly excessive S deposition, either: foliar SO_4^{2-} may reach as high as 50% of total foliar S, but apparently does not exceed this value even in areas of very high S deposition (Johnson 1985).

DECOMPOSITION, NUTRIENT MINERALIZATION, AND NITRIFICATION

Most studies of the effects of air pollution on decomposition have been conducted under conditions of artificial irrigation, often with extremely high inputs over short time periods, raising serious questions about the applicability of these results to actual conditions. For example, would soil organisms be able to adapt to more acidic conditions over longer periods with lower inputs? Moreover, results have been inconsistent between sites and from one study to another. For instance, Like and Klein (1985) found that acid treatments had no effect upon nitrification but stimulated nitrogen mineralization in soil columns from Camel's Hump, Vermont, whereas Strayer et al. (1981) found that acid irrigation had a negative effect on nitrification and either no effect or a stimulative effect upon nitrogen mineralization of soils from Panther Lake in the Adirondacks in New York. Klein et al. (1983) reported that the same treatment enhanced nitrification in one soil from the Adirondacks and inhibited it in two other soils from the same region. Stroo and Alexander (1986) brought forward the suggestion that decreased N mineralization does not necessarily represent a decline in N availability because part of the N supply could be entering with acid precipitation.

Baath et al. (1980) found reduced decomposition rates (measured by the litterbag technique) following acidification of forest soils in Sweden, whereas Hovland et al. (1980) found that acid irrigation caused either increases or decreases in decomposition rates (litterbag technique) depending upon the time of measurement or the amount of acid applied. Similarly, Chang and Alexander (1984) showed that simulated acid rain applied to three northeastern soils enhanced or inhibited decomposition depending on the amount of acid applied and the amount of organic acid leached from soil samples. In another study, sulfuric acid applications to hardwood leaf packs generally stimulated the loss of litter, nutrients, and trace metals from the soil surface (Lee and Weber 1983). Hay et al. (1985) suggested that acidification of the mineral horizons of a podzol soil inhibited the transport of the major classes of soil organic components but increased the transport of nitrogenous substances. Hovland (1981), Kelly and Strickland (1984), and Johnson and Todd (1984) observed little or no effect of artificial acid irrigation upon CO_2 evolution. On the other hand, in one of the few studies that did not involve artificial irrigation, Prescott and Parkinson (1985) found that decomposition was greatly reduced in sites near a sour gas plant that had been emitting sulfur for over 20 years, compared to that in sites farther from the plant.

In vitro studies by Moloney et al. (1983) on the degradation of spruce-fir litter from New England showed that microbial CO_2 evolution was reduced by acidic conditions, and that it was further repressed by the presence of Pb and Zn but not by Al or Cu. From these observations it was suggested that the acidity and heavy metals introduced by polluted rain may adversely affect the metabolism of decomposing microflora.

The effect of trace metals on soil microorganism activity has been examined in a number of laboratory and field studies. Generally, extremely high trace metal concentrations, such as those found in the vicinity of smelters, decrease soil microorganism activity (Zottl 1985). Friedland et al. (1986) reported that levels of Pb, Zn, and Ni below 10^3 mg/kg, levels of Cu below 10^2 mg/kg, and levels of Cu below 120 mg/kg have no measurable effects on decomposition rates. In a long-term field study at

Camel's Hump, Vermont, Friedland et al. (1986) found substantial increases in trace metal and organic matter concentrations of the forest floor over time. However, they suggested that current metal concentrations are not capable of significantly reducing decomposition of organic matter.

Hovland et al. (1980) summarize their results by stating: "The effect of the artificial acid rain appears to be more pronounced on the leaching of metal elements than on the biological activity and the dynamics of N and P." This statement seems appropriate not only for their particular studies but also for the majority of the artificial irrigation studies taken as a whole. It would seem that decomposition and nutrient mineralization must certainly be affected by soil acidification at some point, but this may come after the many decades of acid input usually necessary to change soil acidity. In the initial stages of acid input, however, biological processes in the soil generally do not appear to be as sensitive to acid inputs as chemical processes, namely, soil leaching.

SOIL LEACHING AND NUTRIENT EXPORT

The effects of acid deposition on soil leaching and stream water export have been studied extensively, are reasonably well understood, and have been modeled (e.g., Reuss and Johnson 1986). These effects can be estimated rather easily, even in the absence of control sites, given certain reasonable assumptions about background solution concentrations of SO_4^{2-} and NO_3^-. After the assumed background SO_4^{2-} concentrations are subtracted, a comparison of the total cation or total anion concentration accounted for by SO_4^{2-} with that accounted for by HCO_3^- and organic acids usually provides an approximation of the net effect of atmospheric S deposition on nutrient leaching from soils. Exceptions to this principle, such as sites where significant S-bearing minerals are present or sites where sulfur-bearing fertilizers have been previously applied, are usually clearly defined. The situation with respect to atmospheric N deposition and soil solution NO_3^- concentrations is more complex, because background NO_3^- can be quite high in certain circumstances (Van Miegroet and Cole 1984, Foster 1985), and many different types of ecosystem disturbances other than air pollution can cause an increase in soil solution NO_3^- concentration. The effect of N input on soil leaching also depends on whether NO_3^- or NH_4^+ enters the soil and on whether the latter form is subsequently nitrified. Further complications and uncertainties arise in cases where organic acids are the dominant natural leaching agents because organic acids are commonly estimated through the calculation of an anion deficit (Johnson et al. 1977, Cronan et al. 1978).

The effect of forest soil acidification on element leaching has been shown in soils of southern Sweden (Tyler et al. 1987). Measurements taken over decades show a decline in soil pH. This decline has increased the solubility of elements such as Mg^{2+}, Al^{3+}, Cd^+, and Zn^+, with consequent higher metal concentrations in soil water and increased output of these metals.

The most reliable way to ascertain the effects of atmospheric S or N deposition on soil solution SO_4^{2-} and NO_3^- concentrations is through an analysis of the S and N cycles of the ecosystems in question. Atmospheric S and N deposition in excess of biological demands almost invariably results in an increase in SO_4^{2-} and NO_3^- above background or natural levels. The SO_4^{2-} and NO_3^- can, in turn, leach from the soil if inorganic reactions permit it. Inorganic interactions between NO_3^- and soils are normally minimal, and, as a general rule, atmospheric N input in excess of biological demands leads to increased NO_3^- and associated cation leaching. Fortunately, biological demands for N are often quite high, and many forests are, in fact, N-limited (Cole and Rapp 1981). There are exceptions, however, in which very high rates of NO_3^- leaching occur as a direct consequence of high rates of atmospheric N deposition (Van Breemen et al. 1982) or as a result of excessive N fixation (Van Miegroet and Cole 1984).

On the other hand, biological demands for S are less than 5% of those for N (on a molar basis) and are typically exceeded by atmospheric S inputs in polluted regions (Johnson 1985). Unlike NO_3^-, however, SO_4^{2-} can adsorb to soils (such as many in the southeastern United States) rich in Fe and Al oxides and poor in organic matter. This adsorption can reduce the potential for leaching considerably up to the point where soil adsorption sites equilibrate with incoming SO_4^{2-}(Johnson 1985).

Because solutions have to be electrically neutral, increases in SO_4^{2-} or NO_3^- soil solution concentrations lead to concurrent increases in total cation concentration. The cation composition of the solution in equilibrium with a soil is controlled by both the total anion solution concentration and the composition of the cation exchange complex. These relationships are described fairly accurately by well-known selectivity equations developed more than 50 years ago (e.g., Gapon 1933). They predict, in essence, that the concentration of a given cation in soil solution is governed by the proportion of this cation on the soil exchange complex and the total ionic strength of the soil solution. Reuss (1983) points out one very interesting aspect of these equations regarding the effects of solution concentration changes: as total ionic concentration increases, the ratios of trivalent to divalent to monovalent cations in solution also increase. In other words, as the solution becomes more concentrated, cation concentrations increase in the following descending order: Al^{3+}, Ca^{2+}, Mg^{2+}, K^+, Na^+, and H^+. Thus, Al^{3+} concentrations in soil solution increase not only as the soil acidifies (i.e., as the proportion of Al^{3+} on the exchange complex increases) but also as the total ionic concentration of soil solution increases. These equations also imply that, compared with leaching of the other major cation nutrients, K^+ leaching from soils will be the least affected by acid deposition.

These exchange equations further suggest that the H^+ concentration increases to some extent (though not as much as Al^{3+}, Ca^{2+}, or Mg^{2+}) with any increase in total ionic concentration, even without direct H^+ input from the atmosphere. This increase in H^+ (decrease in pH) causes HCO_3^- and organic anions to protonate (becoming H_2CO_3 and uncharged organic acids), leading to a decrease in the concentrations of these natural anions. This replacement of natural, weak acid anions with atmospherically introduced, strong acid anions (SO_4^{2-} and NO_3^-) is referred to as an "anion shift." It results in a lesser acceleration of total nutrient leaching than would occur with the simple addition of SO_4^{2-} and NO_3^- to the natural leaching of HCO_3 and organic anions. Krug and Frink (1983) argued that this anion shift can be such that no net increase in total cation leaching occurs, even though the anion composition of soil solution changes dramatically. This is an extremely unlikely, if not impossible, scenario in that it would require a H^+ concentration increase equivalent to the addition of SO_4^{2-} and NO_3^-. However, the mitigative effect of the anion shift can be significant in acid soils, where H^+ is a significant cationic solution component.

CHANGES IN SOILS

Since soil nutrient pools are usually large relative to inputs and outputs of nutrients, changes in soil content often occur very slowly and are very difficult to measure. Nonetheless, changes in soils have been noted in certain cases. We found evidence of modern SO_4^{2-} accumulation in soils near Oak Ridge, Tennessee, by sampling beneath and adjacent to old houses (Johnson, et al., 1981). Berden et al. (1987) provide an excellent review of the literature documenting changes in soil acidity over time as well as supply a reasonable evaluation of the potential role of acid deposition in causing these changes. Soil acidity per se is most emphatically not a good marker of air pollution effects; soils can become extremely acid due to natural leaching and plant uptake in unpolluted forests, under either intensive management (Turner and Kelly 1977) or pristine conditions (e.g., Johnson et al. 1977, Ugolini et al. 1977).

Although soils are rather insensitive to changes, we feel strongly that both long-term monitoring of soil chemical properties and rigorous sampling and archival protocols are highly desirable features of any program to monitor markers of air pollution. Had such a program been implemented in the past, much of the current heated debate and often unsupported speculation concerning the effects of air pollution on soils would not exist. Furthermore, the existence of archived soil samples, with proper tests for sample storage effects, would allow measurement and remeasurement of currently unmonitored soil parameters that could be of importance in the future.

CHANGES IN STREAMS

Perhaps the most reliable integrated marker of air pollution effects on forest nutrient cycles is the long-term trend or pattern in stream chemistry. Watershed studies have shown that alteration of solute concentrations in streams and net nutrient losses provide a sensitive indicator of ecosystem stress (O'Neill et al. 1977, Likens et al. 1977, Swank and Douglass 1977). For example, long-term studies in streams draining hardwood forests of the southern Appalachian Mountains show the beginning of delayed response to atmospheric deposition as evidenced by increased SO_4^{2-} concentrations, reduced HCO_3^- concentrations, and changes in levels of other solutes (Swank and Waide 1988). Shifts in stream chemistry have also been related to forest insect infestations and forest harvesting practices (Swank 1986). The use of stream chemistry monitoring as an indicator of air pollution in forests has several disadvantages: (1) a potential lag in response (compared to other ecosystem compartments more directly exposed to pollutants), (2) a lack of long-term records needed to establish trends, and (3) an inability to distinguish cause-and-effect relationships (in the absence of process studies). However, stream chemistry does integrate the spatial and temporal variability of forest nutrient cycles and may provide the most reliable index of cumulative effects of air pollution.

CONCLUSIONS

1. The most sensitive markers of the presence of air pollution are probably the concentrations of certain ions such as SO_4^{2-} and NO_3^- in natural waters. The sensitivity of a given natural water to air pollution declines with the degree to which the water is affected by biological and chemical cycling processes within the ecosystem; thus, the following order of sensitivity is generally found: deposition > throughfall > soil solution > streamflow. With respect to the ease of monitoring, the following order is found: streamflow > wet deposition > soil solution > throughfall > dry deposition.
2. Certain key ion or element concentrations in particular ecosystem components (e.g., SO_4^{2-} in foliage, Al^{3+} in tree rings, lead in forest floor) may also be useful markers of the presence of air pollution.

3. The mere presence of air pollution in a forest ecosystem can by no means be taken as evidence that air pollution is causing any significant damage to the ecosystem.
4. Nutrient pools are most likely to be affected by air pollution. However, due to their relatively large size, they are rather insensitive markers.
5. Sample archiving from nutrient pools of ecosystems with well-documented long-term element budgets is essential for determining long-term changes in ecosystem nutrient pools caused by air pollution as well as by other factors.

ACKNOWLEDGMENT

Research supported by the Electric Power Research Institute under Contract No. RP-2621 with Martin Marietta Energy Systems, Inc., under Contract No. DE-AC05-84OR21400 with the U.S. Department of Energy. Publication No. 3133, Environmental Sciences Division.

References

Amthor, J. S. 1986. An estimate of the 'cost' of nutrient leaching from forest canopies by rain. New Phytol. 102:359-364.

Baath, E., B. Berg, U. Lohn, B. Lundgren, H. Lundkvist, T. Rosswall, B. Soderstrom, and A. Wiren. 1980. Effects of experimental acidification and liming on soil organisms and decomposition in a Scots pine forest. Pedobiologia 20:85-100.

Berden, M., S. I. Nilsson, K. Rosen, and G. Tyler. 1987. Soil acidification: Extent, causes, and consequences. Report 3292. National Swedish Environmental Protection Board, Solna, Sweden.

Bieberdorf, F. W., C. L. Shrewsbury, H. S. McKee, and L. H. Krough. 1958. Vegetation as a measure indicator of air pollution. Part 1. The pine (Pinus taeda). Bull. Torrey Bot. Club 85:197-200.

Chang, F., and M. Alexander. 1984. Effects of simulated acid precipitation on decomposition and leaching of organic carbon in forest soils. Soil Sci. 138(3):226-234.

Cole, D. W., and M. Rapp. 1981. Elemental cycling in forest ecosystems. Pp. 341-409 in D. E. Reichle (ed.), Dynamic Properties of Forest Ecosystems. Cambridge University Press, London.

Cronan, C. W., W. A. Reiners, R. L. Reynolds, and G. Elang. 1978. Forest floor leaching: Contributions from mineral, organic, and carbonic acids in New Hampshire subalpine forests . Science 200:309-311.

Foster, N. W. 1985. Acid precipitation and soil solution chemistry within a maple-birch forest in Canada. For. Ecol. Manage. 12:215-231.

Friedland, A. J., A. H. Johnson, and T. G. Siccama. 1986. Coniferous litter decomposition on Camel's Hump, Vermont: A review. Can. J. Bot. 64:1349-1354.

Gapon, E. N. 1933. On the theory of exchange adsorption in soils. J. Gen. Chem. USSR 3:144-163 (in Russian).

Haines, B., J. Chapman, and C. D. Monk. 1985. Rates of mineral element leaching from leaves of mini plant species from a southern Appalachian forest succession subjected to simulated acid rain. Bull. Torrey Bot. Club 112:258-264.

Hay, G. W., J. H. James, and G. W. Vanloon. 1985. Solubilization effects of simulated acid rain on the organic matter of forest soils: Preliminary results. Soil Sci. 139(5):422-430.

Hovland, J. 1981. The effect of artificial acid rain on respiration and cellulase activity in Norway spruce needle litter. Soil Biol. Biochem. 13:23-26.

Hovland, J., G. Abrahamsen, and G. Ogner. 1980. Effects of artificial rain on decomposition of spruce needles and on mobilization and leaching of elements. Plant Soil 56:365-378.

Johnson, D. W. 1985. Sulfur cycling in forests. Biogeochemistry 1:29-43.

Johnson, D. W., D. W. Cole, S. P. Gessel, M. J. Singer, and R. V. Minden. 1977. Carbonic acid leaching in a tropical, temperate, subalpine, and northern forest soil. Arct. Alp. Res. 9:329-343.

Johnson, D.W., G.S. Henderson, and D.E. Todd. 1981. Evidence of modern accumulation of sulfate in an east Tennessee forested Ultisol. Soil Science 132:422-426.

Johnson, D. W., and D. E. Todd. 1984. Effects of acid irrigation on carbon dioxide evolution, extractable nitrogen, phosphorus, and aluminum in a deciduous forest soil. Soil Sci. Soc. Am. J. 48:664-666.

Kelly, J. M., and R. C. Strickland. 1984. CO_2 efflux from deciduous forest litter and soil in response to simulated acid rain treatment. Water Air Soil Pollut. 23:431-440.

Kelly, J. M., and M. J. Lambert. 1972. The relationship between sulphur and nitrogen in the foliage of Pinus radiata. Plant Soil 37:395-408.

Klein, T. M., J. P. Kreitinger, and M. Alexander. 1983. Nitrate formation in acid soils from the Adirondacks. Soil Sci. Soc. Am. J. 47:506-508.

Krug, E. C., and C. R. Frink. 1983. Acid rain on acid soil: A new perspective. Science 221:520-525.

Lee, J. J., and D. E. Weber. 1983. Effects of sulfuric acid rain on decomposition rate and chemical element content of hardwood leaf litter. Can. J. Bot. 61(3):872-879.

Like, D. E., and R. M. Klein. 1985. The effect of simulated acid rain on nitrate and ammonium production in soils from three ecosystems of Camels Hump Mountain, Vermont. Soil Sci. 140:352-355.

Likens, G. E., F. H. Bormann, R. S. Pierce, J. S. Eaton, and N. M. Jackson. 1977. Biogeochemistry of a Forested Ecosystem. Springer Verlag , New York.

Lovett, G. M., S. E. Lindberg, D. D. Richter, and D. W. Johnson. 1986. The effects of acid deposition on cation leaching from a deciduous forest canopy. Can. J. For. Res. 15:1055-1060.

Moloney, K. A., L. J. Stratton, and R. M. Klein. 1983. Effects of simulated acidic, metal-containing precipitation on coniferous litter decomposition. Can. J. Bot. 61:3337-3342.

O'Neill, R. V., B. S. Ausmus, D. R. Jackson, R. I. Van Hook, P. Van Voris, C. Washburne, and A. P. Watson. 1977. Monitoring terrestrial ecosystems by analysis of nutrient export. Water Air Soil Pollut. 8:271-277.

Prescott, C. E., and D. Parkinson. 1985. Effects of sulphur pollution on rates of litter decomposition in a pine forest. Can. J. Bot. 63:1436-1443.

Prinz, B. 1985. Effects of air pollution on forests. Critical review discussion papers. Prepared discussion. J. Air Pollut. Control Assoc. 35:913-915.

Rehfuess, K. E. 1987. Perceptions on forest diseases in Central Europe. Forestry (in press).

Reuss, J. O. 1983. Implications of the Ca-Al exchange system for the effect of acid precipitation on soils. J. Environ. Qual. 12:591-595.

Reuss, J. O., and D. W. Johnson. 1986. Acid Deposition and the Acidification of Soil and Water. Springer Verlag, New York.

Strayer, R. F., C-J Lin, and M. Alexander. 1981. Effect of simulated acid rain on nitrification and nitrogen mineralization in forest soils. J. Environ. Qual. 10:547-551.

Stroo, H. F., and M. Alexander. 1986. Available nitrogen and nitrogen cycling in forest soils exposed to simulated acid rain. Soil Sci. Soc. Am. J. 50:110-114.

Swank, W. T. 1986. Biological control of solute losses from forest ecosystems. Pp. 85-139 in S. T. Trudgill (ed.), Solute Processes. John Wiley and Sons, Ltd., New York.

Swank, W. T., and J. E. Douglass. 1977. Nutrient budgets for undisturbed and manipulated hardwood forest ecosystems in the mountains of North Carolina. Pp. 343-364 in D. L. Correll (ed.), Watershed Research in Eastern North America: A Workshop to Compare Results . Smithsonian Institution, Edgewater, Maryland.

Swank, W. T., and J. B. Waide. 1988. Characterization of baseline precipitation and stream chemistry and nutrient budgets for control watersheds. pp. 57-79. IN W. T. Swank and D. A. Crossley, Jr. (eds.), Forest Hydrology and Ecology at Coweeta. Ecological Studies 66. Springer Verlag, New York.

Turner, J., and J. Kelly. 1977. Soil chemical properties under naturally regenerated Eucalyptus spp. and planted Douglas-fir. Aust. For. Res. 7:163-172.

Tyler, G., D. Berggren, B. Bergkvist, U. Folkengren-Grerup, L. Folkeson, and A. Rukling. 1987. Soil acidification and metal solubility in forests of south Sweden. Pp. 347-360 in T. C. Hutchinson and K. Meema (eds.), Effects of Atmospheric Pollutants on Forests, Wetlands, and Agricultural Ecosystems. Springer Verlag, New York.

Ugolini, F. C., R. Minden, H. Dawson, and J. Zachara. 1977. An example of soil processes in the Abies amabilis zone of Central Cascades, Washington. Soil Sci. 124:291-302.

Van Breemen, N., P. A. Burrough, E. J. Velthorst, H. F. van Dobben, Toke de Witt, T. B. Ridder, and H. F. R. Reijuders. 1982. Soil acidification from atmospheric ammonium sulphate in forest canopy throughfall. Nature 299:548-550.

Van Miegroet, H., and D. W. Cole. 1984. The impact of nitrification on soil acidification and cation leaching in red alder ecosystem. J. Environ. Qual. 13:586-590.

Wood, T., and F. H. Bormann. 1974. The effects of an artificial acid mist upon the growth of Betula alleghaniensis. Environ. Pollut. 7:259-268.

Zottl, H. W. 1985. Heavy metal levels and cycling in forest ecosystems. Experienta 41:1104-1113.

Human Perturbation of C, N, and S Biogeochemical Cycles: Historical Studies With Stable Isotopes

Brian Fry
The Ecosystems Center Marine Biological Laboratory Woods Hole, Massachusetts 02543

ABSTRACT

Stable isotopes can serve as historical tracers of anthropogenic pollutants. For example, recent anthropogenic inputs of C, N, and S have altered isotopic compositions of tree rings, lake sediments, and components of the atmosphere. Some of these effects are small in magnitude, 1 or less, and difficult to detect without extensive sampling. Examples include carbon isotope changes in tree rings due to local pollution effects or as a result of global changes in atmospheric CO_2 concentrations. Initial sulfur and nitrogen isotope studies suggest larger, more easily detectable changes of 2-7 in precipitation and lake cores. Sulfur isotope changes have been used to establish chronologies of anthropogenic sulfur inputs in some lake sediments. Nitrogen isotope studies of precipitation, lake cores, and tree rings are at a very early stage, but show promise for tracing human nitrogen additions from atmospheric deposition. Use of carbon, nitrogen, and sulfur isotopes as historical pollution markers is appealing because they are natural tracers of, and cycle with, anthropogenic C, N, and S.

INTRODUCTION

The light elements H, C, N, O, and S all are important constituents of organic materials in the environment. These elements have two or more stable isotopes whose variation can be used to trace cycling of water and organic materials in the environment. Here I consider past and potential future uses of these natural tracers in historical studies of anthropogenic pollution. My focus is on carbon, nitrogen, and sulfur isotopes because human activities are causing significant alteration in the cycling of these elements. Studies of hydrogen and oxygen isotopes, in many ways complementary to the studies summarized here, are treated elsewhere (Burk and Stuiver 1981; Brenninkmeijer 1983; White et al. 1985; White 1988; Sternberg 1988).

Because studies of carbon isotope distributions in plants and especially tree rings have been pursued most vigorously to date, I use examples from these investigations to illustrate three different types of isotopic changes that can accompany anthropogenic alteration of element cycles. These three effects are related to changes in sources, in stress-induced metabolism and changes in the concentration of nutrients. Following this short review, I show examples of potential uses of nitrogen and sulfur isotopes to follow anthropogenic changes in the nitrogen and sulfur cycles.

Because of the original and somewhat arbitrary choice of standards, $\delta^{13}C$ values for biological materials are almost always negative (-3 to-100‰) while the $\delta^{15}N$ and $\delta^{34}S$ values can be either positive or negative (-20 to +60 for $\delta^{15}N$ and-60 to +40 for $\delta^{34}S$). One may note that the absolute δ values are usually unimportant; they most often serve as reference points from which to calculate isotopic differences ($\Delta\delta$).

THEORY

Isotopes of an element differ by the number of neutrons in the nucleus. The mass difference caused by extra neutrons slightly alters chemical equilibria and reaction kinetics for different isotopes of the same element. These differences are typically quite small, much less than 1% (e.g., Fig. 1). Using a highly sensitive mass spectrometer, it is possible to measure accurately these small differences, and an extensive theoretical and empirical knowledge of isotopic fractionation now exists (Peterson and Fry 1987). In kinetic reactions, species containing the light isotope react somewhat faster because of lower activation energy. In equilibrium reactions, the light isotope will concentrate in those chemical species where it is less tightly bound. To cite well-known examples of these processes, kinetically-controlled reactions such as photosynthesis favor concentration of light carbon in plants, while CO_2 in the atmosphere is depleted in the heavy carbon isotope during equilibrium exchange with bicarbonate dissolved in the ocean. These kinds of fractionations introduce signals into the natural environment that are present as background isotopic distributions. Human activities often lead to isotopic changes detectable against this natural background.

EXAMPLES FROM 13C STUDIES

1. Source changes. Emission of CO_2 into the atmosphere from burning of fossil fuels and biomass has influenced the isotopic composition of CO_2 in the atmosphere. The preindustrial background isotopic composition of CO_2, recently measured in gas bubbles from Antarctic ice cores, averaged about-6.5 (Fig. 2). In recent decades, anthropogenic activities have added CO_2 with $\delta^{13}C$ =-25 to-30 to the atmosphere, resulting in a $\delta^{13}C$ decline of 1.3 to the current-7.8 value for atmospheric CO_2. In this case, simple mixing of carbon from natural and anthropogenic sources accounts for the observed isotopic changes.

Prior to the ice core studies, many investigations addressed the possible decline in atmospheric CO_2 $\delta^{13}C$ by analysis of tree rings. Carbon in trees typically averages-20 to-35‰, and is depleted in ^{13}C relative to atmospheric CO_2 due to kinetic isotope effects during carbon fixation in photosynthesis. Ideally, trees should maintain a constant offset to the isotopic composition of atmospheric CO_2 if tree rings are to serve as accurate biorecorders of CO_2 isotopic compositions.

Figure 2 shows two examples of the scatter and variability often observed in tree ring studies. The record in one tree from southern Chile (Fig. 2b) is much more variable than the ice core measurements (Fig. 2a), although the same general decline of about 1 is evident in recent years. The second Chilean tree has more consistent $\delta^{13}C$ values, but does not show evidence for a recent decline in $\delta^{13}C$ values (Fig. 2c). This disagreement between tree records is not atypical, and rather elaborate sampling methodology and large sample sizes (many tree ring records) have been used in attempts to obtain accurate representations of net isotopic changes (Tans and Mook 1980; Leavitt and Long 1983; Freyer and Belacy 1983; Stuiver et al. 1984; Leavitt and Long 1986; Leavitt 1987). The averaging process carries implicit assumptions concerning the cause and randomness of influences on $\delta^{13}C$ which, in light of recent studies, have not always been justified (Francey 1985).

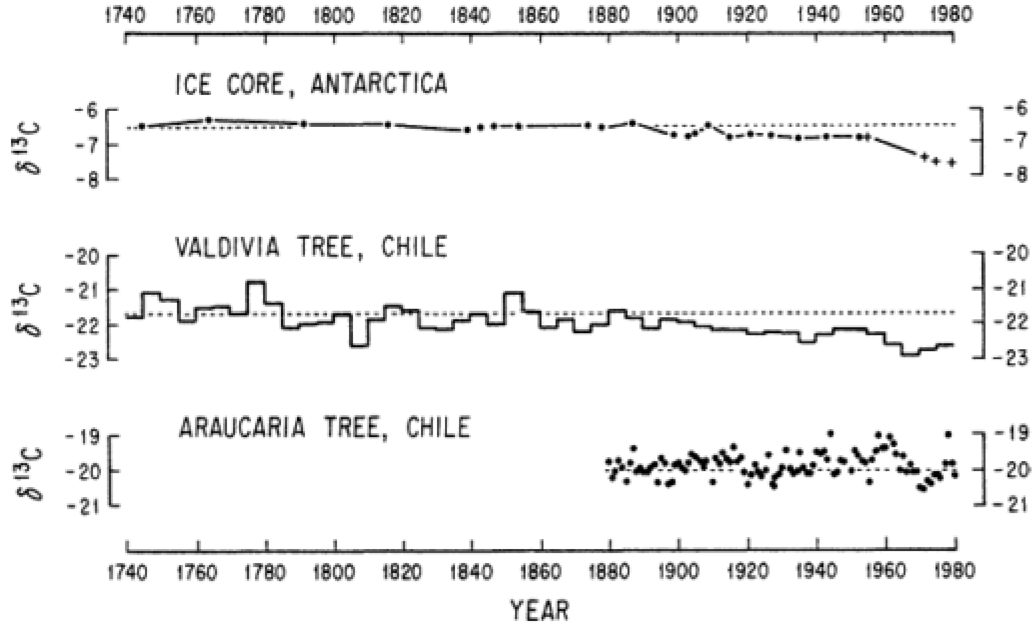


Figure 2.
$\delta^{13}C$ values in three historical records. Upper panel: The isotopic composition of atmospheric CO^2 trapped in Antarctic ice has declined in recent decades (dots = CO_2 extracted from ice, crosses = CO_2 collected directly from the atmosphere at Hawaii). Lower panels: Tree rings record atmospheric CO_2 $\delta^{13}C$, displaced by fractionations during photosynthetic metabolism. Tree ring records from two trees in Chile show typical scatter in $\delta^{13}C$, and in only one of the trees is a recent decline in average $\delta^{13}C$ evident (middle panel). Dashed lines show pre-1900 average $\delta\ ^{13}C$ values for reference.
Sources: Friedli et al. 1986, Stuiver et al. 1984.

2. Physiology and stress. Recent physiological work has emphasized that carbon isotopic compositions of plants respond not only to changes in $\delta^{13}C$ of atmospheric CO_2, but also to other factors such as water supply and, in some cases, light (Fig. 3; Francey 1983). A general physiological model of carbon isotope change in plants has been formulated and extensively tested (O'Leary 1981; Farquhar et al. 1982; Francey and Farquhar 1982) and it is now recognized that environmental variables other than the isotopic composition of CO_2 can significantly influence tree ring isotopic compositions

TERMINOLOGY

Isotopic distributions are measured relative to standard reference materials, and as such are difference δ measurements where

$\delta = (R_{STANDARD}/R_{SAMPLE}-1)1000$

and R = $^{13}C/^{12}C$, $^{15}N/^{14}N$, or $^{34}S/^{32}S$ for carbon ($\delta^{13}C$), nitrogen (δ^{15} N) and sulfur ($\delta^{34}S$) δ values, respectively. By this definition, standards have values of 0, and units are parts per thousand (difference), or ‰. International standards are carbon from the PDB limestone, N_2 gas in air, and sulfur from the Canyon Diablo meteorite.

The δ terminology is in some ways awkward because of the several ratios involved in the δ definition. However, δ values turn out to be related to the percent heavy (or light) isotope content of a sample in a simple, linear manner (Fig. 1). Increases in heavy isotope content cause increases in δ values and samples with high δ values are consequently "heavy" vs. samples with lower values. Conversely, samples with low δ are depleted in the heavy isotope, but enriched in the light isotope, and therefore "light" (Fig. 1).

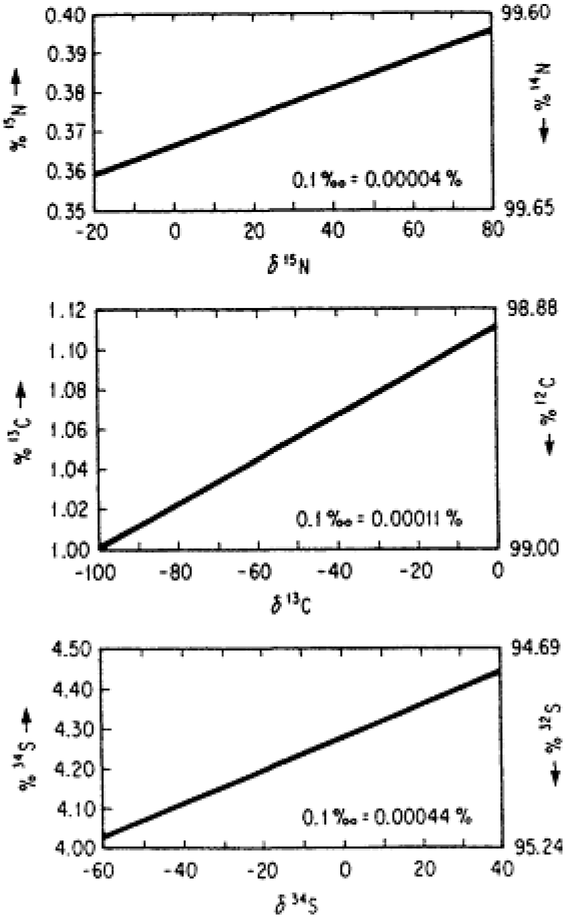


Figure 1.
Relationship of δ values to amounts of heavy and light stable isotopes for carbon, nitrogen and sulfur isotopes. Source: Reproduced, with permission, from the Annual Review of Ecology Systematics Vol. 18 c 1987 by Annual Reviews Inc.

(Stuiver and Braziunas 1987). In simplest form, the model states that isotopic compositions follow the ratio of internal CO_2 concentration to external CO_2 concentration (c_i/c_a). When c_i/c_a decreases due to stomatal closure accompanying water stress, or due to increased assimilation of CO_2 upon strong illumination in water sufficient plants, then $\delta^{13}C$ values increase (or, more generally, when c_i/c_a decreases, δ^{13} C increases). Physiological variations related to CO_2 fixation processes can therefore lead to larger $\delta^{13}C$ changes than the 0.5-1.5 fossil fuel signal (compare Figs. 2 and 3).

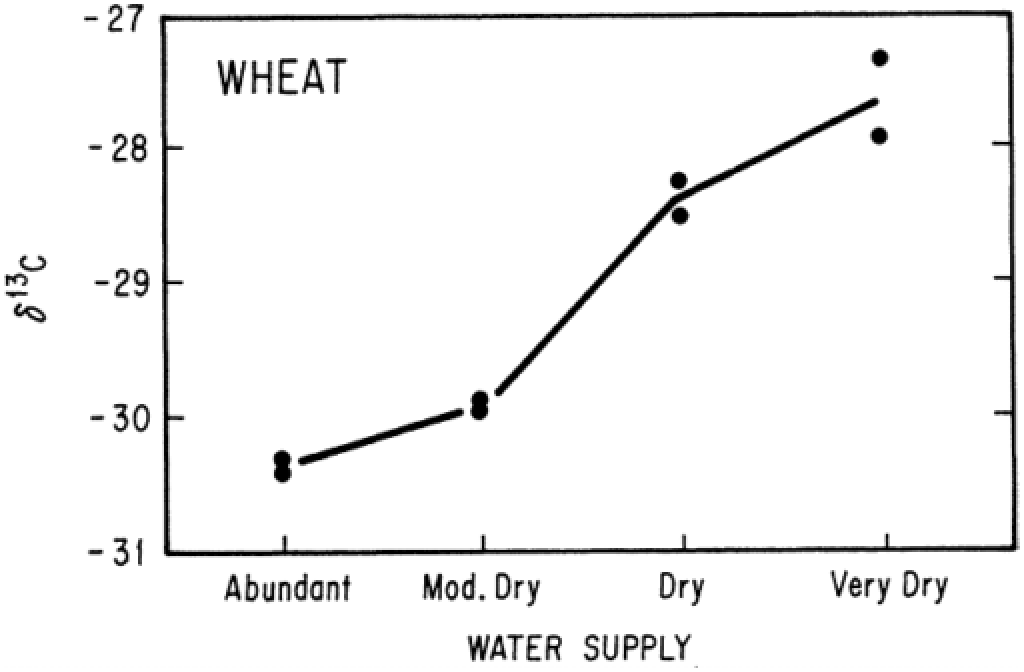


Figure 3.
Increases in $\delta^{13}C$ in response to water supply in wheat. Source: Reprinted with permission of Springer-Verlag N.Y. Inc. from Carbon isotope measurements in baseline air, forest canopy air, and plants 1982. Copyright 1982 by Springer-Verlag N.Y., Inc.

The need for a physiological perspective in tree ring work has been emphasized (Francey 1983, 1985; Francey et al. 1984, 1985), and it is evident that the physiological component of tree rings variation needs to be recognized and "deconvoluted" when using tree rings as substitutes for direct atmospheric CO_2 measurements (Leavitt and Long, in prep.).

Several studies have also specifically investigated the effects of air pollutants on tree rings. Generally, fumigation with SO_2 or ozone causes increases in $\delta^{13}C$ values (Freyer 1979; Leavitt and Long 1987), presumably by affecting the c_i/c_a ratio (Greitner and Winner 1988). These small +1 to +2 isotopic changes are in the opposite direction of the-0.5 to-1.5 anthropogenic fossil fuel signal, and difficult to detect without intensive sampling (Fig. 4).

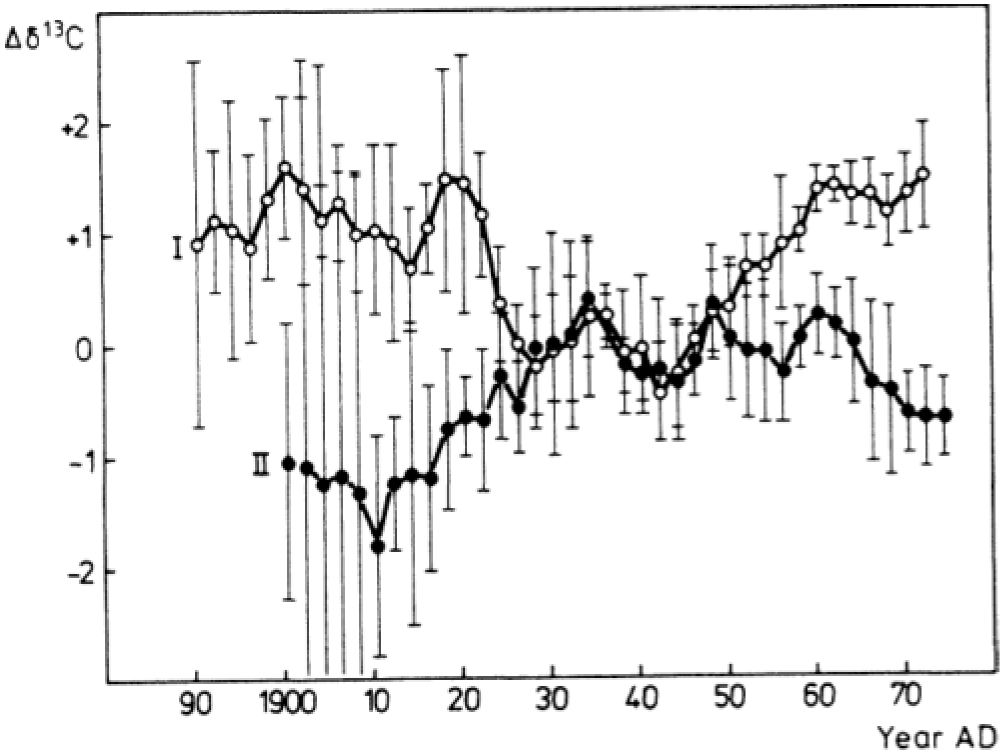


Figure 4.
Isotopic variations in trees 50-100 m from a coal-fired foundry (°) vs. control trees at 2-3 km. distance (●). The foundry was closed from 1929-1949 when tree isotopic compositions are similar. When the foundry was operating from 1890-1929 and 1950-1975, however, heavier values were evident in the polluted trees (higher Δ^{13}C), possibly because of long-term SO_2 fumigation. Data were normalized first by tree by subtracting average 1930-1950 values from individual measurements to yield Δ^{13} C values; means and 90% confidence intervals were obtained by averaging five trees per site. Source: Freyer 1979.

3. CO_2 concentration. Isotopic changes can also result from changing concentrations of nutrients. The effects of increased CO_2 concentrations have not, to my knowledge, been specifically tested in trees, but concentration-related changes have been observed in studies with tomatoes, soybeans, and aquatic algae (Vogel 1980; Smith and Boutton 1981; Degens et al. 1968; Calder and Parker 1973; Mizutani and Wada 1985; Sharkey and Berry 1985). In general, larger carbon isotopic discrimination occurs at higher CO_2 concentrations (Fig. 5). Conversely, as carbon supply becomes more and more limiting, smaller fractionations result, and in the extreme case, no fractionation results when all available carbon diffusing to a plant is taken up without regard for isotopic content.

Present models of isotopic fractionation in trees and other C_3 plants predict that changing atmospheric CO_2 levels will affect isotopic compositions only if the CO_2 concentration gradient from external air to leaf-internal spaces changes significantly (Farquhar et al. 1982). For trees, drought and water relations may often be more important in affecting this concentration gradient than the amount of CO_2 available in the atmosphere.

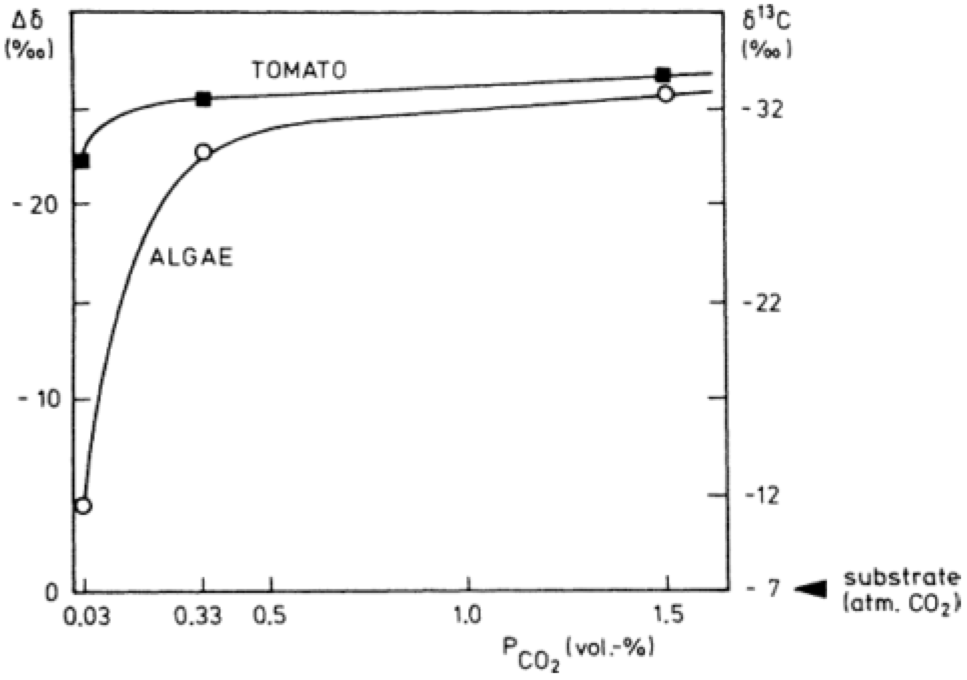


Figure 5.
Isotopic fractionation in mixed algal cultures and for tomato grown with different levels of CO_2. Slow diffusion of CO_2 in water limits the degree of fractionation by algae grown under normal conditions (0.03% CO_2 in air); the roughly 10^4 faster diffusion of CO_2 in air than in water may account for the larger fractionations in tomato. Fractionation $(\Delta^{13}C) = \delta^{13}C_{PLANT} - \delta^{13}C_{FEED\ CO2}$.
Source: Vogel 1980.

SIGNAL-TO-NOISE

The very extensive carbon isotope studies with tree rings have tackled a difficult problem in that the total atmospheric $\Delta\delta$ signal is small at about 1 and there is significant physiological noise that is often of this same magnitude or larger. The signal-to-noise ratio may be more promising for nitrogen and sulfur isotopes, where studies related to tree age and atmospheric pollution are just beginning. The promise of N and S isotope studies comes at the moment from investigations of lake cores (Fig. 6). The background variability (noise) seems similar for the C, N and S records, but the recent isotopic changes for N and S are larger at 2 and 7‰, respectively, than the approximate 1 carbon change. Sulfur and nitrogen isotope studies may, therefore, deserve more attention in the future.

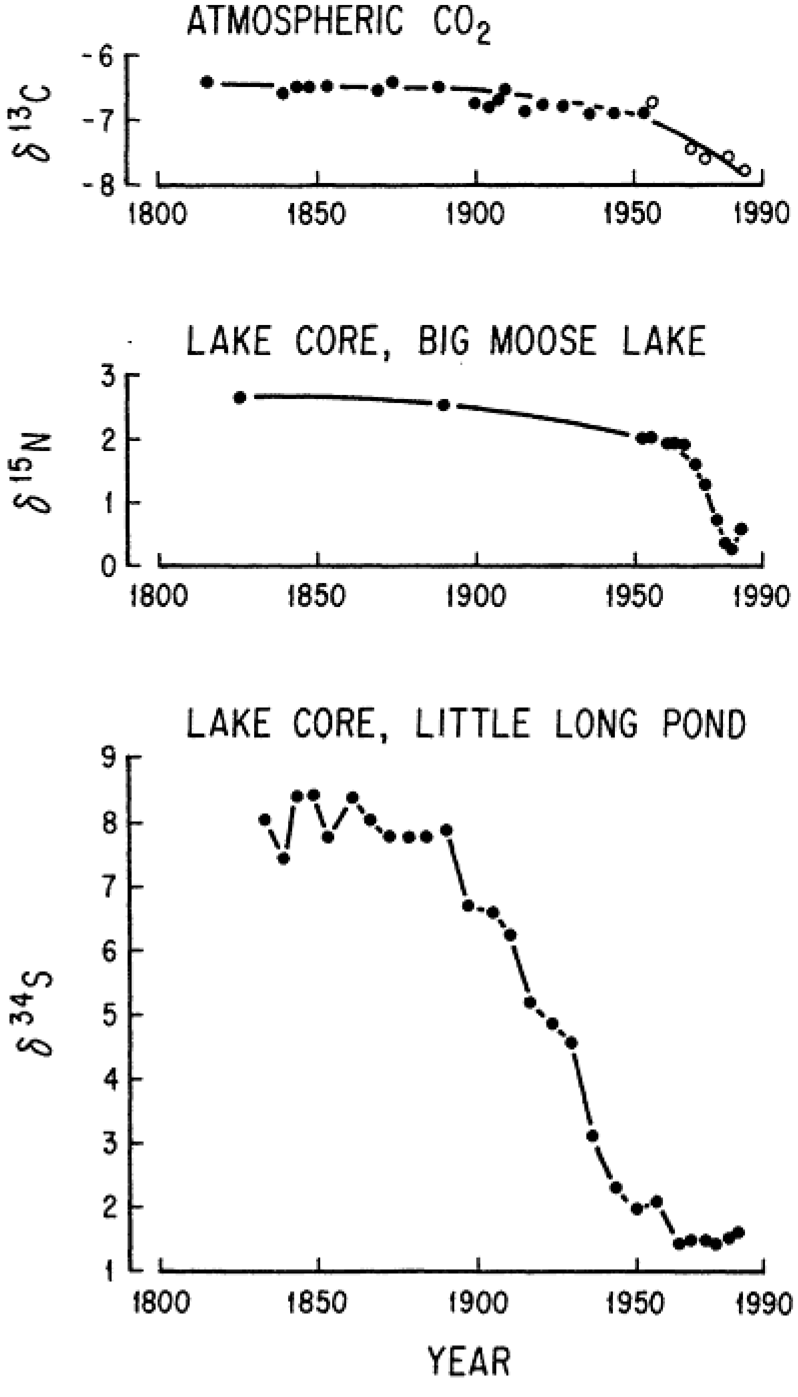


Figure 6.
Comparison of C, N and S isotope historical records. Top: $\delta^{13}C$ of CO_2 from an Antarctic ice core. (Dots = CO_2 from ice core, open circles = CO_2 measured from air at Hawaii). Middle: δ ^{15}N of Big Moose Lake sediments, Adirondack Mountains, New York. Bottom: $\delta^{34}S$ of sediments from Little Long Pond, Maine. Source: Friedli et al. 1986; Fry, unpublished.

NITROGEN ISOTOPES

The initial lake core results of Figure 6 suggest that known recent increases in anthropogenic N loading may be accompanied by a net decline in δ^{15}N. A summary of atmospheric nitrate and ammonium isotopic compositions (Fig. 7) shows a great deal of variation from sample to sample that precludes definitive statements about possible recent δ^{15}N declines. However, it is suggestive that the earliest study (Hoering 1957) from rural and presumably unpolluted Arkansas showed the highest δ^{15}N values for ammonium and nitrate; average values measured in industrial areas are much lower (Freyer 1978). Because tree rings and lake cores can be expected to integrate isotopic compositions of input N, direct study of recent records may be the best way to evaluate whether anthropogenic N differs significantly in δ^{15}N from natural background N. Previous study shows that nitrogen present in tree ring wood is sufficiently abundant for δ^{15}N analysis, and is apparently not translocated after initial fixation in wood (Cotrufo 1983).

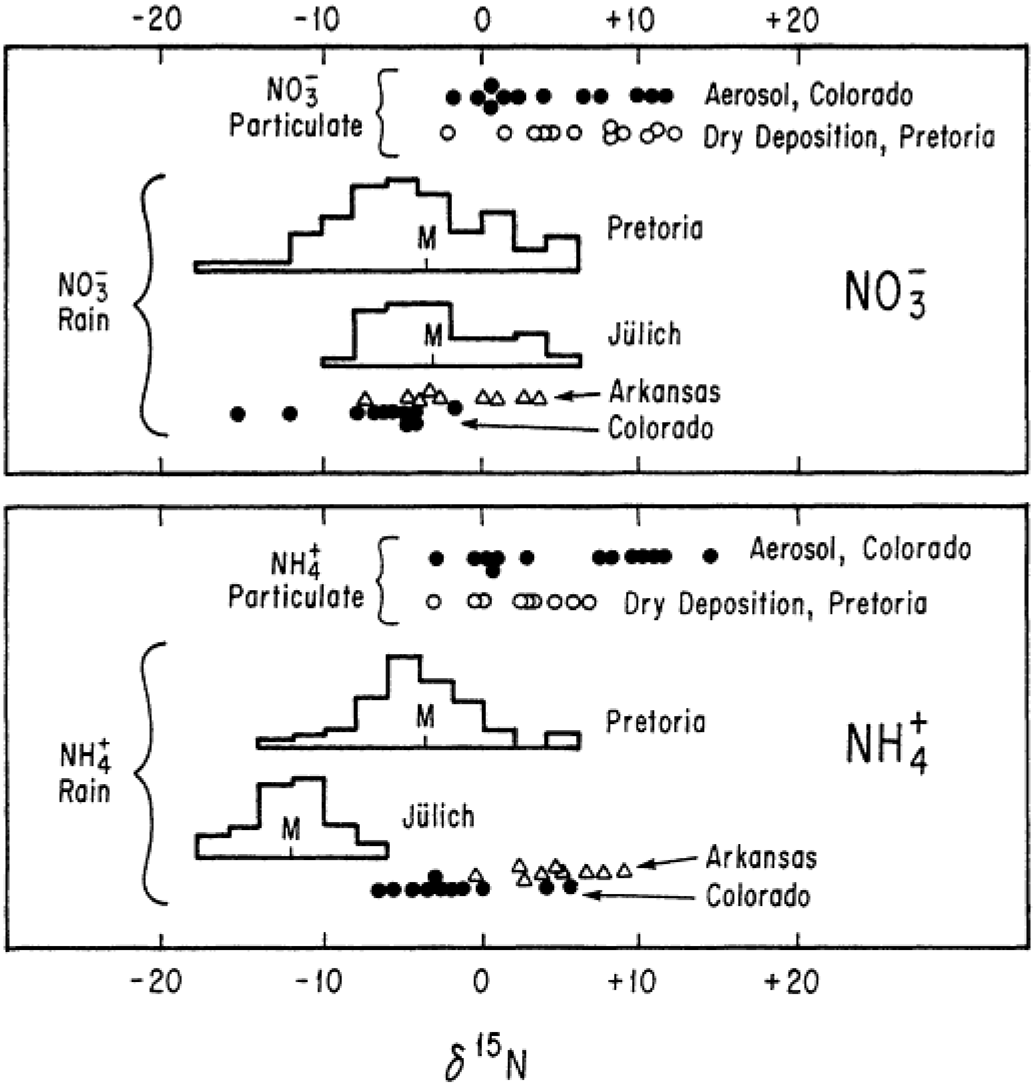


Figure 7.
δ^{15}N values of nitrogen in dry deposition and rainfall. Source: Heaton 1986.

SULFUR ISOTOPES

Several studies of lake cores have shown that large sulfur isotopic changes accompany increased sulfur loading in recently acidified lakes (Nriagu and Coker 1983; Nriagu and Soon 1985). The isotopic record often reflects addition of anthropogenic S with different δ^{34}S, a change, therefore, in source δ^{34}S. For example, in lakes and ponds near the ocean, background values are influenced by +21 sulfate deposited from sea spray, but values decline in more recent sediments towards near zero values (e.g., Little Long Pond in Fig. 7). The decline presumably occurs because of increased anthropogenic sulfur deposition from the atmosphere; δ^{34}S values of atmospheric sulfate currently average +3 to +5 over much of North America (Nriagu and Coker 1978; Saltzman et al. 1983; B. Cook, pers. comm.). In more inland locations, however, background values often average near +3‰, and the new anthropogenic input is small and relatively difficult to detect with δ^{34}S measurements.

Isotopic changes also occur in lake cores because of changing sulfate levels, and separating the source vs. concentration components of isotopic change can be complex (Fry 1986; Peterson and Fry 1987; Fry 1988). As with studies of δ^{13}C in plants, basic physiological investigations need to accompany studies of sulfur isotope change. Sulfur isotopic changes in stressed vegetation have also been documented (Winner et al. 1978, 1981).

No tree ring work with sulfur isotopes has been published, in part because of the difficulty of making the δ^{34}S measurements, and in part because of the very low trace concentrations of sulfur in wood (<0.05%). The strong isotopic signals seen in lake cores, however, suggest that development of new methods of analyzing trace amounts of tree sulfur for δ^{34}S may prove very worthwhile.

CONCLUSIONS

Stable isotope changes in trees that are caused by specific air pollutants have been investigated in only a handful of studies. Initial results from these studies indicate that these changes are generally small and difficult to detect without intensive sampling. Careful comparisons of impacted vs. control trees are necessary to distinguish natural variations from effects associated with anthropogenic pollutants.

Although assessing effects of specific pollutants may thus be labor-intensive, stable isotope studies may function in a wider pollution context as indicators of historical changes in C, N, and S loading to natural systems. Carbon isotope studies of tree rings show relatively small changes because the total increase in CO_2 has been relatively small (<25%) to date and because equilibrium exchange of CO_2 with bicarbonate in the ocean tends to dampen the isotopic signal associated with anthropogenic CO_2 emissions. For sulfur, large isotopic changes occur in recent lake sediments in response to relatively large changes in sulfate loading. This historical record is being compared to records of sulfur emissions on a regional and national scale. Nitrogen isotope studies of tree rings have not yet been performed, but should also provide a record of anthropogenic N additions to forests. This record may be especially interesting since nitrogen often limits growth of forests.

ACKNOWLEDGMENTS

I thank R. J. Francey, S. A. Leavitt, R. H. Waring, and W. E. Winner for helpful discussions during preparation of this paper.

References

Brenninkmeijer, C. A. M. 1983.^{2}H,^{18}O and^{13}C variations in tree rings. Ph.D. thesis, University of Groningen.

Burk, R.L., and M. Stuiver. 1981. Oxygen isotope ratios in trees reflect mean annual temperature and humidity. Science 211:1417-1419.

Calder, J.A., and P.L. Parker. 1973. Geochemical implications of induced changes in ^{13}C fractionation by blue-green algae. Geochim. Cosmochim. Acta 37:133-140.

Cotrufo, C. 1983. Xylem nitrogen as a possible diagnostic nitrogen test for loblolly pine. Can. J. For. Res. 13:355-357.

Degens, E. T., R. R. L. Guillard, W. M. Sackett, and J. A. Hellebust. 1968. Metabolic fractionation of carbon isotopes in marine plankton. I. Temperature and respiration experiments. Deep-Sea Res. 15:1-9.

Farquhar, G.D., M.H. O'Leary, and J.A. Berry. 1982. On the relationship between carbon isotope discrimination and the intercellular carbon dioxide concentration in leaves. Aust. J. Plant Physiol. 9:121-137.

Francey, R. J. 1983. Carbon isotope measurements in baseline air, forest canopy air, and plants. Pp. 160-174 in J. R. Trabalka and D. E. Reichle (eds.), The Changing Carbon Cycle: A Global Analysis. Springer-Verlag, New York.

Francey, R. J. 1985. Tree ring ^{13}C/^{12}C records-an alternate view. Post-conference report on "Carbon Transfer in the Atmosphere, Ocean and Terrestrial System", Lake Arrowhead, May 5-10, 1985.

Francey, R. J., and G. D. Farquhar. 1982. An explanation of ^{13}C/^{12}C variations in tree rings. Nature 297:27-31.

Francey, R. J., M. Barbetti, T. Bird, D. Beardsmore, W. Coupland, J. E. Dolezal, G. D. Farquhar, R. G. Flynn, P. J. Fraser, R. M. Gifford, H. S. Goodman, B. Kunda, S. McPhail, G. Nanson, G. I. Pearman, N. G. Richards , T. D. Sharkey, R. B. Temple, and B. Weir. 1984. Isotopes in Tree Rings. Division of Atmospheric Research Technical Paper No. 4. CSIRO, Australia. 86 pp.

Francey, R. J., R. M. Gifford, T. D. Sharkey, and B. Weir. 1985. Physiological influences on carbon isotope discrimination in huon pine (*Lagarostrobos franklinii*). Oecologia 66:211-218.

Freyer, H. D. 1978. Preliminary ^{15}N studies on atmospheric nitrogenous trace gases. Pageoph 116:393-404.

Freyer, H. D. 1979. On the ^{13}C record in tree rings. Part II. Registration of microenvironmental CO_2 and anomalous pollution effect. Tellus 31:308-312.

Freyer, H.D. and N. Belacy. 1983.$^{13}C/^{12}C$ records in Northern Hemisphere trees during the past 500 years-anthropogenic impact and climatic superpositions. J. Geophys. Res. 88: 6844-6852.

Friedli, H., H. Lötscher, H. Oeschger, U. Siegenthaler, and B. Stauffer. 1986. Ice core record of the $^{13}C/^{12}C$ ratio of atmospheric CO_2 in the past two centuries. Nature 324:237-239.

Fry, B. 1986. Stable sulfur isotopic distributions and sulfate reduction in lake sediments of the Adirondack Mountains, New York. Biogeochem. 2:329-343.

Fry, B. 1988. Sulfate fertilization and changes in sulfur stable isotopic compositions of lake sediments. In P. W. Rundel, J. R. Ehleringer and K. A. Nagy (eds.), Stable Isotopes in Ecological Research. Springer Verlag, New York.

Greitner, C. S., and W. E. Winner. 1988. Increases in $\delta^{13}C$ values of radish and soybean plants caused by ozone. New Phytol., in press.

Heaton, T. H. E. 1986. Isotopic studies of nitrogen pollution in the hydrosphere and atmosphere: A review. Chem. Geol. 59:87-102.

Hoering, T. 1957. The isotopic composition of the ammonia and the nitrate in rain. Geochim. Cosmochim. Acta 12:97-102.

Leavitt, S. W. 1987. Stable-carbon isotopes in tree rings as environmental indicators. Pp. 61-74 in R. W. Hurst, T. E. Davis, and S. S. Augustithis (eds.), The Practical Applications of Trace Elements and Isotopes to Environmental Biogeochemistry and Mineral Resources Evaluation. Theophrastus Publications, Athens, Greece.

Leavitt, S. W., and A. Long. 1983. An atmospheric $^{13}C/^{12}C$ reconstruction generated through removal of climate effects from tree-ring $^{13}C/^{12}C$ measurements. Tellus 35B:92-102.

Leavitt, S. W., and A. Long. 1986. Stable-carbon isotope variability in tree foliage and wood. Ecology 67:1002-1010.

Leavitt, S. W., and A. Long. 1987. Stable-carbon isotopic composition of plants near an Arizona smelter. Pp. 47-57 in R. W. Hurst, T. E. Davis, and S. S. Augustithis (eds.), The Practical Applications of Trace Elements and Isotopes to Environmental Biogeochemistry and Mineral Resources Evaluation. Theophrastus Publications, Athens, Greece.

Mizutani, H., and E. Wada. 1982. Effect of high atmospheric CO_2 concentration of $\delta^{13}C$ of algae. Origins of Life 12:377-390.

Mizutani, H., and E. Wada. 1985. Carbon dioxide and the biosphere. Their historical relationship as inferred from carbon isotope records. Viva Origino 13:25-49.

Nriagu, J. O., and R. D. Coker. 1978. Isotopic composition of sulphur in atmospheric precipitation around Sudbury, Ontario. Nature 274:883-885.

Nriagu, J. O., and R. D. Coker. 1983. Sulfur in sediments chronicles past changes in lake acidification. Nature (London) 303:692-694.

Nriagu, J. O., and Y. K. Soon. 1985. Distribution and isotopic composition of sulfur in lake sediments of northern Ontario. Geochim. Cosmochim. Acta 49:823-834.

O'Leary, M. H. 1981. Carbon isotope fractionation in plants. Phytochemistry 20:553-567.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Ann. Rev. Ecol. Syst. 18:293-320.

Saltzman, E. W., G. W. Brass, and D. A. Price. 1983. The mechanism of sulfate aerosol formation: chemical and sulfur isotopic evidence. Geophys. Res. Lett. 10:513-516.

Sharkey, T. D., and J. A. Berry. 1985. Carbon isotope fractionation of algae as influenced by an inducible CO_2 concentrating mechanism. Pp. 389-401 in W. J. Lucas and J. A. Berry (eds.), Inorganic Carbon Uptake by Aquatic Photosynthetic Organisms. The American Society of Plant Physiologists .

Smith, B.N., and T.W. Boutton. 1981. Environmental influences on $^{13}C/^{12}C$ ratios and C_4 photosynthesis. Pp. 255-262 in G. Akoyunoglou (ed.) Photosynthesis VI. Photosynthesis and Productivity, Photosynthesis and Environment. Balaban Int. Sci. Serv., Philadelphia, PA.

Sternberg, L. daS. L. O. 1988. Oxygen and hydrogen isotope ratios in plant cellulose: mechanisms and applications. In P. W. Rundel, J. R. Ehleringer and K. A. Nagy (eds.), Stable Isotopes in Ecological Research. New York, Springer-Verlag.

Stuiver, M., and T. F. Braziunas. 1987. Tree cellulose $^{13}C/^{12}C$ isotope ratios and climatic change. Nature 327:58-60.

Stuiver, M., R. L. Burk, and P. D. Quay. 1984.$^{13}C/^{12}C$ ratios in tree rings and the transfer of biospheric carbon to the atmosphere. J. Geophys. Res. 89:11731-11748.

Tans, P. P., and W. G. Mook. 1980. Past atmospheric CO_2 levels and the $^{13}C/^{12}C$ ratios in tree rings. Tellus 32:268-283.

Vogel, J.C. 1980. Fractionation of the carbon isotopes during photosynthesis. Sitzungsber. Heidelb. Akad. Wiss. Math.-Naturwiss. K1. Jahrgang 1980, 3. Abh. pp. 111-135. Springer Verlag.

White, J. W. C. 1988. Stable hydrogen isotope ratios in plants: a review of current theory and some potential applications. In P. W. Rundel, J. R. Ehleringer and K. A. Nagy (eds.), Stable Isotopes in Ecological Research. New York, Springer-Verlag.

White, J. W. C., E. R. Cook, J. R. Lawrence, and W. S. Broecher. 1985. The D/H ratios of sap in trees: implications for water sources and tree ring D/H ratios. Geochim. Cosmochim. Acta 49:237-246.

Winner, W. E., J. D. Bewley, H. R. Krouse, and H. M. Brown. 1978. Stable sulfur isotope analysis of SO_2 pollution impact on vegetation. Oecologia 36:351-361.

Winner, W. E., C. L. Smith, G. W. Koch, H. A. Mooney, J. D. Bewley, and H. R. Krouse. 1981. Rates of emission of H_2S from plants and patterns of stable sulphur isotope fractionation. Nature 289:672-673.

Tree-Ring Analysis as an Aid to Evaluating the Effects of Air Pollution on Tree Growth

Edward Cook
Tree-Ring Laboratory Lamont-Doherty Geological Observatory of Columbia University Palisades, New York, 10964
John Innes
Forestry Commission Alice Holt Lodge Wrecclesham Farnham Surrey GU10 4LH United Kingdom

ABSTRACT

Tree-ring analysis has been used to assess the impact of pollution on tree growth near point sources emitting high levels of specific pollutants. However, its use in assessing the impact of lower-level regional air pollutants on forests is more controversial. The plethora of regional pollutants coupled with insufficient physiological understanding of how different tree species respond to pollutants makes any causal link between regional pollution and tree growth difficult to infer. A variety of statistical analysis procedures are available to search for anomalous behavior in tree rings in the form of ring-width decline and changes not explained by climate. Neither of these effects is prima facie evidence for pollution stress in trees. However, the discovery and description of anomalous behavior in tree rings is an important step in understanding the epidemiology of forest decline that may ultimately be found to be caused by pollution.

INTRODUCTION

For many years, air pollutants have been recognized as a factor influencing tree growth, and there is now an extensive literature on the subject (Smith, 1981; McLaughlin, 1985). Since the Industrial Revolution, the presence of industrial plants emitting gases such as sulphur dioxide and hydrogen fluoride or particulates such as soot and heavy metals have caused severe growth reductions and mortality in trees. In extreme cases, large areas have been completely devastated. Such cases are usually well-documented, and the mechanisms and nature of tree growth reductions and mortality are reasonably well understood.

Recently, widespread and severe forest declines have been reported in many parts of the world, especially in Europe (Schütt and Cowling, 1985) and North America (Johnson and Siccama, 1983). These declines cannot be tied directly to any point-source of pollution, but the presence of coincidental high levels of pollutants in the atmosphere suggest that some of the forest declines being observed now have been caused by air pollution. Although many of the currently affected species such as silver fir (*Abies alba* Mill.) and red spruce (*Picea rubens* Sarg.) have experienced large-scale declines in the past (Cramer, 1984; Weiss et al., 1985; Johnson et al., 1986), those past declines do not appear to have occurred on the same scale as the present declines of the same species (Brandl, 1985; McLaughlin et al., 1987). The apparently unique severity and scale of the current forest declines in North America and Europe have been used to bolster the argument that pollution is a primary contributor to these declines.

TREE RINGS AS A POTENTIAL INDICATION OF POLLUTION STRESS IN TREES

Although there is still little convincing evidence for atmospheric pollution being the cause of regional forest declines (Woodman and Cowling, 1987), the search for indicators or markers of pollution stress in trees is being vigorously pursued. One potential indicator, which is available in virtually all temperate forests of the world, is the annual tree-ring increment. Tree rings are an unique source of information on forests. They are the only widely available source of long-term, baseline data on forest growth and productivity that may predate the present era of elevated atmospheric pollution. Insofar as the year to year changes in ring width are an integration and reflection of past environmental influences on tree growth, it may be possible to use tree-ring analysis to detect anomalous changes in tree growth that are characteristic of pollution stress. Thus, by quantitatively comparing past and present tree-ring patterns in forests, an air pollution effect on regional forest productivity may be detectable.

There are several ways in which the annual tree-ring increment can be quantified for study. The simplest way, and the way which will be emphasized in this paper, is to measure the radial ring widths sampled from the breast-height region on the bole of a tree. Because these measures of tree growth can be obtained easily and non-destructively from increment cores, they are very practical for studying certain properties of tree growth. However, as expressions of growth, they are not without their interpretational problems. At breast-height, both cambial age and the distance of the cambium from the photosynthetic centers of the canopy increase with time. These effects, coupled with geometric increases in cambial area each year, frequently cause these ring widths to decrease with age in a curvilinear fashion. This decrease, which is an intrinsic property of tree growth, must not be misinterpretated as an indicator of pollution stress.

There are alternative measures of tree growth based on the annual increment that can reduce the interpretational problems somewhat. One approach is to transform the breast-height ring widths to basal area increments (BAIs) (Phipps, 1984; Hornbeck and Smith, 1985; Phipps and Whiton, 1988). Although BAIs are ideally measured from cross-sections, generally less accurate estimates of BAI can also be obtained from increment cores and tree diameters. If estimated from increment cores, the accuracy of BAI estimates will be effected by the circuit uniformity and symmetry of radial growth on the bole. The transformation of ring width to BAI corrects for allometric growth effects associated with increasing cambial area. Phipps (1984) suggests that BAI increases linearly with time in healthy stands of trees. If this is the case, then a decline in BAI may be indicative of some abnormal stress on tree growth. However, it is not necessarily an indicator of pollution stress.

Other much more informative measures of annual increment can be obtained by detailed stem analysis (Duff and Nolan, 1953; Fritts, 1976; LeBlanc et al., 1987a). This approach requires destructive sampling and analysis of annual increment both radially and with height. However, it is possible to get accurate estimates of annual volume increment and complete growth layer profiles, which are unobtainable any other way. Using detailed stem analysis, LeBlanc et al. (1987a) compared ring-width patterns obtained from breast-height with those obtained from "ring number sequences" (RNSs) that maintained a constant cambial age and position with respect to the crown. They found that the two measures of tree growth were significantly correlated in cases where the sampled trees were dominants in the stand or growing on good sites. Suppressed trees and trees growing on poor sites showed poorer correlation between the two ring-width sequences. LeBlanc et al. (1987a) also noted that RNSs sometimes showed greater changes in radial growth than breast-height ring widths, which suggests that the former may be more sensitive to environmental changes than the latter in some cases. Detailed stem analysis is the best approach for differentiating allometric growth declines from those due to environmental or pollution effects in individual trees. However, the need for destructive sampling and the enormous increase in measurement effort over breast-height ring-width studies will restrict this approach to only a few intensively studied research sites.

Although tree-ring analysis has obvious potential for the study of regional forest decline, its application in such studies is difficult and controversial. The fact that tree rings are an integration of many environmental influences on tree growth means that any pollution signal may be small and embedded in a high level of natural environmental noise, i.e., the signal-to-noise ratio is likely to be low. This problem is especially apparent in the study of regional decline where the level of pollution is comparatively low compared to that near pollution point-sources. Consequently, tree-ring studies of regional forest decline often sample hundreds or even thousands of trees for analysis in order to reduce the noise level (e.g., Schweingruber et al., 1985; McLaughlin et al., 1987).

However, even with very large sample sizes and redundancy in the experimental design, the identification of an unequivocal pollution signal in tree rings is still very difficult. One potential problem in identifying a pollution signal is determining, first, the expectation of normal growth in the absence of pollution (Hyink and Zedacker, 1987). Developing a useful normal growth expectation is extremely difficult for all but the simplest cases, e.g., even-aged, single-species forest plantations. For the closed-canopy forests of eastern North America, which are typically a mixture of tree species and ages, useful normal growth models do not exist (e.g., Hornbeck and Smith, 1985). In such environments, the evolution of tree rings through time is fundamentally stochastic (Cook, 1987a; 1976b) and, therefore, difficult to predict. Although stochastic forecasting methods, such as those based on autoregressive-integrated moving average (ARIMA) time series models (Box and Jenkins, 1970), could be used to produce an expectation of normal growth in a pollution period, the useful forecasting horizon of such methods is likely to be too short for this application. In addition, the determination of an anomalous ring-width departure from expectations of normal growth would be conditional on the validity of those expectations. It is desirable that such an assessment be made as unconditional as possible, i.e., that it not be based on expectations from a potentially flawed normal growth model.

To compound the probable lack of a useful normal growth model, we do not have a good expectation of what a pollution effect looks like in tree rings other than, perhaps, that ring widths should decrease in the presence of air pollution. In itself, an expectation of anomalous ring-width decline is nonunique and, therefore, insufficient for identifying a pollution effect on regional forest growth. Our understanding of the physiological pathways and effects of various pollutants on forests is still poor, especially for relatively low doses of air pollution. We also lack a useful understanding of the interactions between pollution and climate, which may act synergistically or in opposition depending on the way in which climate is affecting tree growth at the time. Thus, without a good pollution effect model for tree rings, there is virtually no possibility of a direct hypothesis test between tree rings and air pollution. Consequently, tree-ring analysis can not be used to prove, in a direct causal sense, that pollution is responsible for forest decline. Rather, it is best suited for eliminating other natural explanations of decline such as climate (Cook, 1987a) and stand dynamics (McLaughlin et al., 1987), and for discovering relationships that may produce testable hypotheses about the interactions between tree growth, climate, and air pollution (Cook et al., 1987; Johnson et al., 1988).

To this end, we will review some methods of tree-ring analysis based solely on ring widths obtained from the breast-height region of the bole, and have been used to assess forest decline and its possible link to air pollution. These methods fall into two basic catagories: testing for anomalous declines in ring width and testing for changes in tree rings that can not be explained by climate. This review will only cover studies of regional forest decline since this problem is much more common than local decline caused by point-sources of pollution (see Thompson, 1981; Fox et al., 1986). All tree-ring studies should be based on the principles of dendrochronology (Fritts, 1976). We will assume a basic familiarity with the science, especially the principle of crossdating upon which the integrity of tree-ring analysis rests. For a comprehensive review of the science, see Fritts (1976), and for a detailed examination of the problems associated with analyzing tree rings for pollution effects, see Cook (1987a) and Wigley et al. (1987).

TESTING FOR ANOMALOUS DECLINES IN RING WIDTHS

In searching for anomalous declines in ring width, the obvious question arises: What is anomalous? The term "anomalous" implies that we know what is not anomalous, i.e., what is normal growth in tree rings. However, as noted in the previous section, we may not have a useful expectation of normal radial growth except for the simplest cases. In order to avoid this obstacle and make the assessment of anomalous decline unconditional in the sense described above, large-scale forest surveys have been used.

In North America, McLaughlin et al. (1987) examined the ring-width patterns obtained from 1012 red spruce trees growing on 48 different sites in the northern and southern Appalachian Mountains. The sites had a wide range of disturbance histories, stocking levels, and age structures. Previous studies of red spruce tree rings (e.g., Johnson and Siccama, 1983; Johnson and McLaughlin, 1986) indicated that red spruce experienced a widespread, synchronous decline in ring width in the northern Appalachians after about 1960. The discovery of this apparently anomalous decline suggested the intervention of some new stress on red spruce growth such as pollution. McLaughlin et al. (1987) sought to test more rigorously and to quantify the existence of this putative anomalous decline using the technique of intervention detection (Downing and McLaughlin, 1987). This technique searches for the occurrence of step-like changes and gross outliers in time series without regard to cause. An example of a pure step-function fitted to a red spruce tree-ring series is shown in Figure 1.

McLaughlin et al. (1987) were able to demonstrate conclusively the existence of a widely synchronous decline in red spruce ring widths after about 1960 in the northern Appalachians. Red spruce in the southern Appalachians showed a less distinct synchronous decline after about 1965. Given the heterogeneity in stand characteristics among the 48 analyzed collections, it is highly unlikely that changes in radial increment due to stand maturation (Zedacker et al., 1987) would predict the observed synchronous declines. Therefore, McLaughlin et al. (1987) concluded that red spruce ring widths have declined anomalously over most of that species' range since 1960.

An analysis of an independent ring-width data set from 2387 red spruce trees growing on (mostly) previously logged, low-elevation sites in New England supports the existence and timing of this synchronous decline (Hornbeck and Smith, 1985). However, Hornbeck and Smith (1985) cautioned that the maturation of these second-growth forests may be responsible for the synchronous decline because many of the stands were logged at about the same time 80-100 years ago. Recent research by Van Deusen (1987b) also supports the stand maturation hypothesis for low-elevation red spruce decline. However, the coincidental decline of red spruce ring widths at all elevations and for a wide range of stand histories argues for a similar influence operating at all locations. Thus, climate and/or pollution may still be responsible for an anomalous ring-width decline of red spruce even at low elevations.

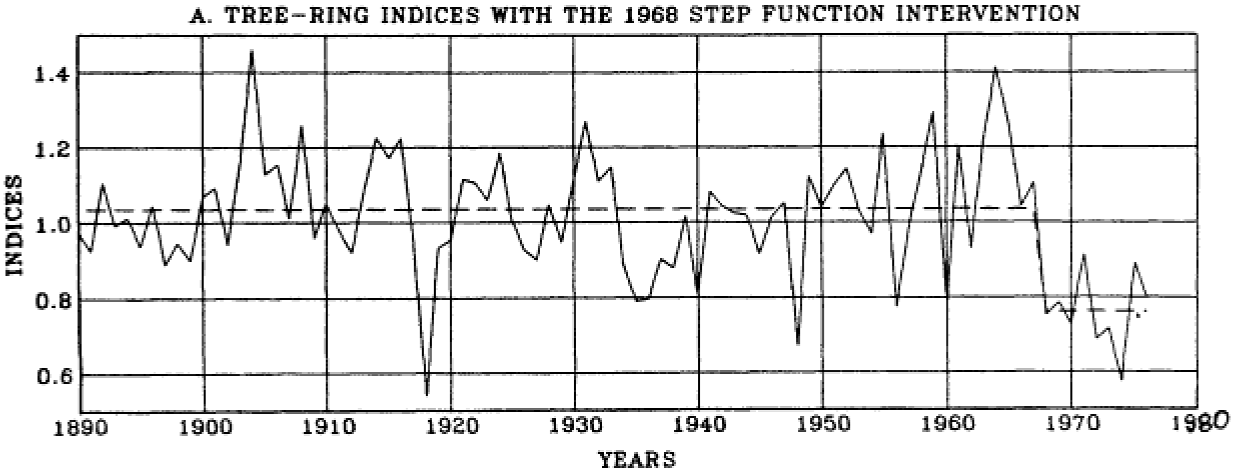


Figure 1.
An example of step-like decline in ring width in red spruce. The dashed line is a simple step-function fitted to the tree-ring series by least squares. These kinds of effects can be objectively searched for in tree rings using the method of intervention detection.

In Europe, Schweingruber et al. (1985) examined the geographic pattern of abrupt, long-term changes in tree rings for several conifer species growing in the Swiss Rhone Valley. The timing of abrupt growth change was determined visually from cross-sections and increment cores of 2500 trees, and the extent of the change was estimated by comparing the mean ring width of the altered growth period with the mean ring width for the same number of rings preceding the period of change. Persistent ring-width changes of 70% or more could be readily determined, although changes of 30% or less could not be reliably identified (Schweingruber et al., 1985). With the exception of one pine species, all of the conifer species showed abrupt ring-width reductions that strongly clustered in the 1970s decade, a time when regional forest decline also began in Europe (Schütt and Cowling, 1985). Schweingruber et al. (1985) were not able to find any clear relationship between the onset of decline and anomalous climate. In addition, the degree of decline was not correlated with site attributes, except for elevation (less decline above 1500 m) and the proximity of the trees to industrial plants (more decline closer to the plants). Schweingruber et al. (1985) concluded that local and regional pollution was the likely cause of the ring-width declines in the Swiss Rhone Valley. For another example of this kind of abrupt growth change analysis, see Schweingruber (1986).

These North American and European studies avoided the need for a normal growth model by examining a very large number of trees from a large number of sites having many different attributes. In so doing, it was possible virtually to eliminate the probability that some stand-level variables, such as stocking level and disturbance history, could explain the observed synchronous ring-width declines. Under these conditions, the statistics supporting the existence of a synchronous decline can be used to assert that the decline is anomalous, in the sense that stand-level variables are an insufficient explanation. However, air pollution cannot generally be regarded as the probable cause of such anomalous declines because meso-scale and synoptic-scale climatic variables and extremes also have the capacity to produce the observed synchronization.

Testing for an anomalous ring-width decline on a single site is much more difficult. Either a justifiable normal growth model or a purely stochastic method, like intervention detection, must be used to test for an anomalous decline. Either way, the possibility that stand-level variables are responsible for the decline will be difficult to eliminate.

The conclusion of Schweingruber et al. (1985) that suggested a forest decline-pollution link is tenable to the extent that climate was not correlated with the onset of decline and the Swiss Rhone Valley is a relatively small area with some point-sources of pollution. The McLaughlin et al. (1987) study was more complicated, covering a much larger area with different climatic regimes, airsheds, and pollution levels. For that reason, no pollution effect could be concluded from the existence of synchronous decline. In the next section, some methods will be described for determining if climate can explain such anomalous ring-width declines.

TESTING FOR CHANGES IN TREE RINGS THAT CANNOT BE EXPLAINED BY CLIMATE

The documentation of an anomalous ring-width decline is an important but insufficient condition for declaring that pollution is contributing to a regional forest decline. Since climate can also reduce the ring widths of trees over broad geographic areas, it is necessary to model and eliminate this potentially confounding effect in the tree rings before a pollution effect can be inferred.

In North America, Cook (1987a) devised a method of testing for the intervention of non-climatic effects in tree rings, such as pollution. This method requires that the ring widths first be reduced to a stationary sequence of relative tree-ring indices via some form of standardization (Fritts, 1976). Cook (1987a) advocated a "stiff" smoothing spline (Cook and Peters, 1981) for estimating and removing the underlying growth trend in ring widths. This method removed very little of the post-1960 ring-width decline in the red spruce tree rings analyzed in that study. However, the determination of the appropriate spline stiffness has been criticized for its subjectivity. As a purely objective alternative, Van Deusen (1987a) suggested using the first-differences of the logarithmically transformed ring widths as the relative tree-ring indices. This method will largely remove any ring-width decline in the resultant indices. The inherent objectivity of trend removal by first differencing is a compelling argument for its use as long as the decline in the ring widths is of little direct interest.

Having standardized the ring widths, Cook (1987a) modeled two slightly different forms of the same red spruce tree-ring chronology with monthly mean temperatures for the years 1890-1950 using stepwise multiple regression analysis. The regression models were able to explain over 50% of the variance in the red spruce tree-ring indices. He then tested the validity of the regression models by predicting tree-ring indices for the 1951-1960 period, which was assumed to be unaffected by factors related to the post-1960 decline. These validation tests were successful, indicating that the temperature-based models could be used to estimate tree rings from climate in the post-1960 decline period. A change in the relationship between tree rings and climate after 1960 would be evidence for the intervention of some new unmodeled variable(s) affecting tree growth. In both cases, the monthly temperature models could not predict the behavior seen in the tree-ring indices after 1960. This included an inability to predict both the decline and the yearly pattern of change in the indices. Thus, there appeared to be a change in the red spruce tree rings after 1960 that could not be explained by climate. An example of this effect, redrawn from Cook (1987), is shown in Figure 2.

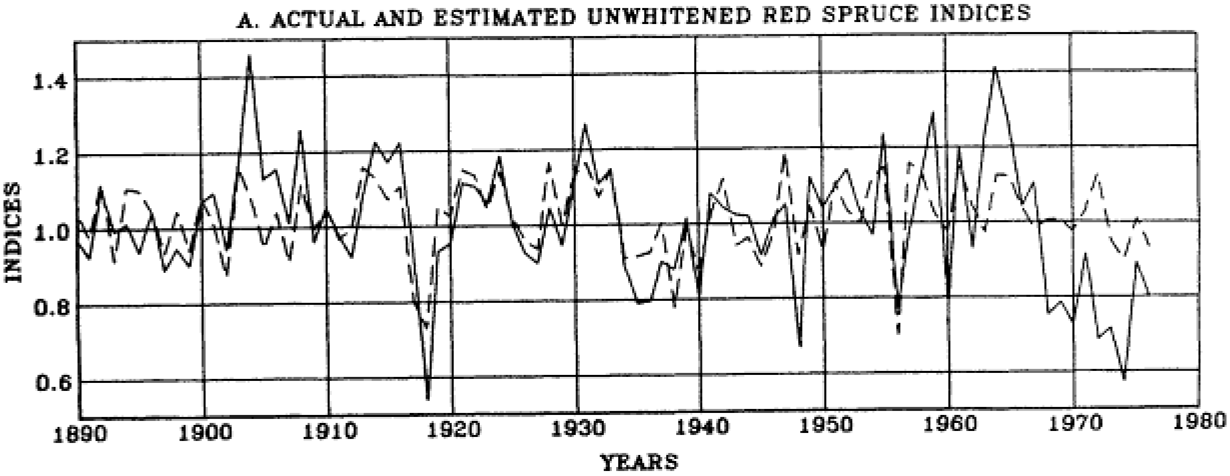


Figure 2.
An example of using empirical climatic response models to search for anomalous effects in tree rings that are not due to climate. The calibration period is the time period used for developing the regression model between tree rings and climate. The resulting model is then used to predict tree rings from climate in the verification period. Note the good predictions up to about 1967 and the breakdown in the model after that date.

With this success, Cook et al. (1987) and McLaughlin et al. (1987) searched for non-climatic changes in tree rings from many red spruce sites. In the northern Appalachians, the results were identical to those just described for all sampled red spruce growing above 800 m elevation. Spruce growing below 800 m elevation sometimes showed a stable response to climate through the post-1960 period. This finding is consistent with data indicating that the red spruce decline is more severe above 800 m (Johnson and McLaughlin, 1986) where, coincidently or not, pollution levels also increase markedly. Cook et al. (1987) also noted a consistent relationship between red spruce growth and climate for all sites above 800 m elevations. They found that red spruce grew better (worse) in the current growing season when August of the previous growing season was cooler (warmer) than average and when the December prior to the current growing season was warmer (cooler) than average. Occurrences of excessively warm Augusts and cold Decembers since the 1820s in the northern Appalachians also correlated with some historical declines of red spruce in that region (Weiss et al., 1985; Johnson et al., 1986). Thus, there may be a relationship between the current red spruce decline and anomalous temperatures, although pollution cannot be eliminated as a contributor.

In Europe, essentially the same method of testing for the intervention of non-climatic effects in tree rings was independently developed and used by Eckstein et al. (1983) and Eckstein (1985) to study the decline of several tree species. Their results also indicate that climate alone could not explain the level of decline seen in the ring widths, although the climatic response appears to change less than it does for red spruce in North America.

The successful use of empirical climatic response models to test for anomalous changes in tree rings, described here and in other studies (e.g., McClenahen and Dochinger, 1985; LeBlanc et al., 1987b), indicates that this approach has considerable potential for the study of forest decline. However, one assumption of this method needs to be investigated further to insure the proper interpretation of its results. The method assumes a stationary relationship between tree rings and climate when there is no intervention of new variables. For this assumption to be correct, systematic changes in climate must not alter the way in which trees respond to climate. This assumption has not been adequately tested even though it is known that climate has changed significantly in the 20th century (Jones et al., 1986; Bradley et al., 1987).

The possibility that changes in the response of trees to climate may be an indicator of pollution stress has lead to the development of another very powerful statistical method based on the Kalman filter. This method, which explicitly allows for time-dependence in the regression coefficients relating tree rings to climate, was developed independently by Van Deusen (1987a) in North America and Visser (1986) in Europe. The method does not require any prior knowledge about the timing of possible interventions. In addition, the complete yearly time-dependence between tree rings and climate is available, which enables the evolution and timing of change to be readily assessed. Finally, the time-dependence in the regression relationships is available separately for each climatic variable in the model. These are obvious advantages over the previous method, which only produces information about the relationship of tree rings with a composite climatic model that is assumed to be time invariant.

A disadvantage of the Kalman filter method is the difficulty in selecting the proper climatic variables for analysis since no subset selection procedures, analogous to stepwise regression, are available. Visser and Molenaar (1986) describe a subset selection method that is based on separately screening each candidate climatic variable for statistical significance with tree rings. Those variables that are significant, even in a time-dependent sense, may be retained in the full model. However, the probability of including spurious variables in the model appears to be great, since there is no way of differentiating time dependence due to spurious association from time-dependence due to physical and biological changes in the trees. The possibility that climatic change is creating any observed time-dependence must also be kept in mind when interpreting the results.

Powerful statistical tools are now available for searching for non-climatic effects in tree rings that could be due to air pollution. The most daunting tasks are in applying these techniques well and in properly interpreting the results.

CONCLUSION

As concern about the effects of air pollution on forests continues to grow, it is likely that tree-ring analysis will play an increasingly important role in the assessment of forest health. The historical perspective available from tree rings is unique, but the proper interpretation of this retrospective look at past tree and forest conditions is by no means simple. Still missing is a useful understanding of how ambient levels of various air pollutants affect the growth of different tree species under natural forest conditions. Until this understanding is obtained, any causal link between air pollution and forest decline that is inferred from tree-ring analysis alone will be very difficult to defend. But to the extent that proper care is taken in developing and statistically analyzing tree-ring data, tree-ring analysis should continue to be an important tool for discovering and characterizing anomalous behavior that may be an indicator of pollution stress in trees.

ACKNOWLEDGMENTS

This research has been supported by a National Science Foundation Division of Climate Dynamics Grant ATM 85-15290. We also acknowledge the support of the Forest Service and National Vegetation Survey in the United States and the Forestry Commission in the United Kingdom. We thank G.C. Jacoby and J. Overpeck for kindly reviewing the manuscript. Lamont-Doherty Geological Observatory Contribution No. 4353.

References

Box, G.E.P., and G. Jenkins. 1970. Time Series Analysis: Forecasting and Control. Holden-Day, San Francisco.

Bradley, R.S., H.F. Diaz, J.K. Eischeid, P.D. Jones, P.M. Kelly, and C.M. Goodess. 1987. Precipitation fluctuations over Northern Hemisphere land areas since the mid-19th century. Science 237:171-175.

Brandl, H. 1985. Zur Bedeutung bestandesgeschichtlicher Untersuchungen in der Forstgeschichte am Beispiel des Tannensterbens im Schwarzwald. Allgemeine Forst und Jagdzeitung 156:142-146.

Cook, E.R., and K.P. Peters. 1981. The smoothing spline: a new approach to standardizing forest interior tree-ring width series for dendroclimatic studies. Tree-Ring Bulletin 41:45-54.

Cook, E.R. 1987a. The use and limitations of dendrochronology in studying the effects of air pollution on forests. Pp. 277-290 In Hutchinson, T.C. and Meema, K.M., eds. Effects of Atmospheric Pollutants on Forests, Wetlands, and Agricultural Ecosystems. Berlin: Springer-Verlag.

Cook, E.R. 1987b. The decomposition of tree-ring series for environmental studies. Tree-Ring Bulletin. 47:37-59.

Cook, E.R., A.H. Johnson, and T.J. Blasing. 1987. Forest decline: modeling the effect of climate in tree rings. Tree Physiology 3(1):27-40.

Cramer, H.H. 1984. On the predisposition to disorders of Middle European forests. Leverkusen Pflanzenschutz Nachrichten 37:208-334.

Downing, D., and S.B. McLaughlin. 1987. Intervention detection-a systematic technique for examining shifts in radial growth rates of forest trees . Pp. 543-554 in Jacoby, G.C. and Hornbeck, J.W., eds. Proceedings of the International Symposium on Ecological Aspects of Tree-Ring Analysis. United States Department of Energy.

Duff, G.H., and N.J. Nolan. 1953. Growth and morphogenesis in the Canadian forest species. I. The controls of cambial and apical activity in *Pinus resinosa* Ait. Canadian Journal of Botany 31:471-513.

Eckstein, D. 1985. On the application of dendrochronology for the evaluation of forest damage. Pp. 287-290 in Schmid-Haas, P., ed. Inventorying and monitoring endangered forests. IUFRO Conference, Zurich 1985. Anstalt fur das forstliche Versuchswesen, Birmensdorf.

Eckstein, D., R.W. Aniol, and J. Bauch. 1983. Dendroklimatologische Untersuchungen zum Tannensterben. European Journal of Forest Pathology 13:279-288.

Fox, C.A., W.B. Kincaid, T.H. Nash III, D.L. Young, and H.C. Fritts. 1986. Tree-ring variation in western larch (*Larix occidentalis*) exposed to sulfur dioxide emissions. Canadian Journal of Forest Research 16:283-292.

Fritts, H.C. 1976. Tree Rings and Climate. Academic Press, London.

Hornbeck, J.W. and R.B. Smith. 1985. Documentation of red spruce growth decline. Canadian Journal of Forest Research 15: 1199-1201.

Hyink, D.M., and S.M. Zedacker. 1987. Stand dynamics and the evaluation of forest decline. Tree Physiology 3(1):17-26.

Johnson, A.H., and T.G. Siccama. 1983. Acid deposition and forest decline. Environmental Science and Technology 17(7): 294A-305A.

Johnson, A.H., and S.B. McLaughlin. 1986. The nature and timing of the deterioration of red spruce populations in Appalachian forests. Pp. 200-230 in Monitoring and assessing trends in acidic deposition. Washington, D.C.: National Academy of Sciences Press.

Johnson, A.H., A.J. Friedland, and J.G. Dushoff. 1986. Recent and historic red spruce mortality: evidence of climatic influence. Water, Air, and Soil Pollution 30:319-330.

Johnson, A.H., E.R. Cook, and T.G. Siccama. 1988. Relationships between climate and red spruce growth and decline in the Northern Appalachians. Proceedings of the National Academy of Sciences. (in press).

Jones, P.D., S.C.B. Raper, R.S. Bradley, H.F. Diaz, P.M. Kelly, and T.M.L. Wigley. 1986. Northern hemisphere surface air temperature variations, 1851-1984. Journal of Climate and Applied Meteorology 25:161-179.

LeBlanc, D.C., D.J. Raynal, and E.H. White. 1987a. Acidic deposition and tree growth: I. The use of stem analysis to study historical growth patterns. Journal of Environmental Quality 16(4):325-333.

LeBlanc, D.C., D.J. Raynal, and E.H. White. 1987b. Acidic deposition and tree growth: II. Assessing the role of climate in recent growth declines. Journal of Environmental Quality 16(4):334-340.

McClenahen, J.R., and L.S. Dochinger. 1985. Tree-ring response of white oak to climate and air pollution near the ohio river valley. Journal of Environmental Quality 14:274-280.

McLaughlin, S.B. 1985. Effects of air pollution on forests: a critical review. Journal of the Air Pollution Control Association 35(5):512-534.

McLaughlin, S.B., D.J. Downing, T.J. Biasing, E.R. Cook, and H.S. Adams. 1987. An analysis of climate and competition as contributors to decline of red spruce in high elevation Appalachian forests of the Eastern United States . Oecologia 72: 487-501.

Phipps, R.L. 1984. Ring-width analysis. Pp. 255-271 in Symposium proceedings: Air pollution and productivity of the forest, October 4-5, 1983. Izaak Walton League, Washington, D.C.

Phipps, R.L., and J.C. Whiton. 1988. Decline in long-term growth trends of white oak. Canadian Journal of Forest Research 18:24-32.

Schütt, P., and E.B. Cowling. 1985. Waldsterben, a general decline of forests in Central Europe: symptoms, development and possible causes. Plant Disease 69:548-558.

Schweingruber, F.H., R. Kontic, M. Niederer, C.A. Nippel, and A. Winkler-Seifert. 1985. Diagnosis and distribution of conifer decay in the Swiss Rhone Valley. In: Turner, H. and Tranquillini, W., eds. Establishment and Tending of Subalpine Forest; Research and Management. Eidgenossische Anstalt fur alas forstliche Versuchswesen, Berichte 270:189-192.

Schweingruber, F.H. 1986. Abrupt growth changes in conifers. IAWA Bulletin 7:277-283.

Smith, W.H. 1981. Air Pollution and Forests. Springer-Verlag, New York.

Thompson, M.A. 1981. Tree rings and air pollution: a case study of *Pinus monophylla* growing in east-central Nevada. Environmental Pollution, Ser. A 26:251-266.

Van Deusen, P.C. 1987a. Some applications of the Kalman filter to tree-ring analysis. Pp. 566-578 in Jacoby, G. C., and Hornbeck, J. W., eds. Proceedings of the International Symposium on Ecological Aspects of Tree-Ring Analysis. United States Department of Energy.

Van Deusen, P.C. 1987b. Testing for stand dynamics effects on red spruce growth trends. Canadian Journal of Forest Research. 17:1487-1495.

Visser, H., 1986. Analysis of tree ring data using the Kalman filter technique. IAWA Bulletin 7:289-297.

Visser, H., and J. Molenaar. 1986. Time-dependent Responses of Trees to weather variations: an Application of the Kalman Filter. Report 50385MOA 86-3041, N.V. KEMA, Arnhem, The Netherlands. 60 pp.

Weiss, M.J., L.R. McGreary, I. Millers, J.T. O'Brien, and M. Miller-Weeks. 1985. Cooperative survey of red spruce and balsam decline and mortality in New Hampshire, New York, and Vermont-1984. Interim report, USDA Forest Service, Forest Pest Management, Durham, NH. 130 pp.

Wigley, T.M.L., P.D. Jones, and K.R. Briffa. 1987. Detecting the effects of acidic deposition and CO_2-fertilization on tree growth. Pp. 239-254 in Kairiukstis, L., Bednarz, Z., and Feliksik, E., eds. Methods of Dendrochronology-1. Systems Research Institute, Polish Academy of Sciences, Warsaw, Poland.

Woodman, J.N., and E.B. Cowling. 1987. Airborne chemicals and forest health. Environmental Science and Technology 21(2): 120-126.

Zedacker, S.M., D.M. Hyink, and D.W. Smith. 1987. Growth declines in red spruce. Journal of Forestry 85:34-36.

Evaluation of Root Growth and Functioning of Trees Exposed to Air Pollutants

J.H. Richards
Department of Range Science and Ecology Center Utah State University Logan, Utah 84322-5230

ABSTRACT

Reduced carbon allocation to roots caused by air pollution exposure results in reduced root growth, turnover and capacity for water and nutrient uptake. Reduced root activity may be a sensitive indicator of the effects of air pollution exposure and should be detectable by the techniques reviewed. Minirhizotrons and rhizotrons allow nondestructive assessment of seasonal patterns of root growth, experimental treatment effects on growth and observation of root development and morphology. The ^{14}C dilution technique may provide a useful alternative to repeated core sampling for long-term study of root turnover. Soil psychrometry is potentially useful for immediate detection of localized water uptake by roots and standard neutron-attenuation techniques can detect cumulative water extraction by roots. Application of a $^{32}P/^{33}P$ dual-isotope labeling technique would allow determination of root and mycorrhizal nutrient uptake capacity in the field as affected by experimental air pollution exposure. Root ingrowth cores enriched with limiting nutrients may be useful in evaluation of the degree of nutrient stress or root growth capacity of trees in the field. The many techniques suitable for field measurement of root functional and morphological parameters would be useful in research situations, but require some development before application to broad-scale monitoring would be feasible. Application of these techniques in the field is dependent on the availability of suitable controls to distinguish pollutant effects from variation due to other stresses or genetic and environmental variation.

Gaseous pollutants have direct effects on photosynthetic capacity and translocation (see reviews by McLaughlin 1985, Mansfield and Jones 1985, Carlson and Bazzaz 1985, Cooley and Manning 1987). Reduced allocation of carbohydrates to root systems as a result of either of these mechanisms will have similar effects. The effects of reduced carbon allocation to roots should be measurable not only as reduced root growth and turnover but also as reduced capacity for nutrient and water uptake.

A recent review of assimilate partitioning in plants exposed to O_3 found that for 20 species root growth was reduced an average of 35% while shoot growth was reduced only 21% (Cooley and Manning 1987). While fumigation with low concentrations of SO_2 caused no visible shoot symptoms in Norway spruce, root growth was reduced by 50% in the year following the fumigation (Keller 1985). Similar results for four broad leaf tree species and red pine were also cited by Keller (1985). The conclusion that root biomass is reduced more than shoot biomass upon exposure to O_3 or SO_2, is also supported by Lechowicz (1987). He noted, however, that NO_2 did not have a differential effect on roots and shoots. While carbohydrate concentrations in roots of plants exposed to pollutants have been determined (see reviews by Cooley and Manning 1987, Lechowicz 1987), pollutant effects on root physiological activity, as distinct from growth, have not been directly investigated. The reviews cited above and others (e.g., Godzik and Krupa 1982) indicate clearly that root production, which reflects the partitioning of assimilates to root growth, is usually reduced before effects are detected in the shoot system or to a greater extent than shoot growth.

Numerous studies on herbaceous plants and tree seedlings suggest that root growth and physiological activity are dependent upon a continuing supply of current assimilate. For example, Thorgeirsson (1988) showed that within 1-2 days after shading or defoliation root respiration of soil-grown perennial grass plants was reduced in proportion to reductions in shoot carbon gain. Hydroponic studies with maize plants indicated that a single day of shading resulted in more than 60% reduction in root respiration and ammonium, nitrate and potassium uptake (Massimino et al. 1981). Following the day of shading, recovery of root respiration and nutrient uptake required 3 days while shoot function recovered within a single day. Studies of both warm-and cool-season grasses demonstrated that root growth ceased within 24 hours of defoliation and did not resume until substantial shoot regrowth had occurred (Crider 1955, Troughton 1957). In these studies, root growth often ceased for several weeks following a single defoliation event. Finally, current photosynthate may be the primary carbon source for new root growth in seedlings of several coniferous forest species (van den Driessche 1987, Ritchie 1982).

In larger plants, such as forest trees, root activity may persist longer on stored reserves. Nevertheless, several studies suggest that root growth is often coupled to current photosynthesis and allocation of carbohydrates. This coupling may be particularly strong between flushing of leaves, when depletion of stored carbohydrates may be substantial (Dougherty et al. 1979, Wargo 1979, McLaughlin et al. 1980), and replenishment of stored reserves. For example, rhizotron studies of mature white oak by Reich et al. (1980) showed that root growth was reduced by more than 50% when competing shoot sinks (shoot, leaf and acorn growth) were most active. Chronic exposure of trees to pollutants may result in reduced allocation to roots and lower carbohydrate reserves, thereby increasing the dependence of root activity on current photosynthate supply. Reduced root growth and impaired functioning may be factors predisposing pollutant-exposed trees to damage from other stresses.

Quantification of the impacts of pollutants on root function in forest trees under field conditions would provide important information for evaluating tree susceptibility to secondary damage. Moreover, because the effects of some gaseous pollutants have been detected in roots prior to shoots, roots may be early and sensitive indicators of damage before damage is detectable in the shoot system (see reviews by Mansfield and Jones 1985, Carlson and Bazzaz 1985). Although root systems may be sensitive indicators, the effects are unlikely to be specific to pollution damage. Other stresses reducing photosynthetic activity or translocation to the root system would have similar effects.

In this paper, I discuss several techniques that have proved useful in measuring root growth and functioning in field situations and may prove applicable to assessing pollutant damage through effects on root systems of forest trees.

ROOT PRODUCTION AND TURNOVER

Fine root production, which has a central role in carbon and nutrient cycles in forest ecosystems, is usually estimated by periodic sampling of the forest floor and mineral soil and determining the mass of live and dead root and soil litter fractions. Fine root production is calculated by a variety of methods with different assumptions concerning transfers of biomass among the root and litter fractions in the belowground system (Persson 1978, McClaugherty et al. 1982, Hansson and Steen 1984).

Depending on the method, calculated values of total fine root production may vary three-to four-fold even when the same sequential harvest data are used (McClaugherty et al. 1982, Singh et al. 1984). Errors in the overall estimates can be large due to variation among samples (Singh et al. 1984), simultaneous root production and mortality (Kurz and Kimmins 1987), or failure to incorporate estimates of losses due to decomposition, herbivory or other processes (Hansson and Steen 1984, Milchunas et al. 1985, Vogt et al. 1986a). Careful tuning of the sampling schedule to the phenology of root growth at the site of interest and appropriate application of statistical constraints are necessary to allow reliable estimates of fine root production (McClaugherty et al. 1982, Persson 1983, Aber et al. 1985, Vogt et al. 1986a, 1986b). For example, root production calculated from sequential sampling may seriously underestimate the true value when fine root production and mortality occur simultaneously and at high rates (Aber et al. 1985, Kurz and Kimmins 1987).

Two other techniques that depend upon modified sequential sampling may be useful for calculating root production. These techniques are based upon ingrowth into root-free soil cores (e.g., Fabiao et al. 1985) or root starch concentration measurements combined with soil temperature monitoring (Marshall and Waring 1985). Analysis of errors in these techniques has been limited (see Caldwell and Virginia 1988).

Because a large investment in sampling and sample processing would be required to obtain the data necessary to calculate fine root production in forests, it is unlikely that this parameter could serve as a practical indicator of air pollution damage. Standardized methods could not be used on many sites because of the need to adapt the sampling scheme to the particular growth conditions of the site. Careful quality control would be required to assure consistent processing of samples through the years that would be necessary to integrate the variation in fine root production due to environmental fluctuations. Even if sampling could be conducted to insure reliable estimates of fine root production through time, large spatial variation, including variation due to stand age, species composition, understory composition and site nitrogen availability (e.g., Persson 1983, Aber et al. 1985, Vogt et al. 1986b), would require simultaneous sampling of interspersed (Hurlbert 1984) exposed or damaged stands and control stands of unexposed or undamaged trees (see Cape, this volume).

An approach to determining root production that requires less intensive sampling than the usual series of sequential harvests involves labeling the structural carbon of the root system with ^{14}C and assessing the subsequent dilution by ^{12}C incorporated into new root structure during the period in which production is to be determined (Caldwell and Camp 1974). A turnover coefficient is derived from the ratios of $^{14}C/^{12}C$ in root structural tissue at the beginning and end of the period. Root production during the period is the product of the turnover coefficient and the total root biomass at the beginning.

This approach has been used successfully in field studies of cold-desert shrubs (Caldwell et al. 1977), but it has not been utilized for forest trees. Application to trees is impractical because of the difficulty of labeling large plants and because of the potential errors resulting from incorporation of ^{14}C from labile carbon pools into new root structural material during the time between the first and final sampling. This incorporation would compromise one of the critical assumptions underlying this technique and, if uncorrected, would result in severely underestimating root production (Milchunas et al. 1985). A second assumption underlying this technique is that ^{14}C and ^{12}C are lost from the root system, by decomposition, herbivory, etc., in the same proportion as they occur in the structural carbon in the root system. Milchunas et al. (1985) and Caldwell and Eissenstat (1987) discussed the errors associated with this technique and concluded that the isotope dilution technique can accurately estimate root production in the field if precautions are taken to insure that both the incorporation and decomposition assumptions are not violated.

Because the isotope dilution technique is sensitive to errors resulting from carbon fluxes from labile to structural pools and the ratio of isotopes in material lost from the root system, it would be more useful as a research tool to study whole-plant carbon allocation patterns than as a monitoring method. Separate turnover coefficients would undoubtedly need to be developed for fine and large roots. The greatest barrier to using this technique in forests is how to label root tissues of large trees practically and adequately. The hazard associated with labeling trees might be alleviated if CO_2 highly enriched or depleted in the stable carbon isotope, ^{13}C, was substituted for $^{14}CO_2$. Highly enriched $NaHCO_3$ (ninety-nine atom percent ^{13}C) is available commercially and could be used to produce $^{13}CO_2$; and some industrial sources of CO_2 are substantially depleted in ^{13}C (B. Fry, pers. comm.). If all the practical problems of labeling trees could be overcome, the isotope dilution technique might be useful in assessing air pollution effects on fine root production in forests. While this technique does not completely alleviate spatial and temporal variation problems associated with sequential harvest sampling, it could be applied to interspersed damaged and healthy trees.

ROOT GROWTH AND DEVELOPMENT

Nondestructive observation of the growth of roots against glass or plastic walls or tubes has been extensively utilized in root system studies for over a century, especially in crop plant root systems. Construction, installation and utilization of large root observation chambers (rhizotrons) and glass or plastic tubes (minirhizotrons) have been reviewed by Böhm (1979), Taylor (1987), and Caldwell and Virginia (1988). Rhizotrons have been effectively used to study the seasonal dynamics of root growth in relation to shoot growth of large trees and to determine the relationships of root growth to soil temperature and soil and plant water status (Reich et al. 1980, Teskey and Hinckley 1981, Kuhns et al. 1985).

Rhizotrons could potentially be used to determine how these relationships might change in trees exposed to air pollution. Rhizotrons could be placed so that roots of both damaged and undamaged trees, or experimentally exposed and control trees, could be monitored on the same site in the same year. In addition root morphology and development (e.g., root elongation rates and the longevity of individual root elements, etc.) could be directly monitored in rhizotrons. As Marshall (1986) showed that fine roots of Douglas fir died as their carbohydrate reserves were depleted, in trees where carbohydrate concentrations of fine root elements are affected by air pollution exposure, longevity of fine root elements should be reduced compared with longevity in undamaged trees. The relationship between root elongation and suberization and development of root hairs as well as mycorrhizal or pathogenic relationships could also be studied with rhizotrons. Fluorescence techniques might be helpful in some of these observations (see review by McMichael and Taylor 1987).

Root observation tubes (minirhizotrons) have advantages over larger rhizotrons in inexpensive replication and increased placement flexibility. Minirhizotrons can generally be used for a longer period of time compared to large glass plates before accumulation of roots dictate replacement. Large numbers of tubes are required, however, to compensate for the reduced area of the observing surface.

Observations in minirhizotrons can be made with instruments ranging from simple, inexpensive periscopes (Richards 1984) to expensive, color-video systems (Upchurch and Ritchie 1984). Minirhizotrons have been used in agronomic situations to determine root depth distributions, root length density and density dynamics (see reviews in Taylor 1987). Applications of minirhizotrons in forests would likely be less important for determining these parameters, however, than for determining root morphological and developmental patterns.

Recording high-resolution, color images in minirhizotrons means that virtually all of the morphological and developmental parameters studied in rhizotrons can also be obtained with minirhizotrons at much less expense. Replicate placements of minirhizotrons would allow researchers to deal with spatial variation problems. Image analysis of video recordings of root systems from minirhizotrons could facilitate data analysis and determination of morphological parameters (Smucker et al. 1987).

Minirhizotrons also provide an efficient way to examine treatment effects on root growth and other root parameters because non-destructive observations can be made on the same root elements before and after treatment. Minirhizotron tubes could be installed in experimental soils of known characteristics to investigate the interaction between roots and soils in the field. Application of minirhizotrons to studying roots of forest trees appears extremely promising. Lack of previous use of minirhizotrons in forests, however, would require much baseline work.

Some morphological and developmental parameters related to pollutant acclimation or damage might better be determined on root samples from cores or on roots of uniform seedlings than through observations of roots growing against transparent barriers. Branching patterns of roots afflicted with aluminum toxicity, including stubby, brittle roots with thickened and brown root tips and an overall reduction in fine branching (Foy et al. 1978), have been found in damaged trees (e.g., Glatzel et al. 1986). Subtle changes in branching patterns of roots could be quantified by utilizing the recently described topological approach of Fitter (1985, 1987). Fitter (1986) showed that root systems exhibiting distinct topologies could be related to their space-filling and water transport efficiencies. Root samples from cores could also be utilized to determine mycorrhizal development in fine roots and element concentrations in fine root tissues. These topics are addressed by other papers in these proceedings (Antibus and Linkins; Shortle; Marx and Sharer). Finally, as root growth potential determined under standard conditions is an accepted measure of seedling physiological status (e.g., Ritchie 1982, 1985, DeWald and Feret 1987), root growth potential of uniform seedlings differentially exposed to air pollutants should provide an excellent, integrative measure of pollutant effects on root system developmental potential.

ROOT FUNCTIONING: WATER UPTAKE

The presence of active roots in soil is commonly inferred by loss of water from the soil as determined by standard techniques utilizing neutron attenuation measures of water content (e.g., van Bavel et al. 1968, Nnyamah and Black 1977, Rambal 1984). This technique can detect subtle differences in the seasonal and depth patterns and extent of water uptake. Unfortunately, it is ineffective in very shallow soil layers and is complicated to some extent by drainage. Nevertheless, if the depth distribution or seasonal pattern of water uptake of forest trees was affected by air pollution, this could be determined through neutron attenuation or other techniques for detecting water uptake by root systems (see Caldwell and Virginia 1988). Here again, as with determinations of root production, temporal and spatial variation and availability of interspersed controls sets a limit on the inference that can be made from these measurements.

Soil psychrometers, which measure water potential in a small volume of soil adjacent to the sensor, can also be used to infer the presence of active roots. Utilizing soil psychrometers, Richards and Caldwell (1987) detected substantial diel fluctuations in the soil water potential of shallow, dry soil layers beneath transpiring sagebrush plants. These fluctuations were related to daytime depletion and nocturnal resupply of water conducted through roots from deep moist soil layers and leaked from active roots into the dry soil layers. The diel fluctuations were not present when active roots were not present. These diel patterns depend on the root morphology of sagebrush, which has a dense shallow root system and a relatively sparse deep root system. For trees with similar types of root systems, growing in situations where the shallow soil dries while the deep root system remains in moist soil, similar transport phenomena would be expected. If found, this phenomena, termed hydraulic lift (Richards and Caldwell 1987), could be used as an indicator of the activity of roots in localized zones in the soil. Since root water uptake can be detected on a daily basis with psychrometers, they would be most useful in short-term pollutant exposure experiments.

ROOT FUNCTIONING: NUTRIENT UPTAKE

Radioactive or rare chemical tracers have been used to determine the locations of active roots in soil (see Caldwell and Virginia 1988 for a review). Reduced allocation of photosynthate to roots because of air pollution exposure could result in reduced overall rooting depth, as has been noted following defoliation of forage species (Crider 1955, Troughton 1957), or temporary cessation of root activity at some depths. Such an effect on grass roots was noted following defoliation when uptake of labeled phosphate from the 15-25-cm soil layer was inhibited for 19-30 days (Oswalt et al. 1959). While techniques such as these do not provide quantitative information about the intensity of root activity, they are easy to use and can be applied repeatedly on the same site, thus allowing evaluation of chronic reductions in root depth or spread.

Dual labeling with radioactive or rare chemical tracers allows efficient determination of the relative importance of roots in different locations or at different times for nutrient uptake (Caldwell and Virginia 1988). These techniques can be applied not only to investigating root depth but more importantly to providing a quantitative measure of the relative intensity of nutrient uptake by roots at different depths.

An advantage of multiple tracer techniques is that the results they provide are an integration of processes in the soil, rhizosphere and plant. This is much more efficient and reliable at providing an ecological perspective than utilizing analyses of individual processes. However, it does not provide information on the mechanisms affecting plant nutrient acquisition at these smaller scales.

A dual-phosphorus-isotope labeling technique has been developed to investigate the relative competitive effectiveness of plants. This technique examines the phosphate uptake of an indicator plant simultaneously competing with two experimental plants (Caldwell et al. 1985, 1987). The effectiveness of phosphate uptake by roots of the indicator plant competing with different plants on opposite sides was determined by the ratio of ^{32}P and ^{33}P in tissues of the indicator plant following labeling with carrier-free phosphate on opposite sides with one or the other isotope. Advantages of this technique were discussed in detail by Caldwell and Eissenstat (1987). This technique could be adapted to investigate the effects of air pollution exposure on the nutrient uptake capacity of roots in trees of moderate size. Using an experimental design similar to that used by Caldwell et al. (1987), the phosphate uptake capacity of fumigated and control trees or trees exposed to ambient versus clean air could be compared. An isotope pair of iron (^{55}Fe, ^{59}Fe), although presenting different radiological hazards, might also be useful in this type of study.

An important process for nutrient uptake by plants is proliferation of roots into nutrient-enriched patches in the soil (St. John et al. 1983, Robinson and Rorison 1985). Recent experiments by Friend et al. (1987) have shown that nitrogen-deficient Douglas fir selectively proliferated roots in nitrogen-enriched ingrowth cores when the enrichment in the core was high enough to stimulate compensatory root growth. Because numerous ingrowth cores can be utilized for the same tree or for damaged and undamaged trees on the same site, this technique has potential for monitoring both the development of nutrient stress in trees that might result from chronic pollutant exposure as well as revealing changes in the capacity to respond to nutrient-enriched ingrowth cores.

If ingrowth cores were enriched in a nutrient that became more limiting as air pollution damage increased (e.g. Mg, Beyschlag et al. 1987, Lange et al. 1987), the relative ingrowth into those cores should increase with damage until the level of damage finally reduced ingrowth capacity. For a nutrient that is already limiting, the capacity to grow roots into ingrowth cores would likely be reduced as the degree of damage increased. Potential advantages of this technique include the internal control provided by analysis of unenriched ingrowth cores, the ability to utilize damaged and undamaged trees within the same stand and the possibility of monitoring the same tree or site through time without substantial disturbance of the soil or plant system.

CONCLUSIONS

The processes of carbon fixation and allocation are directly affected in trees exposed to O_3 and SO_2. Substantial reductions in root production commonly result. In addition, nitrogen deposition on canopies or soil acidification reduces root growth and production. Because of this common response of trees to several of the effects of air pollution, measures of root growth or activity would be useful indicators of forest exposure to potentially damaging levels of air pollution. Root indicators may be particularly important in detection of effects of air pollution damage to trees because the effects on roots have been reported to occur before or with larger magnitudes than effects on shoot systems. While long-term or high intensity exposure of forests causes reductions in root growth, root depth and eventually total root mass, it is more important to detect changes in tree or stand function before severe damage has occurred. Root activity can be measured in the short term by techniques reviewed in this paper, among others, and some of these techniques could be adapted for use as indicators of stress in forests exposed to pollution but not yet apparently damaged. Changes in patterns of root growth or development, water uptake and nutrient uptake would provide clues that damage was occurring. Interpretation of such changes would require concomitant data on tree exposure and studies of whole plant carbon and nutrient balance. Finally, technique development and sampling considerations will require careful attention in the application of root measures as indicators of air pollution damage to forest trees.

SUMMARY

1. Techniques that have been discussed have promise for determining effects of air pollution exposure on roots of forest trees. All of these, however, need validation in research situations before they would be useful in monitoring.
2. Most potential effects of air pollution damage on root function are unlikely to be specifically symptomatic of pollutant damage because they could also result from other stresses. Some root morphological or developmental processes may, however, be symptomatic of toxicity to specific ions in the soil system or indicative of particular physiological perturbations.
3. Of all the techniques and research questions reviewed, four appear most promising for assessing root system damage in large trees due to air pollution exposure. These are:

a. Minirhizotron observations of root growth rates and morphological and developmental parameters.
b. Determination of root growth potential of uniform seedlings.
c. Evaluation of root ingrowth into nutrient-enriched ingrowth cores.
d. Dual-isotope-labeling techniques to evaluate nutrient uptake capacity. Assessments made using these techniques would only be reliable when applied to damaged and undamaged trees or experimentally exposed and control trees on the same sites.

4. Utilizing multiple root measurements will provide much more reliable information than attempting to base root damage assessments on a single measure. Integration of root system studies with study of whole-plant functioning would provide additional benefits.

ACKNOWLEDGMENTS

I appreciate the many helpful comments on this paper provided by R.A. Black and R.T. Richards. Many of the techniques discussed were developed with support from NSF (BSR-8207171 and BSR-8705492) and the Utah Agricultural Experiment Station.

REFERENCES

Aber, J.D., J.M. Melillo, K.J. Nadelhoffer, C.A. McClaugherty, and J. Pastor. 1985. Fine root turnover in forest ecosystems in relation to quantity and form of nitrogen availability: a comparison of two methods. Oecologia (Berlin) 66:317-321.

Beyschlag, W., M. Wedler, O.L. Lange, and U. Heber. 1987. Einfluss einer agnesiumdüngung auf Photosynthese und Transpiration yon Fichten an einem Magnesium-Mangelstandort im Fichtelgebirge. Allgemeine Forst Zeitschrift 27/28/29:738-741.

Böhm, W. 1979. Methods of studying root systems. Ecological Studies Vol. 33. Springer, Berlin. 188 p.

Caldwell, M.M., and L.B. Camp. 1974. Belowground productivity of two cool desert communities. Oecologia (Berlin) 17:123-130.

Caldwell, M.M., R.S. White, R.T. Moore, and L.B. Camp. 1977. Carbon balance, productivity, and water use of cold-winter desert shrub communities dominated by C_3 and C_4 species. Oecologia (Berlin) 29:275-300.

Caldwell, M.M., D.M. Eissenstat, J.H. Richards, and M.F. Allen. 1985. Competition for phosphorus: differential uptake from dual-isotope-labeled interspaces between shrub and grass. Science 228:384-386.

Caldwell, M.M., and D.M. Eissenstat. 1987. Coping with variability: Examples of tracer use in root function studies. In: J.D. Tenhunen, F. Catarino, O.L. Lange and W.C. Oechel (eds.) Plant response to stress — Functional analysis in mediterranean ecosystems. Springer, Berlin.

Caldwell, M.M., J.H. Richards, J.H. Manwaring, and D.M. Eissenstat. 1987. Rapid shifts in phosphate acquisition show direct competition between neighboring plants . Nature 327:615-616.

Caldwell, M.M., and R.A. Virginia. 1988. Root systems. Pp. 95-106 in R.W. Pearcy, J.R. Ehleringer, H.A. Mooney and P. Rundel (eds.) Physiological plant ecology: Field methods and instrumentation. Chapman and Hall, London (in press).

Carlson, R.W., and F.A. Bazzaz. 1985. Plant response to SO_2 and CO_2. Pp. 313-331 in Winner, W.E. et al., Sulfur Dioxide and Vegetation. Physiology, Ecology and Policy Issues. Stanford University Press, Stanford, CA.

Cooley, D.R., and W.J. Manning. 1987. The impact of ozone on assimilate partitioning in plants: a review. Environmental Pollution 47:95-113.

Crider, F.J. 1955. Root-growth stoppage resulting from defoliation of grass. USDA Technical Bulletin No. 1102.

DeWald, L.E., and P.P. Feret. 1987. Changes in loblolly pine root growth potential from September to April. Canadian Journal of Forest Research 17:635-643.

Dougherty, P.M., R.O. Teskey, J.E. Phelps, and T.M. Hinckley. 1979. Net photosynthesis and early growth trends of a dominant white oak (*Quercus alba* L.). Plant Physiology 64:930-935.

Fabiao, A., H.A. Perrsson, and E. Steen. 1985. Growth dynamics of superficial roots in Portuguese plantations of *Eucalyptus globulus* Labill. studied with a mesh bag technique. Plant and Soil 83:233-242.

Fitter, A.H. 1985. Functional significance of root morphology and root system architecture. Pp. 87-106 in A.H. Fitter, D.J. Read, D. Atkinson, and M.B. Usher (eds.) Ecological interactions in soil, Special Publication of the British Ecological Society, No. 4. Blackwell Scientific Publications, Oxford.

Fitter, A.H. 1986. The topology and geometry of plant root systems: influence of watering rate on root system topology in *Trifolium pratense* . Annals of Botany 58:91-101.

Fitter, A.H. 1987. An architectural approach to the comparative ecology of plant root systems. New Phytologist 106:61-77.

Foy, C.D., R.L. Chaney, and M.C. White. 1978. The physiology of metal toxicity in plants. Annual Review of Plant Physiology 29:511-566.

Friend, A.L., S.M. Ohmann, and T.M. Hinckley. 1987. Fine root and hyphal growth in Douglas-fir stands: Response to nitrogen stress. Agronomy Abstracts 1987. p. 256.

Glatzel, G., M. Kazda, and M. Sieghardt. 1986. Zur Frage der Melioration versauerter Böden aus schadstoffbelasteten Buchenwäldern dutch Zufuhr yon Kalk oder halbgebranntem Dolomit. Ein Gefäβversuch mit Rotbuche (*Fagus sylvatica*). Z. Pflanzenernaehr. Bodenk. 149:658-667.

Godzik, S. and S.V. Krupa. 1982. Effects of sulfur dioxide on the growth and yield of agricultural and horticultural crops. Pp. 247-265 in M.H. Unsworth and D.P. Ormrod (eds.), Effects of Gaseous Air Pollution in Agriculture and Horticulture. London: Butterworth Scientific, London.

Hansson, A.C., and E. Steen. 1984. Methods of calculating root production and nitrogen uptake in an annual crop. Swedish Journal of Agricultural Research 14:191-200.

Hurlbert, S.H. 1984. Pseudoreplication and the design of ecological field experiments. Ecological Monographs 54:187-211.

Keller, T. 1985. SO_2 effects on tree growth. Pp. 250-263 in Winner et al., Sulfur Dioxide and Vegetation. Physiology, Ecology and Policy Issues. Stanford University Press, Stanford, CA.

Kuhns, M.R., H.E. Garrett, R.O. Teskey, and T.M. Hinckley. 1985. Root growth of black walnut trees related to soil temperature, soil water potential, and leaf water potential. Forest Science 31:617-629.

Kurz, W.A., and J.P. Kimmins. 1987. Analysis of some sources of error in methods used to determine fine root production in forest ecosystems: a simulation approach. Canadian Journal of Forest Research 17:909-912.

Lange, O.L., H. Zellner, J. Gebel, P. Schramel, B. Köstner, and F.C. Czygan. 1987. Photosynthetic capacity, chloroplast pigments, and mineral content of the previous year's spruce needles with and without the new flush: analysis of the forest-decline phenomenon of needle bleaching. Oecologia 73:351-357.

Lechowicz, M.J. 1987. Resource allocation by plants under air pollution stress: Implications for plant-pest-pathogen interactions. The Botanical Review 53:281-300.

Mansfield, T.A., and T. Jones. 1985. Growth/environment interactions in SO_2 responses of grasses. Pp. 332-345 in Winner et al., Sulfur Dioxide and Vegetation, Physiology, Ecology and Policy Issues. Stanford University Press, Stanford, CA.

Marshall, J.D. 1986. Drought and shade interact to cause fine-root mortality in Douglas-fir seedlings. Plant and Soil 91:51-60.

Marshall, J.D., and R.H. Waring. 1985. Predicting fine root production and turnover by monitoring root starch and soil temperature. Canadian Journal of Forest Research 15:791-800.

Massimino, D., M. Andre, C. Richaud, A. Daguenet, J. Massimino, and J. Vivoli. 1981. The effect of a day at low irradiance of a maize crop. I. Root respiration and uptake of N, P and K. Physiologia Plantarum 51:150-155.

McClaugherty, C.A., J.D. Aber, and J.M. Melillo. 1982. The role of fine roots in the organic matter and nitrogen budgets of two forested ecosystems. Ecology 63:1481-1490.

McLaughlin, S.B., R.K. McConathy, R.L. Barnes, and N.T. Edwards. 1980. Seasonal changes in energy allocation by white oak (*Quercus alba* L.). Canadian Journal of Forest Research 10:379-388.

McLaughlin, S.B. 1985. Effects of air pollution on forests. A critical review. Journal of the Air Pollution Control Association 35:512-534.

McMichael, B.L., and H.M. Taylor. 1987. Applications and limitations of rhizotrons and minirhizotrons. Pp. 1-13 in H.M. Taylor (ed.), Minirhizotron observation tubes: Methods and applications for measuring rhizosphere dynamics. ASA Special Publ. No. 50. ASA-CSSA-SSSA, Madison, WI.

Milchunas, D.G., W.K. Lauenroth, J.S. Singh, C.V. Cole, and H.W. Hunt. 1985. Root turnover and production by ^{14}C dilution: implications of carbon partitioning in plants. Plant and Soil 88:353-365.

Nnyamah, J.U., and T.A. Black. 1977. Rates and patterns of water uptake in a Douglas fir forest. Soil Science Society of America Journal 41:972-979.

Oswalt, D.L., A.R. Bertrand, and M.R. Teel. 1959. Influence of nitrogen fertilization and clipping on grass roots. Soil Science Society of America Proceedings 23:228-230.

Persson, H. 1978. Root dynamics in a young Scots pine stand in Central Sweden. Oikos 30:508-519.

Persson, H.A. 1983. The distribution and productivity of fine roots in boreal forests. Plant and Soil 71:87-101.

Rambal, S. 1984. Water balance and root water uptake by a Quercus coccifera L. evergreen shrub. Oecologia (Berlin) 62:18-25.

Reich, P.B., R.O. Teskey, P.S. Johnson, and T.M. Hinckley. 1980. Periodic root and shoot growth in oak. Forest Science 26:590-598.

Richards, J.H. 1984. Root growth response to defoliation in two *Agropyron* bunchgrasses: Field observations with an improved root periscope. Oecologia (Berlin) 64:21-25.

Richards, J.H., and M.M. Caldwell. 1987. Hydraulic lift: substantial nocturnal water transport between soil layers by *Artemisia tridentata* roots. Oecologia (Berlin) 73:486-489.

Ritchie, G.A. 1982. Carbohydrate reserves and root growth potential in Douglas-fir seedlings before and after cold storage. Canadian Journal of Forest Research 12:905-912.

Ritchie, G.A. 1985. Root growth potential: principles, procedures and predictive ability. Pp. 93-104 in M.L. Duryea (ed.), Evaluating seedling quality: principles, procedures, and predictive abilities of major tests. Proceedings of a workshop, Oregon State University, Corvallis.

Robinson, D., and I.H. Rorison. 1985. A quantitative analysis of the relationships between root distribution and nitrogen uptake from soil by two grass species. Journal of Soil Science 36:71-85.

Singh, J.S., W.K. Lauenroth, H.W. Hunt, and D.M. Swift. 1984. Bias and random errors in estimators of net root production: a simulation approach. Ecology 65:1760-1764.

Smucker, A.J.M., J.C. Ferguson, W.P. DeBruyn, R.K. Belford, and J.T. Ritchie. 1987. Image analysis of video-recorded plant root systems. Pp. 67-80 in H.M. Taylor (ed.), Minirhizotron observation tubes: Methods and applications for measuring rhizosphere dynamics. ASA Special Publ. No. 50. ASA-CSSA-SSSA, Madison, WI.

St John, T.V., D.C. Coleman, and C.P.P. Reid. 1983. Growth and spatial distribution of nutrient-absorbing organs: selective exploitation of soil heterogeneity. Plant and Soil 71:487-493.

Taylor, H.M. (ed.) 1987. Minirhizotron observation tubes: Methods and applications for measuring rhizosphere dynamics. ASA Special Publ. No. 50. ASA-CSSA-SSSA, Madison. 144 p.

Teskey, R.O., and T.M. Hinckley. 1981. Influence of temperature and water potential on root growth of white oak. Physiologia Plantarum 52:363-369.

Thorgeirsson, H. 1988. The modeling and measurement of respiratory carbon use and net carbon gain of two *Agropyron* bunchgrasses. Ph.D. Dissertation, Utah State University, Logan, Utah.

Troughton, A. 1957. The underground organs of herbage grasses. Commonwealth Bureau of Pastures and Field Crops, Bulletin No. 44, Hurley, Berkshire.

Upchurch, D.R., and J.T. Ritchie. 1984. Battery-operated color video camera for root observations in minirhizotrons. Agronomy Journal 76:1015-1017.

van Bavel, E.H.M., K.J. Brust, and G.B. Stirk. 1968. Hydraulic properties of a clay loam soil and the field measurement of water uptake by roots. II: The water balance of the root zone. Soil Science Society of America Proceedings 32:317-321.

van den Driessche, R. 1987. Importance of current photosynthate to new root growth in planted conifer seedlings. Canadian Journal of Forest Research 17:776-782.

Vogt, K.A., C.C. Grier, S.T. Gower, D.G. Sprugel, and D.J. Vogt. 1986a. Overestimation of net root production: a real or imaginary problem? Ecology 67:577-579.

Vogt, K.A., C.C. Grier, and D.J. Vogt. 1986b. Production, turnover, and nutrient dynamics of above-and belowground detritus of world forests. Advances in Ecological Research 15:303-377.

Wargo, P.M. 1979. Starch storage and radial growth in woody roots of sugar maple. Canadian Journal of Forest Research 9:49-56.

The Use of Remote Sensing for the Study of Air Pollution Effects in Forests

Barrett N. Rock
James E. Vogelmann
Nancy J. Defeo
Institute for the Study of Earth, Oceans and Space Science and Engineering Research Building University of New Hampshire Durham, NH 03824

ABSTRACT
Remote sensing techniques employing satellite and airborne multispectral data sets provide an accurate means of detecting, quantifying, mapping and monitoring damage in high elevation spruce/fir forests in the northeastern United States (U.S.) and the Federal Republic of Germany (FRG). A gradient of montane conifer damage has been detected using Landsat Thematic Mapper data acquired for the Adirondack Mountains of New York (most severe), Green Mountains of Vermont (moderate damage), and the White Mountains of New Hampshire (limited damage). Landsat Multispectral Scanner data have been used to detect a drop in near infrared reflectance between 1973 and 1984 in transition zone forests (dominated by red spruce and balsam fir) and portions of hardwood forests in the Green Mountains, while reflectance has not changed in upper elevation fir zones. Based on *in situ* spectrometry, such a drop in reflectance has characterized increasing levels of forest decline damage in red spruce. Advanced high-spectral resolution airborne imaging spectrometers have detected highly-diagnostic spectral features associated with *in situ* spectral measurements of spruce damage in both the U.S. and FRG. Current and future work on diagnostic spectral signatures may allow such advanced sensors to identify specific kinds of damage (i.e., determine causes of damage).

INTRODUCTION

The northeastern United States have been experiencing a decline in red spruce and balsam fir since approximately 1960 ([1]). At present, no specific causes have been identified as responsible for the damage. The Forest Response Program of the USDA Forest Service has asked a number of specific questions in order to clarify cause-and-effect issues better. Some of the specific questions for which remote sensing studies may provide input are as follows:

Are changes in growth and mortality in spruce-fir forests in the eastern United States greater than can be attributed to typical trends and levels of natural variability?

What spatial patterns, if any, exist in growth and mortality changes in spruce-fir forests in the eastern United States and how do these patterns relate to spatial patterns of potential pollutant exposure?

What are the effects of sulfur and/or nitrogen derived pollutants alone or in combination with oxidants on spruce and fir morphology?

Previous forest damage and decline studies of Camels Hump in the Green Mountains of Vermont have identified three components of a spectral signature associated with decline in red spruce (*Picea rubens*): a blue shift of the chlorophyll well/red edge; a drop in reflectance of the near infrared (NIR) plateau; and a relative increase in the short wave infrared (SWIR) reflectance values ([2],[3]). A damage mapping technique has been developed which utilizes a ratio of SWIR/NIR reflectance values as measured by aircraft and satellite spectral bands ([3],[4]). This technique has been shown to be an extremely accurate means of detecting, quantifying and monitoring forest damage in conifer stands in both the northeastern and southeastern United States ([5],[6]). Presently, researchers at the University of New Hampshire are involved in several remote sensing studies that build on this work. One study involves the use of satellite data to determine the change in NIR reflectance associated with amount of damage in the Green Mountains between 1973 and 1984. A second study examines spatial patterns of damage which exist across the Adirondacks, Green Mountains and White Mountains. A third study uses a high-spectral resolution airborne sensor to look at spectral signatures characterizing various types of damage. These studies are summarized below.

CHANGE DETECTION STUDIES

Remote sensing investigations employing NS-001 Thematic Mapper Simulator (TMS) and Landsat Thematic Mapper (TM) data ([3],[4],[5],[6]) have shown excellent correlations between ground-based estimates of conifer forest damage and 1.65/0.83 micrometer band (TM 5/4) ratios. Figure 1 shows a damage assessment image made using the TM band 5/band 4 ratio along with a near infrared band (band 5) and a visible band (band 2), color coded red, green, blue, respectively. Red areas in the image represent damaged conifer areas, while dark blue represents healthy conifers and turquoise represents deciduous zones. Numbered and lettered areas are sites for which ground assessments have been made ([2],[3],[4]). Although images produced using this ratio are extremely accurate in mapping and quantifying forest damage levels, it is often difficult to ascertain what proportion of the damage detected is a result of a general forest decline phenomenon ("unnatural" damage) and what is attributed to "natural" conditions, such as those related to poor growing conditions, ice and wind storms, and other natural stresses.

One can begin to address the question of what proportion of damage is natural vs. unnatural by using multitemporal remote sensing data sets to monitor forest conditions through time. The following is a summary of a study to evaluate the potential of using Landsat Multispectral Scanner (MSS) data to detect long-term reflectance changes indicative of high-elevation coniferous forest health ([7]). Data from August 29, 1973 (Landsat 1) and August 21, 1984 (Landsat 5) from the Green Mountains of Vermont were used in this study. Sun elevation was 48° for both data sets, and solar azimuth was similar for both scenes (134° and 136° for the 1973 and 1984 data sets, respectively).

Multispectral Scanner data were computer-processed at the Jet Propulsion Laboratory (Pasadena, CA, U.S.A.) using the VICAR processing system installed on a VAX 11/780 computer. Bands used in the study were centered at 0.65 (0.60-0.70; MSS Band 5) and 0.95 (0.80-1.10; MSS Band 7) micrometers. Following co-registration of portions of the data sets including coverage of the Green Mountains, data sets were standardized by use of 20 forested targets. These sites represented relatively mature stands, most of which were located at low elevations, and were presumed to have undergone minimal spectral change between 1973 and 1984. Sites were field-checked in August of 1987 to verify that these areas had not been logged or selectively thinned between 1973 and 1984. Standardization targets included six coniferous sites and 14 deciduous sites. Mean digital numbers were extracted from each site for the 0.95

Figure 1.
Damage assessment image made using NS-001 Thematic Mapper Simulator data. Numbered and lettered areas are study sites. Red areas indicate heavy forest damage. Modified from Rock et al. (3). Reprinted with permission from Rock et al. 1986. Copyright 1986 by American Institute of Biological Sciences.

micrometer band from both 1973 and 1984 data sets. The 1973 vs. 1984 values regressed against each other yielded an r^2 value of 0.971 for the 0.95 μm band. This indicates that an essentially linear relationship exists between 1973 and 1984 data sets for this band, and implies that the MSS band 7 spectral properties for these standardization sites had not changed significantly during the time period.

The linear regression equation derived from the relationship between the 1973 vs. 1984 vegetation standardization targets was then used to convert digital number values from 1984 data into units comparable in value to 1973 data for the 0.95 μm band. Following standardization of the 1984 data set for the 0.95 μm band, a difference image was produced by subtracting 1984 from 1973 data sets, and adding an offset of 100 to eliminate negative numbers. Pixels for which values were greater than 100 showed a decrease in NIR reflectance from 1973 to 1984 relative to the 20 vegetation standardization targets. Pixels for which values were less than 100 showed an increase in NIR reflectance between these dates, relative to the standardization targets.

A three-color composite using the 1973 0.65 μm and 0.95 μm bands in conjunction with the 0.95 μm difference data set (Figure 2) in the order of blue, green and red was produced. The 0.65 and 0.95 μm bands were linearly stretched using standard methodology to enhance contrast, and the difference data set was linearly stretched to enhance decreases in reflectance between 1973 and 1984. This image not only indicates where major reflectance decreases have occurred but also depicts topographic relief. Areas of red or dark orange generally indicate where coniferous areas decreased in reflectance, whereas yellow to light orange areas indicate where deciduous vegetation decreased in reflectance, in relation to the standardization targets. Areas that are blue to green showed either no major near-infrared reflectance changes, or increases in reflectance. Field and laboratory spectral data acquired for red spruce at the Camels Hump study area and for Norway spruce in West Germany suggest that a decrease in the near infrared reflectance accompanies an increase in needle damage associated with forest decline ([8],[9],[10]). Within the montane coniferous areas, decreases in reflectance were most apparent in the transition zone forests on the western lower slopes, where balsam fir and red spruce dominate. Near-infrared reflectance at the upper elevations, where balsam fir dominates, was relatively unchanged. A general trend of decreasing basal area and inferred biomass loss through time has been documented for the montane forest on the west facing slopes of Camels Hump (the northernmost mountain seen in Fig. 2) ([11],[12]). It is presumed that this decrease in basal area and inferred loss of green leaf biomass and the concomitant increase in amounts of dead branches/trees results in the observed decreases in reflectance in the coniferous portions of the difference image.

It should be noted that it has been found that lower reflectance in the near-infrared implies lower amounts of biomass as estimated by leaf area index (LAI) measurements for some species ([13]). However, it has not been documented that lower levels of biomass (or LAI) correlate well with near-infrared reflectance for conifer species ([14],[15]). Therefore, at present, it cannot be stated that decreases in the near-infrared reflectance noted for much of the conifer zone at Camels Hump are directly related to decreases in green leaf biomass, or with the increases in dead branches and trees which accompany loss of biomass, or both.

The data sets being compared were acquired during approximately the same time of year (late August), and thus potential problems due to different solar angles and azimuths have been negated. However, it should be noted that annual phenological differences due to rainfall and temperature variations represent potential problems in multitemporal studies. Phenological differences may be minimized, but not totally eliminated as factors contributing to reflectance differences between data sets, by selection of data from the same date from year to year. Rainfall and temperature data for the areas and dates in question should be used to determine if annual phenological

Figure 2.

False color composite image from Multispectal Scanner (MSS) data of a portion of the Green Mountains of Vermont using 1973 0.65 and 0.95 µm bands, and the 0.95 µm difference data set. Areas of red, orange or yellow indicate where near-infrared reflectance has decreased from 1973 to 1984 in relation to 20 deciduous and coniferous targets. Modified from Vogelmann ([7]).

differences are likely to be major factors influencing the remotely sensed data sets being compared.

It is likely that the lower reflectance of the deciduous vegetation in the higher elevations in 1984 vs. 1973 was due at least in part to rainfall differences affecting green leaf biomass production. More rainfall occurred during the growing season of 1973 as compared with that of 1984. However, it is not felt that rainfall differences alone can explain the reflectance differences noted in the high elevation coniferous regions. Some of these areas showed reflectance changes (e.g., the west facing transition zone), whereas adjacent regions (e.g., high elevation areas dominated by balsam fir) that presumably were under similar climatic conditions did not. The differences in rainfall might be expected to have different effects on conifer leaf flush in 1984 vs. 1973. However, since conifers retain their needles for several years, these first-year needles make up only a portion of the total conifer foliage influencing reflectance. Such rainfall differences would not be expected to affect greatly the phenologic state of the older needles. Thus, coniferous vegetation would not be as susceptible to annual variation in rainfall patterns as would deciduous vegetation.

At present, it is felt that the decrease in near-infrared reflectance noted in the 1984 data set as compared to the 1973 data set for the high-elevation coniferous regions is attributed to the general forest decline process, being related to the increased levels of mortality and decreased levels of green biomass that have been documented in this region.

SPATIAL STUDIES

The detection and quantification of spatial patterns of conifer forest damage in the eastern United States may be done accurately and objectively using remote sensing techniques. Remote sensing data can be used to detect large, regional variations in forest condition that can then be correlated with patterns of pollutant exposure, soil types, geology and other factors that may affect the condition of forest communities.

Previous studies have found that the ratio of TM band 5 to band 4 is strongly correlated with ground-based measurements of forest damage in the northeastern United States. The higher the level of forest damage, the higher the ratio value ([3],[4],[5],[6]). A Thematic Mapper scene (that included coverage of the Green Mountains and the Adirondack Mountains) acquired August 4, 1984, and a second scene (that included the Green Mountains and the White Mountains) acquired June 10, 1984 were used. From these data sets, values of the damage assessment ratio (TM band 5 / band 4) were compared among the three mountain ranges.

The two TM scenes were standardized by calibrating pixel values based on homogeneous ground targets and by using the same parameters to stretch the band 5 / band 4 ratio over the full 0-255 dynamic range. Coniferous portions of the image were isolated from non-coniferous forest regions using a method in which a mask was placed over all regions of the image which did not correspond to coniferous forest. A complete description of the method can be found in Vogelmann and Rock ([6]).

A damage rating scale was developed using the TM band 5 / band 4 ratio to assess relative damage levels of montane conifer areas among selected mountains in the Green Mountains of Vermont and the White Mountains of New Hampshire ([6]). The same procedure was used to assess relative damage levels of conifer areas in the Adirondack Mountains. Low, medium and high damage study sites located on Camels Hump in the Green Mountains were used as standards of reference. Damage levels for each of these reference sites were determined by visually assessing percentage foliar loss at each study site ([4],[6]).

Ranges of ratio values corresponding to low, medium and high damage categories were defined, and numbers of conifer pixels falling within each damage category were totaled. The level of damage for each mountain was then summarized using the following equation:

Damage / Rating = (100-% Low Damage Pixels + % High Damage Pixels) /2

Table 1. Conifer damage in the Adirondack Mountains, Green Mountains and White Mountains.

Damage Pixels	Damage Rating	Site	Elevation	Low% Damage	% Medium Damage	% High
Pixels				**(meters)**	**Pixels**	
ADIRONDACKS						
	Whiteface Mt.	1484	8.6	12.7	78.7	85.1
	High Peaks Area	1268	2.3	6.8	90.4	94.3
GREEN MOUNTAINS						
	Camels Hump	1244	26.5	20.7	52.8	63.2
	Mt. Abraham	1260	25.6	25.6	48.9	61.7
	Breadloaf Mt.	1165	37.9	24.6	37.5	49.8
WHITE MOUNTAINS						
	Mt. Moosilauke	1464	72.3	16.8	10.9	19.3
	Lafayette Mt.	1585	63.9	19.0	17.0	26.6

Table 1 is a summary of conifer damage for several high elevation areas in the Adirondack Mountains, Green Mountains and White Mountains. It should be made clear that these damage ratings are relative measures of forest health based on the field work done at Camels Hump. Thus, a damage rating does not correspond to percent mortality, but is merely a relative measure that can be used to compare damage levels among individual mountains.

It is apparent that there is a trend of decreasing damage from the westernmost range (Adirondacks) to the easternmost range (the White Mountains). It is also evident from the table and from field studies that elevation, slope and aspect alone are not factors which account for the relative levels of damage in coniferous forests in New York and New England. The National Acid Precipitation Assessment Program sponsored studies to determine the spatial patterns of wet deposition pH values in North America.

Results are shown in Figure 3. The pattern of pH values found in the mountains of the northeastern United States correlates with our damage assessment: the lowest pH values in North America are centered over the Adirondacks and pH values increase (indicating less acidic conditions) in all directions from this area. The lowest pH values (in the Adirondack Mountains) correspond to the highest damage ratings, while higher pH values (in the White Mountains of New Hampshire) correspond to the lower damage ratings of the three ranges studied. The Green Mountains in Vermont, which have intermediate damage levels, are inferred to have intermediate pH values.

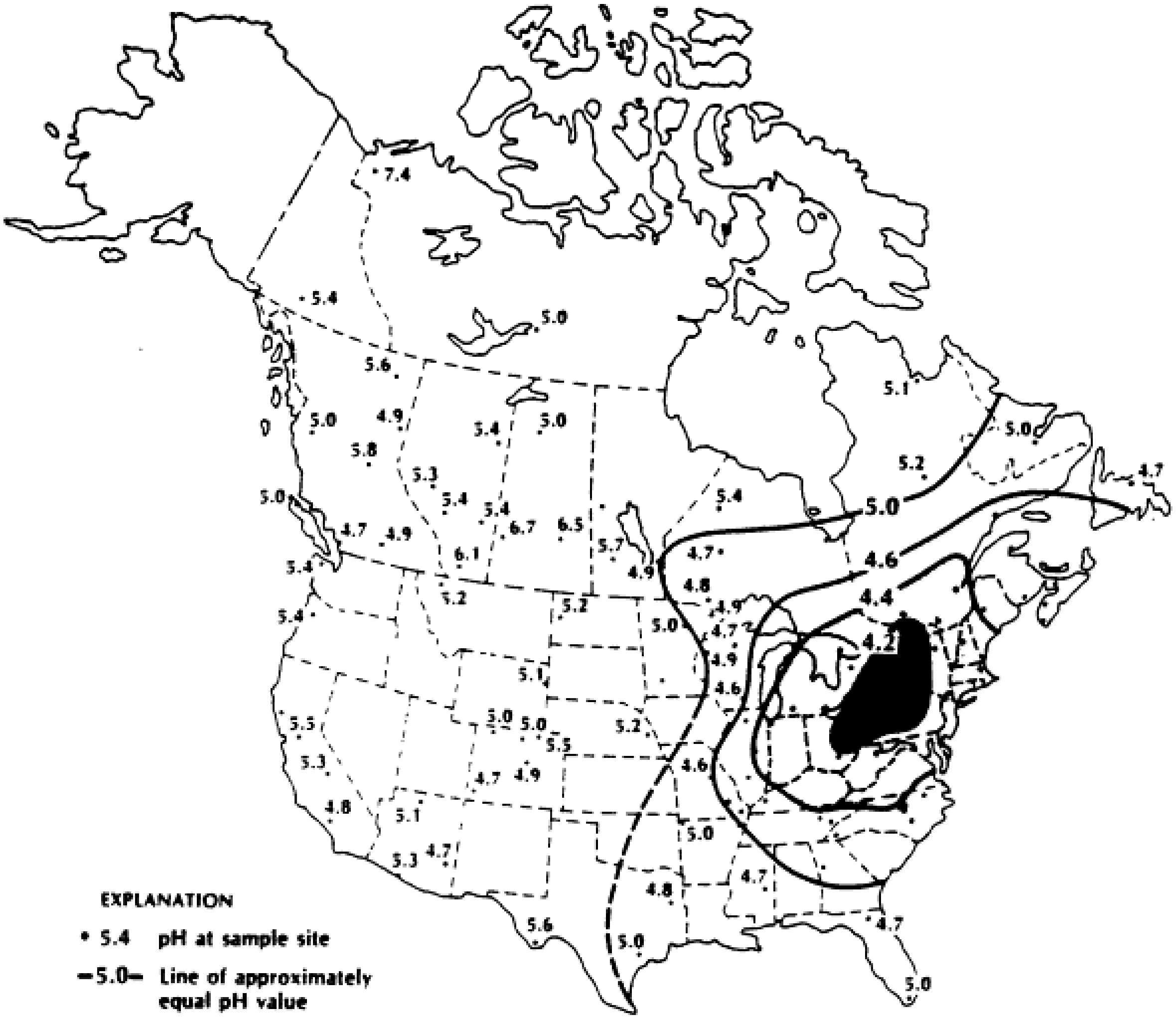


Figure 3.
pH measurements for North America, measured in 1982 (16).

FLUORESCENCE LINE IMAGER STUDIES

To date, high-spectral resolution *in situ* and airborne sensor data sets have been acquired for forest decline sites in the northeastern United States (3,9), and the Federal Republic of Germany (8,10). Although these high-resolution data sets provide a great deal of fine-spectral feature information relating to specific symptoms of forest decline (chlorosis, canopy dryness, and foliar loss), as yet such symptoms have not been related to exposure to specific pollutants such as sulfur and/or nitrogen compounds, either alone or in combination with oxidants. To develop spectral signatures characteristic of spruce response to specific pollutant exposure, high-resolution *in situ* spectral assessment studies must be conducted in association with controlled-exposure experiments. As a means of portraying the power of airborne high-spectral resolution data in assessing types of forest damage, work presented in detail elsewhere ([9]) is cited below.

An airborne imaging spectrometer, known as the Fluorescence Line Imager (FLI), and owned and operated by Moniteq, Ltd., Toronto, Canada [1], has been used to detect reflectance features associated with the chlorophyll well/red edge blue shift characteristic of *in situ* spectral measurements ([9]). These spectral fine features are not detected by broad-band sensor systems such as the MSS, TM or TMS, but rather require the high-spectral resolution capabilities of imaging spectrometers such as the FLI and NASA's Airborne Visible Infrared Imaging Spectrometer (AVIRIS).

Figure 4 presents *in situ* and FLI reflectance data acquired for a low and a high damage site on Camels Hump (sites 1 and 7, respectively, Fig. 1). In both, normalized plots are presented and the blue shift is readily seen. In addition, spectral reflectance in the visible green and red regions of the electromagnetic spectrum (0.50-0.69μm) characteristic of chlorosis is also seen in both data sets. The FLI data acquired from several forest decline (Waldsterben) study sites in the Federal Republic of Germany are currently being assessed. The blue shift is also seen in these European data and appears to be highly diagnostic of levels of damage in Norway spruce and white fir.

Using red edge parameters, a false color image of the FLI flight line may be compared with a similar portion of the TMS image for the same area. A comparison of the two images suggests that the broad-band TMS data are detecting generic damage in both red spruce in the transition zone forest (lower elevation) and balsam fir (higher elevation) in the conifer forest zone on Camels Hump ([9]). The FLI image appears to be mapping only the damage, based on red edge parameters, occurring in the transition zone red spruce. Winter damage and fir wave damage is known to occur in the upper elevation, fir-dominated conifer forests above the transition zone on Camels Hump. It has also been shown that balsam fir on the mountain has undergone a less severe (although statistically significant) decline in vigor and biomass than has the red spruce ([12]). This suggests that the use of both sensor systems (TM/TMS and FLI) may provide information which allows separation of different damage types: forest decline damage in red spruce and winter damage/fir-wave damage in balsam fir.

High-spectral resolution remote sensing systems currently available on airborne platforms will eventually be available on orbital platforms such as the NASA/ESA Earth Observing System (Eos). Once *in situ* spectral signatures have been identified which are diagnostic of specific natural or pollutant damage, such airborne and/or spaceborne sensor systems may provide forest assessment capabilities which will relate specific spectral signatures (effects) to specific causal agents so that direct cause-and-effect relationships may be remotely detected and monitored on a regional/global scale.

SUMMARY

Each remote sensing system has limitations based on spatial/spectral resolution, band placement, software availability, and other complicating factors. Used alone, each sensor can only be used to solve a portion of the forest damage and decline/atmospheric pollutant puzzle. When data are acquired with many sensors over the same region, cause-and-effect issues can be addressed more readily.

In the above studies we found that the changes in health in certain portions of the spruce-fir forests in the eastern United States were greater than can be attributed to

---

[1] Reference to specific manufacturers is for clarity and does not constitute endorsement of product by NASA or the University of New Hampshire

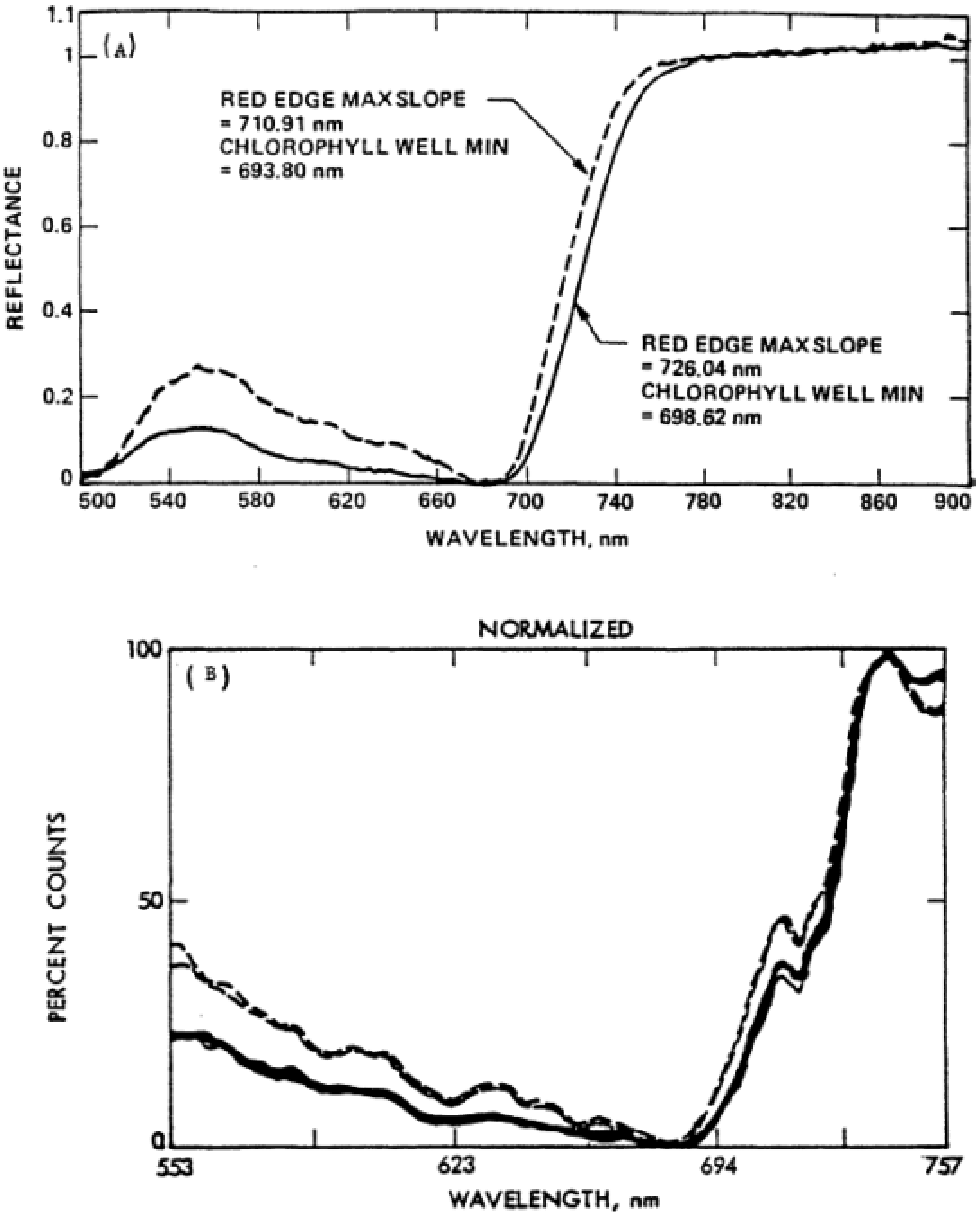


Figure 4.
Ground spectral data (A) and aircraft spectral data (B) for high and low damage sites on Camels Hump, Vermont. In each, the solid curves are from low damage areas (site #1 in Fig. 1) and the dashed curves are from high damage areas (site # 7 in Fig. 1). Both sets of curves show a diagnostic "Blue Shift" in the position of the chlorophyll red edge located at approximately 710-740 nm.

typical trends and natural variability. Also, a distinct spatial pattern of greater damage in the Adirondack Mountains, decreasing to the east, has been detected and this pattern of damage corresponds to spatial patterns of wet deposition pH values. Finally, current research indicates that spectral signatures characteristic of damage exist, and these can be used to identify various damage symptoms.

The work described in this paper was not funded by the U.S. Environmental Protection Agency and therefore the contents do not necessarily reflect the views of the Agency and no official endorsement should be inferred.

References

1. U.S. Department of Agriculture, Forest Service. 1985. Cooperative survey of red spruce and balsam fir decline and mortality in New York, Vermont and New Hampshire, 1984, Broomall, PA: U.S. Dept. of Agriculture, Forest Service, Northeastern area, 53 pp.
2. Rock, B.N., Williams, D.L., and Vogelmann, J.E. 1985. Field and airborne spectral characterization of suspected acid deposition damage in red spruce (*Picea rubens*) from Vermont. Proceedings of the 11th International Symposium on Machine Processing of Remotely Sensed Data, Purdue University, West Lafayette, IN, pp. 71-81.
3. Rock, B. N., Vogelmann, J.E., Williams, D.L., Vogelmann, A.F., and Hoshizaki, T. 1986. Remote detection of forest damage. BioScience, 36:439-445.
4. Vogelmann, J. E,. and Rock, B. N. 1986. Assessing forest decline in coniferous forests of Vermont using NS-001 Thematic Mapper Simulator data. Int. J. Remote Sensing, 7:1303-1321.
5. Rock, B. N., Defeo, N. J., and Vogelmann, J. E. 1987. Vegetation survey pilot study: detection and quantification of forest decline damage using remote sensing techniques. Final Report to the USDA Forest Service, Jet Propulsion Laboratory Document D-4669, Pasadena, California, 30 pp, plus appendices.
6. Vogelmann, J. E., and Rock, B. N. 1988. Assessing forest damage in high-elevation coniferous forests in Vermont and New Hampshire using Landsat Thematic Mapper data. Remote Sens. Environ., 24:227-246.
7. Vogelmann, J. E. 1988. Detection of forest change in the Green Mountains of Vermont using Multispectral Scanner data. Int. J. Remote Sensing, 9:1187-1200.
8. Rock, B. N., Hoshizaki, T., Lichtenthaler, H., and Schmuck, G. 1986. Comparison of *in situ* spectral measurements of forest decline symptoms in Vermont (USA) and the Schwarzwald (FRG). Proc. of Int. Geosci. and Remote Sensing Symposium (IGARSS'86), IEEE 86CH2268-1, IEEE, New York, Vol 3: 1667-1672.
9. Rock, B. N., Hoshizaki, T., and Miller, J. R. 1988. Comparison of *in situ* and airborne spectral measurements of the blue shift associated with forest decline. Remote Sens. Environ., 24:109-127.
10. Herrmann, K., Rock, B. N., Ammer, U., and Paley, H. N. 1988. Preliminary assessment of Airborne Imaging Spectrometer and Airborne Thematic data acquired for forest decline areas in the Federal Republic of Germany. Remote Sens. Environ., 24: 129-149.
11. Vogelmann, H. W., Bliss, M., Badger, G. and Klein, R. M. 1985. Forest decline on Camels Hump, Vermont. Bull. Torrey Bot. Club, 112:274-287.

12. Vogelmann, H. W., Perkins, T., Badger, G. and Klein, R. M. 1988. A 21-year record of forest decline on Camels Hump, Vermont. Eur. J. For. Path.: in press.
13. Wiegland, C. L., Richardson, A. J., and Kanemasu, E. T. 1979. Leaf area index estimates for wheat from Landsat and their implications for evapotranspiration and crop modeling. Agron. J., 71:336-342.
14. Peterson, D. L., Spanner, M. A., Running, S. W., and Teuber, K. B. 1987. Relationship of Thematic Mapper Simulator data to leaf area index of temperate coniferous forests. Remote Sens. Environ., 22:323-341.
15. Franklin, J. 1986. Thematic mapper analysis of coniferous forest structure and composition. Int. J. Remote Sensing, 7:1287-1301.
16. NAPAP (National Acid Precipitation Assessment Program). 1983. Annual report to the President and Congress, Washington, D.C.

Indigenous and Cultivated Plants as Bioindicators

Leonard H. Weinstein
John A. Laurence
Boyce Thompson Institute Ithaca, NY 14853

ABSTRACT

In most geographical regions, indigenous or cultivated species of plants are present for use as indicators of pollution from ozone, sulfur dioxide, hydrogen fluoride, hydrochloric acid, chlorine, and other phytotoxicants. Where they do not occur, portable "gardens" or lichen "boards" can be distributed in appropriate areas, or gardens can be planted *in situ* Careful selection of indicator species can not only identify the pollutant or pollutant mixture, but can provide approximate estimates of geographic distribution, source strength, pollutant dose, and can aid in locating particular sources. A useful adjunct is measurement of pollutant accumulation by chemical analyses, especially for metals and fluoride. Evaluations are made by: (a) Studies of species depletion, such as lichen deserts. (b) specific types of foliar lesions and field distribution of sensitive species. Plant bioindicators are low in cost and maintenance, and are applicable to urban and rural areas, to wide geographical areas, and to remote areas where electrical power is unavailable.

INTRODUCTION

Plant bioindicators or biomonitors are living entities that respond, usually at the organismal level, in characteristic and reliable ways to physical or chemical factors in their environment. The occurrence, distribution, vigor, and appearance of indigenous or cultivated plants, or their capacity to accumulate toxicants, have been used for more than 75 years to detect the presence and amount of airborne pollutants ([44]).

Plants range in sensitivity to airborne pollutants from highly sensitive to highly tolerant. Often there is significant range in sensitivity between cultivars of the same species. In ecosystems, plants are often the most susceptible receptors of toxicants, and their response, whether in the production of foliar lesions, change in form, or altered metabolism, may be more easily measured than chemical or physical detection of pollutants.

Generally, by the response of one or more bioindicators/biomonitors can help to identify ecological problems, to document changes and trends in the general quality of forest ecosystems, and to predict effects on wildlife habitats. Specifically, they have been .used to (i) establish the presence of a pollutant, (ii) aid in its identification, (iii) relate dose of the pollutant to response of the receptor, (iv) delineate the spatial and temporal distribution of the pollutant, and (v) measure pollutant accumulation (gases, particles, heavy metals, etc.). Since the general methods of using plant receptors were first introduced, many different approaches have been used for each type or species and many of these will be reviewed briefly. The minor differences in the methods used to biomonitor forest, agricultural, or urban ecosystems, will not be discussed.

Plant bioindicators can be compared to the use of litmus paper to indicate that a substance is acidic. However, little quantitative information, except perhaps the identity of the toxicant through the production of specific symptoms, is provided. Plant biomonitors, on the other hand, may provide a quantitative estimate of the amount of a toxicant accumulated by a selected species, as a pH electrode quantitates acidity. Under appropriate conditions, biomonitors can substitute for instruments, and they have the advantage that, unlike instruments, they integrate the effects of the toxicants over a range of environments ([20], [28], [39], [52]). Thus, in this context, they are biointegrators, expressing the biological effect of a dose of pollutant and integrating climatic, cultural, and other biological factors into their response. In some cases, bioindicators and biomonitors may substitute for mechanical collectors or analytical instruments, especially where access to utilities is limited. In addition, biomonitoring programs may be less costly than physical or chemical systems. In other cases, however, the use of receptor organisms may be preferred, since they are "tuned" to the ecosystem and will respond to climatic changes taking place. The State of Maryland has chosen to include plant biomonitoring, by assessing foliar fluoride concentration, in its standard to evaluate effects of fluorides on plants.

GENERAL CONSIDERATIONS

To an ecologist, a bioindicator is a species of plant or animal that responds characteristically to the conditions that occur in a particular region or habitat ([31]). In air pollution studies, it is used to define a plant which exhibits a specific symptomology when exposed to a phytotoxicant ([14], [15]).

The useful bioindicator is a plant that is (a) genetically uniform to minimize natural variability ([14], [15]); (b) sensitive to a specific pollutant by producing a characteristic and easily recognizable symptom; (c) abundant and with a large geographical distribution ([13]); (d) capable of growth throughout the field season ([19]); and (e) capable of absorbing a pollutant in a predictable manner if it is to be used as a measure of accumulation ([14], [15], [17]).

Selection of Plant Materials

Lists of plant species exhibiting varying degrees of sensitivity to air pollution have been catalogued ([3], [4], [5], [7], [14], [15], [24], [25], [26], [29], [34], [49], [50], [51], [54], [55]). In general, those sensitive species with the widest distribution in an area of concern are the preferred bioindicators ([12]). If the purpose of a survey is to determine a simple "yes" or "no" answer that one or more pollutants are causing a problem, all sensitive vegetation should be inspected regardless of species. If the purpose of the survey is to provide information for mapping, for example, based upon the degree of injury observed, a single, widely distributed species should be used. Often, lower forms of plant life are employed, including mosses and lichens. These lower forms may not only be widely distributed but they may also have other attributes, such as extreme sensitivity to the pollutant(s), e.g., lichens and sulfur dioxide and fluoride. One major drawback to the use of lichens is that they are very slow-growing and severe damage or destruction can eliminate them from an ecosystem. But the absence of lichens in a particular area is also valuable information, and the lichen "deserts" in cities and around certain industrial sources are well known ([27], [28], [41]). If there are no sensitive indigenous species present, surrogate species may be introduced at selected sites. Where a single pollutant is known or suspected to be responsible for an ecological problem, only a single species may be needed. Where the problem might be caused by more than one pollutant, an array of species is selected and distributed in key sites.

Indicator Species

Indicator plant species may be used to detect and evaluate all important ecotoxicants by exhibiting extreme sensitivity, e.g., the production of characteristic symptoms in the form of necrotic or chlorotic lesions, distortion of plant growth or other changes in form, alterations in pigment development, or the capacity for accumulation. It is rare that the indigenous or cultivated species in an area do not include some suitable bioindicators in agricultural or forest ecosystems. It is often possible to use a dominant crop or tree species as the principal bioindicator. For example, white pine shows great sensitivity to ozone and sulfur dioxide, and the symptoms are different ([23]). In Ontario, silver maple is used as a biomonitor through accumulation of airborne fluoride, where measured values above a given concentration in the foliage indicate a polluted condition. If one or more native species are not available, introduced species may be used. Outstanding examples are the use of gladiolus plants as an indicator of fluoride pollution ([54], [55]), or of Bel-W3 tobacco ([21], [22]) and Japanese morning glory as indicators of photochemical oxidant pollution ([32], [38]). Many species of mosses and lichens are used to evaluate air purity ([16], [18], [33], [35], [36], [37], [42], [43], [48]) or accumulation of heavy metals ([11], [41]). White ash, wild cherry, yellow poplar, and many other deciduous trees are sensitive to ozone; birch, beech, catalpa, and many maples are examples of trees sensitive to sulfur dioxide; most pines, boxelder, apricot, peach, and many other deciduous and coniferous tree species are sensitive to fluoride. Goldenrods, a widely distributed indigenous weed, are excellent accumulators of PCBs ([10]).

Kinds of Symptoms

The most common characteristic of an indicator species is the production of foliar lesions in response to a given pollutant. Measurements of injury can be very simple, e.g., injured vs. non-injured, or more complex, e.g., assignment of subjective or objective numerical values to denote severity. Additional variables used to assess the effects of pollutants have included percentage of leaves injured, percentage of leaf area injured, growth in length, fresh mass, dry mass, photosynthesis, respiration, transpiration, or chlorophyll content ([19], [40]).

Plants are also used as accumulators of specific toxicants and many of these systems are in use throughout the world to measure fluorine, sulfur, heavy metals, PCBs, etc. ([17], [19], [30], [40], [41]). The presence or absence of certain characteristic traits or diseases can also be used. For example, the relative abundance of tarspot of maple ([6]) or blackspot of rose ([45]), both diseases caused by fungi, have been used to judge air quality.

Field Monitoring Systems

Several field biomonitoring systems have been developed and deployed. Several are described below.

Indicator Gardens-Perhaps the simplest system is the indicator garden, consisting of species of plants that respond differentially to pollutants ([3], [14], [15], [40], [53]). Such a bioindicator garden is in use in Minnesota to monitor sulfur dioxide and ozone concentrations ([1]) (Fig. 1). Indicator gardens are grown in indigenous soil or in a soil mixture of known composition and they utilize species of known and defined sensitivity. For instance, genetic isolines of tobacco, which differ in their sensitivity to ozone, have been used. By comparing the slopes of injury (size of foliar lesions) vs. time curves for line Bel-W3 (sensitive) to line Bel-B (tolerant), air quality can be categorized as poor, moderate, fair, or good (15).

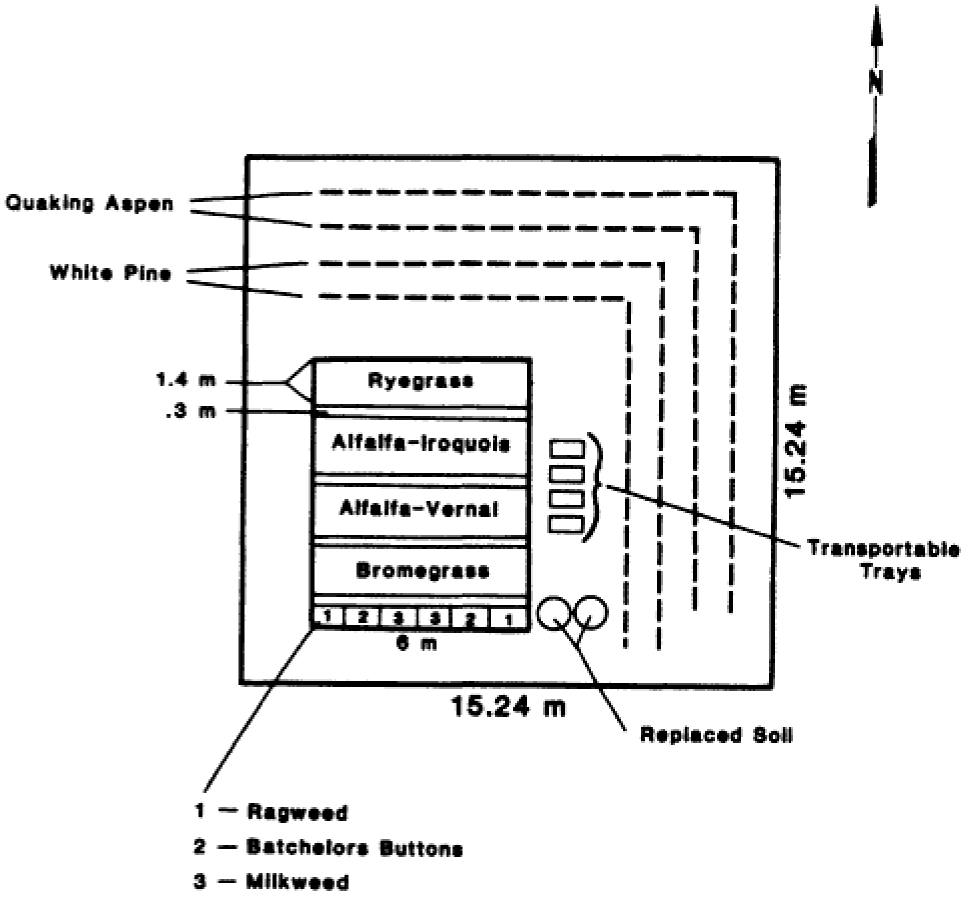


Figure 1.
Bioindicator plot design used in Minnesota to monitor ozone and sulfur dioxide.

Portable Exposure Benches- Often locations where bioindicators are used are isolated, maintenance (watering, weeding, fertilizing, etc.) becomes a concern. This problem is partly solved by the use of special growth benches ([2], [3]). These benches not only support potted indicator plants above the ground for protection from animals, but also provide an automatic watering system (Fig. 2). Exposure benches are used extensively in Europe for supporting indicator trees or other sensitive species.

Lichen Transplants- Disks of corticolous lichen thalli with their bark substrate have been transplanted from areas of clean air to trees growing in polluted air ([8], [9]). A more successful system, where lichen disks are placed in holes in boards and mounted on posts, is in use in Germany. Lichens may also be transplanted to small blocks of wood that are attached to the vanes of an anemometer, thus insuring continuous exposure of the lichen transplant to the prevailing winds ([3], [41], [47]). One disadvantage of using lichens is that they respond slowly to changes in air quality and seldom recover once damaged severely.

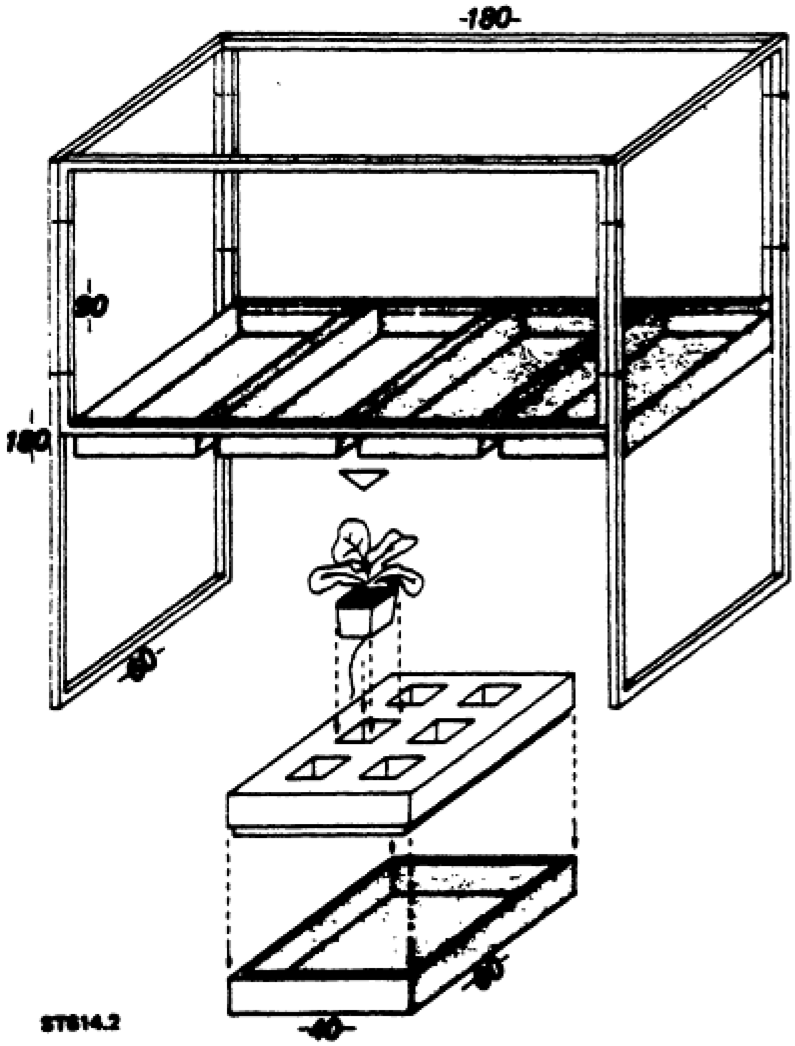


Figure 2.
Exposure bench for multiple bioindicator plants, supported by a galvanized steel frame and with automatic watering. The water reservoir, styrofoam support, and potted plant are shown.
Source: Reprinted with permission of Staub-Reinh from Luft 1985. Copyright 1985 by Staub-Reinh.

Grass Cultures- Standard methods have been devised for determinating the accumulation of several air pollutants over short-and long-term periods using ryegrass cultures ([3], [46]) (Fig. 3). The methods are primarily used in Germany for monitoring fluoride, sulfur, chloride, lead, cadmium, zinc, copper, nickel, vanadium, and other elements. In general, the cultures consist of seedlings of ryegrass growing in a defined medium, provided with a water supply, and mounted above the ground. By removing samples at specific intervals for elemental analysis, the rate of uptake, geographical distribution, and total accumulation of toxicant can be determined.

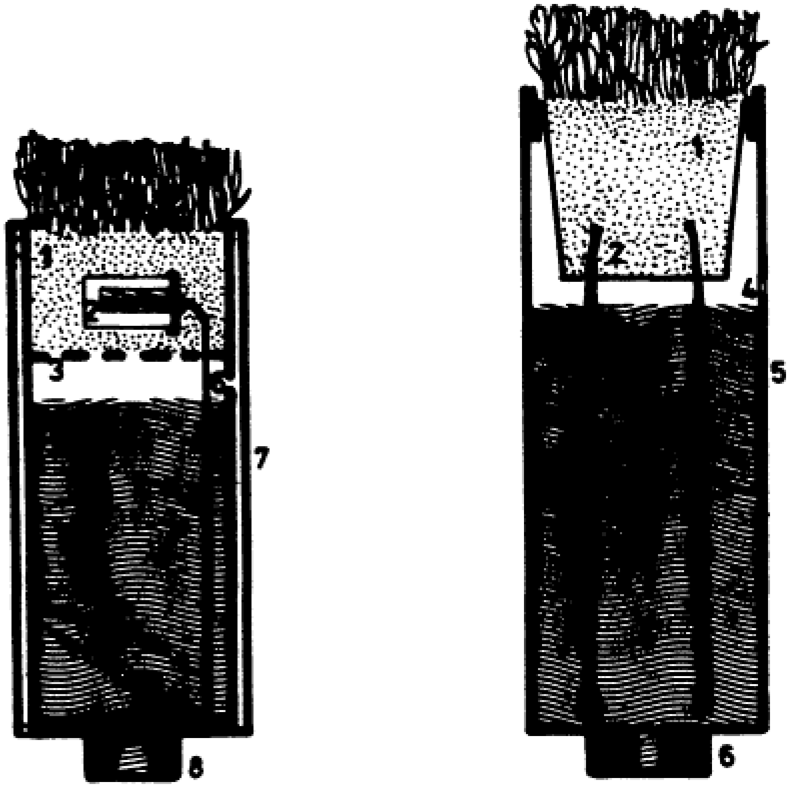


Figure 3.
Self-watering grass culture containers for biomonitoring of heavy metals, fluoride, etc. Left: ([1]) standard soil mix, ([2]) ceramic cylinder, ([3]) filter plate, ([4]) wick, ([5]) water reservoir, ([6]) overflow hole, ([7]) double-walled container, ([8]) connector flange. Right ([1]) standard soil mix, ([2]) glass fiber wick, ([3]) water reservoir,)[4]) overflow hole, ([5]) outer container, ([6]) connector flange.

Conclusions- Biomonitor systems can be attractive alternatives to the usual physical-chemical methods for detecting and measuring air pollutants. The success of biomonitor programs is dependent on the geographic area where they are used, and the amount of research devoted to developing and calibrating the system. Biomonitors have some advantages over more traditional monitoring systems. They are biointegrators, expressing the biological effects of the dose of pollutant. They are generally low cost and low maintenance systems, applicable to urban and rural areas, to wide geographical areas, and to remote forest ecosystems. They are, perhaps, the best early warning systems available.

References

1. Anonymous. 1984. Development of a Biological Air Quality Indexing System. A Report to the Minnesota Air Quality Board. 380 pp. St. Paul, MN 55101.
2. Arndt, U., Erhardt, W., Keitel, A., Michenfelder, K., Nobel, W., and C. Schlüter. 1985. Standardisierte Exposition yon pflanzlichen Reaktionsindikatoren. Staub-Reinh. Luft 45:481-483.
3. Arndt, U., Nobel, W., and B. Schweizer. 1987. Bioindikatoren. Möglichtkeiten, Grenzen und neue Erkenntnisse. 388 pp. Eugen Ulmer GmbH & Co., Stuttgart, FRG.
4. Benedict, H.M., and W.H. Breen. 1955. The use of weeds as a means of evaluating vegetation damage caused by air pollution. Proc. Natl. Air Pollution Symp., 3rd, pp. 177-190.
5. Berge, H. 1973. Plants as indicators of air pollution. Toxicology 1:79-89.
6. Bevan, R.J., and G.N. Greenhalgh. 1976. Rhytissma acerinum as a biological indicator of air pollution. Environ. Pollut. 10:271-285.
7. Brandt, C.S., and W.W. Heck. 1968. Use of plants for pollutant identification and field monitoring. Pp. 428-443 in A.C. Stern, (ed.), Air Pollution, Vol. 1, 2nd ed. Academic Press, New York.
8. Brodo, I.M. 1961. Transplant experiments with corticolous lichens using a new technique. Ecology 42:838-841.
9. Brodo, I.M. 1966. Lichen growth and cities: A study on Long Island, New York. Bryologist 69:427-449.
10. Buckley, E. H. 1987. PCBs in the atmosphere and their accumulation in foliage and crops. Chapter 7. Pp. 175-201 in Phytochemical Effects of Environmental Compounds, J.A. Saunders, L. Kosak-Channing, and E. Corm (eds.). Plenum Publ. Corp.
11. Clough, W.S. 1975. The deposition of particles on moss and grass surfaces. Atmos. Environ. 9:1113-1119.
12. Cole, G.A. 1958. Air pollution with relation to agronomic crops. III. Vegetation survey methods in air pollution studies. Agron. J. 50:553-555.

13. Darley, E.F. 1960. Use of plants for air pollution monitoring. J. Air Pollut. Control Assoc. 10:198-199.
14. Feder, W.A. 1978. Plants as bioassay systems for monitoring atmospheric pollutants. Environ. Health Perspect. 27:139-147.
15. Feder, W.A., and W.J. Manning. 1979. Living plants as indicators and monitors. In W.W. Heck, S.V. Krupa, and S.N. Linzon, Handbook of Methodology for the Assessment of Air Pollution Effects on Vegetation. TE-2 Agricultural Committee, Air Pollution Control Association, Pittsburgh, PA.
16. Gilbert, O.L. 1968. Bryophytes as indicators of air pollution in the Tyne Valley. New Phytol. 67:15-30.
17. Guderian, R. 1977. Air pollution: Phytotoxicity of acidic gases and its significance in air pollution control. Chapter 4 in Ecological Studies, Vol. 22, Springer-Verlag, Berlin.
18. Hawksworth, D.L., and F. Rose. 1971. Lichens as litmus for air pollution: A historical review. Int. J. Environ. Stud. 1:281-296.
19. Heck, W.W. 1966. The use of plants as indicators of air pollution. Air Water Pollut. 10:99-111.
20. Heck, W.W., Dunning, J.A., and I.J. Hindawi. 1966. Ozone: Nonlinear relation of dose and injury in plants. Science 151:577-578.
21. Heck, W.W., and A.S. Heagle. 1970. Measurement of photochemical air pollution with a sensitive monitoring plant. J. Air Pollut. Control Assoc. 20:97-99.
22. Heggestad, H.E., and H.A. Menser. 1962. Leaf spot-sensitive tobacco strain Bel-W3, a biological indicator of the air pollutant ozone. Phytopathol. 52:735.
23. Hepting, G.H. 1966. Air pollution impacts to some important species of pine. J. Air Pollut. Control Assoc. 16:63-65.
24. Hindawi, I.J. 1968. Injury by sulfur dioxide, hydrogen fluoride, and chlorine as observed and reflected in vegetation in the field. J. Air Pollut. Control Assoc. 18:307-312.
25. Jacobson, J.S. 1977. Plants as indicators of photochemical oxidants in the U.S.A. VDI-Berichte Nr. 270:191-196.
26. Jacobson, J.S., and A.C. Hill. (eds.) 1970. Recognition of Air Pollution Injury to Vegetation: A Pictorial Atlas. Air Pollution Control Association, Pittsburgh, PA. 102 pp.
27. LeBlanc, F. 1969. Epiphytes and air pollution. Pp. 211-221 in Air Pollution, Proc. First European Congress on the Influence of Air Pollution on Plants and Animals, Wageningen, 1968.

28. LeBlanc, F., and J. DeSloover. 1970. Relation between industrialization and the distribution and growth of epiphytic lichens and mosses in Montreal. Can. J. Bot. 48:1485-1496.
29. Leone, I.A., Brennan, E., and R.H. Daines. 1964. Plant life as air pollution indicators. Proc. Northeast. Weed Control Conf. 18:451-457.
30. Lihnell, D. 1969. Sulphate contents of tree leaves as an indicator of SO_2 air pollution in industrial areas. Pp. 341-352 in Air Pollution.
31. Mellanby, K. 1978. Biological methods of environmental monitoring. Pp. 1-13 in J. Lenihan and W.W. Fletcher, eds. Environment and Man, Vol. 7: Measuring and Monitoring the Environment. Academic Press, New York.
32. Nakamura, H., and S, Matsunaka. 1974. Indicator plants for air pollutants. Susceptibility of morning glory to photochemical oxidants: Varietal difference and effect of environmental factors. Proc. Crop Sci. Soc. Jpn. 43:522.
33. Nash, T.H., III. 1973. Sensitivity of lichens to sulfur dioxide. Bryologist 76:333-339.
34. Nash, T.H., III. 1976. Lichens as indicators of air pollution. Naturwiss. 63:364-367.
35. Nash, T.H., III, and E.H. Nash. 1974. Sensitivity of mosses to sulfur dioxide. Oecologia 17:257-263.
36. Nash, T.H., III, and L.L. Sigal. 1980. Sensitivity of lichens to air pollution with an emphasis on oxidant air pollutants. Pp. 117-124 in Proc. Symp. on Effects of Air Pollutants on Mediterranean and Temperate Forest Ecosystems. June 22-27, 1980, Riverside, CA.
37. Nash, T.H., III, and L. Sigal. 1981. Ecological approaches to the use of lichenized fungi as indicators of air pollution. Chapter 25. Pp. 481-497 in The Fungal Community. D.T. Wicklow and G.C. Carrol, eds. Marcel Decker, Inc., New York.,
38. Nouchi, I., and K. Aoki. 1979. Morning glory as a photochemical oxidant indicator. Environ. Pollut. 18:289-303.
39. Oshima, R.J. 1974. A viable system of biological indicators for monitoring air pollutants. J. Air Pollut. Control Assoc. 24:576-578.
40. Posthumus, A.C. 1976. The use of higher plants as indicators for air pollution in the Netherlands. Proc. Kuopio Meeting on Plant Damages Caused by Air Pollution, Sci. Pap. Symp., 1976, pp.115-120.
41. Puckett, K.J. 1988. Bryophytes and lichens as monitors of metal deposition. In Assessing Air Quality With Lichens and Bryophytes. T.H. Nash III and V. Wirth, eds. Bibliographica Lichenographica. Stuttgart, FRG.
42. Rao, D.N., and F. LeBlanc. 1965. Effect of sulfur dioxide on the lichen alga, with special reference to chlorophyll. Bryologist 69:69-75.
43. Rao, D.N., and F. LeBlanc. 1967. Influence of an iron-sintering plant on corticolous epiphytes in Wawa, Ontario. Bryologist 70:141-157.

44. Ruston, A.G. 1921. The plant as an index of smoke pollution. Ann. Appl. Biol. 7:390-403.
45. Saunders, P.J.W. 1966. The toxicity of sulphur dioxide to *Diplocarpon rosae* Wolf causing black spot of roses. Ann. Appl. Biol. 58:103-114.
46. Scholl, G. 1971. Die Immissions rate von Fluor in Pflanzen als Masstab für eine Immissionsbegrenzung. VDI Berichte Nr. 164. pp. 39-45.
47. Schönbeck, H. 1969. A method for determining the biological effects of air pollution by transplanted lichens. Staub-Reinh. Luft 29:17-21.
48. Showman, R.E. 1975. Lichens as indicators of air quality around a coal-fired power generating plant. Bryologist 78:1-6.
49. Skye, E. 1979. Lichens as biological indicators of air pollution. Annu. Rev. Phytopathol. 17:325-341.
50. Thomas, M.D. 1951. Gas damage to plants. Annu. Rev. Plant Physiology 2:293-322.
51. Thomas, M.D. 1961. Effects of air pollution on plants. Pp. 233-278 in Air Pollution. World Health Organization Monograph Series #46. Columbia University Press, New York.
52. Treshow, M. 1965. Evaluation of vegetation injury as an air pollution criterion. J. Air Pollut. Control Assoc. 15:266-269.
53. van Raay, A. 1969. The use of indicator plants to estimate air pollution by SO_2 and HF. Pp. 319-335 in Air Pollution. Proc. First European Congr. on the Influence of Air Pollution on Plants and Animals, Wageningen, 1968.
54. Weinstein, L.H. 1977. Fluoride and plant life. J. Occup. Med. 19:49-78.
55. Zimmerman, P.W., and A.E. Hitchcock. 1956. Susceptibility of plants to hydrofluoric acid and sulfur dioxide gases. Contrib. Boyce Thompson Inst. 18:263-279.

Experiments and Observations on Epiphytic Lichens as Early Warning Sentinels of Forest Decline

Martha G. Scott
Thomas C. Hutchinson
Department of Botany and Institute for Environmental Studies University of Toronto Toronto, Ontario, Canada. MSS IAI.

ABSTRACT

Forest declines are now widely reported in Europe and eastern North America, especially in high elevation areas where fogs, oxidants and SO_2 frequently co-occur. Analyses of cloudwater indicate that high-altitude fogs are more acidic ($\bar{x}^{\#}$ pH of 3.8) than ambient precipitation at the same sites and may be present for up to 50% of the year. A combination of high humidity and nutrient enrichment normally allows for a prolific, species-rich epiphytic lichen flora on coniferous trees in the transition zones.

In previous field studies, we have shown that boreal forest-floor lichens such as *Cladina rangiferina* respond to seasonal sprays of simulated, acidic rain of less than pH 3.5 by producing abnormal morphological and cytological structures. We have, therefore, selected a number of high-altitude sites showing varying degrees of forest dieback in Quebec, Vermont and New York (North America) and in the Black Forest and Hartz Mountains (Germany) to determine whether similar abnormalities occur in lichen populations exposed to ambient levels of acidity. We shall discuss the suitability of two, widely-distributed epiphytic lichens, *Hypogymnia physodes* and *Pseudevernia* sp., as early warning indicators of forest dieback, based on a combination of morphological, cytoplasmic and chemical data.

INTRODUCTION

The nature of the forest decline, which is currently affecting tree species in Europe, Scandinavia and eastern North America, is complicated both by the plethora of hypotheses which have been advanced to explain the diebacks and by the complexity of the forest ecosystem itself. A delicate balance exists between above-ground foliar processes such as gas exchange, cuticular integrity and stomatal mechanisms and less visible below-ground factors such as soil chemistry, decomposer organisms, mycorrhizal associations and nutrient availability. Superimposed upon these interrelationships are disease vectors, climatic variables, community and stand dynamics and anthropogenically-derived pollutants, all of which may play a vital role in determining the health of trees within narrowly-defined geographical limits.

In order to understand the dynamics of specific dieback scenarios and the possible involvement of atmospheric pollutants such as acid rain, acid fogs and oxidants, it is necessary to document links between known levels of inputs (e.g., $SO_4^=$ and NO_3^-) and regional declines of specific species. Unfortunately, the long-term monitoring of ambient precipitation chemistry is time-consuming and expensive and, to date, only a handful of such programmes exist. The CHEF project (Chemistry of High Elevation Fog-Schemenauer 1986), currently being implemented at two sites in Quebec, and a similar study at Whiteface Mountain in New York, are two such programmes which provide accurate information about atmospheric chemistry in high-elevation forests where significant declines of balsam fir and red spruce are occurring.

This report presents the results of studies conducted both in Germany and at the high-elevation locations noted above, on two widely-distributed species of lichens, *Hypogymnia physodes* and *Pseudevernia* sp. which show good potential as sentinels of forest dieback.

Historically, there are distinct advantages in predicting the severity of atmospheric pollution on the basis of changes in 'simple' organisms such as lichens and mosses, which have neither roots nor the waxy cuticle which protects higher plants. Traditionally, the presence or absence of key indicator species has been used as a kind of 'litmus paper' to map levels of SO_2 emitted from point-source industrial processes (Leblanc and Rao 1966; Seaward 1987). The disadvantages of this technique lie in the fact that expertise in lichen taxonomy is required to interpret the data and that the response of a particular lichen to a gaseous pollutant may be an "all-or-nothing phenomenon," open to a number of alternative explanations.

However, the acidophilic nature of lichen thalli, in particular of the fungal partner in the symbiosis, suggests that these organisms may be much more tolerant of acid rain and fogs than they are of gaseous pollutants (Scott and Hutchinson, 1987). In fact, lichen populations epiphytic on declining Norway spruce in Germany are actually flourishing. Tolerant lichen species which gradually change in some aspect of their biology over a long period have potential value as bioindicators of tree dieback in impacted areas.

Accordingly, the objectives of this current study are two-fold:

1. To assess the effect on lichen tissue chemistry of elevated levels of sulphate, nitrate, ozone and possibly metals. In 1986, the mean fog pH at high elevation sites in eastern Quebec was 3.8, whereas the mean pH of precipitation at the same sites was 4.3 (Schemenauer 1986). Modelling techniques suggest that fogs and clouds blanket these mountaintops for up to 50% of the year.
2. To determine whether specific morphological or cellular aberrations exist in species of *Hypogymnia* and *Pseudevernia* epiphytic on declining balsam fir and red spruce at these sites. Our previous research in a boreal forest ecosystem showed that branching pattern abnormalities and changes in starch and lipid content of algal cells could be induced in *Cladina* species by seasonal sprays of simulated rain of pH less than 3.5.

METHODOLOGY

Only a brief summary of our experimental design and methodology will be presented here. Data are preliminary and have not yet been statistically analysed. Our study sites are listed in the following table. To date, all of the high elevation locations have been sampled. However, low elevation sites experiencing acidic fogs near the Bay of Fundy, Nova Scotia and a northern boreal forest site with low pollutant inputs will be visited in 1988. A range of altitudes is included for each mountain.

Site	Elevation
Germany:	
Black Forest Region -4 sites	500-1300 m
Hartz Mountains -2 sites	800-1000 m
Czechoslovakian Border -1 site	up to 1200 m
North America:	
Mt. Tremblant (Quebec)	100 m, 590 m, 860 m
Mt. Sutton (Quebec)	845 m, 970 m
Whiteface Mountain (N.Y.)	884 m, 1160 m
Camel's Hump (Vermont)	884 m, 1067 m
Low Elevation Sites:	
Sudbury, Ontario (near smelting operations)-*Cladina* sp.	
Burt Lake, Ontario-boreal forest ecosystem	
Bay of Fundy, Nova Scotia-low elevation with acidic fog monitoring station	

Two species of lichen were present at most of the study locations. *Hypogymnia physodes*, a foliose epiphyte of conifers, has a ubiquitous distribution and is semi-tolerant of gaseous pollutants such as SO_2. Two species of the subfruticose *Pseudevernia* (*P. consocians* in NA and *P. furfuraceae* in Europe) were chosen also because of irregular morphological features noted during preliminary site visits in 1987.

Because of the wide geographical disparities between the study sites, appropriate control sites were difficult to establish. Although the boreal forest location in northern Ontario is unaffected by air currents from the southwest, it is a low-elevation site. However, boreal and high-altitude transitional forests are quite similar in structure and contain many of the same understory species. Low-elevation sites at the base of the mountains are inappropriate because the mixed hardwood forests do not support the same lichen flora and microhabitat factors are completely different. At two elevations on Mount Tremblant and Mount Sutton, therefore, we selected paired sites of different exposures to prevailing winds, in order to test the hypothesis that atmospheric inputs from the SW were more damaging to lichens than inputs from an eastern or northern source.

At each site, 10 balsam fir and red spruce trees, with a DBH of approximately 50 cm, were selected at random from within a defined area. Crown dieback was rated subjectively using a scale of 1 to 5, with I being healthy (less than 20% dead branches and chlorotic needles) and 5 being dead. The percentage cover of all lichen species present on a one-metre segment of the bole of the trees (measured upwards from DBH) was also recorded. From within this 1 metre zone, even-aged samples of *H. physodes* and *Pseudevernia* were then harvested for laboratory analyses.

The following tests were performed for each collection:

(1) Lichen thalli were examined and photographed using a dissecting microscope. Unusual branching or lobation patterns were noted, along with abnormal production of sexual and asexual reproductive structures (Sigal and Nash 1983). This information will be quantified.

(2) Tissue, containing both the algal and fungal symbionts, was fixed for TEM using a previously-published protocol (Scott and Larson 1984).

(3) Following digestion with nitric acid, the tissue chemistry of unwashed lichens was analysed using either ICP (inductively-coupled plasma emission spectroscopy) or atomic absorption spectroscopy. Data were obtained for a number of elements and expressed on a ug/g dry weight basis (n=5-8).

(4) Pieces of lichen thalli similar in dry weight and exposed surface area were soaked in double-distilled water for one hour. Aqueous extracts were then filtered and analysed for cations (Na^+, NH_4^+, K^+) and anions (Cl^-, F^-, NO_3^-, $PO_4^{\equiv}$, $SO_4^{=}$) using DIONEX (n=6 reps/study site).

RESULTS AND DISCUSSION

Our results clearly demonstrate that both morphological and chemical indicators of air pollution effects can be seen in lichen epiphytes of declining trees. As seen in Table 1, the percentage cover of the dominant epiphyte *H. physodes* decreases with increasing altitude at all but the most northern location (Mt. Tremblant). Ideally, lichen growth should be extremely vigorous in such a cool, moist habitat. The lowest percentage cover of dominant lichen species on boles of balsam fir was recorded near the summit of Whiteface Mountain, where the average crown dieback rating was 4.1, or extremely severe. On-site observations also indicated that many lichens were white or pinkish in appearance, a symptom which may be the result of membrane damage resulting in destruction of chlorophyll.

Table 1. Percentage Cover of lichens on boles (DBH) of Balsam Fir growing at high elevation sites in eastern North America.

Site	% Hypogymnis	physodes	Total Dominant species	Total Lichen Cover		Crown Dieback Rating
	$\bar{x}$	S.E.		$\bar{x}$	S.E.	
Mt. Tremblant:						
590 m	26	8	55	66	27	2.8
860 m	36	5	77	84	7	2.6
Mt. Sutton:						
845 m	29	5	83	103	11	3.3
970 m	26	5	65	94	25	3.1
Whiteface (N.Y.):						
884 m	35	5	66	90	8	3.3
1160 m	21	5	44	72	15	4.1
Camel's Hump:						
884 m	35	6	71	97	12	3.4
1067 m	29	3	49	89	12	3.2

Micrographs of *Cladina rangiferina* (boreal), *H. physodes* and *P. furfuraceae* are presented in Plates I and 2. Figures 1-3 show podetia of *C. rangiferina* which received periodic sprays of acidic rain (a field simulation experiment) (Hutchinson et al., 1987). As compared to the normal situation in which branching occurs close to the tip (Fig. 1), affected lichens produced numerous stunted branches on fully-elongated portions of the thallus which usually do not initiate new growth (Fig. 2). These short branches dichotomize repeatedly and rapidly produce sexual structures (Fig. 3). In addition, there is an increase in the number of algal clusters as compared to the volume of the thallus comprised of fungus. At high elevation sites, thalli of a number of species showed either branching pattern abnormalities (Figs. 4,5,7) or they produced enormous numbers of asexual structures. German populations of *Pseudevernia furfuraceae* from both the Schwarzwald regions and the Hartz Mountains (acid fog, ozone and SO_2) produced club-shaped structures which failed to dichotomize at the growing tips (Fig. 4 arrow). Asexual structures known as isidia, which contain both the algal and the fungal symbiont (Fig. 5,6) are the normal reproductive propagule of this genus. However, thalli harvested from severely declining sites in both Europe and North America produced large numbers of these highly branched structures with little subsequent elongation of the 'internodal' regions (Fig. 5). This phenomenon occurred on both large and small diameter thalli in the populations. Lobes of *Hypogymnia physodes* (not shown) were also convoluted and had prolific marginal soralia (another form of asexual structure). Morphological changes in lichen thalli exposed to air pollution have been reported in the literature for *Parmelia* species exposed to high dosages of SO_2 near Sudbury, Ontario (LeBlanc and Rao 1966) and for *H. enteromorpha* from the heavily-impacted San Bernardino Mountains in California (Sigal and Nash 1983). These authors also observed an abnormally high production per mm^2 of asexual pycnidia. Factors which control branching patterns in healthy lichenized fungi are very poorly understood. In non-lichenized Ascomycetes, sources of carbon, nutrients and especially nitrogen (Griffin, 1981) have been reported to control the degree of 'hyperbranching' of hyphae. Since the stemflow of declining trees may be both acidic and enriched with canopy leachates, altered nutritional factors may, indeed, play a role in stimulating branch production in lichens at high elevation sites. A fertilization effect from the nitrate component of the acidic fogs may also be related to the vigorous lichen growth.

At the cellular level (Plate 2), Figures 8 and 9 show transmission electron micrographs of *C. rangiferina* exposed to simulated rains in a boreal forest ecosystem (Hutchinson et al., 1987). Compared to the normal appearance of the cytoplasm (Fig. 8, pH 5.6), thalli sprayed with rain of pH 2.5 or 3.0 contained algal cells with huge peripheral lipid bodies (Fig. 9) and large deposits of intrathylakoidal starch. This phenomenon has been reported in lichens exposed to high levels of industrial pollution, including SO_2 near urban centres in Madrid (Silva-Pando and Ascaso 1982) and in higher plants exposed to salt stress (Winter 1982).

In high elevation populations of *Hypogymnia* and *Pseudevernia*, starch and lipid are also accumulated by algal cells (Figs. 10, 13). This apparent sequestering of photosynthetic products may represent a failure to translocate sugar alcohols to the fungal partner and might ultimately result in decreased growth of the hyphae and possibly saprophytism. Within the population of algal cells in an affected lichen, healthy and senescent algal cells co-occur. However, the most severely declining sites had the highest percentage of algae with cytoplasmic damage. Deterioration of the chloroplast and cellular membranes was commonly found, along with extensive cell wall degradation. The degree of cellular damage to the lichens appeared to be correlated with the tree dieback rating for the site.

Figure 1. Light micrograph of growing tips of *C. rangiferina* from a boreal forest ecosystem. Podetia sprayed with 'rain' of pH 5.6 had numerous terminal apothecia (Ap). (Hutchinson et al., 1987).

Figure 2. Initialtion of numerous small branches on a mature lower portion of *C. rangiferina* sprayed periodically for five years with artificial rain of pH 2.5.

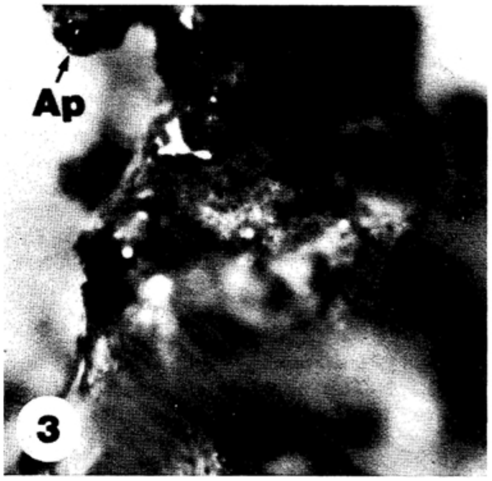


Figure 3. High magnification of a newly-initiated branch with a developing sexual structure (Ap - apothecium). The dark coloration is a result of numerous, large algal cell clusters.

Figure 4. *Pseudevernia furfuraceae* from the Schwarzwald region (greater than 1000m), West Germany. Note the heavily isidiate regions (large black arrow), which failed to dichotomize.

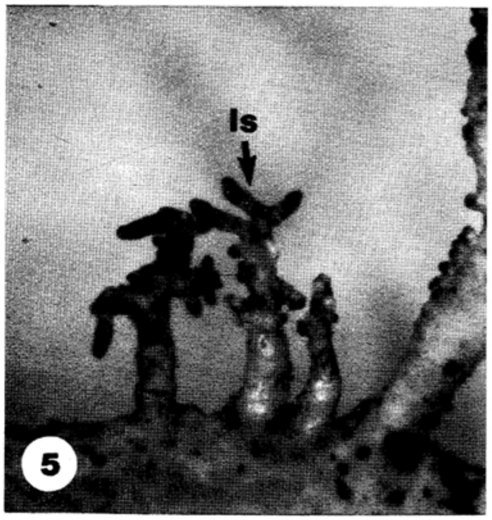


Figure 5. Higher magnification of a portion of the thallus shown in Figure 4. Isidia (Is) are highly branched.

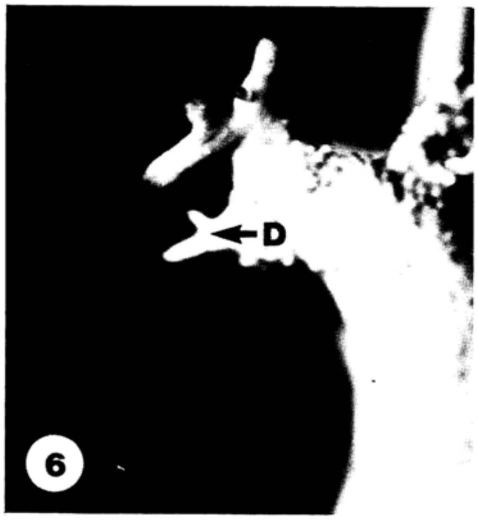


Figure 6. A portion of the same species (P. furfuraceae) from a lower elevation collection (500m) in the Schwarzwald. The thallus is relatively smooth, with fewer numbers of isidia.

Figure 7. P. furfuraceae from the Hartz mountains. Branched isidia are short and thickened. Algal cells (A) are darkened and necrotic-looking, as is much of the upper surface of the thallus.

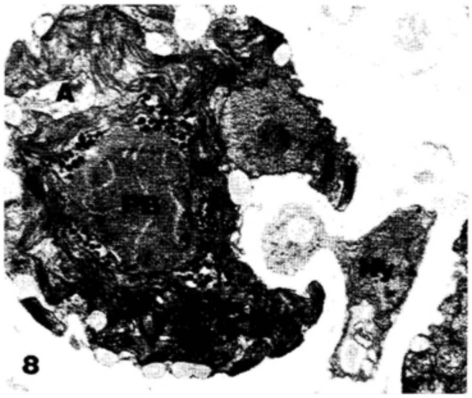


Figure 8. Cytoplasm of the unicellular alga, Trebouxia, in C. rangiferina from the boreal forest simulation experiment. this micrograph is representative of either unsprayed podetia or podetia sprayed with rain of pH 4.0-5.6. A-algal layer. PB-pyrenoid body (starch organizing centre). Ch-Chloroplast. Hy-Hyphae. (Hutchinson et al., 1987).

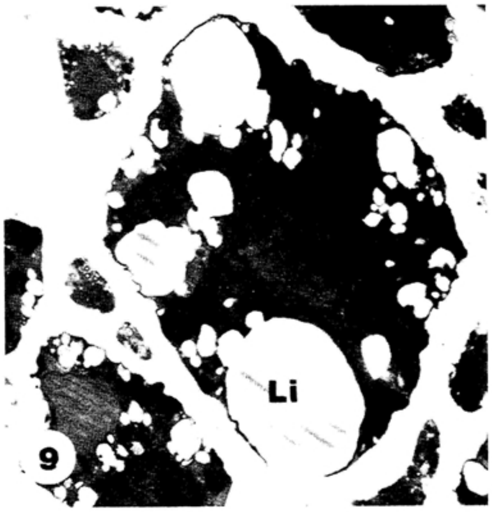


Figure 9. Algal cell from the same species sprayed with simulated rain of pH 2.5. Note the large deposits of peripheral lipid (Li).

Figure 10. Algal cells of *P. consocians* from a high elevation site on Camel's Hump, Vermont. Cells are relatively normal in appearance, except that they contain numerous starch grains (St) and poly-phosphate type bodies (Pp).

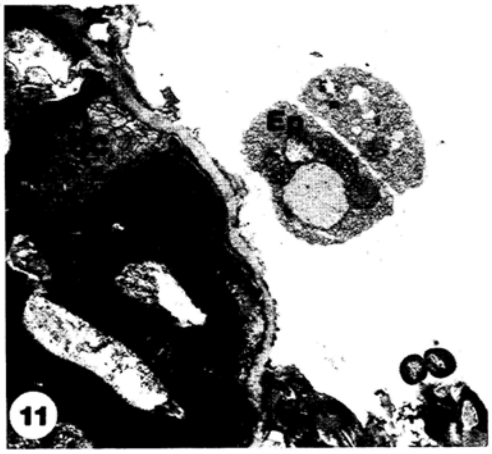


Figure 11. Epiphytes (Ep), with well-preserved cytoplasm are commonly found on the surface of lichens harvested from the Hartz mountains in Germany. Although many of the lichenized algae were senescent, there were an abundance of micro-organisms on the upper cortex.

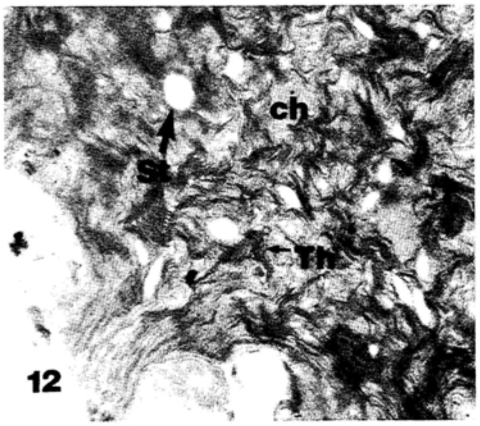


Figure 12. High magnification of the thylakoid membranes (Th) in the chloroplast (Ch) of an alga from *H. physodes* (Schwarzwald region). Note the interthylakoidal starch grains (St) and the disruption of membrane structure.

Figure 13.
The alga of H. physodes from a high elevation collection on Whiteface Mountain, N.Y. Note the large, peripheral lipid bodies (Li) and the accumulation of starch bodies (arrow).

Two strong patterns emerge from examination of the tissue chemistry data in Table 2. First, at heavily impacted sites such as Whiteface Mountain, there is a general pattern of loss of important plant nutrients, such as P, Mg and Mn, with increasing elevation. At sites with intermediate dieback, some of these nutrients, especially Ca, may be substantially elevated, possibly because of foliar leaching or mobilization of cations from bark substrates exposed to acidified stemflow. In the case of calcium, two values are presented for each site, one with all trees included in the sample, and a separate mean from which values for dead trees were removed. It is apparent that a tremendous flush of Ca is released from senescent trees, thus substantially elevating the lichen tissue content of elemental Ca. Even with the dead trees removed, however, Ca levels are elevated at sites with a SW exposure, compared to sheltered sites at an equivalent altitude on the same mountain. Calcium content of epiphytic lichens may, therefore, be a useful marker of tree senescence.

As far as the metals are concerned, lichens from heavily-impacted high-elevation sites contain high levels of A1, Fe, Pb and Cu (not shown). All of these metals may, in some way, be related to by-products of industrial processes. Aluminum has been reported by Scherbatskoy (1982) and Scherbatskoy and Klein (1983) to occur in cores taken from declining red spruce at high elevation sites. It is interesting to note the pattern for Pb which is positively correlated with increasing elevation, with the exception of sheltered sites which contain only background levels of Pb.

Although the data for anions and cations in aqueous extracts of lichen tissues are incomplete, the pattern for ammonia is similar to that observed for the metals. Nitrate and sulfate are also substantially elevated, especially in lichens from the collection near the Czechoslovakian border.

In conclusion, epiphytic lichens appear to have potential as bioindicators of forest decline. To be useful as early warning indicators, however, it is important to identify morphological and chemical markers in advance of severe tree dieback. Both the growth pattern abnormalities and the accumulation of Ca and metals occur in species of *Hypogymnia* and *Pseudevernia* epiphytic on apparently healthy conifers. If, in fact, we can demonstrate that good correlations exist between atmospheric inputs and changes in lichen flora, then these studies may provide a faster, more economical way to assess ambient air quality in the absence of permanent atmospheric monitoring stations.

Table 2. Tissue chemistry (ug/g) of Hypogymnia physodes from high elevation sites.

Site (Elevation)	P	Ca	A1	Fe	Pb	S	N
Germany:							
Black Forest (1000-1300 m)	590	2248	850	1038	71	2023	17,400
Hartz (900-1000)	558	2348	463	1032	60	1786	16,300
Czechoslovakia (1000-1200 m)	1390	2322	1147	1606	56	2856	-
Mt. Tremblant:							
100 metres	1323	87,120	604	750	26	2630	-
590 metres	884	6409 (2110)*	591	807	67	1947	13,200
860 metres	1285	5650 (2347)*	507	548	92	1702	14,100
860 m (sheltered)	686	2069	173	184	28	1237	11,300
Mt. Sutton:							
845 metres	1422	6597 (2012)*	649	928	138	1996	16,900
845 m (sheltered)	1938	3346	715	1025	65	1600	12,300
970 metres	754	10,406 (3935)*	690	818	99	1237	12,100
Whiteface (N.Y.):							
884 metres	1765	4471 (3468)	608	779	48	1393	9,300
1160 metres	850	9568 (1016)	870	1382	87	1720	13,800
Camel's Hump (VA):							
884 metres	1341	14,657 (2409)	890	629	92	1896	11,100
1067 metres	1836	14,836 (2665)	548	650	78	1478	8,100

ACKNOWLEDGMENTS

Our thanks are due to Dr. R. Schemenauer of the Atmospheric Environment Service of Environment Canada for his considerable help and encouragement in initiating this project. We also thank field workers on the Chemistry of High Elevation Fog (CHEF) project. Marilyn Feth and Catriona Gordon provided invaluable technical assistance. The project was funded by the Wildlife Toxicology Fund to whom we are most grateful. Travel funds were supplemented by the Canadian Forestry Service.

References

GRIFFIN, D.H. 1981. "Growth and Development of the Thallus" in Fungal Physiology. John Wiley and Sons, New York. Pp. 117-130.

HUTCHINSON, T.C., M. SCOTT, C. SOTO, and M. DIXON. 1987. The effect of simulated acid rain on boreal forest floor feather moss and lichen species. Pp. 411-426 in Effects of Atmospheric Pollutants on Forests, Wetlands and Agricultural Ecosystems, T.C. Hutchinson and K.M. Meema (eds.), Springer-Verlag Berlin Heidelberg.

LEBLANC, F., and D.N. RAO. 1966. Reaction de quelques lichens and mousses epiphytiques a l'anhydride sulfureux dans la region de Sudbury, Ontario. The Bryologist 69:338-346.

SCHEMENAUER, R.S. 1986. Acidic Deposition to Forests: The 1985 Chemistry of high elevation fog (CHEF) project. Atmosphere-Ocean (24)4:303-328.

SCHERBATSKOY, T. 1982. Changes in Aluminum and heavy metal concentrations in *Picea rubens* wood in Northern Vermont. Cambial Activities Increment 7:2-3.

SCHERBATSKOY, T., and R.M. KLEIN. 1983. Response of spruce and birch foliage to leaching by acidic mists. J. Environ. Qual. 12:189-195.

SCOTT, M.G., and D.W. LARSON. 1984. A correlated light and electron microscope study of *Umbilicaria* lichens. Can. J. Bot. 62 (9):1947-1964.

SCOTT, M.G., and T.C. HUTCHINSON. 1987. Effects of a simulated acid rain episode on photosynthesis and recovery in the caribou-forage lichens, *Cladina Stellaris* (Opiz.) Brodo and *Cladina rangiferina* (L.) Wigg. New Phytologist 107:567-575.

SEAWARD, M.D. 1987. Effects of quantitative and qualitative changes in air pollution on the ecological and geographical performance of lichens. Pp. 439-448 in The Response of Forests, Crops and Wetlands to Atmospheric Pollution, T.C. Hutchinson and K. Meema (eds.) Springer-Verlag. Berlin, Heidelberg.

SIGAL, L.L., and T.H. NASH III. 1983. Lichen communities on conifers in southern California mountains: an ecological survey relative to oxidant air pollution. Ecology 64(6): 1343-1354.

SILVA-PANDO, F.J., and C. ASCASO. 1982. Modificaciones ultraestructurales de liquenes epifitos transplantados a zonas urbanas de Madrid. Collectanea Botanica 13(1):351-374.

WINTER, E. 1982. Salt tolerance of *Trifolium alexandrinum* L. III. Effects of salt on ultrastructure of phloem and xylem transfer cells in petioles and leaves. Australian J. Plant Physiol. 9:239-250.

Fungal and Bacterial Symbioses as Potential Biological Markers of Effects of Atmospheric Deposition on Forest Health

Donald H. Marx
USDA Forest Service Southeastern Forest Experiment Station Athens, GA 30602
Steven R. Shafer
USDA Agricultural Research Service NC State University Raleigh, NC 27695-7616

ABSTRACT

The ecological role and physiological functions of the different microbial symbiotic associations, specifically ectomycorrhizae, endomycorrhizae, and actinorhizae, which occur naturally on roots of forest trees will be discussed.

The effects of man-made and natural stress on tree physiological and soil processes which may cause change in (i) microbial symbiont species composition in the root zone; (ii) microbial symbiont species succession in developing stands of trees; and (iii) morphological and physiological attributes of the symbiotic associations will be presented. Essential methodologies needed to study these interacting chemical and biological factors on tree seedlings and on more mature trees will be proposed.

Microorganisms are present in great numbers on and near the feeder roots of trees, and they play vital roles in numerous physiological processes. These dynamic processes are mediated by associations of microorganisms participating in saprophytic, pathogenic and symbiotic root activities. The major symbiotic associations on tree roots are mycorrhizae and actinorhizae.

MYCORRHIZAE

The term mycorrhiza (fungus-root) is used to described a structure that results from a mutually beneficial association between the fine feeder roots of plants and species of highly specialized, root-inhabiting fungi. Mycorrhizae are active, living components of the soil and have some properties like those of roots and some like those of microorganisms. The mycorrhizal fungi derive most if not all of their needed organic nutrition (carbohydrates, vitamins, amino acids) from their symbiotic niche in the primary tissues of roots. Evidence suggests that the mycorrhizal habit evolved as a survival mechanism for both partners of the association, allowing each to survive in the existing environments of low soil fertility, drought, disease, and temperature extremes. Because of this coevolutionary process, mycorrhizae are as common on the root systems of trees and other plants as are chloroplasts in their leaves. In examining plants in a natural environment, the question is not "are the plants mycorrhizal" because they all are, but rather "what type of mycorrhiza is present and what is the degree of mycorrhizal development on the roots?" There are over 4000 publications on mycorrhizae in the plant science literature.

Endomycorrhizae

This type of mycorrhiza is the most widespread and comprises three groups, Ericaceous mycorrhizae occur on four or five families in the Ericales. Orchidaceous mycorrhizae are a distinct type that occur only in the plant family Orchidaceae. These two groups of endomycorrhizae are not widespread and will not be discussed.

Vesicular-arbuscular mycorrhizae (VAM) form the third group of endomycorrhizae. They occur on more plant species than do all other types of mycorrhizae combined and have been observed in roots of over 1000 genera of plants representing some 200 families. It has been estimated (Kendrick and Berch 1985) that over 90% of the 300,000 species of vascular plants in the world form VAM. VAM fungi are ubiquitous in all natural soils throughout the world except where they have been eliminated by man's activities. Inoculum density and fungal species, however, differ in different soils supporting different plants.

The fungi forming YAM belong to the class Zygomycetes and the family Endogonaceae, which includes the genera *Glomus, Gigaspora, Acaulospora, Sclerocystis, Entrophospora*, and *Endogone*. These fungi have very little host specificity. One VAM fungus may have a plant host range including trees, vines, grasses, legumes, and desert plants. The main characteristic of VAM is the presence of vesicles and/or arbuscules in the primary root cortex. The endodermis, stele, and root meristems are not colonized. Inter-and intracellular hyphae present in the cortex are connected to the external mycelium that spreads and ramifies in soil. Some VAM produce large sporocarps (5-10 mm in diameter) containing many spores, and others form large (100-600 um in diameter), single, thick-walled spores on the root surface, in the rhizosphere, or in the root tissues. VAM fungi cannot grow saprophytically in soil and, therefore, can only grow while in symbiotic association with their plant hosts. They may, however, survive for decades in soil as dormant spores without plant associations.

VAM increase a plant's uptake of certain nutrients, particularly P, Cu, and Zn. These elements are relatively immobile in soil, and zones of depletion develop near feeder roots. The extramatrical growth of hyphae from VAM fungi can extend beyond the feeder roots and increase the volume of soil from which these elements are absorbed. The additional nutrient absorption due to VAM fungi can result in several-fold growth increases in plants. The degree of plant benefit appears to be related to the plant's P requirement, its ability to absorb nutrients from soil, the amount of available P in soil, and the species of VAM fungus involved. Additions of available P to soil may eliminate the plant's dependence on VAM (Baylis 1970, 1972). However, it takes from 50 to 100 times the normal P levels found in forest soils to eliminate the VAM dependency of hardwood trees (Kormanik et al., 1982).

There are other significant benefits of VAM to plants. VAM are capable of reducing the effects of various fungal pathogens and suppressing the effects of parasitic nematodes (Schenck 1981). VAM have also been shown to enhance water uptake, increase tolerance to heavy metals, saline soils and drought, decrease transplant shock, and bind soil into semistable aggregates.

Ectomycorrhizae

This type of association occurs on about 10% of the world flora. Trees belonging to the Pinaceae (pine, fir, larch, spruce, hemlock), Fagaceae (oak, chestnut, beech), Betulaceae (alder, birch), Salicaceae (poplar, willow), Juglandaceae (hickory, pecan), Myrtaceae (eucalyptus), Ericaceae (*Arbutus*), and a few others form ectomycorrhizae.

Some tree genera such as *Alnus, Eucalyptus, Casuarina, Cupressus, Juniperus, Tilia, Ulmus*, and *Arbutus* will form both ectomycorrhizae and VAM, depending on soil conditions and tree age.

Numerous fungi have been identified as forming ectomycorrhizae. In North America alone it has been estimated that more than 2100 species of fungi form ectomycorrhizae with forest trees. Worldwide, there are over 5000 species of fungi that can form ectomycorrhizae on some 2000 species of woody plants. Among the basidiomycetous fungi, species of Hymenomycetes (mushrooms) in the genera Boletus, *Cortinarius, Suillus, Russula, Gomphidius, Hebelorna, Tricholoma, Laccaria*, and *Lactarius* and species of the Gasteromycetes (puffballs) in the genera *Rhizopogon, Scleroderma*, and *Pisolithus* form ectomycorrhizae. Certain orders in the Ascomycetes such as Eurotiales (*Cenococcum geophilum*), Tuberales (truffles), and Pezizales have species that form ectomycorrhizae on trees.

In ectomycorrhizae, intercellular hyphae surround cortical cells forming the Hartig net, and several hyphal layers cover the outside of the feeder root forming the fungus mantle. Ectomycorrhizal colonization normally changes the feeder root morphology and color. They may be unforked, bifurcate, nodular, multi-forked (coralloid), or in other shapes. Their color, which is usually determined by the color of the mycelium of the fungal symbiont, may be jet-black, red, yellow, brown, white, or blends of these colors. Unfortunately, with the exception of the above mentioned fungi, few of the hundreds that exist in any given forest stand can be identified based on characteristics of the ectomycorrhizae. The only sure way of identifying ectomycorrhizal fungi is with their fruit bodies. As with VAM, ectomycorrhizal colonization is limited to the primary cortex and does not spread beyond the endodermis or into meristem tissues of the feeder root. Unlike VAM, however, many ectomycorrhizal fungi can be grown routinely in pure culture. An important aspect of both VAM and ectomycorrhizal fungi is that neither group of fungi can exist saprophytically in nature without a plant-host association. Spores or resistant hyphae may survive long periods in soil without a plant host, but the fungi from these propagules will not grow independent of their plant host as saprophytes.

Ectomycorrhizal fungi aid the growth and development of trees. For some trees, such as *Pinus*, they are indispensable for growth under natural conditions. The obligate requirement of pine for ectomycorrhizae in a natural environment has been clearly shown by numerous workers in tree regeneration trials in former treeless areas and in countries without native ectomycorrhizal trees (Marx 1980, Mikola 1973). Pine or other obligate tree species can be grown from seed in aseptic culture, hydroponics, or elsewhere without ectomycorrhizae. In these nonmycorrhizal conditions however, the seedlings must be furnished with the factors (high nutrients, water) that are normally supplied by the fungal symbiont and must be kept in a stress-free environment or they will die or not grow normally. Mycorrhizae, especially ectomycorrhizae, appear to be the first line of biological defense against stress for trees.

Trees with abundant ectomycorrhizae have a much larger, physiologically active, root-fungus area for nutrient and water absorption than trees with few or no ectomycorrhizae. This increase in surface area comes both from the multi-branching habit of most ectomycorrhizae and from the extensive vegetative growth of hyphae of the fungal symbionts from the ectomycorrhizae into the soil. These extramatrical hyphae function as additional nutrient and water-absorbing entities and assure maximum nutrient capture from the soil by the host. Ectomycorrhizae are able to absorb and accumulate nitrogen, phosphorus, potassium, and calcium in the fungus mantles more rapidly, and for longer periods of time, than nonmycorrhizal feeder roots. Ectomycorrhizae also appear to increase the tolerance of trees to drought, high soil temperatures, soil toxins (organic and inorganic), and extremes of soil acidity caused by high levels of sulfur or aluminum. Ectomycorrhizae deter infection of feeder roots by root pathogens, such as species of *Pythium* or *Phytophthora* (Marx and Krupa 1978). Hormone relationships induced by fungal symbionts cause ectomycorrhizal roots to have greater longevity (length of physiological activity) than nonmycorrhizal roots (Slankis 1973, Ng et al. 1982, Ek et al. 1993). Not all species of fungi form ectomycorrhizae that have equal benefit to their hosts. Some are more effective than others.

Many species of fungi are normally involved in the ectomycorrhizal associations of a forest stand, a single tree species, an individual tree, or even a small segment of lateral root. As many as three species of fungi have been isolated from an individual ectomycorrhiza (Zak and Marx 1964). Even as a single tree species can have numerous species of fungi capable of forming ectomycorrhizae on its roots at any given time, a single fungus can enter into ectomycorrhizal association with numerous tree species on the same. site. A single species of ectomycorrhizal fungus can develop numerous biotypes or clones in a very limited area of a pure stand (Fries 1987). Some fungi are apparently host-specific; others have broad host ranges and form ectomycorrhizae with members of numerous tree genera in diverse families.

Ectendomycorrhizae are intermediate types with features of both ecto- and endomycorrhizae. In comparison to other mycorrhizal types, little research has been done on them. They appear to have limited distribution in forest soils and tree nurseries and are found on roots of normally ectomycorrhizal trees. Little is known of their importance to trees.

Factors Affecting Mycorrhizal Development

Many factors affect mycorrhizal development. It is necessary, however, to separate those that affect the tree from those that affect the fungal symbionts. Generally, any soil or above-ground condition that influences root growth (i.e., carbon allocation) also influences mycorrhizal development. The first prerequisite to mycorrhizal development is that a susceptible feeder root be preformed by the plant host. Second, there must be viable inoculum of a mycorrhizal fungus present in the rhizoplane to colonize the root. Third, soil chemical, physical, and biological conditions must be favorable for successful symbiotic colonization. The main factors influencing susceptibility of tree roots to mycorrhizal infection appear to be photosynthetic potential and soil fertility. High light intensity and low-to-moderate soil fertility enhance mycorrhizal development; the other extremes of these conditions (light intensity below 20% of full sunlight and excessively high soil fertility) reduce, or may even prevent, mycorrhizal development. However, it normally takes 10 to 50 times the nitrogen and phosphorus normally found in most forest soils to significantly suppress mycorrhizal development of forest trees (Cline et al. in press). Mechanical defoliation that reduces photosynthetic surfaces reduces mycorrhizal development (Last et al. 1979). Increased photosynthesis due to CO_2 enrichment of the atmosphere increases mycorrhizal development (O'Neill et al. 1987). Light intensity and fertility appear to influence either the biochemical status of feeder roots, such as controlling levels of simple sugars, or the synthesis of new feeder roots, both of which are products of carbon allocation (Ekwebelam and Reid 1983). Roots growing rapidly because of high soil fertility contain few simple sugars and they are not highly susceptible to symbiotic colonization (Marx et al. 1977). The supply of photosynthates from the tree host to the fungal symbiont is of paramount importance to the development, function, and maintenance of mycorrhizae. This supply not only furnishes the energy and carbon for fungal growth but is intimately connected with nutrient uptake of mycorrhizal roots (France and Reid 1983).

The factors that affect the fungal symbionts directly are those which regulate survival of the fungi in the soil or their growth on roots. Extremes of soil temperatures, pH, moisture, etc., and presence of antagonistic soil microorganisms can affect the survival of symbionts and thereby influence the mycorrhizal fungus inoculum potential of the soil (Marx et al. 1984). Unfortunately, with the exception of recent work on fungicides (Marx et al. 1986, Marx and Cordell 1987), factors affecting survival of inoculum of specific mycorrhizal fungi in soil have not been studied.

Actinorhizae

The term actinorhizae refers to the nodules that *Frankia* spp. form with roots of their host plant. The nodules are lobed, coralloid, infection-induced lateral roots exhibiting restricted apical growth but profuse branching.

Actinorhizal plants are fast-growing pioneer plants, mostly trees and shrubs, and are important in the nitrogen economy of temperate forests. Typically, they colonize barren, nutrient-poor soils. Roots of these plants are nodulated by filamentous bacteria in the genus *Frankia* of the Actinomycetales. Taxonomically, actinorhizal plants are distributed through at least 170 species among 22 genera in 8 plant families (Betulaceae, Casuarinaceae, Coriaraceae, Datiscaceae, Elaeagnaceae, Myricaceae, Rhamnaceae, and Rosaceae). However, not all species in all genera are affected. Like leguminous plants with *Rhizobium* spp. or *Bradyrhizobium* spp., actinorhizal plants fix atmospheric N_2 into plant-available forms. Actinorhizal plants can fix up to 362 kg N/ha/yr, an amount similar to that fixed by leguminous plant-rhizobial systems. The actinorhizal relationship is beneficial to both organisms. Actinorhizal plants normally form mycorrhizae and actinorhizae at the same time in natural environments. Some species like *Alnus* and *Casuarina* can form tripartate symbioses, i.e., actinorhizae, VAM, and ectomycorrhizae, on the same tree at the same time.

The characteristics of the actinorhizal infection reflect the great degree of interaction between the symbionts. *Frankia* spp. are identified as thin, usually nonseptate hyphae 0.8 to 1.2 um in diameter, forming intercalary or terminal sporangia bearing spores and vesicles. Some strains have not been observed to sporulate. Some isolates exhibit degrees of host-specificity while others do not. Hyphae of a strain of *Frankia* that are compatible with the host penetrate a root hair and grow within the root hair cell. Cortical cells in the vicinity begin to divide, even though they are not yet infected. Hyphae then invade these cortical cells and begin to form vesicles. A localized thickening, called the primary nodule, develops. A lateral root forms adjacent to the primary nodule, and hyphae grow into the cortical cells from the primary nodule. Thus, while prospective cells for infection in the primary nodule arise from de-differentiation of cortical cells, cells for infection in the "true nodule" that develops from the lateral roots arise from continued production of cells by the apical meristem of the lateral root. The meristem itself is not infected. Once a cortical cell has differentiated behind the meristem and starts to grow, hyphae infect the cell, coil around the nucleus, and fill the cell. As an increasing number of cells in the true nodule become infected and other apical meristems become active, the true nodule takes on a lobed, coralloid appearance. Branched hyphae borne in intracellular clusters near the host cell wall form swollen vesicles at the tips. These vesicles are probably the site of N_2 fixation. In alder nodules, each infected host cell contains one cluster of several hundred vesicles in the upper tips of the nodule lobes. Lower parts of the lobes contain cells with degenerating endophyte, which seems to be digested eventually by the host cells. Spores form in the late stages of the infection cycle, and decaying nodules release spores into the soil. The pattern of cortical cells infected around the stele is host-dependent. The same isolate in roots of different hosts may exhibit a different infection pattern. Nodules last an average of 3 to 4 years but may persist for as long as 8 years. They may be over 3 cm in diameter and each can contain billions of spores.

Actinorhizae have great ecological and commercial significance. Many actinorhizal plants (e.g., *Alnus* spp. in the northern hemisphere) are important colonizers of N-poor soils. Leguminous plants and other N-fixation symbioses are absent from large areas in cool climates, such as in Canada and Scandinavia, so a large proportion of soil N in those areas is derived through actinorhizae. Some alder spp. produce 15 to 25 tons of biomass/ha/yr and are among the highest-yielding tree species in the temperate zone.

The N_2-fixation activities of actinorhizae give rise to the ecological and commercial importance of the relationship. Almost all *Frankia* spp. can use NH_4^+, NO_3^-, and selected amino acids as sources of N and many exhibit growth on N-free cultural media. In nodules, *Frankia* fixes N_2 from the air into NH_4^+. However, more N_2 is fixed than is required by the endophyte, so some NH_4^+ is exported to the host cell cytoplasm, converted to the amino acid citrulline, and translocated from the nodule to other parts of the plant. Thus, a net accretion of N to the plant and to the site occurs.

Most factors affecting mycorrhizal development also affect actinorhizal development. The nodule-forming process seems to be more sensitive than plant growth to soil acidity. Most isolates of *Frankia* grow best in culture in pH 6.5-7.0 as do most of their tree hosts. Vesicle formation and N_2-fixation in culture can be suppressed with increases in the availability of N, and nodulation is also suppressed by increased concentrations of NH_4^+-N. This latter effect may be attributable to lowered C availability to the nodules under high-N conditions. As with mycorrhizae, actinorhizae represent a C sink to the host. Nodulation and N_2-fixation, therefore, are affected by host photosynthetic rate. The activity of nodules is linked to the supply of photosynthate and is altered by changes in light intensity, foliar area, girdling, mycorrhizal status (P absorption), and C partitioning by the host. Any factor that supresses photosynthesis, stimulates respiration, decreases photosynthetic area, and suppresses C transport to roots affects actinorhizal development and function. For further information on actinorhizae see Akkermans and Roelofsen (1980), Akkermans and Van Dijk (1981), Akkermans et al. (1984) and Stowers (1987).

ECOLOGICAL ASPECTS OF TREE SYMBIOSES

Other than the limited information presented above, there is a paucity of research or observational data available on the ecology of endomycorrhizal and actinorhizal associations of trees. The following discussion is, therefore, restricted to ectomycorrhizal associations.

Fungal Succession and Forest Development

Terrestrial plant communities tend to be dominated by either endomycorrhizal or ectomycorrhizal plants (Moser 1967). During ecosystem succession to climax forest in most temperate regions, the fungal associates of higher plants change from endomycorrhizal or VAM dominance in herbaceous and scrub communities to ectomycorrhizal dominance associated with trees (Rose 1980). Tropical plant succession may progress from the nonmycorrhizal state of pioneer plants to an endomycorrhizal status of climax hardwoods, such as those found in rain forest (Janos 1980).

In ectomycorrhizal fungus succession in forests, it is now recognized that there are distinct early-and late-stage fungi. The pioneering work on this concept was done in Scotland. Dighton and Mason (1985) suggested that early-stage fungi are similar to "r" plant species (Harper 1977), since they spend most of their time in acts of colonization then give way to other species in natural succession. They view late-stage fungi as similar to "K" plant species since the latter are specialists in a resource-limited environment with intense interference from their associates. In aseptic culture (i.e., without competition and other stresses) early-and late-stage fungi form ectomycorrhizae on seedlings equally well. However, only the early-stage fungi are able to rapidly colonize seedlings in natural, nonsterile soil that harbors competitors and other stresses. Early-stage fungi may not totally disappear from mature stands, but they are supplanted by more dominant species (Dighton et al. 1986a) or suppressed in reproduction due to canopy characteristics. Last and Fleming (1985) suggest that the differences in root physiology and rhizosphere populations between seedlings and mature trees might be beneficial to volunteer seedlings. Late-stage ectomycorrhizae on the mature trees may transfer carbon to these seedlings through the mycorrhizal bridge (Reid and Woods 1969) and benefit seedling growth in the suboptimal light conditions in closed canopy stands. This carbon transfer to seedlings may also function in clearcuts where ectomycorrhizae are still viably attached to cut stumps and sprouts.

As trees become larger internal recycling of nutrients within the tree increases, and demand for nutrients from soil generally decreases (Miller 1979). The amount of carbon available to support a mycorrhizal fungal symbiont may differ with tree size, with small trees having limited supplies and supporting fungi with limited carbon demands, and larger trees supporting more carbon demanding fungi (Mason et al. 1985, Last et al. 1985). They concluded that the stage of development of the ecosystem would strongly influence the resources available to mycorrhizal fungi, and that successional stages of fungi occur due to their variable requirements for host carbon.

Associated with ectomycorrhizal fungus succession is increased fungal species diversity with increasing stand age and increasing number of host species in the stand (Mason et al. 1982, Last et al. 1984). There is also evidence that the succession of ectomycorrhizal fungi is influenced by a tree x soil interaction (Last et al. 1984); seed source, tree vigor and leaf retention (Mason et al. 1982); and forest fertilization (Hora 1959, Shubin et al. 1977). Fruit body production by these fungi, which is the main parameter used to observe succession, is also strongly influenced by season of year, rainfall amount and frequency, organic content of soil, root density, and degree of ectomycorrhizal development (Wilkins and Harris 1946). It is assumed that as ectomycorrhizal development increases so does fruit body production as long as all other factors are equal. The amount of fruit body production is an accurate surrogate for degree of ectomycorrhizal development in bare-root tree nurseries (Marx et al. 1984). Whether this relationship holds true in complex ecosystems is unknown at this time.

Ectomycorrhizae and Net Primary Production

Ectomycorrhizae are important organs for the accumulation and storage of soil-derived and host carbon-based nutrients. The first are essential for the tree host and the latter for growth of hyphae and production of fruit bodies by the fungi. There are no actual measurements of the quantities and types of plant carbon compounds used by ectomycorrhizal fungi as a proportion of total photosynthesis. Some estimates, however, have been made in field situations.

The amount of photosynthate required to support a healthy and vigorous population of ectomycorrhizal fungi on roots has been estimated in only a few cases. These estimates, based on measured carbon content of fruit bodies and estimated carbon content of the fungal mantle and extramatrical mycelia (turn-over rate), vary from a high of over 50% in Douglas-fir forests in Oregon (Fogel and Hunt 1979) to a more likely 15% for the same tree species (Vogt et al. 1982). The first report did not verify that all fungal components measured were ectomycorrhizal in origin; the latter report did.

Odum and Biever (1984) considered the ectomycorrhizal association a major cybernetic control subsystem in terrestrial plant communities and concluded that it has not been adequately considered in energy flow models. They based this conclusion on measurements of the partitioning of net primary production between 4-year-old loblolly pine and fruit bodies, ectomycorrhizae, and mycelia of specific fungi on severely eroded soils in the Copper Basin of Tennessee. They estimated that from 15 to 25% of net primary production flowed along the ectomycorrhizal pathway of trees on this site.

Production of fruit bodies by ectomycorrhizal fungi can be quite large in forest stands. In Sweden, several thousand fruit bodies of over 70 species of ectomycorrhizal fungi were collected during a 3-year period in a 5-ha stand of pure beech (Tyler 1984). In Scotland, a 5-year average of between 240,000 and 490,000 fruit bodies of ectomycorrhizal fungi/ha were produced yearly under Scots pine (Richardson 1970). In Georgia, Thacker (1971) collected fruit bodies of ectomycorrhizal fungi for I year from areas of about 3 ha in three different forest types. Collected fresh weights were 4812 g of 31 fungal species in a 25-year-old pine plantation, 6560 g of 56 species in a 70-year-old pine stand, and 9991 g of 74 species in a 70-year-old pine-oak stand. This report is the only one in the literature suggesting that fungal succession and increased species diversity may occur in aging stands in the southern U.S.

ECTOMYCORRHIZAE AND ATMOSPHERIC DEPOSITION

Several controlled-exposure studies in recent years have shown that ambient or above-ambient levels of atmospheric deposition can affect ectomycorrhizal development on seedlings. Direct exposure of specific ectomycorrhizae of loblolly pine to O_3 and SO_2 caused a significant reduction in their respiration, but respiration in nonmycorrhizal roots was reduced more. Certain ectomycorrhizae were tolerant of O_3, while others were more tolerant of SO_2 (Carney et al. 1978, Garrett et al. 1982). Sharer et al., (1985) found that simulated rain treatments of pH 4.0 and 3.2 inhibited ectomycorrhizal development of loblolly pine seedlings, but that rain treatment of pH 2.4 stimulated development. Mahoney et al., (1985) found that root growth of loblolly pine was more heavily impacted by O_3 and SO_2 in nonmycorrhizal than in mycorrhizal seedlings. Their results suggested that ectomycorrhizae altered the pollution effects on root and shoot growth resulting in more root growth and elimination of the effect of pollutants. Reich et al. (1985, 1986) found that seedlings of white pine and northern red oak produced more short roots and higher percentages of ectomycorrhizae after exposure to O_3, and that SO_2 and acid rain treatments decreased both root parameters.

One could predict that any significant change in carbon allocation patterns to roots caused by air pollution would affect root growth and, subsequently, mycorrhizal development (Reich et al. 1987, Winner et al. 1987). Also, any significant change in soil chemistry causing increased levels of available metals (A1, Zn, Pb, etc.) in solutions could cause dysfunction in one or both partners in the symbiotic association (McCreight and

Schroeder 1982, Jones et al. 1986, Wasserman et al. 1987). Recently, Dixon and Buschena (1988) showed that growth of nonmycorrhizal pine seedlings was reduced and that ectomycorrhize protected the pine seedlings from toxicity caused by Cd, Cu, Ni, Pb, and Zn. In Poland, Kowalski (1987) found distinct qualitative and quantitative changes in ectomycorrhizae of various tree species planted on a prepared and fertilized site damaged by industrial emission (SO_2, Zn, and Pb). The percentage of living ectomycorrhizae decreased with increasing pollution levels, but ectomycorrhizae were prevalent on even the most pollution-damaged living trees. He concluded that reduced photosynthetic activity, strongly altered soil chemistry, and high concentrations of heavy metals were responsible for ectomycorrhizal depression. In the Netherlands, Termorshuizen and Schaffers (1987) found three times more ectomycorrhizae, 20 times more fruit bodies, and much greater fungus diversity in 5 to 10-year-old *Pinus sylvestris* than in 50-to 80-year-old trees. This observation appears to conflict with the succession and diversity concept discussed earlier. However, in the older stand, these fungus parameters were negatively correlated with concentrations of SO_2, O_3, and NH_3. They were positively correlated with the mean number of needleyears of these older trees. There were no differences in soil chemistry between the two stands of trees. They concluded that the lack of fungal species diversity on the old trees could cause a decreased resistance to both man-made and natural stress. In Scotland, Dighton et al., (1986b) applied 1500 mm/yr of pH 3.0 rain for 5 years to *P. sylvestris* trees and found a reduced abundance of ectomycorrhizae and short roots. No abnormality in morphology was found in any ectomycorrhizae. They concluded that increased levels of Al and Mn in the acid treated soils reduced root growth, which caused a reduction in ectomycorrhizae. Numerous other researchers in other parts of Europe have reported similar depressions in ectomycorrhizae on trees with various degrees of foliar symptoms of air pollution damage (Blaschke 1981, 1982, Schlechte 1986).

BETTER METHODS ARE NEEDED TO STUDY SYMBIOSES

Basically, we know only that unhealthy trees seem to have depressed root symbiotic development compared to healthy trees. Biologically, this suppression is predictable. The major problem is separating cause from effect — the old chicken-and-egg dilemma. At this time, we do not know what normal or abnormal populations of mycorrhizae or actinorhizae are for any tree species, of any age, on any site, anywhere in the world. How many species of symbionts are normal? What proportion of the short roots should be symbiotically colonized? Is microbial succession from young to older trees a worldwide phenomenon? Does it occur in actinorhizal and VAM tree systems? Is succession caused directly by changing root physiology, soil characteristics, or rhizosphere populations? If so, could atmospheric deposition alter succession? Or is succession happening simply because it has had time to happen? Can normal succession be interrupted by any form of plant stress? For ectomycorrhizae, is the presence of late-stage fungi the result of physiological change in aging trees? Or is it simply due to the fact that late-stage fungi do not proliferate or reproduce as rapidly, do not produce spores in a manner that ensures rapid dissemination, do not produce as many spores as do early-stage fungi and, therefore, take longer to appear in the system? Are the late-stage fungi more or less susceptible to dysfunctions in physiology of roots or foliage? Do exposure experiments on tree seedlings with early-stage ectomycorrhizae contribute meaningfully to our understanding of the reaction of late-stage/mature trees to the same exposure. Since we cannot recognize "normal", how are we to recognize "abnormal" populations of symbiotic associations? The answer is very simple — we can't! Answers to these simple questions exist only for a few very isolated situations that may not apply elsewhere. The biggest problem is that reliable and standardized methods to study symbiotic relationships in the field do not exist (Schenck 1982). Only in recent years have we been able to develop reasonably standardized methods for mycorrhizal research on small tree seedlings with specific ectomycorrhizal fungi in a rather controlled environment, the tree nursery.

Cline et al. (in press) discussed briefly the inherent problems in mycorrhizal assessments and concluded that these assessments must be coupled to the root growth rate, or to existing root biomass. Percent short root colonization is a widely used and reasonably reliable assessment parameter for seedlings of comparable sizes, but it has serious limitations elsewhere. It is important to know the total number of short roots that are available for potential colonization. A percent of short roots colonized tells us little because one seedling may have 4000 short roots, and another 400. A visual estimation of 50% infection tells little unless the 10-fold difference in total number of short roots is also known. Number of mycorrhizae/cm of lateral root has little value unless total number and length of lateral roots are also known. The diverse morphology of ectomycorrhizae also creates assessment problems. One mature ectomycorrhiza of *Pisolithus tinctorius* may have more surface area and more fungus tissue (more carbon demand) than 20 ectomycorrhizae formed by *Cenococcum geophilum* on the same seedling. How do we accurately measure the amount of fungal biomass? Gravimetric (France et al. 1985) and catalytic potential (Iyer 1978) methods have been developed but neither method measures lateral root length or short roots.

Growth rate of lateral roots may have profound effects on mycorrhizal assessments. For example, mycorrhizal status may appear to decrease in a situation that stimulates lateral root growth. In the presence of a lateral root growth stimulator, mycorrhizal infection may lag behind lateral root extension and short root proliferation, thereby, suggesting a depression in mycorrhizae. Examples of conditions or agents that stimulate lateral root growth include fertilization (especially with phosphorus), warming of soils in spring, and water becoming available after prolonged deficit.

Conversely, mycorrhizal status may appear to increase in the presence of a lateral root growth inhibitor. If root growth slows or stops, the growth rate of the fungi and roots may be more closely matched or the mycorrhizae may develop faster than new short roots can be produced. The result is a net increase in numbers of mycorrhizae over time and a higher percentage of short roots colonized without a significant increase in total numbers of short roots. A few inhibitors of lateral root growth are rapid defoliation (storm damage), moderate soil water deficits, soils getting colder in fall, soil compaction, and depleted available soil phosphorus. Thus, the time of root sampling for mycorrhizal assessments can influence greatly the perception of mycorrhizal status.

Very few methods have been developed to study survival of inoculum of microbial symbionts in soil. Depression of symbiotic associations could be caused by depressed survival of their inocula in soil. None of these symbionts can be isolated directly from soil but, in the case of ectomycorrhizal and actinorhizal symbionts, they can be reisolated from roots with considerable effort. Reisolation results, which vary from I to 10% success, however, do not relate directly to survival potential of the specific microorganisms. Techniques have been developed to study the effects of various soil treatments on survival of vegetative inoculum of *Pisolithus tinctorius* in nursery soil (Marx et al. 1986, Marx and Cordell 1987). This procedure uses nonmycorrhizal roots of pine seedlings as a fast "trap" of viable inoculum and is reasonably quantitative. This technique could be modified to test survival of other propagules of these microorganisms, such as spores and sclerotia. A problem remains, however, because of the apparent physiological differences between early-stage (like *P. tinctorius*) and late-stage fungi. With this technique, can seedling roots be used as a "trap" for inocula of late-stage fungi? Can nonmycorrhizal seedlings be transplanted into variously treated soil collected from mature stands and form mycorrhizae with late-stage fungi? Another ecological aspect of symbiotic associations, especially ectomycorrhizae, which has been completely ignored is what can happen to the airborne spores of these fungi while in flight. During fruit body production by these fungi, thousands of spores/m^3 of air/day (LeTacon et al., 1987) are airborne for various periods of time. What effects do gaseous pollutants, such as O_3 and SO_2, have on their viability during this spore-flight period? What effect does acidic rain have during spore washout from the air?

There is no easy way to assess accurately root symbiotic associations on tree seedlings, on small trees, or in natural soil. Reliable and reproducible methods to qualitatively and quantitatively assess root symbioses must be developed if we are ever to understand their reaction to man-made and natural stresses in either experimental or real-world situations. When we realize that the "abnormal" environment of today may be the "normal" environment of tomorrow, we must not delay in developing these methods.

CONCLUSION

Common biological sense indicates that root symbiotic associations are responsive to effects of atmospheric deposition on forests. Unfortunately, at this time, base-line data does not exist as to the symbiotic status of healthy forests. Techniques to assess quantitatively and qualitatively symbiotic associations on forests containing trees of different age and species, do not currently exist and must be developed before this information can be produced. Because of these limitations, it is doubtful that symbiotic associations can be used today as potential biological markers of the effects of atmospheric deposition on forest health.

SUMMARY

1. Root symbioses (mycorrhizae or actinorhizae) are as common on roots of plants in forest ecosystems as are chloroplasts in leaves of plants in the ecosystems.
2. A forest ecosystem, a stand of mixed tree species, a plantation of trees, a single tree, or a small segment of lateral root on a tree can have many types of symbioses with multiple microbial species.
3. Susceptible short roots on lateral roots must be formed by the tree host before root colonization by microbial symbionts can occur.
4. Viable inoculum of the symbiotic microorganisms must be present near susceptible roots before symbiotic colonization can occur.
5. Edaphic factors can strongly influence root colonization by affecting root growth and, probably, inoculum survival and viability of the microbial symbionts.
6. Vitality of root symbiotic associations is strongly dependent on tree host physiology, mainly photosynthesis. Reduced photosynthesis equals reduced root symbioses.
7. Ectomycorrhizal fungal succession apparently occurs during the development of forest stands. Increasing age of stands and increasing number of tree species in the stand increase ectomycorrhizal fungus species diversity.

8. Current methods to measure root symbionts or their function have severe limitations, especially for ecological studies, and better methods must be developed.

References

Akkermans, A.D.L., D. Baker, K. Huss-Danell, J.D. Tjepkema. (eds.). 1984. Frankia Symbioses. Developments in Plant and Soil Sciences, Vol. 12. Martinus Nijhoff/Dr W. Junk Publ., The Hague. 258 pp.

Akkermans, A.D.L., W. Roelofsen. 1980. Symbiotic nitrogen fixation by actinomycetes in *Alnus*-type root nodules. Chap. 12 in Stewart, W.D.P.; Gallon, J.R. Nitrogen Fixation. Academic Press, London. 451 pp.

Akkermans, A.D.L., C. van Dijk. 1981. Non-leguminous root-nodule symbioses with actinomycetes and *Rhizobium*. Pp. 57-103 in Broughton, W.J. (ed.). Nitrogen Fixation. Vol. 1: Ecology. Clarendon Press, Oxford. 306 pp.

Baylis, G.T.S. 1970. Root hairs and phycomycetous mycorrhizas in phosphate-deficient soil. Plant Soil 33:713-716.

Baylis, G.T.S. 1972. Minimum levels of phosphorus for nonmycorrhizal plants. Plant Soil 36:233-234.

Blaschke, H. 1981. Schadbild und Atiologie des Tannensterbens. II. Mycorrhizastatus und pathogene Vorgange im Feinwurzelbereich als Symptome des Tannessterbens. Eur. J. For. Pathol. 11:375-379.

Blaschke, H. 1982. Schadbild und Atiologie des Tannensterbens. III. Das Vorkommen einer Phytophthora-Faule an Feinwurzeln der Weisstanne (*Abies alba* Mill.). Eur. J. For. Pathol. 12:232-238.

Carney, J.L., H.E. Garrett, H.G. Hedrick. 1978. Influence of air pollutant gases on oxygen uptake of pine roots with selected ectomycorrhizae. Phytopathology 68:1160-1163.

Cline, M.L., R.J. Stephans, and D.H. Marx. (In press). Influence of atmospherically deposited nitrogen on mycorrhizae: A critical literature review. Ann. Rev. Physiology.

Dighton, J., P.A. Mason. 1985. Mycorrhizal dynamics during forest tree development. Pp. 117-139 in Moore, D.; Casselton, L.A.; Wood, D.A., Frankland, J.C. (eds.). Developmental Biology of Higher Fungi. Cambridge Univ. Press, Cambridge.

Dighton, J., J.M. Poskitt, D.M. Howard. 1986a. Changes in occurrence of basidiomycete fruit bodies during forest stand development: With specific reference to mycorrhizal species. Trans. Br. Mycol. Soc. 87:163-171.

Dighton, J., R.A. Skeffington, K.A. Brown. 1986b. The effects of sulphuric acid (pH 3) on roots and mycorrhizas of *Pinus sylvestris* . Pp. 739-743 in Gianinazzi-Pearson, V., S. Gianinazzi. (eds.). Physiological and Genetical Aspects of Mycorrhizae, CNRS-INRA, Dijon, France.

Dixon, R.K., C.A. Buschena. 1988. Response of ectomycorrhizal Pinusbanksiana and Picea glauca to heavy metals in soil. Plant and Soil 105:265-271.

Ek, M., P.O. Ljungquist, E. Stenstrom. 1983. Indole-3-acetic acid production by mycorrhizal fungi determined by gas chromatography-mass spectrometry. New Phytol. 94:401-407.

Ekwebelam, S.A., C.P.P. Reid. 1983. Effect of light, nitrogen, fertilization, and mycorrhizal fungi on growth and photosynthesis of lodgepole pine seedlings. Can. J. For. Res. 13:1099-1106.

Fogel, R., G. Hunt. 1979. Fungal and arboreal biomass in a western OregonDouglas fir ecosystem: Distribution patterns and turnover. Can. J. For. Res. 9:245-256.

France, R.C., M.L. Cline, C.P.P. Reid. 1985. Gravimetric determination of ectomycorrhizal infection. Soil Bio. Biochem. 17:381-382.

France, R.C., C.P.P. Reid. 1983. Interactions of nitrogen and carbon in the physiology of ectomycorrhizae. Can. J. Bot. 61:964-984.

Fries, N. 1987. Somatic incompatibility and field distribution of the ectomycorrhizal fungus *Suillus luteus* (Boletaceae). New Phytol. 107:735-739.

Garrett, H.E., J.L. Carney, H.G. Hedrick. 1982. The effects of ozone and sulfur dioxide on respiration of ectomycorrhizal fungi. Can. J. For. Res. 12:141-145.

Harper, J.L. 1977. Population biology of plants. Academic Press, New York.

Hora, F.B. 1959. Quantitative experiments on toadstool production in woods. Trans. Br. Mycol. Soc. 42:1-14.

Iyer, J.G. 1978. Enzymatic content of feeder roots of nursery stock as indicator of their mycorrhizal infestation. Univ. Wisconsin For. Res. Notes #219.

Janos, D.P. 1980. Vesicular-arbuscular mycorrhizae affect tropical rain forest plant growth. Ecology 61:151-152.

Jones, M.D., M.H.R. Browning, T.C. Hutchinson. 1986. The influence of mycorrhizal associations on paper birch and jack pine seedlings when exposed to elevated copper, nickel or aluminum. Water, Air, and Soil Pollution 31:441-448.

Kendrick, B., S. Berch. 1985. Mycorrhizae: Applications in agriculture and forestry. Pp. 109-152 in Robinson, C.W. (ed.). Comprehensive Biotechnology, Vol. 4. Pergamon Press, Oxford.

Kowalski, S. 1987. Mycotrophy of trees in converted stands remaining under strong pressure of industrial pollution . Angew. Botanik 61:65-83.

Last, F.T., L.V. Fleming. 1985. Factors affecting the occurrence of fruitbodies of fungi forming sheathing (ecto-) mycorrhizas with roots of trees. Proc. Indian Acad. Sci. (Plant Sci.) 94:111-127.

Last, F.T., P.A. Mason, K. Ingle, L.V. Fleming. 1984. Succession of fruit-bodies of sheathing mycorrhizal fungi associated with Betula pendula. For. Ecol. Manage. 9:229-234.

Last, F.T., P.A. Mason, J. Wilson, K. Ingleby, R.C. Munro, L.V. Fleming J.W. Deacon. 1985. 'Epidemiology' of sheathing ectomycorrhizas in unsterile soils: A case study of Betula pendula. Proc. Royal Soc., Edinburgh, 85B:299.

Last, F.T., J. Pelham, P.A. Mason, K. Ingleby. 1979. Influence of leaves on sporophore production by fungi forming sheathing mycorrhizas with *Betula* spp. Nature (London) 280:168-169.

LeTacon, F., J. Garbaye, G. Carr. 1987. The use of mycorrhizas in temperate and tropical forests. Symbiosis 3:179-206.

Mahoney, M.J., B.I. Chevone, J.M. Skelly, L.D. Moore. 1985. Influence of mycorrhizae on the growth of loblolly pine seedlings exposed to ozone and sulfur dioxide. Phytopathology 75:679-682.

Marx, D.H. 1980. Ectomycorrhizal fungus inoculations: A tool for improving forestation practices. Pp. 13-71 in Mikola, P. (ed.). Tropical Mycorrhiza Research, Clarendon Press, Oxford.

Marx, D.H., C.E. Cordell. 1987. Triadimefon affects *Pisolithus* ectomycorrhizal development, fusiform rust, and growth of loblolly and slash pines in nurseries. USDA For. Serv. Res. Paper SE-267, Asheville, NC.

Marx, D.H., C.E. Cordell, R.C. France. 1986. Effects of triadimefon on growth and ectomycorrhizal development of loblolly and slash pines in nurseries. Phytopathology 76:824-831.

Marx, D.H., C.E. Cordell, D.S. Kenney, J.G. Mexal, J.D. Artman, J.W. Riffle, R. Molina. 1984. Commercial vegetative inoculum of *Pisolithus tinctorius* and inoculation techniques for development of ectomycorrhizae on bare-root tree seedlings . For. Sci. Monogr. 25.

Marx, D.H., A.B. Hatch, J.F. Mendicino. 1977. High soil fertility decreases sucrose content and suceptibility of loblolly pine roots to ectomycorrhizal infection by *Pisolithus tinctorius*. Can. J. Bot. 55:1569-1574.

Marx, D.H., S.V. Krupa. 1978. Mycorrhizae. A. Ectomycorrhizae. Pp. 373-400 in Interactions Between Nonpathogenic Soil Microorganisms and Plants. Elsevier Scientific Publ. Co., Amsterdam.

Mason, P.A., F.T. Last, J. Pelham, K. Ingleby. 1982. Ecology of some fungi associated with an aging stand of birches (Betula pendula and B. pubescens). For. Ecol. Manage. 4:19-39.

McCreight, J.D., D.B. Schroeder. 1982. Inhibition of growth of nine ectomycorrhizal fungi by cadmium, lead, and nickel in vitro. Environmental and Experimental Bot. 22:1-7.

Mikola, P. 1973. Application of mycorrhizal symbiosis in forestry practice. Pp. 383-411 in Marks, G.C.; Kozlowski, T.T. (eds.). Ectomycorrhizae their ecology and physiology. Academic Press, New York.

Miller, H.G. 1979. The nutrient budgets of even-aged forests. Pp. 221-256 in Ford, E.D.;

Malcolm, D.C.; Atterson, J. (eds.). The Ecology of Even-aged Forest Plantations. Inst. Terrestrial Ecology, Cambridge.

Moser, M. 1967. Die ektotrophe ernahrungsweke an der Waldgrenze. Mitteilungen Forstlichen Bundesversuchsanstalt Wien 75:357-380.

Ng, P.P., A.L.J. Cole, P. Jameson, J.A. McWha. 1982. Cytokinin production by ectomycorrhizal fungi. New Phytol. 91:57-62.

Odum, E.P., L.J. Biever. 1984. Resource quality, mutualism, and energy partitioning in food chains. The American Naturalist 124:360-376.

O'Neill, E.G., R.J. Luxmoore, R.J. Norby. 1987. Increases in mycorrhizal colonization and seedling growth in *Pinus echinata and Quercus alba* in an enriched CO_2 atmosphere. Can. J. For. Res. 17:878-883.

Reich, P.B., A.W. Schoettle, H.F. Stroo, R.G. Amundson. 1986. Acid rain and ozone influence mycorrhizal infection in tree seedlings. J. Air Pollut. Control Assoc. 36:724-726.

Reich, P.B., A.W. Schoettle, H.F. Stroo, J. Troiano, R.G. Amundson. 1985. Effects of O_3, SO_2, and acidic rain on mycorrhizal infection in northern red oak seedlings. Can. J. Bot. 63:2049-2055.

Reich, P.B., A.W. Schoettle, H.F. Stroo, J. Troiano, R.G. Amundson. 1987. Effects of ozone and acid rain on white pine (*Pinus strobus*) seedlings grown in five soils. I. Net photosynthesis and growth. Can. J. Bot. 65:977-987.

Reid, C.P.P., F.W. Woods. 1969. Translocation of ^{14}C-labelled compounds in mycorrhiza and its implications in interpreting nutrient cycling. Ecology 50:179-181.

Richardson, M.J. 1970. Studies of *Russula emetica* and other agarics in a Scots pine plantation. Trans. Br. Mycol. Soc. 55:217-229.

Rose, S.L. 1980. Mycorrhizal associations of some actinomycete nodulated nitrogen-fixing plants. Can. J. Bot. 58:1449-1454.

Schenck, N.C. 1981. Can mycorrhizae control root disease? Plant Dis. 65:230-234.

Schenck, N.C. (ed.). 1982. Methods and Principles of Mycorrhizal Research. Am. Phytopathol. Soc., St. Paul, MN.

Schlechte, G. 1986. Zur mykorrhizapilzflora in geschadigten Forstbestanden. Zeitschrift fur Mykologie 52:225-232.

Sharer, S.R., L.F. Grand, R.I. Bruck, A.S. Heagle. 1985. Formation of ectomycorrhizae on Pinus taeda seedlings exposed to simulated acidic rain. Can. J. For. Res. 15:66-71.

Shubin, V.I., N.I. Ronkonen, A.V. Saukkonen. 1977. The effect of fertilizers on the fructification of macaromycetes on young birch trees. Miko. Fitopatol. 11:294-303.

Slankis, V. 1973. Hormonal relationships in mycorrhizal development. Pp. 231-298 in Marks, G.C.; Kozlowski, T.T. (eds.). Ectomycorrhizae their ecology and physiology. Academic Press, New York.

Stowers, M.D. 1987. Collection, isolation, cultivation, and maintenance of *Frankia*. Pp. 29-53 in Elkan, G.H. (ed.). Symbiotic Nitrogen Fixation Technology. Marcel Dekker, Inc., New York. 440 p.

Termorshuizen, A.J., A.P. Schaffers. 1987. Occurrence of carpophores of ectomycorrhizal fungi in selected stands of *Pinus sylvestris* in the Netherlands in relation to stand vitality and air pollution. Plant and Soil 104:209-217.

Thacker, S.W. 1971. Weight, species, and time of occurrence of mushrooms in three common forest types in the Piedmont region of Georgia. M.S. thesis, School of Forest Resources, Univ. Georgia, Athens. 35 p.

Tyler, G. 1984. Macrofungi of Swedish beech forest. Univ. Lurid, Sweden. 117 p.

Vogt, K.A., C.C. Grier, C.E. Meier, R.L. Edmunds. 1982. Mycorrhizal role in net primary production and nutrient cycling in *Abies amabilis* (Dougl.) Forbes ecosystems in Western Washington. Ecology 63:370-380.

Wasserman, J.L., L. Mineo, S.K. Majumdar, C. Van Tyne. 1987. Detection of heavy metals in oak mycorrhizae of northeastern Pennsylvania forests, using x-ray microanalysis. Can. J. Bot. 65:2622-2627.

Wilkins, W.H., G.C.M. Harris. 1946. The ecology of the larger fungi. V. An investigation into the influence of rainfall and temperature on the seasonal production of fungi in a beechwood and a pinewood. Ann. Appl. Biol. 33:179-188.

Winner, W.E., I.S. Cotter, H.R. Powers, Jr., J.M. Skelly. 1987. Screening loblolly pine seedling responses to SO_2 and O_3: Analysis of families differing in resistance to fusiform rust disease. Environ. Pollut. 47:205-220.

Zak, B., D.H. Marx. 1964. Isolation of mycorrhizal fungi from roots of individual slash pines. For. Sci. 10:214-222.

Microbial and Rhizosphere Markers of Air Pollution Induced Stress

R.K. Antibus
A.E. Linkins III
Department of Biology Clarkson University Potsdam, N.Y. 13676

ABSTRACT
The rhizosphere is a soil region of intense biological activity and differs significantly from bulk soil in terms of numbers and types of microorganisms. The structural and functional diversity of the rhizosphere is maintained by input of root derived carbon sources. Rhizosphere activity has been shown to exert either positive or negative effects on plant growth through effects on nutrient availability, production of growth regulators or phytotoxic substances, or by suppression of pathogens. Rhizosphere structure and activity has been throughly studied in crop plants grown in agricultural soils; much less is known about rhizospheres of mature trees in forest soils. Research approaches tend to emphasize either: 1) the isolation, enumeration and identification of rhizosphere species, or 2) the characterization of rhizosphere activities or products. Advantages and disadvantages of the use of such approaches as biomarkers are discussed. Rhizosphere studies under natural conditions are greatly complicated by the high spatial and temporal variability found in soil properties and root development.

Air pollutants might influence rhizosphere organisms by: 1) affecting plant carbon fixation thus altering carbon availability in the rhizosphere, or 2) through direct effects on soil chemical or physical properties. Research results, obtained largely with potted plants, are available to support both of the above mechanisms. However, the paucity of information does not allow for generalizations about potential rhizosphere biomarkers of pollution induced stress. Long-term data on the occurrence of fruitbodies of ectomycorrhizal fungi in Europe suggest that air pollution may be involved in reducing the species diversity and abundance of these endorhizosphere organisms. Data on acid phosphatase production by field-collected ectomycorrhizae suggest that different fungi may have different functional roles relative to host nutrition. Air pollution induced changes in species composition of tree ectomycorrhizae could potentially affect soil nutrient cycles and tree growth.

INTRODUCTION

The rhizosphere is considered to be that zone of the soil environment influenced by plant roots. It is widely believed that this zone will be variable in extent and be directly influenced by root physiology and by soil environmental factors. Available evidence suggests that plants and rhizosphere organisms function in an interdependent fashion.

Rhizosphere organisms depend on plants for a continuous supply of reduced carbon and are recognized to play a significant role in nutrient cycling, thus exerting an influence on plant growth. Air pollutants or other stresses which affect carbon fixation may influence rhizosphere populations through an effect on carbon supply; alternatively rhizosphere populations may be influenced by direct effects of air pollutants on the soil environment. Changes in rhizosphere structure or function would in turn affect nutrient cycling and exert a feedback effect on plant growth. Much of what is presently known about rhizosphere structure and function has been obtained by using crop plants grown in agricultural soils and caution should be used in extrapolating these results to forest ecosystems.

RHIZOSPHERE STRUCTURE AND MAINTENANCE

Numerous studies have shown that bacterial numbers are greatly increased in rhizosphere soils. Dilution plate counts indicate that the ratio of bacterial numbers in rhizosphere compared to nonrhizosphere soil varies from 10:1-50:1 (Richards 1987). Numbers of fungi are also increased in rhizosphere compared to nonrhizosphere soil; however, the magnitude of this increase is often smaller than observed for bacteria. Comparison of rhizosphere and nonrhizosphere soils indicate that significant qualitative shifts occur in the bacterial and fungal species detected (Gerhardson and Clarholm 1986). Rhizosphere species composition is influenced by numerous factors, including plant species and genotype, plant nutrient status, presence and type of mycorrhizae, soil type, soil moisture, light supply and other factors.

The continued maintenance of a "normal" rhizosphere is mediated by the release of a wide variety of organic carbon compounds. Available data, obtained from crops and tree seedlings, suggest that 40-50 % of the net carbon fixed may be exuded or rapidly released to the rhizosphere (Perry et al. 1987), more complex carbon compounds may enter the rhizosphere more slowly resulting from root aging. The rhizosphere contains a diverse array of metabolic substrates such as exudates, secretions, plant mucilages, mucigel, and lysates (Rovira et al. 1979). The diversity and complexity of released compounds is likely to be an important factor contributing to the high species diversity of rhizosphere microorganisms.

Attempts to describe the rhizosphere have emphasized the spatial locations of organisms within the root zone. Whipps and Lynch (1986) recognized three regions supporting microbial growth: endorhizosphere organisms live within the root, rhizoplane organisms live on the root surface, and ectorhizosphere organisms inhabit a zone around the root. The terms mycorrhizosphere and mycosphere have also been applied to the zones associated with mycorrhizae and fungal hyphae respectively. Although such regions and their component species may be difficult to characterize operationally, such a classification has heuristic value. Microorganisms can be envisioned as existing along a gradient in terms of plant and soil influences. Endorhizosphere species are in contact with the plant and have direct access to reduced carbon. Organisms in distal portions of the ectorhizosphere will be more directly influenced by soil factors. We would expect organisms to show metabolic adaptations to the resources available in their surroundings. Our inability to grow vesicular-arbuscular (VA) fungi in pure culture and the limited capacities for complex carbohydrate utilization demonstrated by ectomycorrhizal (ECM) fungi suggest that endorhizosphere species are adapted to root supplied carbon (Harley and Smith 1983). Rhizosphere bacterial isolates demonstrate growth and amino acid utilization patterns suggesting adaptation to root products. Air pollutants affecting tree carbon fixation or allocation patterns have potential to influence rhizosphere organisms; however, our knowledge of these effects in forest species is limited.

RHIZOSPHERE FUNCTIONS

Rhizosphere organisms may exert either positive or negative effects on plant growth. These effects are determined by the rhizosphere's capacity to: 1) influence nutrient availability, 2) produce plant growth regulators or phytotoxic compounds, or 3) suppress pathogenic organisms. A complex set of microbial interactions determines the structure of the rhizosphere community, these include: antagonism, commensalism, parasitism, and predation. Bacteria may exhibit inhibitory or stimulatory effects on the formation of mycorrhizal associations, which in turn affect plant growth and mineral nutrition (Bowen and Theodorou 1979). Formation of various types of mycorrhizae can subsequently produce quantitative and qualitative alterations in rhizosphere bacteria and fungi (Katznelson et al. 1962, Meyer and Linderman 1986). These changes observed in rhizosphere composition may be mediated by a diverse array of metabolites produced by rhizosphere organisms (Lynch 1987).

Nye and Tinker (1977) listed the mechanisms by which rhizosphere organisms might influence plant nutrient availability, including alterations in root morphological and physiological properties, effects on phase equilibria of soil nutrients, effects of soil chemical composition and organic matter mineralization, direct transfer of nutrients, and competition for soil nutrients. Potentially favorable effects on root morphology and respiration have been obtained with several rhizosphere bacteria on crop plants (Hadas and Okon 1987). Work with vesicular-arbuscular species under controlled conditions suggests that the increased surface area provided by extramatrical hyphae provides a satisfactory explanation for observed increases in uptake of immobile nutrients like phosphate. Under natural conditions, however, the situation becomes more complex and organisms such as nitrogen fixers and hyphal grazers will influence rhizosphere composition and nutrient cycling (Coleman 1985).

Some workers have suggested that root and rhizosphere phosphatases and phosphate solubilizing bacteria may contribute to P nutrition under field conditions. Recent studies (Tarafdar and Jungk 1987, Kroehler and Linkins 1988) suggest that root surface and rhizosphere phosphatase activities will hydrolyze organic phosphorus forms at rates sufficient to meet plant needs. These studies do not imply a necessary role for rhizosphere organisms, however. Ectomycorrhizae are the most common form of mycorrhizae on many important temperate tree species and can increase the uptake of macronutrients (Harley and Smith 1983). Ectomycorrhizal fungi alter root morphology, and control the movement of nutrients from rhizosphere to plant by surrounding plant roots with a fungal sheath. Phosphatases produced by ECMs are active against a variety of organic phosphate sources found in forest soils (Bartlett and Lewis 1973) and likely play key roles in phosphorus cycling. We have shown that ECMs formed by different fungi exhibit different levels of phosphatase activity (Antibus et al. 1981). Activity appears to vary seasonally for a given host-fungus combination (Antibus and Linkins, unpublished data), and we feel that phosphatase activity reflects the general physiological activity of ECMs at given times. Further research could determine the efficacy of using phosphatases or other enzyme assays as general indicators of root and rhizosphere activity. It may also be possible to use ratios of enzymes such as the ratio of acid alkaline phosphatases to determine the relative contributions of bacteria to rhizosphere enzyme activity.

EXPERIMENTAL APPROACHES

Rhizosphere workers usually attempt either to dissect the rhizosphere and study the workings of its components or to examine the physiology of the intact zone. Studies of the former type emphasize isolation, quantification and identification of rhizosphere organisms, whereas the latter type emphasize the measurement of specific rhizosphere activities or products. Both approaches are valid, but are subject to numerous limitations and drawbacks.

Techniques employed in the dissection and study of the rhizosphere include procedures such as plate counting, enrichment cultures, end-point dilutions, direct visualization and many others. These have the advantage of allowing identification of specific organisms or groups of organisms. However, the selective nature of isolation techniques has led to severe criticisms (Brock 1987). Direct visualization procedures overcome some of these limitations but often do not allow identification. The use of fluorescent antibodies may permit identification, but requires increased technical expertise. Another technically complex but potentially useful method to study populations of specific rhizosphere bacteria involves the use of specific DNA probes (Holben et al. 1988). Biomass estimates suffer from difficulties in separating live and dead organisms, or from an inability to separate various specific groups of organisms. The use of biochemical markers specific to certain species or functional groups may allow the *in situ* estimation of rhizosphere organisms (Pace et al. 1986). One such study has been conducted with rhizosphere bacteria under greenhouse conditions (Tunlid et al. 1985). The application of specific biochemical markers requires a thorough knowledge of biochemistry, technical expertise and elaborate equipment.

Workers attempting to understand the physiological aspects of the rhizosphere have examined such processes as nitrogen fixation or enzyme activities in the root and surrounding soil. A disadvantage of these studies is that the key organisms involved in the process of interest are usually not identified. These techniques may thus fail to detect perturbation-induced population shifts which buffer against physiological change. Enzyme studies offer the advantage of studying processes, such as nutrient mineralization, which are crucial to plant growth. The procedures are sensitive and assays are easy to perform using commonly available equipment. Because enzyme assays are integrated measures, they demonstrate less variability than microbial counts (Burns 1978); however, they may also demonstrate time delays in response to perturbations.

Rhizosphere characteristics change over extremely short distances making study of this soil region difficult. In addition to methodological difficulties mentioned, interpretation of results under natural conditions is complicated by a high amount of spatial and temporal variability in root growth and soil properties.

AIR POLLUTION EFFECTS

Air pollutants might affect rhizosphere activity by plant mediated effects or through direct effects on the soil environment. Using pine seedlings Luxmoore et al. (1986) showed that carbon dioxide enrichment resulted in plant-induced decreases in rhizosphere pH and increased solubility of certain cations. Firestone et al. (1984) found the effects of simulated acid precipitation on rhizosphere composition resulted from an influence on root characteristics rather than changes in soil properties. In this study solubilization of cations by acid precipitation was an important factor affecting bulk soil microorganisms, and various pollutants can, under controlled conditions, affect bulk soil microbiology, soil processes and enzyme activities (Wilke 1987). Dighton et al. (1986) found numbers of root tips and types of ECMs formed on *Pinus sylvestris* were affected when seedlings were grown in soils previously exposed to artificial acid rain for 5 years. Evidence based on production of fruitbodies of known ECM fungi strongly suggests that the mycorrhizal fungus flora of the Netherlands has changed in the last 80 years. It has been suggested that fruitbody productivity has decreased because of air pollution-induced stress of the host species *Pinus sylvestris* (Termorshuizen and Schaffers 1987), and that ECM species frequency and diversity have been reduced through air pollution induced changes in soil chemistry (Arnolds 1988). The latter study indicates that some species may have been lost completely from the fungal flora. These data, and work by Haselwandter et al. (1988) on radiocaesium accumulation in fruitbodies, suggest that the occurrence of ECM fungi may provide a valuable and easily obtained record of the effects of air pollution on the endorhizosphere of ECM tree species. Unfortunately, little data of a mycosociological nature are available for North American forests. Our work (Fig. 1) shows that naturally occurring ECM morphotypes formed by several species of ECM fungi produce significantly different acid phosphatase activities and may exert differential influences on organic phosphorus mineralization. Data on fruitbody occurrence suggest that ECM populations in Europe have changed; a concomitant shift in mycorrhizal root tips would likely influence enzyme production and rhizosphere composition. Shifts in the relative abundance of these morphotypes, whether due to air pollution effects on trees or soils, could affect phosphorus cycling and other soil processes. Alternatively, changes in soil properties might result in ecotypic differentiation. Woolhouse (1969) showed that ecotypes of plants growing on metal-contaminated soils produced acid phosphatases differing in susceptibility to cation inhibition; the development of such changes in ECM fungi might serve as a marker of air pollution-induced changes in forest soils.

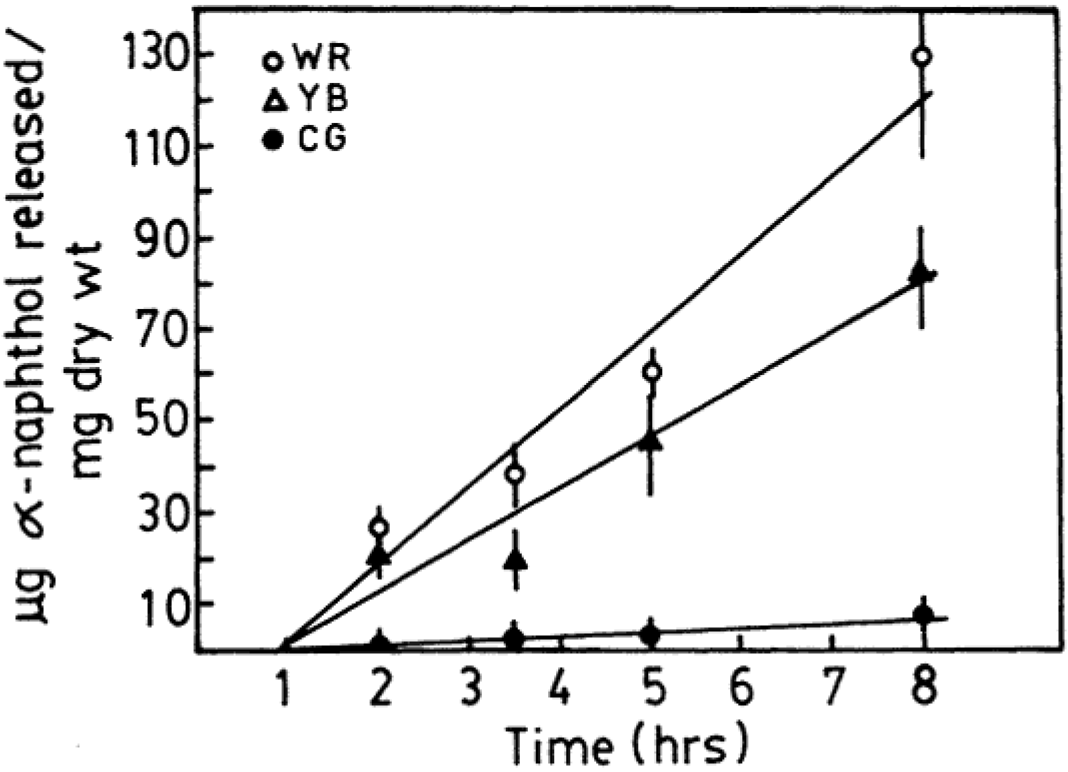


Figure 1.
Acid phosphatase activity of field-collected Douglas fir ectomycorrhizal morphotypes: WR - white rhizomorph producer, YB - yellow brown smooth type and CG-Cenococcum geophilum. Assays were conducted at 25C in pH 5.0 citrate buffer. Values are means with standard deviation bars (n=5).

A review of available literature indicates that air pollutants can affect structure and function of tree rhizospheres. These effects may relate to changes in root supplied carbon or changes in soil environment. Too few data are available to suggest that any particular rhizosphere organisms or functions may serve as a simple biomarker of pollution induced stress. We suggest that the measure of root and rhizosphere enzyme activities can provide a simple and useful approach to the study of air pollution effects on rhizosphere physiology. More data will be needed before rhizosphere physiology can be interpreted in terms of tree growth. However, such information on the function of rhizosphere organisms is crucial if we are to understand the significance of air pollution effects on the occurrence of rhizosphere organisms.

SUMMARY

- - The rhizosphere is an area of intense biological activity driven by root-derived carbon.
- - Rhizosphere activity may exert positive or negative influences on plant growth through effects on nutrient availability, production of growth regulators or phytotoxic substances, or by suppression of pathogens. Most of what is known about rhizosphere effects comes from studies of crop plants in agricultural soils.
- - Air pollutants might influence rhizosphere structure or function by effects on plant carbon fixation or allocation, or by direct effects on the soil environment. Evidence to support both mechanisms is available.
- - Rhizosphere studies usually emphasize either: 1) isolation, enumeration and identification of component species, or 2) the characterization of activities or products. The former approach would allow detection of air pollution-induced shifts in key rhizosphere organisms, whereas the latter would detect shifts in important rhizosphere processes. Advantages and limitations of these approaches are discussed.
- - Potentially useful approaches to rhizosphere study using techniques from molecular biology are appearing; however, these often require technical expertise and sophisticated equipment.
- - The study of the response of the rhizosphere under natural conditions is complicated by great spatial and temporal variability in soil properties and root development.
- - Occurrence and composition of ectomycorrhizal fruitbodies could potentially serve as a biomarker in forests of ECM trees. Studies of ECM fruitbody occurrence in the Netherlands suggest that air pollution has reduced the frequency and diversity of these endorhizosphere organisms.
- - ECMs formed by different fungi on a single tree species can demonstrate significantly different acid phosphatase activities. Shifts in ECM species composition in relation to air pollution could potentially affect forest soil nutrient cycles.

References

Antibus, R.K., J.G. Croxdale, O.K. Miller, and A.E. Linkins. 1981. Ectomycorrhizal fungi of *Salix rotundifolia* Trautv. III. The surface phosphatase activities of resynthesized mycorrhizal complexes. Can. J. Bot. 59:2458-2465.

Arnolds, E. 1988. The changing macromycete flora in the Netherlands. Trans. Br. Mycol. Soc. 90:391-406.

Bartlett, E.M., and D.H. Lewis. 1973. Surface phosphatase activity of mycorrhizal roots of beech. Soil Biol. Biochem. 5:249-257.

Bowen, G.D., and C. Theodorou. 1979. Interactions between bacteria and ectomycorrhizal fungi. Soil Biol. Biochem. 11:119-126.

Brock, T.D. 1987. The study of microorganisms *in situ*: progress and problems. Pp. 1-17 in Ecology of microbial communities, M. Fletcher, T.R.G. Gray and J.G. Jones(eds.). Cambridge University Press, Cambridge.

Burns, R.G. 1978. Enzyme activity in soil some theoretical and practical considerations. Pp. 295-340 in Soil enzymes, R.G. Burns (ed.). Academic Press, New York.

Coleman, D.C. 1985. Through a peal darkly: an ecological assessment of root-soil-microbial-faunal interactions. Pp. 1-21 in Ecological interactions in soil, A.H. Fitter (ed.). Blackwell, Oxford. pp. 1-21.

Dighton, J., R.A. Skeffington, and K.A. Brown. 1986. The effects of sulfuric acid (pH 3) on roots and mycorrhizas of *Pinus sylvestris.* Pp. 739-743 in Physiological and genetical aspects of mycorrhizae, V. Gianinazzi-Pearson and S. Gianinazzi (eds.). INRA, Versailles. pp. 739-743.

Firestone, M.K., J.G. McColl, K.S. Killham, and P.D. Brooks. 1984. Microbial response to acid deposition and effects on plant productivity. Pp. 51-63 in Direct and indirect effects of acid deposition on vegetation. R.A. Linthurst. Butterworth, Stoneham, MA.

Gerhardson, B, and M. Clarholm. 1986. Microbial communities on plant roots. Pp. 19-34 in Microbial communities in soil, V. Jensen, A. Kjoller and L.H. Sorensen (eds.). Elsevier, New York.

Hadas, R., and Y. Okon. 1987. Effect of *Azospirillum brasilense* inoculation on root morphology and respiration in tomato seedlings. Biol. Fertil. Soils 5:241-247.

Harley, J.L., and S.E. Smith. 1983. Mycorrhizal symbiosis. Academic Press, New York.

Haselwandter, K., M. Berreck, and P. Brunner. 1988. Fungi as bioindicators of radiocaesium contamination: pre-and post-Chernobyl activities. Trans. Br. Mycol. Soc. 90:171-174.

Holben, W.E., J.K. Jansson, B.K. Chelm, and J.M. Tiedje. 1988. DNA probe method for the detection of specific microorganisms in the soil bacterial community. Appl. Environ. Microbiol. 54:703-711.

Katznelson, H., J.W. Rouatt, and E.A. Peterson. 1962. The rhizosphere effect of mycorrhizal and non-mycorrhizal roots of yellow birch seedlings. Can. J. Bot. 40:377-382.

Kroehler, C.J., and A.E. Linkins. 1988. The root surface phosphateses of *Eriophorum vaginatum*: Effects of temperature, pH, substrate concentration and inorganic phosphorus. Plant Soil 105:3-10.

Luxmoore, R.J., E.G. O'Neill, J.M. Ellis, and H.H. Rogers. 1986. Nutrient uptake and growth responses of Virginia pine to elevated atmospheric carbon dioxide. J. Environ. Qual. 15:244-251.

Lynch, J.M. 1987. Biological control within microbial communities of the rhizosphere. Pp. 55-82 in Ecology of microbial communities, M. Fletcher, T.R.G. Gray and J.G. Jones (eds.). Cambridge University Press, Cambridge.

Meyer, J.L., and R.G. Linderman. 1986. Selective influence on populations of rhizosphere or rhizoplane bacteria and actinomycetes by mycorrhizas formed by *Glomus fasiculatum*. Soil Biol. Biochem. 18:191-196.

Nye, P.H., and P.B. Tinker. 1977. Solute movement in the soil-plant system. Blackwell, Oxford.

Pace, N.R., D.A. Stahl, D.J. Lane, and G.J. Olsen. 1986. The analysis of natural microbial populations by ribosomal RNA sequences. Pp 1-55 in Advances in microbial ecology. Vol. 9, K.C. Marshall (ed.). Plenum Press, New York.

Perry, D.A., R. Molina, and M.P. Amaranthus. 1987. Mycorrhizae, mycorrhizospheres, and reforestation: current knowledge and research needs. Can. J. For. Res. 17:929-940.

Richards, B.N. 1987. The microbiology of terrestrial ecosystems. Longman, Essex.

Rovira, A.D., R.C. Foster, and J.K. Martin. 1979. Origin, nature and nomenclature of the organic materials in the rhizosphere. Pp. 1-4 in The soil-root interface, J.L. Harley and R.S. Russell (eds.). Academic Press, New York.

Tarafdar, J.C., and A. Jungk. 1987. Phosphatase activity in the rhizosphere and its relation to the depletion of soil organic phosphorus. Biol. Fertil. Soils 3:199-204.

Termorshuizen, A.J., and A.P. Schaffers. 1987. Occurrence of carpophores of ectomycorrhizal fungi in selected stands of *Pinus sylvestris* in the Netherlands in relation to stand vitality and air pollution. Plant Soil 104:209-217.

Tunlid, A., B.H. Baird, M.B. Trexler, S. Olsen, R.H. Findlay, and D.C. White. 1985. Determination of phospholipid ester-linked fatty acids and poly betahydroxybutyrate for estimation of bacterial biomass and activity in the rhizosphere of the rape plant *Brassica napus* L. Can J. Microbiol. 31:1113-1119.

Whipps, J.M., and J.M. Lynch. 1986. The influence of the rhizosphere on crop productivity. Pp. 187-244 in Advances in microbial ecology, Vol. 9, K.C. Marshall (ed.). Plenum Press, New York.

Wilke, B.M. 1987. Fluoride-induced changes in chemical properties and microbial activity of mull, moder and mot soils. Biol. Fertil. Soils 5:49-55.

Woolhouse, H.W. 1959. Differences in the properties of the acid phosphatases of plant roots and their significance in the evolution of edaphic ecotypes. Pp. 357-380 in Ecological aspects of the mineral nutrition of plants, I.H. Rorison (ed.). Blackwell, Oxford.

THE WORKSHOP PAPERS BIOCHEMICAL/CELL-TISSUE SESSION

Foliar Nitrate Reductase: a Marker for Assimilation of Atmospheric Nitrogen Oxides[1]

Richard J. Norby
Environmental Sciences Division Oak Ridge National Laboratory Oak Ridge, Tennessee 37831-6034

ABSTRACT

Atmospheric deposition of nitrates to forest canopies has been implicated as predisposing trees to environmental stresses. A first step in the evaluation of this hypothesis is the determination of whether atmospheric nitrates are absorbed and metabolized by tree foliage. The enzyme nitrate reductase (NR) is an appropriate marker for nitrate metabolism because it is the rate-limiting step in the assimilation of nitrate into organic compounds and it is substrate-inducible. In laboratory studies, NR was not induced in red spruce foliage exposed to NO_3^- in acid mist, but NR activity increased dramatically in spruce seedlings exposed to NO_2 or HNO_3 vapor, suggesting that gaseous nitrogen oxides can be assimilated by spruce foliage. Nitrate reductase activity also can be measured in the field and may be a useful marker for trees that are impacted by nitrogen pollution, but extensive characterization of diurnal and seasonal variation in foliar NR activity is an important prerequisite.

THE NITROGEN HYPOTHESIS OF FOREST DECLINE

One of the hypotheses that has been put forth to explain forest decline in eastern North America and western Europe is the so-called "nitrogen hypothesis." This hypothesis states in part that elevated levels of nitrogen deposition in a form available for direct foliar uptake by tree canopies (e.g., HNO_3 vapor, NO, NO_2) disrupts normal nutrient cycles and physiological processes, resulting in increased susceptibility to other stresses (Lindberg et al. 1987). Foliar absorption of gaseous nitrogen oxides (NO_x), in contrast to root uptake from the soil solution, circumvents the plant's regulatory control of N uptake, increasing the possibility of physiological imbalances. The possible physiological imbalances include disruption of the metabolic and growth processes required for winter hardening (Nihlgard 1985, Friedland et al. 1984, Waring 1987) and changes in carbon allocation patterns (McLaughlin 1985). An unfavorable balance between transpiring leaves and water-absorbing roots can increase drought susceptibility

---

[1] . Research sponsored by the USDA, National Acid Deposition Assessment Program Interagency Agreement 40-1647-45 with the U.S. Department of Energy under contract DE-AC05-84OR21400 with Martin Marietta Energy Systems, Inc. Publication No. 3113, Environmental Sciences Division.

(Norby et al. 1986). Imbalances in other nutrients can also result (Mohren et al. 1986, Waring 1987).

A BIOLOGICAL MARKER FOR EVALUATION OF NITROGEN HYPOTHESIS

The nitrogen hypothesis is difficult to test experimentally because of the number of steps between the predisposing stress (NO_x) and the response to the inciting stress (drought or winter conditions). A co-occurrence of NO_x deposition and forest decline clearly is insufficient evidence for a causal relationship. An efficient approach might be to investigate the critical links in the logical sequence of necessary events linking atmospheric deposition to changes in whole-plant physiology (Lindberg et al. 1987). One such link is the metabolism of foliar absorbed NO_x: if the nitrogen compounds are not reduced and assimilated into organic compounds, an alteration of carbon-nitrogen relations and the attendant effects on stress susceptibility are unlikely.

The reduction of nitrate to nitrite by the enzyme nitrate reductase (NR) is the rate-limiting step in the assimilation of nitrate into organic compounds (Guerrero et al. 1981). The activity of this enzyme may, then, be an appropriate marker for determining whether foliar-absorbed nitrogen oxides are assimilated by tress. This paper will explore the possible use of nitrate reductase as a marker in manipulative laboratory studies as well as in field surveys, with a focus on the impact of nitrogen deposition to high elevation red spruce (*Picea rubens*) forests.

CHARACTERISTICS OF NITRATE REDUCTASE

Nitrate reductase is potentially a valuable biological marker because it is substrate-inducible and responsive to environmental parameters. The presence of the substrate (nitrate) causes the *de novo* synthesis of the enzyme, an uncommon phenomenon in higher plants (Zielke and Filner 1971). Because the turnover rate of the enzyme is rapid (4-h half-life under noninducing conditions, Zielke and Filner 1971), the amount of active enzyme in tissue is low unless its synthesis is induced. The presence of NR in tissue, however, is not diagnostic for nitrate uptake because nitrate is not an obligatory inducer (Guerrero et al. 1981). There is a certain low level of NR always present ("constitutive NR") that is functional in nitrate reduction but not influenced by nitrate concentration (Beevers and Hageman 1983, Rajasekhar and Oelmüller 1987). Furthermore, other N compounds can induce NR, including ammonium (Rajasekhar and Oelmüller 1987), nitrite, urea, and certain amino acids (Haynes and Goh 1978). The activity of NR is influenced by a number of other environmental factors, including light, temperature, drought, and time of day, and physiological factors, such as hormones, carbohydrates, leaf water potential, and tissue age (Beevers and Hageman 1983). Rapid variations in NR activity in response to these factors are not due to long-term changes in the amount of enzyme, but are the result of inactivating proteins (Guerrero et al. 1981). With these precautions and exceptions in mind, it can, nevertheless, be stated that the level of NR is generally high in organisms grown on nitrate and low in organisms grown on ammonium (Guerrero et al. 1981). Although nitrate is not an absolute requirement for the induction of NR, the presence of active NR is an absolute requirement for the reduction, and, hence assimilation, of nitrate. There are many conflicting generalizations in the literature regarding the N nutrition of forest trees, and it is difficult to predict the level of NR that might be present in the foliage of a given tree. There appears to be no universal correlation between environmental factors and root or leaf assimilation of nitrate (Smirnoff and Stewart 1985). Plants vary in their dependence on ammonium vs. nitrate, in large part because of conditions in the rooting media, but most plants, including trees, can induce NR and assimilate nitrate if nitrate is present (Smirnoff et al. 1984). Under low levels of nitrate uptake by trees, most of the nitrate may be reduced in the roots (Andrews 1986); nevertheless, NR activity commonly is found in the leaves of many woody plants (Smirnoff et al. 1984, Al Gharbi and Hipkin 1984). Foliar NR activity in gymnosperms generally is low, but it can be induced by nitrate feeding (Smirnoff et al. 1984). Yandow and Klein (1986), however, reported that NR was not measurable in needles of red spruce seedlings growing with or without nitrate. If red spruce needles have no or very low NR activity, then NR would need to be induced before foliar-absorbed nitrate could be assimilated. The research question becomes whether NR is indeed induced in red spruce needles when they are exposed to nitrogen oxides.

EXPERIMENTAL USE OF NITRATE REDUCTASE AS A MARKER

One characteristic of NR that is important to its potential use as a biological marker is that it is fairly easy to assay. The so-called *in vivo* assay, which actually uses chopped tissue and not whole intact plants, obviates difficult enzyme extraction and purification steps. The protocol, which is derived from the approach described by Jaworski (1971), should be optimized for each species or tissue type (Al Gharbi and Hipkin 1984). A spectrophotometer is the only instrument required, so the assay could be done in the field. Alternatively, branch samples can be brought back to the laboratory if certain precautions are taken.

It is important to note that the NR assay does not determine the rate at which nitrate is being reduced in intact tissue. Total extractable NR activity greatly exceeds the actual *in situ* rate; the enzyme is present in excess and is operating below its Vmax. The *in vivo* assay predicts the capability for reduction (Huffaker and Rains 1978, Andrews 1986).

We have conducted a series of laboratory experiments to determine whether NR is induced in red spruce needles after exposure of the plants to NO_2, HNO_3 vapor, and NO_3^- in mist (Norby et al., in prep.). NR activity in needles of seedlings exposed to 75 ppb NO_2 increased rapidly within 1 day after the fumigation began, stayed at a level about twice that of control seedlings during the fumigation, and dropped quickly back to control levels 1 to 2 days after the NO_2 was withdrawn. No nitrate or nitrite accumulated in the needles. Very similar responses were obtained with exposure to HNO_3 vapor (about 75 ppb), except recovery was slower. The slower recovery possibly was due to continued movement (and hence continued induction of NR) of surface-adsorbed HNO_3 into the leaf interior (Marshall and Cadle 1987). There was an indication of nitrate (but not nitrite) accumulation in the needles exposed to HNO_3 vapor. Unlike the gaseous nitrogen oxides, nitrate applied in pH 3.5 mist did not increase NR activity over that in seedlings exposed to pH 5.0 mist.

The results of the experiment with NO_2 fumigation are similar to the results of Wingsle et al. (1987), who exposed Scots pine seedlings with 85 ppb NO_2 and detected a large increase in foliar NR activity after 1 day, with maximum activity after 2-4 days of exposure. Similar experiments with HNO_3 vapor have not been reported. These results show that red spruce has the capability to assimilate gaseous nitrogen oxides. Foliar NR was a useful marker for this purpose.

EVALUATION OF NITRATE REDUCTASE AS A MARKER IN THE FIELD

The use of NR as a biological marker has not been tested in the field. We excavated several red spruce seedlings from an intensively-studied field site in the Great Smoky Mountains National Park (McLaughlin et al. 1988), and exposed them to NO_2 along with the greenhouse-grown plants described above. Although the level of NR activity in both treated and control plants was lower than in the greenhouse-grown seedlings, the pattern of response to NO_2 was quite similar (Norby et al., in prep.).

Nitrate reductase also was assayed on cut shoots of mature spruce trees from two sites in the Smoky Mountains. The samples collected from the higher elevation site had 50% higher NR activity on September 24 and 30% higher on October 15 compared to samples from the lower elevation site (Y. Weerasuriya and R. J. Norby, unpublished data). The high elevation samples also exhibited a greater capacity for NR induction, as shown by putting the cut stems in KNO_3 solution. The NR activity of noninduced needles ranged from 20 to 110 nmol NO_2^-/(h·g DW), which is similar to that reported for noninduced needles of other conifers (Smirnoff et al. 1984) and is typical for constitutive NR (Al Gharbi and Hipkin 1984). The level of NR activity of samples collected on November 11 was much lower than that of the previous collections, and there was no difference between sites.

There is, not yet, a firm basis for speculation about why the spruce trees differed between sites in their levels of NR or in NR inducibility. The differences could be attributable to different carbon economies, phenology, soil characteristics, nitrogen deposition, or random variation. One of these sites is adjacent to a site described by Johnson et al. (1988) as having very high levels of nitrate in the soil solution, although foliar N concentrations of the red spruce trees are only about 1%. Much more extensive monitoring of the trees during the entire growing season will be necessary before an explanation might emerge. The results do show, however, that mature red spruce trees have NR in the needles, albeit at very low levels, that foliar NR is inducible, and that foliar NR activity in seedlings is responsive to NO_2 and HNO_3 vapor.

Whether pollutant NO_x gases cause a detectable increase in foliar NR at realistic concentrations and under field conditions remains a key research question. The literature on NR suggests that it is unlikely that a simple measurement of NR activity in the foliage of a tree, or even a more extensive monitoring of changes in foliar NR of a tree during the season or during a suspected pollution episode, will provide an unambiguous marker that the tree is impacted by nitrogen oxides. There are too many other environmental and physiological factors in addition to nitrate concentration that also induce NR or regulate its activity. Furthermore, some level of NR can be expected always to be present (constitutive NR), which may be sufficient to metabolize most foliar-absorbed NO_x without altering the marker.

SUMMARY

1. Nitrate reductase, which is the key enzyme involved in assimilation of NO_3^- by plants, is relatively easy to assay in the foliage of trees, can be detected at very low levels in the foliage of mature, forest-grown trees, and is known to respond to the amount of nitrate in tissue. It may provide an appropriate marker for evaluation of an important aspect of the "nitrogen hypothesis."
2. Nitrate reductase is an appropriate biological marker in controlled, manipulative experiments to test whether foliage has the capacity to metabolize foliar-deposited nitrogen oxides.
3. In such experiments it has been demonstrated that red spruce seedlings do have the capability for foliar assimilation of NO_2 and HNO_3 vapor, but wet-deposited nitrate did not affect the marker. Additional experiments on the effect of foliar-absorbed N on

whole-plant physiology are, therefore, appropriate for evaluating the "nitrogen hypothesis."

4. Extensive monitoring of NR activity during a growing season and in relation to realistic levels of NO_x pollutants will be necessary to evaluate the utility of NR as a biological marker in the field, but it probably will not prove to be an unambiguous marker for an impact of nitrogen pollutants.

References

Al Gharbi, A., and C.R. Hipkin. 1984. Studies on nitrate reductase on British angiosperms. New Phytol. 97: 629-639.

Andrews, M. 1986. The partitioning of nitrate assimilation between root and shoot of higher plants. Plant Cell Environ. 9: 511-519.

Beevers, L., and R.H. Hageman. 1983. Uptake and reduction of nitrate: Bacteria and Higher Plants. Pp. 351-375 in A. Läuchli and R.L. Bieleski (eds.), Encyclopedia of Plant Physiology, New Series Vol. 15A. Springer-Verlag, Berlin.

Friedland, A.J., R.A. Gregory, L. Karenlampi, and A.H. Johnson. 1984. Winter damage to foliage as a factor in red spruce decline. Can. J. For. Res. 14: 963-965.

Guerrero, M.G., J M. Vega, and M. Losada. 1951. The assimilatory nitrate-reducing system and its regulation. Ann. Rev. Plant Physiol. 32:169-204.

Haynes, R.J., and K M. Goh. 1978. Ammonium and nitrate nutrition of plants. Biol. Rev. 53: 465-510.

Huffaker, R.C., and D.W. Rains. 1978. Factors influencing nitrate acquisition by plants; assimilation and fate of reduced nitrogen . Pp. 1-43 in D.R. Nielson and J.G. MacDonald (eds.), Nitrogen in the Environment, Vol. 2. Academic Press, New York.

Jaworski, E.G. 1971. Nitrate reductase assay in intact plant tissue. Biochem. Biophys. Res. Commun. 43: 1274-1279.

Johnson, D.W., A.J. Friedland, H. Van Miegroet, R.B. Harrison, E. Miller, S.E. Lindberg, D.W. Cole, D.A. Schaefer, and D.E. Todd. 1988. Nutrient status of some contrasting high-elevation forests in the eastern and western United States. In G.D. Hertell, chairman, Effects of Atmospheric Pollutants on the Spruce-Fir Forests of the Eastern United States and Federal Republic of Germany. Proceedings of a symposium; 1987 October 19-23; Burlington VT. Gun. Tech. Rep. NE-??, U.S. Dept. Agric., For. Serv., Northeastern For. Expt. Sta., Broomall, PA. in press.

Lindberg, S.E., G.M. Lovett, and K-J. Meiwes. 1987. Deposition and forest canopy interactions of airborne nitrate. Pp.117-130 in T.C. Hutchinson and K.M. Meema (eds.), Effects of Atmospheric Pollutants on Forests, Wetlands and Agricultural Ecosystems. NATO ASI Series, Vol. G16. Springer-Verlag, Berlin.

McLaughlin, S.B. 1985. Effects of air pollutants on forests. J. Air Pollut. Control Assoc. 35: 512-534.

McLaughlin, S.B., C.P. Andersen, P.J. Hanson, R.J. Norby, N.T. Edwards, and R.R. Tardiff. 1988. Interactive effects of natural and anthropogenic factors on growth and physiology of southern red spruce. In G.D. Hertell, chairman, Effects of Atmospheric Pollutants on the Spruce-Fir Forests of the Eastern United States and Federal Republic of Germany. Proceedings of a symposium; 1987 October 19-23; Burlington VT. Gen. Tech. Rep. NE-??, U.S. Dept. Agric., For. Serv., Northeastern For. Expt. Sta., Broomall, PA. in press.

Marshall, J.D., and S.H. Cadle. 1987. Cuticular deposition of nitric acid vapor and its assimilation by eastern white pine. General Motors Research Report EV-294. Warren, MI.

Mohren, G.M.J., J. Van den Burg, and F.W. Burger. 1986. Phosphorus deficiency induced by nitrogen input in Douglas fir in the Netherlands. Plant Soil 95: 191-200.

Nihlgård, B. 1985. The ammonium hypothesis-an additional explanation to the forest dieback in Europe. Ambio 14: 2-8.

Norby, R.J., G.E. Taylor, Jr., S.B. McLaughlin, and C.A. Gunderson. 1986. Drought sensitivity of red spruce seedlings affected by precipitation chemistry. Pp. 34-41 in C.G. Tauer and T.C. Hennessey (eds.), Proceedings, Ninth North American Forest Biology Workshop, Stillwater, Oklahoma .

Rajasekhar, V.K., and R. Oelmüller. 1987. Regulation of induction of nitrate reductase and nitrite reductase in higher plants. Physiol. Plant. 71:517-521.

Smirnoff, N., P. Todd, and G.R. Stewart. 1984. The occurrence of nitrate reduction in the leaves of woody plants. Ann. Bot. 54: 363-374.

Smirnoff, N., and G.R. Stewart. 1985. Nitrate assimilation and translocation by higher plants: comparative physiology and ecological consequences. Physiol. Plant. 64: 133-140.

Waring, R.H. 1987. Nitrate pollution.' a particular danger to boreal and subalpine coniferous forests. Pp. 93-105 in T. Fujimori and M. Kimura (eds.), Human Impacts and Management of Mountain Forests. Forestry and Forest Products Research Institute, Ibaraki, Japan.

Wingsle, G., T. Nasholm, T. Lundmark, and A. Ericsson. 1987. Induction of nitrate reductase in needles of scots pine by NO_x and NO_3^-. Physiol. Plant. 70: 399-403.

Yandow, T.S., and R.M. Klein. 1986. Nitrate reductase of primary roots of red spruce seedlings. Plant Physiol. 64: 723-725.

Zielke, H.R., and P. Filner. 1971. Synthesis and turnover of nitrate reductase induced by nitrate in cultured tobacco cells. J. Biol. Chem. 246: 1772-1779.

Free-Radical Mediated Processes As Markers of Air Pollution Stress in Trees

Curtis J. Richardson
Richard T. Di Giulio
Norman E. Tandy
School of Forestry and Environmental Studies Duke University

ABSTRACT

Some of the air pollutants believed responsible for the decline of forests, particularly O_3, NO_x, H_2O_2, and SO_2 can generate toxic oxygen-free radicals when introduced into biological systems. Free radicals can be defined simply as molecules containing an unpaired electron. The generation of radical species can occur through oxidations, reductions or ionizing radiation. Dioxygen reduction commonly occurs in both biotic and abiotic systems and the intermediates of this reduction (oxyradicals) are the superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), and the very reactive hydroxyl radical (OH). The extremely reactive hydroxyl radical oxidizes organic molecules, including biomolecules which results in enzyme breakdown, membrane damage (lipid peroxidation), and DNA alterations. The primary defense against the oxygen compounds superoxide and hydrogen peroxide involves the enzymes superoxide dismutase (SOD), peroxidase (Px) and catalase. In addition, non-enzymatic defenses include such compounds as vitamin E (ã-tocopherol), β-carotenes, glutathione and ascorbic acid.

We hypothesize that an increase in the production of these antioxidant enzymes by some genotypes in the population will result in increased tree resistance to air pollutants. Changes in the levels of production of these antioxidants and an increase of malondialdehyde (an index of lipid peroxidation) could also be used as biomarkers of oxidant stress in trees. Current air pollution research on 3 loblolly pine families (*Pinus taeda*) subjected to 5 ozone and 2 acid level treatments has shown increases in SOD, peroxidase, total glutathione and malondialdehyde at high O_3 levels in conjunction with significant decreases in net photosynthesis. Our preliminary work and that of others suggest that we may be able to utilize antioxidants as early warning biomarkers of oxidative stress in trees.

INTRODUCTION

Photochemical oxidants such as SO_2, NO_x, and ozone are recognized as having phytotoxic effects on plants via the generation of free radicals (Mehlhorn et al. 1986, Castillo et al. 1987, Tandy et al. 1987, Wellburn 1987). A free radical is defined as any atom, group of atoms or molecule in a particular state with one unpaired electron occupying an outer orbital (Del Maestro 1980). The one electron oxidation or reduction of compounds results in the production of either cation or anion radicals, respectively (Thomas and Aust, 1986). The univalent pathway for reduction of molecular oxygen results in the generation of the superoxide anion radical (O_2^-), hydrogen peroxide, (H_2O_2) and hydroxyl radical (OH) intermediates as shown in Figure 1. In addition to direct oxidative degradation of lipids, destruction of intercellular and extracellular proteins, including enzyme inactivation, or DNA damage, indirect toxic effects like mutagenicity and carcinogenicity have been related to oxygen radical formation (Kappus 1987).

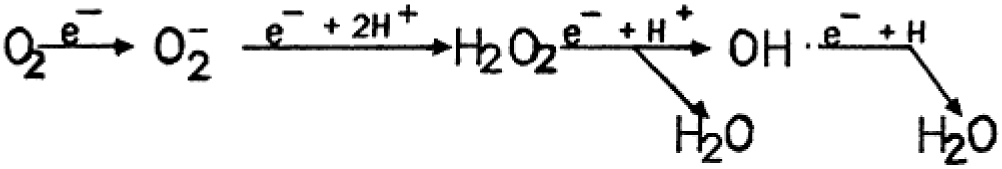


Figure 1.
The univalent pathway for reduction of molecular oxygen (from Del Maestro, 1980).

Aerobic cells have evolved a variety of enzymatic mechanisms against the toxic effects of the intermediates of oxygen reduction (oxyradicals) such as superoxide dismutase (SOD), peroxidase (Px), and catalase shown in Figure 2. In addition non-enzymatic defenses include such compounds as glutathione, vitamin E (ã-tocopherol), β-carotenes, and ascorbic acid (Di Giulio and Richardson 1987).

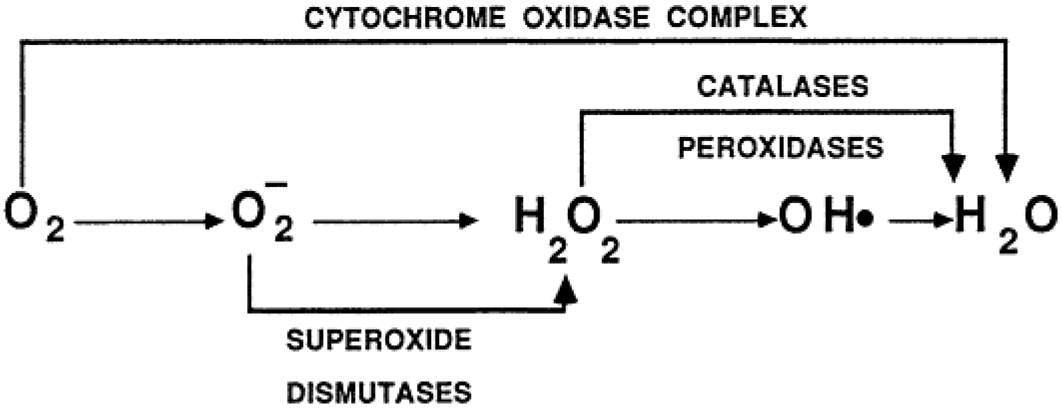


Figure 2.
Enzymatic defense mechanisms to prevent accumulation of reactive free radicals (from Del Maestro 1980).

In this paper we briefly review the literature concerning the use of antioxidants as biomarkers of photochemical oxidant air pollution stress in trees as well as present some of our preliminary findings on free radicals and antioxidants in loblolly pine. Our research was designed to test critically the hypothesis that a key mechanism of action underlying "air emission" stress involves the generation of toxic free radical intermediates. We propose that the key gaseous pollutants associated with acid precipitation (i.e., NO_2, SO_2, and ozone) are the principal sources of this free radical-mediated toxicity in trees and that acidity *per se* exacerbates this toxicity.

The specific goal of this phase of our research was to establish dose-response relationships between ozone, acid rain and selected free radical-related biochemical responses, photosynthesis, and growth in *Pinus taeda* (loblolly pine).

MATERIALS AND METHODS

Plant Material and Treatments:

Detailed information outlining our experimental design, treatment levels and methods are found in Richardson and Di Giulio (1987). Three half-sib families of loblolly pine seedlings were exposed to 5 ozone treatments in conjunction with 2 acid rain treatments in open-top chambers (Richardson and Di Giulio 1987). Results were statistically analyzed as a split plot design with 2 replicates.

Harvests and Physiological Measurements:

Monthly harvests were conducted at approximately mid-month during the growing season. At each harvest at least six seedlings per chamber were examined for *in situ* physiological responses and subsequently harvested for biochemical assays. Immediately prior to harvest, rates of photosynthesis, and stomatal resistance were measured *in situ* with a LI-COR 6200 portable photosynthesis system.

Biochemical Measurements:

Immediately following the harvest of each seedling, needles were clipped into vials and immediately immersed in liquid nitrogen. These samples were shipped to the Ecotoxicology Laboratory at Duke and stored at-70° C until analysis. Activities of SOD were determined by the methods of McCord and Fridovich (1959), Beauchamp and Fridovich (1971), and Asada et al. (1974). Peroxidase (Px) was analyzed following the methods of Putter and Becker (1983). Reduced and oxidized glutathione (GSH and GSSG) were measured by a modification of the spectrophotometric assay of Smith (1985). Lipid peroxidation was determined by the malondialdehyde (MDA) method of Uchiyama and Mihara (1978).

REVIEW, RESULTS AND DISCUSSION

Rabinowitch and Fridovich (1983) in an excellent review of superoxide radicals, superoxide dismutases (SOD), and oxygen toxicity in plants argued that superoxide dismutases found in plants resembles those found in other organisms and that varying forms of SOD (Cu/Zn and Mn) might provide a defense against SO_2, sunscald, herbicides, and photooxidative injury. Our lab has also found varying types of SOD's in red spruce (Tandy et al. 1987). It is interesting to speculate that a nutrient deficiency of Cu, Mn or Zn, as a result of acid rain leaching, might lead to a decrease in the ability of the plant to produce various forms of SOD. A model of how SO_2 effects plant chloroplasts and cytoplasm was proposed by Schulz in 1985 (Fig. 3). Once SO_2 enters the leaf it is converted to bisulfite and sulfite. Bisulfite is photooxidized to less toxic SO_4 and the superoxide radical (O_2^-). The superoxide radical can be dismutated by SOD, to H_2O_2 and O_2. H_2O_2 is converted to H_2O and O_2 by Px and catalase (Figs. 2 and 3).

Toxicologically, O_2^- and H_2O_2 are considered particularly important as precursors for the highly reactive and destructive OH radical. Cell injury can be severe if even a small portion of the superoxide radical is converted to OH.

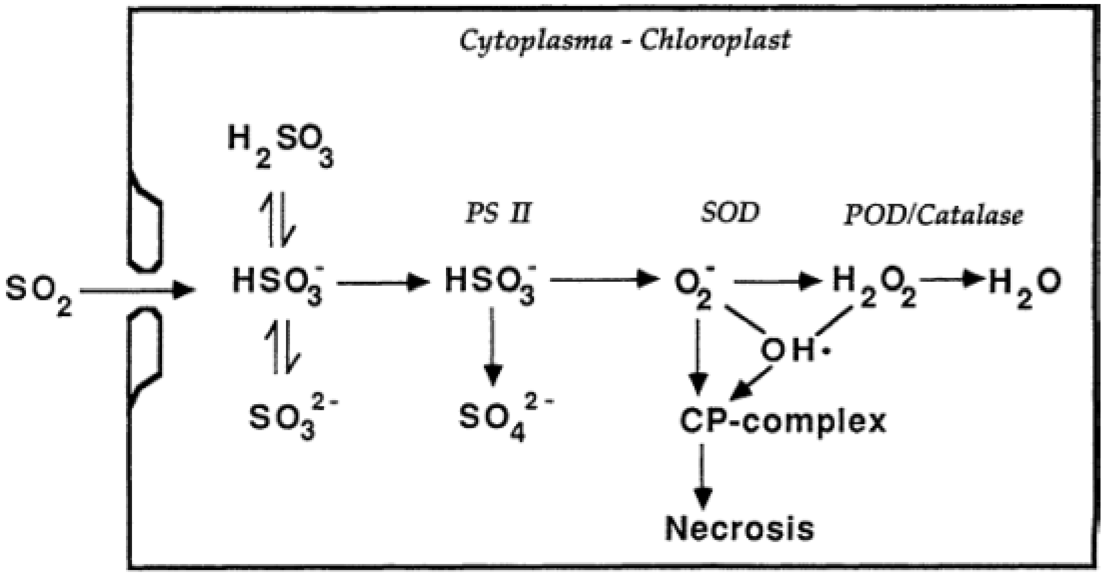


Figure 3.
A model of how SO_2 affects plant chloroplasts and cytoplasm as proposed by Schulz (modified form Schulz 1986)/

Activity of Px, SOD, and catalase in needles of *Pinus sylvestris* (scotch pine) after 240 hours of exposure to 0.3 ppm of SO_2 was shown to depend on needle age and reached a maximum in November (Fig. 4, from Schulz 1986). In addition he reported no visible needle injury or necrosis but a significant increase of Px and SOD over controls (Fig. 4). Catalase did not show an increase over controls. The intercellular fluid of *Picea abies* (Norway spruce) also displayed a significant increase in SOD after 30 days of 7-hour exposures with 300 ug.m^{-3} of ozone (Fig. 5, data from Castillo et al. (1987). The 30-day fumigation doubled the SOD activity in cell material as compared to the 2 day treatment. They also noted a decrease in ascorbic acid content and attributed this to the scavenging of toxic-free radicals by ascorbate-specific peroxidase. Glutathione and vitamin E were reported to increase significantly in both *Abies alba* (white fir) and Norway spruce after exposure to O_3, SO_2 and $SO_2 + O_3$ treatments (Mehlhorn et al. 1986). Treatment of these trees with SO_2 gave a higher increase of antioxidants in needles than those exposed to O_3 or charcoal filtered air. In addition, a combination of both gases resulted in the maximum increase in both vitamin E and total glutathione, significantly above the ozone and filtered treatment alone.

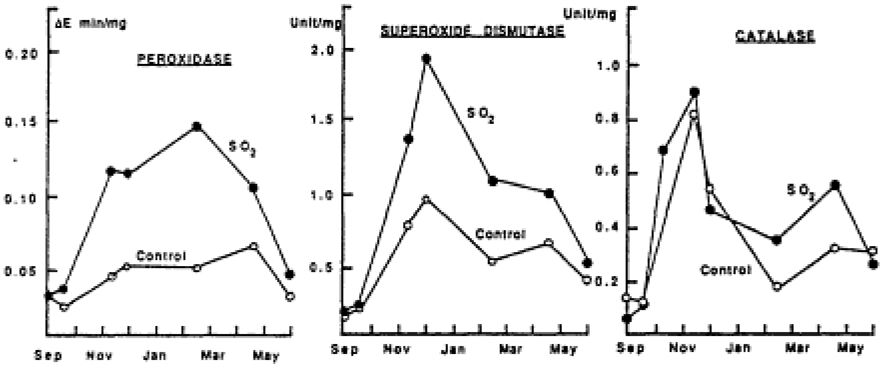


Figure 4.
Activity of peroxidase (POD, note: Px in our paper) superoxide dimutase (SOD) and catalase in needles of Pinus sylvestris after 240 hours of exposure to 0.8 ppm of SO_2 (modified from Schulz, 1986).

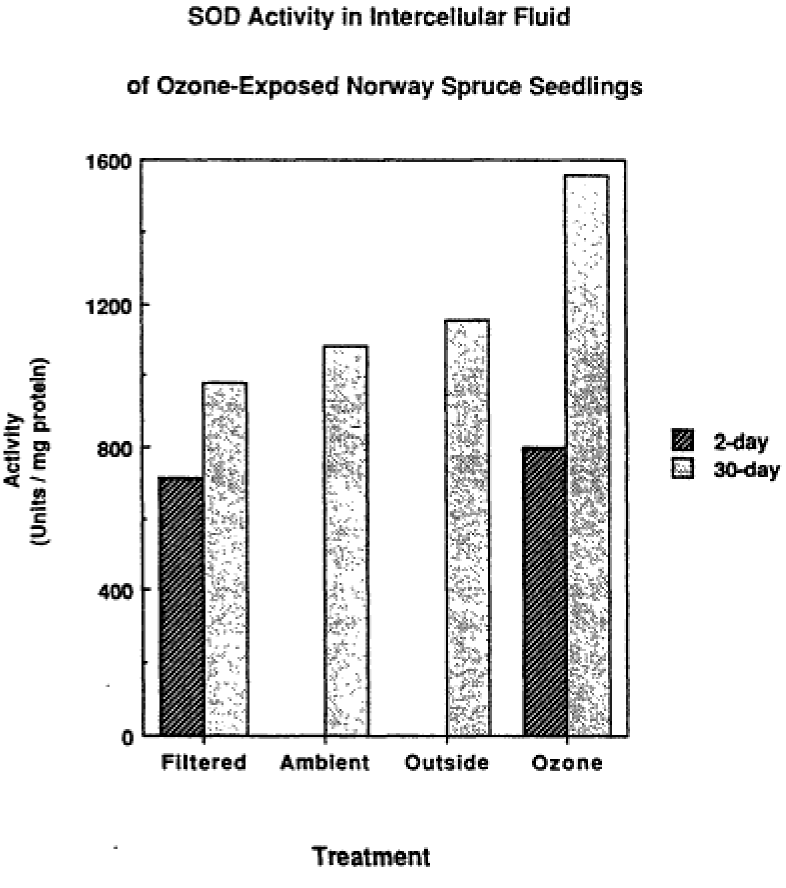
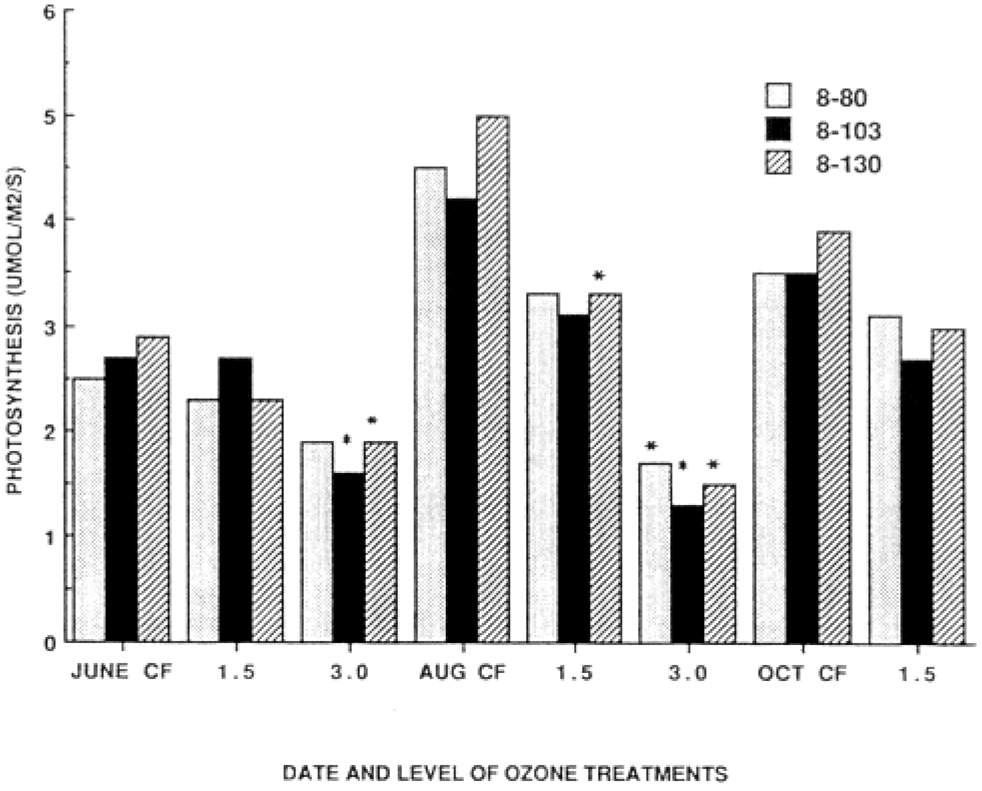


Figure 5.
Superoxide dismutase activity in intercellular fluid of picea abies after 30 days of 7-hour exposures with 300 ug.m^{-3} of ozone (after Castillo et al., 1987).

In our Ioblolly pine study first-flush 1987 needles did not show a significant response after 90 days of exposure at the 1.5 level of 24 hour/day ozone treatments (from March to June); photosynthesis was reduced only 1% ,8%, and 20% at 1.5 x ambient ozone (66 ppb) for families 8-103, 8-80, and 8-130, respectively (Fig. 6). Ambient ozone was 48 ppb on a seasonal average. The seasonal peak for photosynthesis was found in August for all 3 families in the control chambers (CF). In August, after 150 days of treatment, photosynthesis was reduced by an average of 29% in the 3 families treated with 66 ppb of ozone as compared to charcoal filtered controls with 20 ppb ozone. Photosynthesis was significantly affected by 24-hour ozone exposure at the 1.5 treatment above ambient levels only in family 8-130 in August, and for all 3 families at the 3.0 level; there were no statistically significant effects of acid rain. By 210 days of exposure (October) all the seedlings had a reduced photosynthetic rate from the August high values. The 3.0 treatment could not be measured since the seedlings displayed premature senescence of the 1987 first flush fascicles, which normally function for 2 complete growing seasons.



(note: * = significantly different from control at 0.05 level)

Figure 6.
Seasonal trends of photosynthesis in the 1987 first-flush needles for loblolly pine families, 8-130, 8-80, and 8-103 exposed to charcoal-filtered (control), 1.5 and 3.0 times ambient ozone for 24 hours per day. Each bar represents the mean of 18 plants. Premature senescence of needles removed the 3.0 treatment from analysis in October.

A preliminary biochemical analysis (data from all 3 families combined) of August needle tissue exposed to 1.5 X ambient ozone and collected concurrently with gas exchange measurements showed that MDA increased about 20% above controls (Fig. 7). while photosynthesis decreased 29% on average in the 3 families (Fig. 8). Malonaldehyde levels were increased by 100% at 3.0 X ambient ozone compared to charcoal-filtered (CF) treated plants, indicating a significant increase in lipid peroxidation, a common result of oxidant damage (Fig. 7). Photosynthesis dropped an average of 67% at the 3.0 treatment level in the 3 families. The responses of Px, a key enzymatic component of the anti-oxidant defense, showed almost the same pattern of increases as MDA under increased exposure from 1.5 to 3.0 X ambient ozone compared to CF (Figs. 7 and 8). Reduced glutathione (GSH) increased only at the highest ozone treatment.

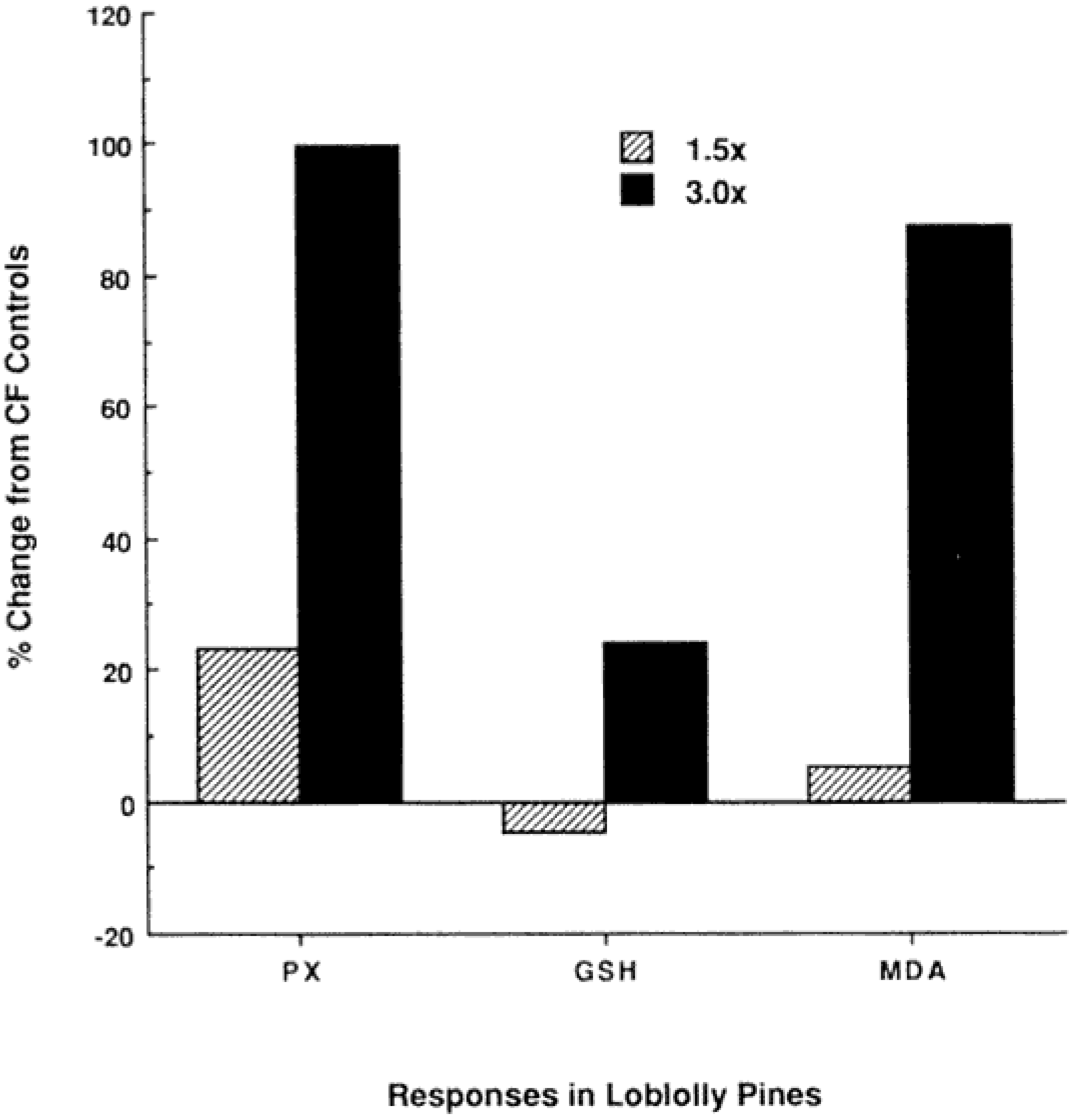


Figure 7.
Biochemical analysis of malonaldehyde (MDA), peroxidase (PX) and glutathione (GSH) as compared to controls (percent increase above charcoal filter treatment) in lablolly pine exposed to 24 hours of ozone at 1.5 and 3.0 times ambient in August 1987.

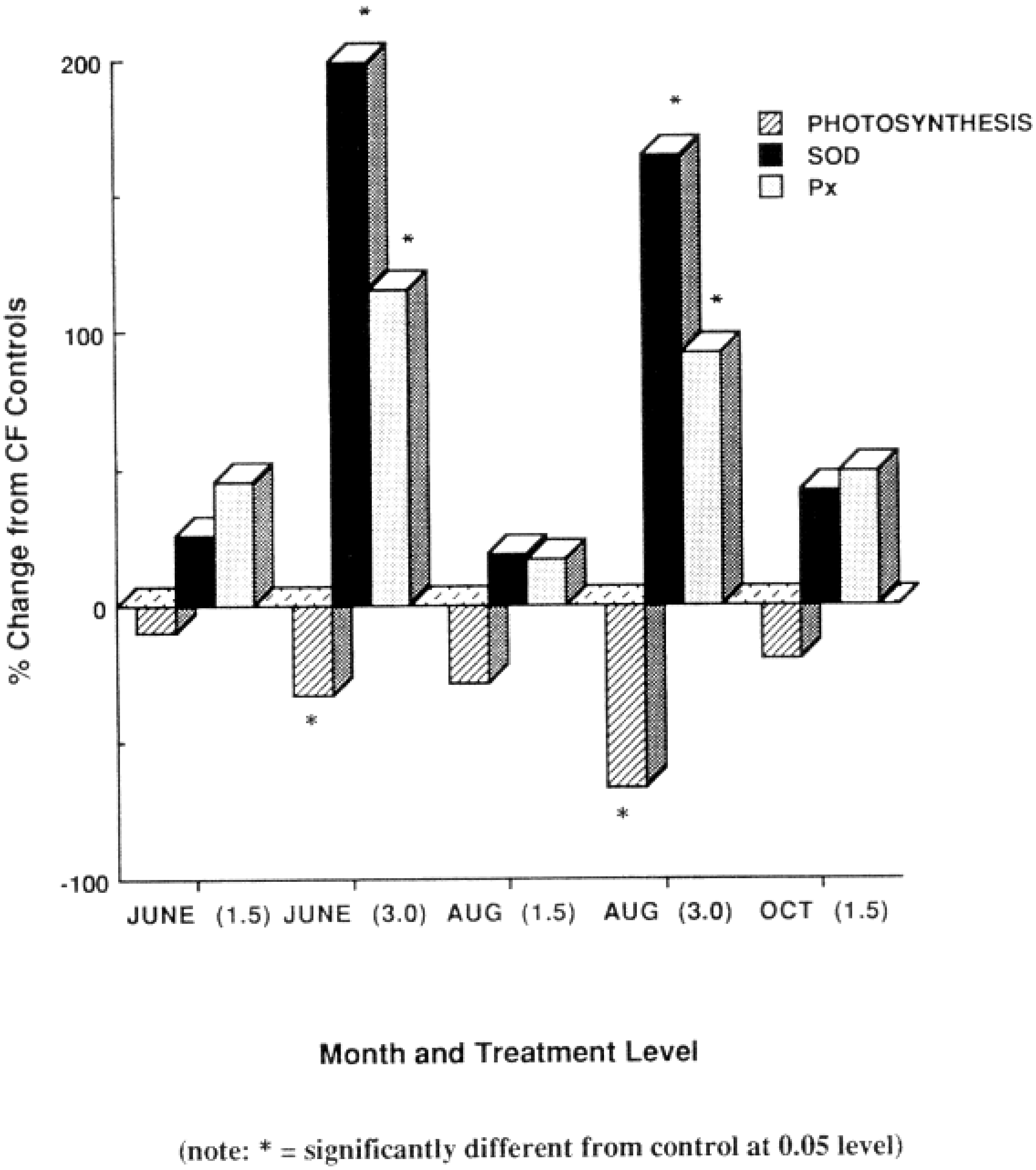


Figure 8.
A seasonal comparison of the change from controls in photosynthetic rates and antioxidants SOD and Px in loblolly pine in response to 24 hours of exposure to 1.5 and 3.0 times ambient ozone levels found in Durham, North Carolina.

A seasonal comparison of photosynthetic rates, SOD and Px response averaged over all 3 families reveals that highest levels of these antioxidants are found under the 3.0 treatments in June and August when photosynthesis was reduced by 33% and 67%, respectively (Fig. 8). SOD increased nearly 200% over controls in June and 165% in August, while Px averaged nearly a 100% increase over controls in both months. At the 1.5 X treatment level in June, SOD and Px increased by 26% and 46% over controls while photosynthesis decreased by 10%. These data suggest that antioxidants may be used as an indicator of oxidative stress in trees early in the growing season. In August, photosynthesis decreased by 29% and SOD and Px increased by 19% and 17%, respectively. By fall SOD and Px both had increased over early summer values and treated plants had higher values than controls by nearly 50%; photosynthesis was decreased by 20% in the exposed plants (Fig. 8).

These data show that antioxidants and MDA increased significantly over controls while photosynthesis simultaneously decreased. Our data are too preliminary to establish direct relationships, dose response curves or even family differences, but they do indicate that antioxidants may be useful biomarkers for indexing oxidant air pollutant stress in trees.

CONCLUSIONS

The possibility of tracking oxidant stress from these biochemical markers is encouraging. However, much more experimental work is needed to establish mechanism of action, molecular basis of antioxidant increase, seasonal effects, effects of multiple gases, as well as dose response relationships for individual species and ecotypes.

Further work is also needed to test adequately the hypothesis that air pollutant oxidants significantly increase antioxidants *in vivo* . Future research should employ combinations of ozone with other free radical generating pollutants, such as sulfur dioxide, nitrogen dioxide, and hydrogen peroxide to test the effect of complex gas mixtures on plant biochemical response.

ACKNOWLEDGMENTS

The loblolly pine research was funded by a USDA Forest Service grant through the Southern Commercial Forest Research Cooperative (29-260). The funding for the development of assays for antioxidants in spruce and pine came from a grant through the USDA Forest Service Spruce-Fir research Cooperative (23-020). We kindly thank Dr. Tom Sasek, Dave Keen, and Ed Fendick for assistance with photosynthesis and statistical analysis.

References

Asada, K., M. Takahashi, and M. Nagate. 1974. Assay and inhibitors of spinach superoxide dismutase. Agric. Biol. Chem. 38:471-473.

Beauchamp, C., and I. Fridovich 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44:276-287.

Castillo, F. J., P. R. Miller, and H. Greppin. 1987. Extracellular biochemical markers of photochemical oxidant air pollution damage to Norway spruce. Experimentia 43: 111-115.

Del Maestro, R. F. 1980. An approach to free radicals in medicine and biology. Acta Physiol. Stand. 492: 153-168.

Di Giulio, R. T., and C. J. Richardson. 1987. Effects of atmospheric deposition on red spruce: A free radical-based approach. Annual report to Spruce/fir Coop. Broomall, Penn.

Kappus, H. 1987. Oxidative stress in chemical toxicity. Arch. Toxieol. 60: 144-149.

McCord, J.M., and I. Fridovich 1969. Superoxide Dismutase: an enzymic function of erythrocuprein. J. Biol. Chem. 244: 6049-6055.

Mehlhorn, H., G. Seaufart, A. Schmidt, and K.J. Kurnert. 1986. Effect of SO_2 and O_3 on production of antioxidants in conifers. Plant Physiol. 82: 336-338.

Putter, J., and R. Becker. 1983. Peroxidases. In Bergmeyer, H.U. (ed.), Methods of Enzymatic Analysis. Verlag Chamie, Deerfield Beach, Florida.

Rabinowitch, H. D., and I Fridovich. 1983. Superoxide radicals, superoxide dismutases and oxygen toxicity in plants. Photochem. and photobiol. 37:679-690.

Richardson, C. J., and R. T. Di Giulio. 1987. Effects of gaseous pollutants and acid precipitation on open-top chambered loblolly seedlings in Duke Forest: physiology and biochemistry. Annual report (SC07) to SCFRC, Raleigh North Carolina.

Schulz, H. 1986. Biochemical and factor analytical examinations for interpretations of SO_2-indications in needles of *Pinus sylvestris*. Biochem. Physiol. Pflanzen 81: 241-256.

Smith, I.K. 1985. Stimulation of glutathione synthesis in photorespiring plants by catalase inhibitors. Plant Physiol. 79:1044-1047.

Tandy, N. E., R. T. Di Giulio, and C. J. Richardson 1987. Isozymes of superoxide dismutase in red spruce and their importance in protecting against oxidative stress. (In Press, International Symposium on the Effects of Atmospheric pollutants on Spruce/Fir forests of Germany and the U. S.).

Thomas, C. E., and S.D. Aust. 1986. Free radicals and environmental toxins. In Anals. of Emergency Medicine. 15:1075-1083.

Uchiyama, M., and M. Mihara. 1978. Determination of malonaldehyde precursor in tissues by thiobarbituric acid test. Anal. Biochem. 86:271-278.

Wellburn, A. R. 1987. Biochemical mechanisms of combined action of atmospheric pollutants upon plants. Pp. 813-829 in V. B. Vouk, G. C. Butler, A. C. Upton, D. V. Parke and S. C. Asher (eds). Methods For Assessing the Effects of Mixtures of Chemicals. SCOPE 1987.

Biochemical Indicators of Air Pollution Effects in Trees: Unambiguous Signals Based on Secondary Metabolites and Nitrogen in Fast-Growing Species?

Clive G. Jones
Institute of Ecosystem Studies The New York Botanical Garden Mary Flagler Cary Arboretum Millbrook, NY 12545
James S. Coleman
Department of Biological Sciences Stanford University Stanford, CA 94305

ABSTRACT

Various perturbations such as air pollution, shading, low nutrients, herbivore and pathogen attack cause stress and damage to plants. Stresses and damage may be distinguished on the basis of their effects on function and structure of the plant respectively, and on the time frame with which they occur. Different stresses may be classified on the basis of their relative or absolute effect on carbon (C) or nutrient acquisition. At the leaf tissue level, stress effects on carbon-based secondary metabolites (CSM) and nitrogen (N) may be predicted from the relative or absolute availability of C and N resources. In addition, stress often results in mobilization of N. The primary determinant of the magnitude and rate of difference in stress response between plant species is the inherent growth rate of plants. Fast-growing species may show plastic, dynamic responses in mobile CSM because allocation to growth takes priority over allocation to these compounds. Slow growers may not show such plastic and dynamic responses. Tissue damage initiates processes of repair and defense, which may result in mobilization of C and N to the site of damage for repair, and polymerization of CSM at the site of damage.

A predictive model based on the above factors is presented with different perturbations as dependent variables and mobile and polymerized CSM, total and mobile N and time as independent variables. No single variable has a unique value for particular types of stress or damage, but combinations of two or more variables predict that signals will be distinguishable. If the predictions are correct, they will permit air pollution stress and/or damage to be relatively unambiguously identified. Preliminary data from ozone exposure of a fast-growing species, cottonwood, support the predictions of the model.

INTRODUCTION

Ideally, a biochemical indicator of air pollution effects in trees should provide an unambiguous, easily measured signal, specific to air pollution and occurring in most species. It is unlikely that such a silver bullet will be found because plants are exposed to multiple, simultaneous abiotic and biotic perturbations that are not distinguishable solely on the basis of which external force is acting on the plant. The end result of these perturbations is partially the effect of the particular stress or damage agent, and partially the result of plant adjustment. The particular nature of the plant response is determined by inherent plant characteristics that differ between species. Nevertheless, we will argue that it is possible to: distinguish between perturbations that are stresses versus those that damage the plant; categorize different stresses in terms of their effects on plant function; categorize the response of plants by applying plant physiological concepts; and categorize plant species on the basis of their inherent growth rates. These concepts can be linked together and used to predict changes in classes of biochemicals that are dynamic and sensitive to changes in plant function in certain species. Our arguments extend and derive from concepts we have presented regarding the nature of physiological and biochemical responses of plants to different stresses (Jones and Coleman, 1988a). A predictive model will be presented for selected perturbations. We will focus attention on carbon-based secondary metabolites (CSM) and total and mobile nitrogen (N) in leaves. We will then describe a preliminary test of this model, using data from studies on the biochemistry of cottonwood following acute ozone exposure (Jones and Coleman, in prop.). Of necessity, this short paper will be an outline of these ideas, rather than a detailed exposition.

COMPONENTS OF A PREDICTIVE MODEL

Distinguishing Stress from Damage

Plants are exposed to a diversity of abiotic and biotic perturbations, in addition to air pollution. These perturbations often occur simultaneously and include shading, nutrient and water deficiencies, and herbivore and pathogen attack (Chapin et al., 1987; Jones and Coleman, 1988a; Mooney et al., 1988). Distinguishing the outcome of these perturbations on the plant requires that we first understand in what ways the effects of different perturbations to the plant are similar or different. Perturbations can be classified as resulting in stress-defined here as *interference with plant function*, or damage-defined here as *interference with plant structure* (cf., Grime, 1979). The perturbations listed above can result in stress or damage (Fig. 1). Stress may lead to subsequent damage (Pell, 1979) (e.g., drought may increase susceptibility to herbivores; Mattson and Haack, 1987), and damage may lead to subsequent stress (Bassman and Dickmann, 1985) (e.g., oxidant injury reduces carbon gain and growth; Reich, 1987), but this is not inevitable. Furthermore, certain perturbations cause stress but not damage, but all perturbations causing damage have the potential also to cause stress. Interestingly, air pollution, herbivore and pathogen attack can similarly result in both stress and damage (Norris, 1979; Williams, 1979; Bassman and Dickmann, 1985; Hawkins et al., 1986a,b; Tissera and Ayers, 1986; Jones and Coleman, 1988a). Stress and damage tend to occur on different time frames (Grime, 1979; Pell, 1979; Pell and Dan, 1988). While stress effects may be short (i.e., acute) or long (chronic), effects of damage are usually comparatively short-lived after initial damage (Kimmerer and Kozlowski, 1982; Edwards et al., 1986). Distinguishing between stress and damage is critical to the use of bioindicators because certain plant biochemicals show different responses, depending on whether stress and/or damage occurs (Jones and Coleman, 1988a).

Whole Plant Partitioning of Resources in Response to Stress

The overall effect of stress is a reduction in the acquisition of resources-the dominant plant function (Mooney, 1972). Plants respond to stress by adjusting partitioning of existing and subsequent resources to ameliorate the effects of stress (Bloom et al., 1985; Chapin et al., 1987; Szaniawski, 1987). For example, when carbon (C) acquisition is limited by shading or air pollution, plants subsequently partition proportionately more C to shoots (Mooney and Winner, 1988). This results in a shoot C gain relative to nutrient acquisition, and this C may be used to produce proportionately more leaf area or photosynthetic machinery, which presumably then restores the C balance by increasing photosynthetic capacity. On the other hand, plants exposed to nutrient (N) limitation partition more C to root growth (Robinson, 1986; Hunt and Nicholls, 1986; Mooney and Winner, 1988). This enables plant roots to grow and explore a greater soil area for resources, and creates a greater surface area for absorption of nutrients and water (Ingestaad and Agren, 1988). The overall result of adjustment to stress is a balancing of the C:N ratio around some optimal value (Bryant et al., 1983; Bloom et al., 1985; Chapin et al., 1987; Agren and Ingestaad, 1987; Ingestaad and Agren, 1988). Using this approach, we can derive a primary classification of stresses in terms of their relative effects on C or nutrient acquisition. Thus, air pollution, herbivory and pathogen attack (on leaves) can result in C stress, whereas nutrient limitation results in N stress (Jones and Coleman, 1988a). This primary classification is an essential component of the predictive model.

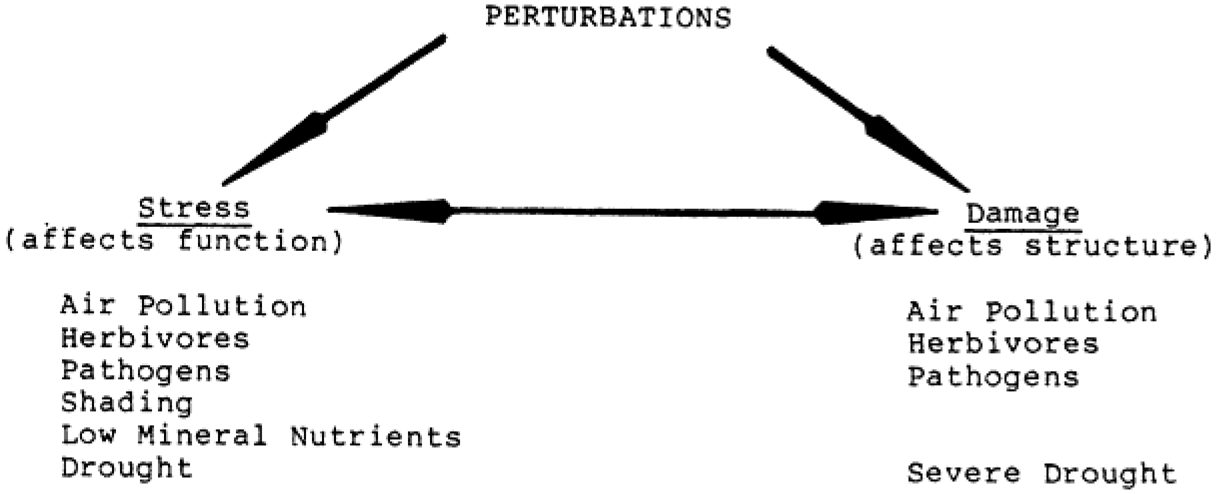


Figure 1.
Some perturbations to plants that may result in stress and/or damage.

Allocation of Resources within Tissues

We can apply the concept of C and nutrient balance at a lower level of organization-the tissues (i.e., leaves)-by applying the resource availability hypothesis (Bryant et al., 1985). This hypothesis focuses primarily on changes in plant secondary metabolites (e.g., phenolics, terpenoids) and nutrients such as protein and soluble N. The hypothesis predicts that plants allocate C or N to these compounds as a function of their availability in the environment. In this paper we will restrict our comments to carbon-based secondary metabolites (CSM) (e.g., phenolics, terpenoids) and organic N. Evidence substantiating the general applicability of this hypothesis is now quite considerable (Rhoades, 1983; Bryant et al., 1985; Larsson et al., 1986; Bryant, 1987) and, therefore, we will generalize in the context of selected stresses, rather than present a detailed exemplification. A more detailed treatment examining physical characteristics and a broad suite of chemical changes in leaves is presented in Jones and Coleman (1988a). When C gain is limited relative to nutrient availability (e.g., air pollution, shading), allocation to CSM is predicted to decline, and leaves become relatively enriched in nitrogen. Nutrient stress is predicted to result in an increase in CSM because of a relative increase in the availability of C, while N concentrations are predicted to decline.

Stress-Induced Changes in the Form of Nitrogen

Nutrient stress results in mobilization of N, with increases in concentrations of amino acids, imino acids, poly-and di-amines (Stewart and Larher, 1980; Erickson and Dashek, 1982; Smith, 1984; White, 1984). This occurs because nutrient stress induces catabolism of plant protein and subsequent retranslocation of N to shoot tips (Stewart and Larher, 1980).

Plant Determinants of the Magnitude and Rate of Stress Responses

Not all plants show dynamic changes in CSM or N following stress. The inherent growth rate of plants has been invoked to explain these differences (Coley et al., 1985). This concept has three major components. First, fast-growing species that tend to occur in resource-rich environments are predicted to allocate proportionately fewer resources to the production of CSM compared to slow-growing species from resource-poor environments, presumably because the value of individual leaves to a plant decreases as relative growth rate increases. Second, fast-growing species are predicted to make small amounts of mobile CSM with high turnover rates and metabolic costs (e.g., phenolic glycosides, monoterpenes); whereas slow-growers should construct large amounts of relatively immobile CSM with relatively low turnover rates and metabolic costs (e.g., tannins). Third, under conditions of stress, fast-growers should show extensive plasticity in the production of CSM, because allocation to growth is predicted to be a higher priority than allocation to CSM. For example, a reduction in C gain due to shading reduces mobile phenolics (Waring et al., 1986; Larsson et al., 1986; Bryant et al., 1987; Mole and Waterman, 1988). On the other hand, allocation to immobile CSM should be a high priority in slow-growers, so there should be less plasticity when these plants are stressed (Lincoln and Mooney, 1984; Bryant et al., 1985; Coley et al., 1985). Inherent growth rate is thus a critical predictor of the expected response of different plant species to stress.

Plant Responses to Damage

While stress results in adjustments of the primary plant function of resource acquisition, damage at the tissue level activates processes of repair and defense (McLaughlin and Shriner, 1980; Putritch and Jensen, 1982; Shigo, 1984). Repair requires C and N to be moved to the site of damage as resources for synthesis of membranes, cell walls, enzymes and other metabolites, and to remove any further damage agents (such as free radicals) (Lee and Bennett, 1982; Pell and Dan, 1988). Consequently, damage results in increases in mobile forms of N (e.g., amino acids, soluble enzymes e.g.; Green and Ryan, 1972) and C (e.g., sugars Craker and Starbuck, 1972; Heath, 1984; Koziol and Whatley, 1984; Guderian et al., 1985; Tallamy and Raupp, 1989). Defense occurs to prevent subsequent invasion by pathogens or attack by herbivores of vulnerable tissues. A frequent response of plants to damage is the deposition of phenolic materials (e.g., lignin, polyphenolics) into damaged or adjacent tissues (Rhoades, 1979; Deverall, 1982; Daly, 1984; Kemp and Burden, 1986). This requires mobilization of polyphenolic precursors to the site of damage or *in situ* biosynthesis, rapidly followed by polymerization (Howell, 1974; Tingey et al., 1975, 1976; Curtis et al., 1976). The recognition that damage can result in local increases in mobile N and polymerized forms of CSM over short time frames (hours, days) is critical to the predictive model.

PREDICTING STRESS AND DAMAGE RESPONSES IN FAST-GROWING TREE SPECIES

The Model

The predicted relationships between stress, damage and the biochemical responses on plants are shown for fast-growing plant species in Table 1, and are based on the previous considerations. The independent variable is the perturbation. Dependent variables are the % of total leaf C allocated to mobile CSM (e.g., mobile phenolics), and polymerized CSM (lignin, polyphenolics), either as absolute values compared to unstressed or undamaged plants, or as a relative value compared to total foliar N; total foliar N, either absolute or relative to total C; % of total N in mobile, low molecular weight forms (amino, imino acids, di-amines, polyamines) either absolute or relative to total C; and the duration of the response-short (hours, days) or long (weeks, season, years).

Table 1. Predicted relationships between perturbation, duration of effect of perturbation and foliar biochemistry. Mobile and polymerized CSM can be a % of total C or absolute concentrations or relative to total N. Mobile N can be a % of total N or absolute concentrations. Alternatively, total and mobile N may be relative to total C. +: increase; -: decrease; O: no change.

Perturbation	Duration of Effects	Mobile CSM	Polymerized CSM	Total N	Mobile N
Damage (Air pollution, Herbivores, pathogens)	Short	+	+	+	+
Carbon Stress (Air pollution, Long shading, prior defoliation by herbivores)	Short/Long	-	O	+	O
Mineral Nutrient Stress	Long	+	O	-	+

It can be seen that each single dependent chemical variable shows increases, decreases or no change, depending on the type of stress. No single type of stress, nor damage, has a unique value for the response of each variable, with the exception of increases in polymerized CSM with damage. However, combinations of two or three variables are predicted to show unique, stress-or damage-dependent signals. For example, if the mobile CSM is plotted against the total foliar N (Fig. 2), a clearer separation of the different stresses or damage is obtained. Damage due to air pollution, herbivores or pathogens is distinguished by the high values for mobile CSM and N, and the presence of polymerized CSM. It is probably reasonable to suppose that the specific damage agents of herbivores or pathogen attack can be distinguished visibly, at least in the case of foliar chewing, mining, galling or leaf-rolling herbivores. On the other hand, carbon stress (air pollution, shading) produces high values for N and low values for CSM, and no polymerized CSM is present. Different sources of carbon stress (air pollution, shading, prior defoliation) should be distinguishable if canopy dominant, unshaded trees are sampled and if there are reasonable records of prior defoliation by insects. Nutrient stress has low values for total N and high values for mobile CSM, with no polymerized CSM present.

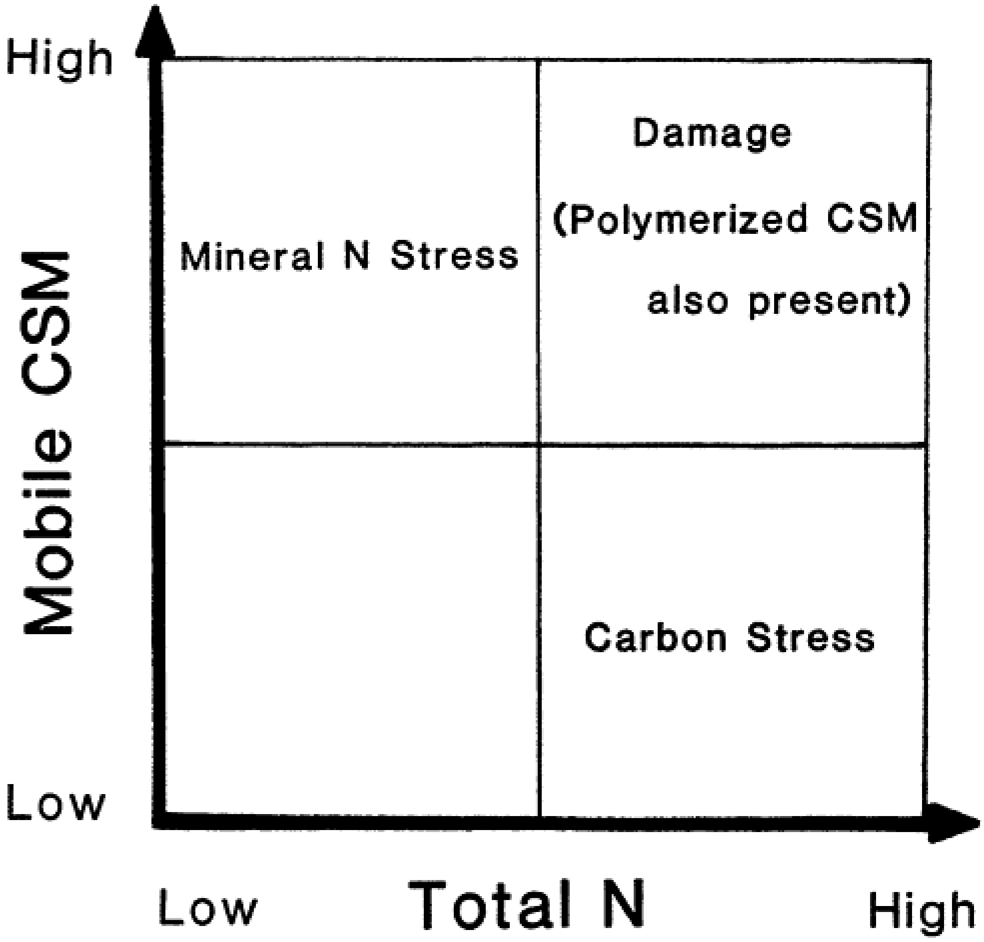


Figure 2.
Predicted values for, and relationships between, mobile CSM, and total N with different types of stress and damage. The biaxis plot shows separation of carbon stress from nutrient stress and damage.

Our model suggests that one approach to find markers of air pollution effects in forests would be to determine the relative or absolute allocation of foliar C to mobile CSM (e.g., phenol glycosides, low molecular weight phenolics, terpenoids) and polymerized forms of CSM (e.g., polyphenols, tannins, lignins) and the absolute or relative total foliar N and % of total nitrogen in mobile N (polar, low molecular weight such as amino and imino acids, di-and polyamines), for canopy-dominant, fast-growing species. Sampling these predicted changes in allocation over reasonable time periods (2-3 years), or comparing allocation with plants growing under similar conditions at lower pollutant concentrations, may facilitate relatively unambiguous determination of both damage and stress effects due to air pollution.

A PRELIMINARY TEST OF THE PREDICTIVE MODEL

Jones and Coleman (in prep) exposed saplings of two clones of cottonwood, *Populus deltoides*, to an acute dose of ozone (20 pphm, 5 hr). This species is an indeterminate fast-grower. The ozone dose had no significant effect on the growth of the plant, and did not cause visible injury to the leaves that were chemically analyzed, compared to charcoal-filtered air controls (Coleman et al., 1987). The concentration of one class of mobile CSM, phenol glycosides, polymerized phenolics, total N and polar (mobile) N were determined in leaves. Although the selected ozone dose had no direct effect on growth, and did not cause visible injury to assayed leaves, both damage and short-term stress to the plant occurred for the following reasons. Leaves older than those assayed frequently showed visible ozone injury. These doses are also known to reduce photosynthetic rates and carbon gain (Reich, 1983), biomass partitioning (Reich and Lassoie, 1985) and water use (Reich and Lassoie, 1984) in poplars. Lastly, both clones showed significant changes in subsequent resistance to insects and diseases (Coleman et al., 1987; Jones and Coleman, 1988b; Coleman and Jones, 1988), indicating changes in structure and/or function in leaves that were not visibly injured.

Table 2. Predicted changes in biochemical characteristics of P. deltoides exposed to stress, damage, and both stress and damage when exposed to ozone (20 pphm, 5 hr.). Abbreviations as in Table 1.

		Mobile CSM Phenolic Glycosides	**Polymerized CSM Phenolics**	**Total N**	**Mobile N**
Damage Only		+	+	+	+
Stress Only		-	O	+	O
Damage	Damage>Stress	+	+	+	+
+	Damage=Stress	O	+	+	+
Stress	Stress>Damage	-	+	+	+

The model predicts that for this short-term perturbation (Table 2), stress should result in a reduction in the mobile CSM phenol glycosides, an increase in total N, and no change in mobile N. Damage should be indicated by the presence of polymerized CSM phenolics, an increase in total N and an increase in mobile N. Since both stress and damage occurred in the experiment, we would expect an increase in total N and mobile N and the presence of polymerized CSM phenolics. If stress exceeded damage in intensity, mobile CSM phenol glycosides should decrease. If damage exceeded stress in intensity, mobile CSM should increase and stress would not be detectable from a sample taken at only one time, because the other discriminatory variable, total N, is also predicted to increase with both stress and damage. If damage and stress were both equal in intensity, mobile CSM phenolic glycosides should not have changed. Table 3 shows that mobile CSM phenol glycosides decreased; polymerized phenolics were present; total N increased in both clones, but significantly so, in only one clone; and mobile N increased. The model predicts that the changes in the parameters we measured indicate that indeed both stress and damage occurred. In addition, if the model is correct, stress effects were greater in intensity than damage effects, because mobile CSM phenol glycosides showed an overall decline.

Table 3. Leaf characteristics of 2 clones of P. deltoides exposed to an acute ozone dose (20 pphm, 5 hr) that had no effect on growth and did not produce visible injury.* p > 0.1;** p > .0005. Residue phenolics are Folin=Denis positive phenols remaining in the plant after extraction with solvents. Polar N is nitrogen extracted into butanolic or aqueous fractions. Data from Jones and Coleman, 1989.

	Clone O_3	**ST109 CFA**	**Clone O_3**	**ST66 CFA**
Phenol glycosides, % DW as glucose equivalents (≡ Mobile CSM)	7.44*	10.57	7.89*	10.71
Residue Phenolics, % DW (≡ Polymerized CSM)	0.28	0	0.64	0
Total N, %DW	1.92	1.81	2.02**	1.68
Polar N, % of (≡ Mobile N)	22	12	17	13

CONCLUSION

The above data were measured prior to the development of this model, but were not considered in constructing the predictions of the model. The experiment was by no means a rigorous test of the predictions-for example, we did not measure photosynthetic rates or examine leaves histologically to confirm independently that stress and damage occurred in this particular experiment. Nevertheless, the findings do not contradict the predictions of the model. This is encouraging, and suggests that tests of the model in greenhouse and field chamber experiments, as well as the field, are warranted.

ACKNOWLEDGMENTS

Contribution to the program, Institute of Ecosystem Studies, The New York Botanical Garden. We thank NSF (BSR-85-16679), The NRC and the Mary Flagler Cary Charitable Trust, for support of CGJ, and EPRI and Hal Mooney for support of JSC.

References

Agren, G.I., and Ingestaad, T. 1987. Root/shoot ratios as a balance between nitrogen productivity and photosynthesis. Plant, Cell and Environ. 10:579-586.

Bassman, J.H., and Dickmann, D. 1985. Effects of defoliation in the developing leaf zone of young *Populus* X *euramericana* plants. III. Distribution of ^{14}C photosynthate after defoliation. For. Sci. 31:358-366.

Bloom, A.J., Chapin, F.S. III, and Mooney, H.A. 1985. Resource limitation in plants-an economic analogy. Annu. Rev. Ecol. Syst. 16:363-392.

Bryant, J.P. 1987. Feltleaf willow-snowshoe hare interactions: Plant carbon/nutrient balance and floodplain succession. Ecology 68:1319-1327.

Bryant, J.P., Chapin, F.S. III, and Klein, D.R. 1983. Carbon/nutrient balance of boreal plants in relation to vertebrate herbivory. Oikos 40:357-368.

Bryant, J.P., Chapin, F.S. III, Reichardt, P., and Clausen, T.P. 1985. Adaptation to resource availability as a determinant of chemical defense strategies in woody plants. Rec. Adv. Phytochem. 19:219-237.

Bryant, J.P., Chapin, F.S. III, Reichardt, P.B., and Clausen, T.P. 1987. Response of winter chemical defense in Alaskan paper birch and green alder to manipulation of plant carbon/nitrogen balance. Oecologia 72:510-514.

Chapin, F.S. III, Bloom, A.J., Field, C.B., and Waring, R.H. 1987. Plant responses to multiple environmental factors. BioScience 37:49-57.

Coleman, J.S., and Jones, C.G. 1988. Plant stress and insect performance: Cottonwood, ozone and a leaf beetle. Oecologia 76:57-61.

Coleman, J.S., Jones, C.G., and Smith, W.H. 1987. Effects of ozone on cottonwood-leaf rust interactions: Independence of abiotic stress, genotype and leaf ontogeny. Can. J. Bot. 65:949-953.

Coley, P.D., Bryant, J.P., and Chapin, F.S. III. 1985. Resource availability and plant antiherbivore defense. Science 230:895-899.

Craker, L.E., and Starbuck, J.S. 1972. Metabolic changes associated with ozone injury of leaves. Can. J. Plant Sci. 52: 589-597.

Curtis, C.R., Howell, R.K., and Kremer, D.R. 1976. Soybean peroxidases from ozone injury. Environ. Pollut. 11:189-194.

Daly, J.M. 1984. The role of recognition in plant disease. Annu. Rev. Phytopathol. 72:273-307.

Deverall, B.J. 1982. Introduction. Pp. 1-20 in Bailey, J.A., and Mansfield, J.W. (eds.). Phytoalexins. Blackie, Glasgow.

Edwards, P.J., Wratten, S.D., and Cox, H. 1986. Wound-induced changes in the acceptability of tomato for larvae of *Spodoptera littoralis* . A laboratory bioassay. Ecol. Entomol. 10:155-158.

Erickson, S.S., and Dashek, W.V. 1982. Accumulation of foliar soluble protein in sulphur-dioxide stressed *Glycine max* c.v. "Essex" and *Hordium vulgare* cvs. "Proctor" and "Excelsior" seedlings. Env. Pollut. Ser. A 28:89-108.

Green, T.R., and Ryan, C.A. 1972. Wound-induced proteinase inhibitors in plant leaves. Science 175:776-777.

Grime, J.P. 1979. Plant Strategies and Vegetation Processes. J. Wiley & Sons, Chichester, UK.

Guderian, R., Tingey, D.T., and Rabe, R. 1985. Effects of photochemical oxidants on plants. Pp. 129-333 in Guderian, R. (ed.). Air Pollution by Photochemical Oxidants. Springer-Verlag, Berlin.

Hawkins, C.D.B., Aston, M.J., and Whitecross, M.I. 1986a. Short term effects of infestation by two aphid species on plant growth and shoot respiration of three legumes. Physiol. Plant. 68:329-334.

Hawkins, C.D.B., Whitecross, M.I., and Aston, M.J. 1986b. Interactions between aphid infestation and plant growth and uptake of nitrogen and phosphorus by three leguminous host plants. Can. J. Bot. 64:2362-2367.

Heath, R.L. 1984. Air pollutant effects on biochemicals derived from metabolism: Organic, fatty and amino acids. Pp. 275-290 in Koziol, M.J., and Whatley, F.R. (eds.). Gaseous Air Pollutants and Plant Metabolism. Butterworths, London.

Howell, R.K. 1974. Phenols, ozone and their involvement in pigmentation and physiology of plant injury. Pp. 94-105 in Dugger, M. (ed.). Air Pollution Effects on Plant Growth. ACS Syrup. Ser. 3. Washington, D.C.

Hunt, R., and Nicholls, A.D. 1986. Stress and coarse control of root-shoot partitioning in herbaceous plants. Oikos 47:149-158.

Ingestaad, T., and Agren, G.I. 1988. Nutrient uptake and allocation at steady state nutrition. Physiol. Plant. 72:450-459.

Jones, C.G., and Coleman, J.S. 1988a. Plant "stress" and insect herbivory: Toward an integrated perspective. In Mooney, H.A., Winner, W.E. and Pell, E.J. (eds.). An Integrated Response of Plants to Stress. Academic Press, New York (in press).

Jones, C.G., and Coleman, J.S. 1988b. Plant stress and insect behavior: Cottonwood, ozone and the feeding and oviposition preference of a beetle. Oecologia 76:51-56.

Kemp, M.S., and Burden, R.S. 1986. Phytoalexins and stress metabolism in the sapwood of trees. Phytochem. 25:1261-1269.

Kimmerer, T.W., and Kozlowski, T.T. 1982. Ethylene, ethane, acetaldehyde and ethanol production by plants under stress. Plant Physiol. 69:840-847.

Koziol, M.J., and Whatley, F.R. (eds.). 1984. Gaseous Air Pollutants and Plant Metabolism. Butterworths, London.

Larsson, S., Wiren, A., Lundgren, L., and Ericsson, T. 1986. Effects of light and nutrient stress on leaf phenolic chemistry in *Salix dasyclados* and susceptibility to *Galerucella lineola*. Oikos 47:205-210.

Lee, E.H., and J.H. Bennett. 1982. Superoxide dismutase: A possible protective enzyme against ozone injury in snapbeans (*Phaseolus vulgaris*). Plant Physiol. 69:1444-1449.

Lincoln, D.E., and Mooney, H.A. 1984. Herbivory on *Diplaucus auranticus* shrubs in sun and shade. Oecologia 64:173-176.

Mattson, W.J., and Haack, R.A. 1987. The role of drought stress in provoking outbreaks of phytophagous insects. Pp. 365-407 in Barbosa, P., and Schultz, J.C. (eds.). Insect Outbreaks. Academic Press, San Diego.

McLaughlin, S.B., and Shriner, D.S. 1980. Allocation to defense and repair . Pp. 407-431 in Horsfall, J.G., and Cowling, E.B. (eds.). Plant Disease: Vol. V. Academic Press, New York.

Mole, S., and Waterman, P.G. 1988. Light-induced variation in phenolic levels in foliage of rain forest plants. I. Chemical changes. J. Chem. Ecol. 14:1-22.

Mooney, H.A. 1972. The carbon balance of plants. Ann. Rev. Ecol. Syst. 3:315-346.

Mooney, H.A., and Winner, W.E. 1988. Effect of environmental stress on the partitioning of resources between plant roots and shoots. In Mooney, H.A., Winner, W.E. and Pell, E.J. (eds.). Integrated Response of Plants to Stress. Academic Press, New York (in press).

Mooney, H.A., Winner, W.E., and Pell, E.J. (eds.). 1988. An Integrated Response of Plants to stress. Academic Press, New York (in press).

Norris, D.M. 1979. How insects induce disease. Pp. 239-256 in Horsfall, J.G., and Cowling, E.B. (eds.) Plant Disease: Vol. IV. Academic Press, New York.

Pell, E.J. 1979. How air pollutants induce disease. Pp. 273-292 in Horsfall, J.G., and Cowling, E.B. (eds.) Plant Disease: Vol. IV. Academic Press, New York.

Pell, E.J., and Dan, M.F. 1988. Multiple stress and plant senescence. In Mooney, H.A., Winner, W.E. and Pell, E.J. (eds.). Integrated Response of Plants to Stress. Academic Press, New York (in press).

Putritch, G.S., and Jensen, G.D. 1982. Non-specific host-tree processes occurring in bark in response to damage and their role in defense. Pp. 94-102 in Heybroek, H.M., Stephen, B.R., and Weissenberg, K. yon (eds.). Resistance to Diseases and Pests of Forest Trees. Center for Agric. Publ., Wageningen, Neth.

Reich, P.B. 1983. Effects of low concentrations of O_3 on net photosynthesis, dark respiration and chlorophyll contents in aging hybrid poplar leaves. Plant Physiol. 73:291-296.

Reich, P.B. 1987. Quantifying plant responses to ozone: a unifying theory. Tree Physiol. 3:63-91.

Reich, P.B., and Lassoie, J.P. 1984. Effects of low level O_3 exposure on leaf diffusive conductance and water-use efficiency in hybrid poplar. Plant Cell and Envir. 7:661-668.

Reich, P.B., and Lassoie, J.P. 1985. Influence of low concentrations of ozone on growth, biomass partitioning and leaf senescence in young hybrid poplar plants. Envir. Pollut. Ser. A 39:39-51.

Rhoades, D.F. 1979. Evolution of plant chemical defenses against herbivores. Pp. 3-54 in Rosenthal, G.A., and Janzen, D.H. (eds.). Herbivores: Their Interaction with Secondary Plant Metabolites. Academic Press, New York.

Rhoades, D.F. 1983. Herbivore population dynamics and plant chemistry. Pp. 155-200 in Denno, R.F., and McClure, M.S. (eds.). Variable Plants and Herbivores in Natural and Managed Systems. Academic Press, New York.

Robinson, D. 1986. Compensatory changes in the partitioning of dry matter in relation to nitrogen uptake and optimal variations in growth. Annals Bot. 58:841-848.

Shigo, A.L. 1984. Compartmentalization: A conceptual framework for understanding how trees grow and defend themselves. Annu. Rev. Phytopathol. 22:273-307.

Smith, T.A. 1984. Putrescine and inorganic ions. Rec. Adv. Phytochem. 18:7-54.

Stewart, G.R., and Lather, F. 1980. Accumulation of amino acids and related compounds in relation to environmental stress. Pp. 609-635 in Miflin, B.J. (ed.). The Biochemistry of Plants. Vol. 5. Amino Acids and Derivatives. Academic Press, New York.

Szaniawski, R.K. 1987. Plant stress and homeostasis. Plant Physiol. Biochem. 25:63-72.

Tallamy, D., and Raupp, M. (eds.). 1989. Phytochemical Induction by Herbivores. J. Wiley, New York (in press).

Tingey, D.T., Fites, R.C., and Wickliff, C. 1975. Activity changes in selected enzymes from soybean leaves following ozone exposure. Physiol. Plant. 33:316-320.

Tingey, D.T., Fites, R.C., and Wickliff, C. 1976. Differential foliar sensitivity of soybean cultivars to ozone associated with differential enzyme activities. Physiol. Plant. 37: 69-72.

Tissera, P., and Ayres, P.G. 1986. Transpiration and water relations of faba bean (*Vicea faba*) infected by rust (*Uromyces vicea-fabae*) . New Phytologist 102:385-395.

Waring, R.H., McDonald, A.J.S., Larsson, S., Ericsson, T., Wiren, A., Ericsson, A., and Lohammar, T. 1986. Differences in chemical compositions of plants grown at constant relative growth rates with stable mineral nutrition. Oecologia 66: 157-160.

White, T.C.R. 1984. The abundance of invertebrate herbivores in relation to the availability of nitrogen in stressed food plants. Oecologia 63:90-105.

Williams, P.H. 1979. How fungi induce disease. Pp. 163-180 in Horsfall, J.G. and Cowling, E.B. (eds.) Plant Disease: Vol. IV. Academic Press, New York.

Metals in Roots, Stem, and Foliage of Forest Trees

Walter C. Shortle
USDA Forest Service Northeastern Forest Experiment Station Durham, NH 03824

ABSTRACT
Eight metals (Ca, K, Mg, Mn, Fe, Zn, Cu, Mo in decreasing molar concentrations) are considered essential for tree growth and function. They account for less than one percent of tree mass. They must be obtained from soils derived from the earth's crust in which the eight most abundant metals are Al, Na, Ca, Fe, Mg, K, Ti, Mn. To obtain the essential metals from among the common metals requires mechanisms for selectively accumulating some metals, while discriminating against others, by biologically regulated energy-consuming processes. Of major concern are forests in which soil pH is in the 3 to 4.5 range. In this range Al ions can be released from soil solids by inputs of strong inorganic ions, such as sulfate and nitrate. Increased vulnerability of large trees may be triggered as Al ions attain equimolar concentration with Ca ions in the absorbing fine roots.

Awareness that a fatal tree disease was developing in the spruce-fir forests of Germany began in the mid-1970s with descriptions of a dieback and decline of silver fir (*Abies alba* Mill.) generally referred to as "fir-dying." Trees with dying crowns had a marked decrease in sapwood basal area (portion of the transverse area of secondary xylem that is functional sapwood) in the lower stem, and a marked increase in "wetwood" (wound initiated infection) in the lower stem and upper secondary roots (Bauch et al. 1979). Firs with dying crowns and decreasing sapwood basal areas due to the spread of wetwood had been declining in cambial activity since the 1950s, long before crown symptoms were observed. Dendroclimatological studies indicated that this suppression of cambial growth was not related to weather patterns (Eckstein et al. 1983).

By the early 1980s, a dieback and decline syndrome was observed in Norway spruce (*Picea abies* (L.) Karst), although the symptoms were not as pronounced as in silver fir (Schütt and Cowling 1985). Spruce accounts for 40 percent of the commercial forest in West Germany, fir only about 2 percent. Concern now changed from "fir-dying" to "forest-dying" in central Europe and in other countries including the United States and Canada. The relationship of this sudden loss of tree health to stresses created by air pollution associated with the 20th century economy has been the subject of many scientific investigations and a matter of great public interest. Although no definite conclusions have yet been reached about the actual impact of air pollution and acidic deposition on forests, the possibility clearly exists that forests of central Europe and eastern North America are at risk from air pollution, either by direct chronic effects on foliage or indirect cumulative effects on fine roots in soil.

A mechanism has been proposed that links some soil related effects of acidic deposition to suppression of cambial growth, reduction of sapwood basal area, crown degradation, and eventual mortality (Shortle and Smith 1988). The mechanism involves the gradual replacement of essential metal cations, calcium and magnesium, in the rooting zone of spruce-fir stands with aluminum, which is released into soil solution by adding strong anions to acidic soils (pH 3 to 4.5) where spruce and fir commonly grow (Ulrich 1983). The molar ratio of aluminum to calcium in absorbing fine root tissue increases and at an equimolar ratio the uptake of calcium becomes limited (Bauch and Schroeder 1982, Stienen et al. 1984, Bauch et al. 1985a, Shortle and Stienen 1988). Adding calcium to decrease the ratio in soil and in fine roots restored short-term calcium uptake in isotope studies (Schroeder, et al. 1988), partially reversed damage in seedling studies (Stienen and Bauch 1988), and reversed the declining cambial growth of mature trees in liming studies (Bauch et al. 1985b). The timing and dose of calcium additions are obviously important factors in obtaining a favorable response.

Calcium is the most abundant metal in spruce, fir, and northern hardwood trees (Young and Carpenter 1967). The high calcium requirement for mature trees was recognized as a major difference between trees and crop plants (Rennie 1955). Unlike other major essential elements (nitrogen, phosphorus, and potassium), calcium is not recovered from older living sapwood as it is converted into a heartwood core in which calcium will be sequestered until the wood decays (Bamber and Fukazawa 1985). Although the uptake of calcium and other major essential cations may vary considerably from site to site, the amount incorporated into functional sapwood appears to remain relatively constant (Shortle and Bauch 1986). If calcium uptake is restricted by interference from aluminum, and the rate of incorporation is conserved, then a prolonged suppression of cambial growth is the expected outcome. Prolonged cambial suppression would be seen as a series of narrow growth rings. The conversion of wider growth rings into the heartwood core at the inner sapwood boundary is no longer balanced by the addition of new sapwood rings at the outer sapwood boundary (the vascular cambium), thus the sapwood basal area decreases as observed in declining spruce and fir trees. Mature spruce and fir trees with smaller sapwood basal areas have smaller crowns (Marchand 1984). Large trees with small crowns and low sapwood basal areas are more vulnerable to attack by pathogens and insects (Shigo 1985a,b). Such trees would also have less energy available to compensate for the adverse aluminum to calcium ratio through additional fine root production.

The range of soil pH values observed in the humus and underlying mineral soil in spruce-fir stands across northern New England (Shortle and Stienen 1988) was similar to those observed in many stands in Germany (Stienen et al. 1984). Extracts of humus (organic soil horizons) collected from Camels Hump, Vermont, and Mount Abraham (25 km to the south), where red spruce are dying, had a molar Al:Ca ratio of 13.0 (Taylor et al. 1986) and 6.0 (Shortle and Stienen 1988), respectively; those from Acadia National Park, Maine, and Beddington (60 km to the north), where red spruce appeared healthy, had a molar Al:Ca ratio of 1.2 and 0.8, respectively. When the humus layers from Camels Hump and Acadia were used in an experiment with rain chemistry, mist chemistry, and ozone, soil was the only treatment affecting both the above-ground and below-ground biomass of red spruce seedlings (Taylor et al. 1986). No treatment specific symptoms of visible needle injury were observed. Experiments with spruce seedlings under various cultural conditions in which the molar Al:Ca ratio was 1 indicated that this condition can affect the development of fine roots and shoots (Schier 1985, Stienen and Bauch 1988).

Molar ratios of Al:Ca in spruce and fir fine root tissue were determined from collections made from humus and mineral soil sampled across New England in 1985 and

1986. In brief summary, at Mount Abraham, Vermont, Al:Ca in fine root tissue taken from the lower humus, the major rooting zone of spruce and fir, was 3.4 (spruce, 1985), 1.7 (fir, 1985), and 4.1 (spruce, 1986). Fine root tissue in the underlying mineral soil at Mount Abraham was 3.1 (spruce, 1986). At Beddington, Maine, Al:Ca in fine root tissue taken from the lower humus was 0.1 (spruce, 1985), 0.3 (fir, 1985), and 0.1 (spruce, 1986). Fine root tissue in the underlying mineral soil at Beddington was 1.3 (spruce, 1986). Fine root production was sparse in the mineral soil from both sites. Values for seven additional locations between Mount Abraham and Beddington were intermediate, but more like Beddington than Mount Abraham at this time. However, at the Crawford Notch, New Hampshire, location where the largest spruce are dead and dying, spruce fine root samples taken in 1987 had an Al:Ca ratio of 1.1 for humus and 3.7 for mineral soil.

Limited growth of yellow birch (*Betula alleghaniensis* Britton), is attributed to aluminum in the podzols of New. England (Hoyle 1971). The humus layer supported good growth of younger, smaller trees, but did not compensate for the inadequacy of the mineral subsoil to support growth of larger trees, especially in the presence of aluminum. At high elevation sites, it appears that aluminum may now be affecting the uptake in the lower humus, which is usually a safe place for fine root absorption.

The molar Al:Ca ratio of fine root tissue may be a useful indicator of the influence of acidic deposition on spruce-fir forests on acid soils (pH 3 to 4.5). Under existing acidic conditions, forests are at risk from the continued input of strong anions derived from emissions of sulfur and nitrogen. Above-ground tissues are highly conserved with respect to major metal ion concentrations with stemwood being more stable than foliage. These parts of the tree appeared to respond to changing soil conditions by changing rates of tissue development from cambium and buds. Changes in cambial activity, sapwood basal area, and the spread of infection in forest trees can be monitored by electrical measurements (Shigo and Shortle 1985).

Sampling absorbing fine roots (0.2 mm diam X<5 mm length) in amounts sufficient to determine molar ion ratios is not feasible on a routine basis over large geographic areas. However, some simpler analyses applied in sequence on samples of soil from the zone of major fine root activity may be useful as an indicator of atmospheric effects in the forest. Presumptive tests for low acidity and high ionic strength could be made by simple electrical measurements followed by determination of soluble or exchangeable metal ion ratios, such as Al:Ca and Al:Mg on acidic soils, or Ca:K on less acidic soils, by routine chemical analysis. Coupling such measurements with periodic electrical measurements of cambial activity and internal infections could help locate areas of progressive decline and impending mortality, or of recovery following episodes of decline and mortality.

References

Bamber, R.K., and K. Fukazawa. 1985. Sapwood and heartwood: A review. Forestry Abstracts 46:567-580.

Bauch, J., P. Klein, A. Fruhwald, and H. Brill. 1979. Alteration of wood characteristics in *Abies alba* Mill. due to "fir-dying" and considerations concerning its origin. Eur. J. For. Pathol. 9:321-331.

Bauch, J., P. Rademacher, W. Berneike, J. Kroth, and W. Michaelis. 1985a. Breite und Elementgehalt der Jahrringe in Fichten aus Waldschadensgebieten. Pp. 943-959 in Waldschaden-Einflussfaktoren und ihre Bewertung. Dusseldorf, FRG: VD1 Berichte 560.

Bauch, J., and W. Schroeder. 1982. Zellularer Nachweis yon Elementen in den Feinwurzeln gesunder und erkrankter Tanne (*Abies alba* Mill.). Forstwissenschaftliches Centralblatt 101:285-294.

Bauch, J., H. Stienen, B. Ulrich, and E. Matzner. 1985b. Einfluss einer Kalkung bzw. Dungung auf den Elementgehalt in Feinwurzeln und das Dickenwachstum yon Fichten aus Waldschadensgebieten. Allgemeine Forst Zeitschrift 43:1148-1150.

Eckstein, D., R.W. Aniol, and J. Bauch. 1983. Dendroklimatologische Untersuchungen zum Tannesterben. Eur. J. For. Pathol. 13:279-288.

Hoyle, M.C. 1971. Effects of the chemical environment on yellow birch root development and top growth. Plant and Soil. 35:623-633.

Marchand, P. J. 1984. Sapwood area as an estimator of foliage biomass and projected leaf area for *Abies balsamea* and *Picea rubens*. Can. J. For. Res. 14:85-87.

Rennie, P. J. 1955. The uptake of nutrients by mature forests growth. Plant and Soil 7:49-95.

Schier, G. A. 1985. Response of red spruce and balsam fir seedlings to aluminum toxicity in nutrient solutions. Can. J. For. Res. 15:29-33.

Schroeder, W.H., J. Bauch, J., and R. Endeward. 1988. Microprobe analysis of Ca exchange and uptake in the fine roots of spruce: influence of pH and aluminum. Trees 2(3): (in press).

Schütt, P., and E.B. Cowling. 1985. Waldsterben, a general decline in forests in central Europe: symptoms, development and possible causes. Plant Disease 69:548-558.

Shigo, A. L. 1985a. Compartmentalization of decay in trees. Sci. American 252:96-103.

Shigo, A. L. 1985b. Wounded forests, starving trees. J. Forestry 83(11):668-673.

Shigo, A. L., and W.C. Shortle. 1985. Shigometry. A reference guide. USDA For. Serv. Agric. Handb.646. 48 p.

Shortle, W. C., and J. Bauch. 1986. Wood characteristics of *Abies balsamea* in New England states compared to *Abies alba* from sites in Europe with decline problems. IAWA Bull. n.s. 7:375-387.

Shortle, W.C., and K.T. Smith. 1988. Aluminum-induced, calcium deficiency syndrome in declining spruce-fir forests Science 240:1017-1018.

Shortle, W.C., and H. Stienen. 1958. Role of ions in the etiology of spruce decline. In Proc. Research Symposium, The effects of atmospheric pollution on spruce and fir forests in the eastern United States and the Federal Republic of Germany, October 18-23, 1987. Burlington, VT. USDA For. Serv. Gen. Tech. Rep. NE-(in press).

Stienen, H., R. Barckhausen, H. Schaub, and J. Bauch. 1984. Mikroskopische und rontgenenergie dispersive Untersuchungen an Feinwurzeln gesunder und erkrankter Fichten (*Picea abies* (L.) Karst.) verssschiedener standarte. Forstwissenschaftliches Centralblatt 103:262-274.

Stienen, H. and J. Bauch. 1988. Element determination in tissues of spruce and fir seedlings from hydropanic and soil cultures simulating acidification and deacidification. Plant and Soil 106:231-238.

Taylor, G.E., Jr., R.J. Norby, S.B. McLaughlin, A.H. Johnson, and R.S. Turner. 1986. Carbon dioxide assimilation and growth of red spruce (*Picea rubens* Sarg.) seedlings in response to ozone, precipitation chemistry and soil type. Oecologia (Berlin) 70:163-176.

Ulrich, B. 1983. A concept of forest ecosystem stability and of acidic deposition as a driving force for destabilization. Pp. 1-32 in Ulrich, B., Pankrath, J. (eds.). Effects of air pollution in forest ecosystems. Dordrecht, Holland: D. Reidel Publishing Co.

Young, H.E., and P.M. Carpenter. 1967. Weight, nutrient element and productivity studies of seedlings and saplings of eight tree species in natural ecosystems. Tech. Bull. 28. Orono, ME: Maine Agric. Expt. Stn., Univ. of Maine. 39 p.

The Potential of Trees to Record Aluminum Mobilization and Changes in Alkaline Earth Availability

E. A. Bondietti
C. F. Baes III
S. B. McLaughlin
Environmental Sciences Division Oak Ridge National Laboratory Oak Ridge, Tennessee 37831-6038

ABSTRACT

The mobilization of exchangeable soil cations by atmospheric deposition of mineral acid anions and the distribution of polyvalent cations in the xylem are discussed to provide the basis for interpreting both radial concentration and concentration ratio patterns of polyvalent cations in annual growth rings of trees. There is strong circumstantial evidence that the increases in Al:Ca ratios in the annual rings of red spruce and eastern hemlock in the Great Smoky Mountains National Park have occurred in recent decades and are related to aluminum mobilization. Decreases in radial growth rates as well as changes in the storage patterns of alkaline earth elements may also be linked to aluminum mobilization. Thus, the same mechanism responsible for surface water acidification may be important in the growth decline phenomenon associated with trees growing on very acidic soils. Conclusions as to the current utility of radial concentration patterns in evaluating air pollution effects on trees are discussed and further research needs are outlined.

BACKGROUND

One of the consequences of increasing the deposition of sulfate and nitrate to forests is the mobilization of exchangeable soil cations to maintain the charge balance in the soil solution. Depending on the base saturation or acidity of the soil, increased leaching of Al^{3+}, base cations, or both can occur [1]. Mineral cycling rates may also increase [1]. An important question related to this workshop is whether annual growth rings contain a record of the impact of atmospheric deposition on the availability of exchangeable soil cations, particularly in soils low in exchangeable bases.

The movement and retention of cations in the transpiration stream are influenced by adsorption to charged sites in the cell walls of the xylem. Cation binding in the cell wall follows well established charge/ionic radius relationships so that the stem xylem can be thought of as an ion exchange column [2,3] with most of the sites occupied by four elements—K, Na, Ca, and Mg [4]—as well as lesser amounts of Mn, Sr, Al, etc. Each successive annual ring, therefore, can be thought of as a newly formed ion exchange column, and the sapwood can be considered a moving zone of cation exchange columns which conduct water and solutes at varying efficiencies because of age, blockages, and other factors.

Radiotracer studies indicate that calcium taken up from soil concentrates near the phloem [5]; thus, newly formed cell walls are incorporating recently assimilated calcium. Although radial concentrations of nitrogen, phosphorus, and even monovalent cations often change greatly at the heartwood-sapwood boundary, divalent cations generally do not, exhibiting a radial concentration decline from the pith to the cambium [6,7,8,9,10]. However, discontinuities in cation concentration trends in tree rings can occur in response to wounding. The potential importance of the radial migration of polyvalent cations has been recognized [11] but appears to be a minor factor affecting radial distributions [9], at least when compared to the uncertainty in how many annual rings are conducting solutes at any given time.

The Stem as a Recorder of Changes in Cation Availability

The exchangeable cation composition of soils or the composition of nutrient solutions affects the bulk composition of woody stems and automobile lead and smelter emission histories are recorded in tree rings [11]. However, there are very little empirical data relating changes in soil, solution chemistry at a given site to changes in the radial concentration patterns of Ca^{2+}, Mg^{2+}, Al^{3+}, Mn^{2+}, and other cations in xylem from that site. Differences in cation availability between field sites is easier to demonstrate. To compare trees of the same species but of different ages, it is necessary to normalize for the pith-to-cambium decline in concentrations of divalent cations. Normalizing to calcium, an essential cell wall constituent, allows comparisons based on relative differences rather than on absolute concentrations.

When the Al:Ca ratios in the xylem from three sets of five red spruce (*Picea rubens* Sarg.) trees cored at 550 m, 790 m and 975 m elevation on Camels Hump Mountain in Vermont [10] are compared, the Al:Ca ratios increase with elevation (Fig. 1). This finding is consistent with the increase in soil acidity which occurs on Camels Hump with increasing elevation [12]. The Mn:Ca ratios also increase with elevation [10]. For comparison, the average Al:Ca ratio (0.0035) in increments from 22 red spruce cores from the Great Smoky Mountains National Park (GSMNP) [9,10] is also plotted as a solid line in Figure 1. Because the calcium concentrations in the growth increments of red spruce of comparable age from Vermont and from GSMNP are similar [9,10], Figure 1 illustrates not only that differences in soil pH are apparently reflected in xylem but more importantly, that these differences between elevations existed in the past. For example, soils at 975 m on Camels Hump have apparently been more acid than soils at 550 m or soils in GSMNP for a very long time.

Several examples illustrate that changes in cation availability during the life of a tree are reflected in radial concentrations in wood. When yellow-poplar trees were cored 20 years after a dolomite soil application, elevated calcium levels remained in the xylem formed immediately after the treatment. The authors concluded that tree rings could be used to understand historical changes in the chemical environment of trees [13]. A slightly different and more universal perspective on how accurately different tree species record changes in cation availability can be obtained by using fallout ^{90}Sr, a calcium analog with a known input history. For example, Figure 2a illustrates the distribution of ^{90}Sr in GSMNP red spruce radial growth increments measured by the senior author. Also plotted is the deposition history (in New York). Although ^{90}Sr does appear in wood formed prior to the fallout period, reflecting either conduction in active sapwood or radial migration, the amount and number of years affected is relatively small. This and other measurements of ^{90}Sr in different species indicate that 5-or 10-year increments are satisfactory for determining changes in the availability of calcium.

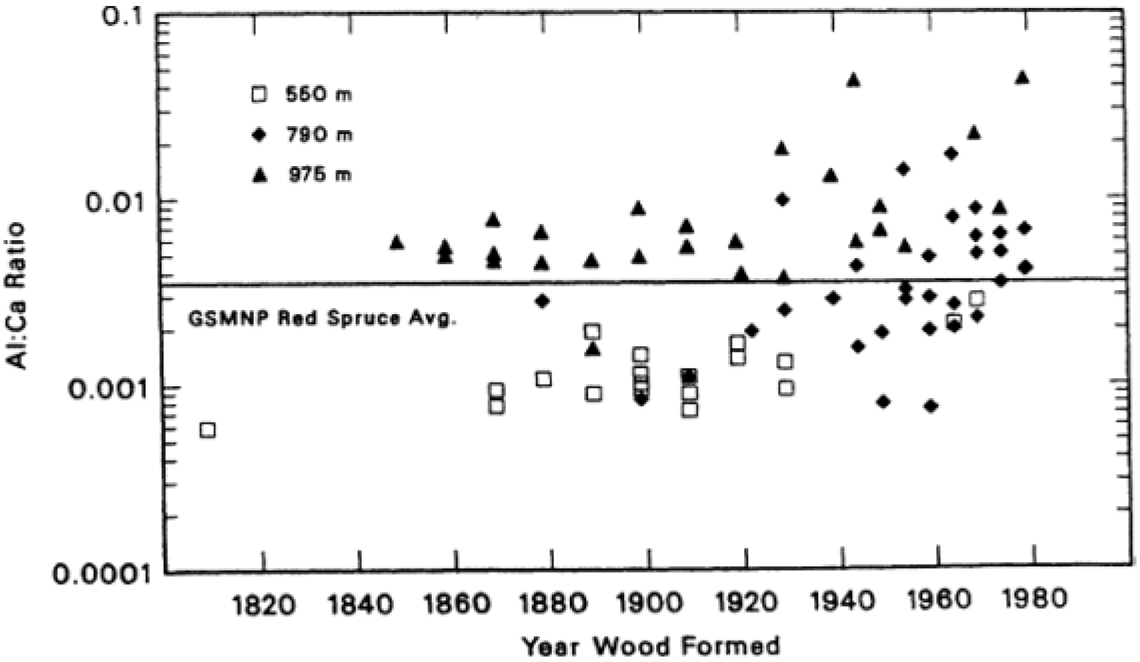


Figure 1.
Ratios of Al:Ca in red spruce increment cores sampled at three elevations on Camels Hump Mountain in Vermont. The line is the average Al:Ca ratio in 22 red spruce cores sampled in the Great Smoky Mountains National Park.

Aluminum behavior in xylem is illustrated by the Ca, Mg, and Al concentration trends in an increment core from a red spruce sampled by C. F. Baes III at a site in the GSMNP where a fire occurred in the early 1920s (Fig. 2b). The radial concentration

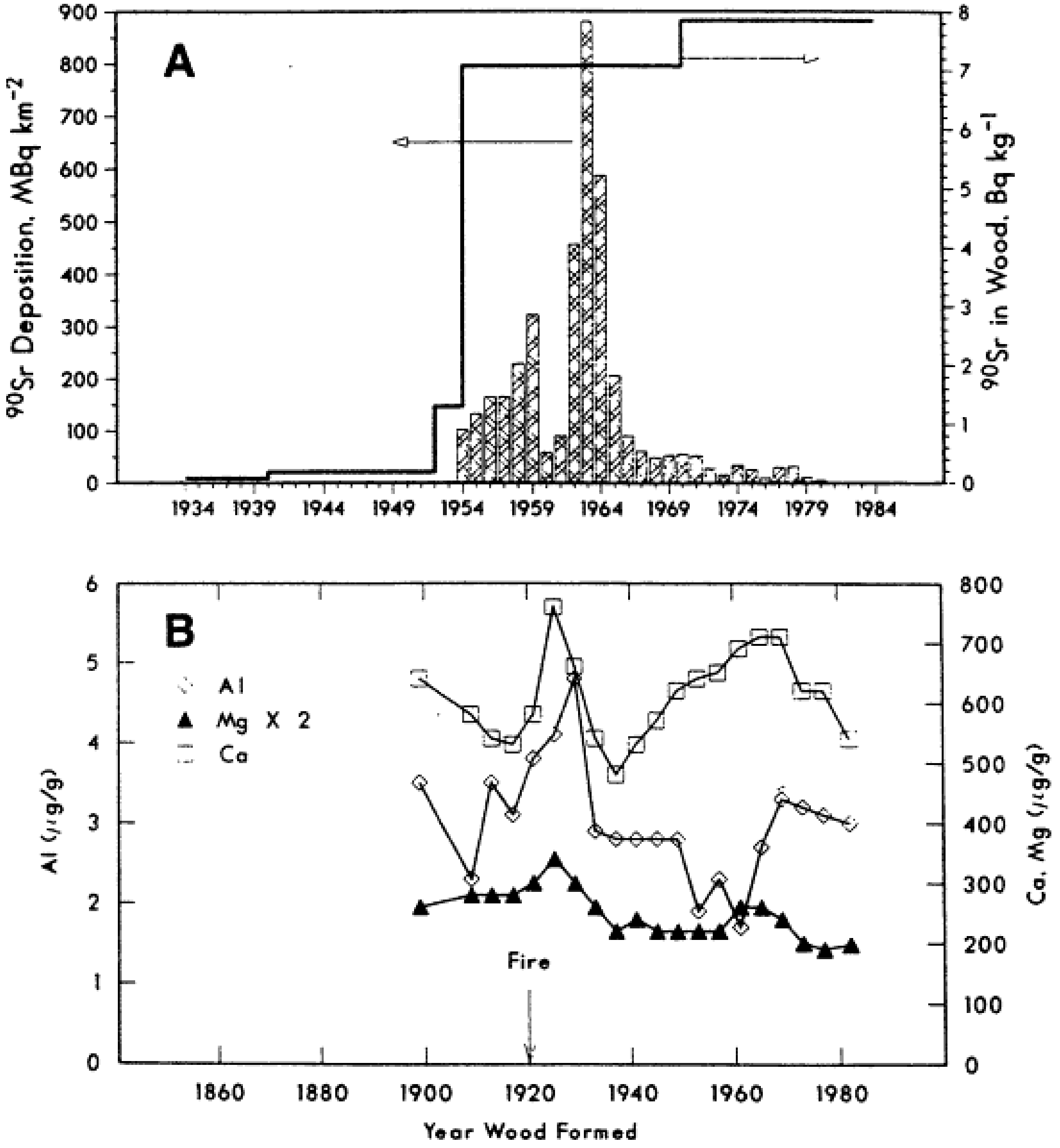


Figure 2
Examples of historical changes in cation availability: (A) the concentrations of fallout ^{90}Sr in increments of a red spruce stem section from the Great Smoky Mountains National Park (solid line) compared to the deposition history of ^{90}Sr in New York (bars): (B) the concentration trends of Al, Ca, and Mg in a red spruce core sampled in 1982 at Double Springs Gap, Great Smoky Mountains National Park, where a fire occurred in the 1920s.

trends in this core show rather pronounced Al, Ca, and Mg maxima in wood formed shortly after the fire. While this pattern is probably a consequence of the fire (NO_3^--induced cation mobilization?), it also illustrates that fluctuations in polyvalent cation concentrations are preserved. The aluminum and calcium trends after 1940 will be discussed below.

Aluminum:Calcium Ratios in Red Spruce and Eastern Hemlock in the Great Smoky Mountains National Park

In 1983, Baes and McLaughlin sampled coniferous trees at various locations in GSMNP and measured a number of elements in 12-mm-diameter increment cores using inductively coupled plasma-optical emission spectroscopy [9]. They reported that aluminum concentrations in xylem began increasing in all species in the mid-1900s even though the concentrations of the alkaline earths, particularly Ca, were often declining during this same period. When Al:Ca ratios were calculated [10], the 1940s stood out as the period when xylem aluminum began increasing at various high-elevation locations in the Park. Our hypothesis is that aluminum mobilization in the soil probably accounts for these increases because regional SO_x and NO_x and emissions also increased substantially in the 1940s.

Soils at high elevations in the GSMNP are very acid, with much less than 10% of the exchange complex saturated with base cations [14]. Figure 3a illustrates that under these conditions the Al^{3+}:Ca^{2+} ratio in the soil solution will increase as mineral acid anion concentrations increase [1]. The ratio also increases as base saturation decreases [exchangeable acidity increases). This generic example was derived using the soil chemistry model described in the appendix of Reference [1].

Fig 3b shows estimated regional SO_x and NO_x emissions upwind from GSMNP [15], illustrating the timing of increasing anion inputs into these soils. Figures 3c and 3d illustrate the trends in Al:Ca earth ratios in three GSMNP red spruce cores and an eastern hemlock (*Tsuga canadensis* L.) core representative of the data set [9,10]. In 18 of 22 red spruce and 14 of 17 hemlock cores, the average Al:Ca ratio was greater in wood formed after 1938 (a common date where a split in the growth increments was made [9]) than before. A transitory increase in the Al:Ca ratio was frequently found in wood formed around the turn of the century [10]. This increase is contemporaneous with large SO_2 emissions from open pit roasting of sulfide ore at Ducktown, Tennessee, 90 km upwind from the GSMNP [16]. These increases are consistent with the idea that this ecosystem contains soils which are very sensitive to Al^{3+} mobilization by mineral acid anion inputs. A comparable example may exist in red cedar measurements made in the lead-mining region of Missouri, where increases in wood aluminum coincide with PbS ore production histories [17].

Calcium concentrations in the spruce core illustrated in Fig. 2b begin increasing in the 1940s, possibly reflecting the mobilization of a Ca^{2+} pool originally released by the fire (Fig. 2b). Aluminum begins increasing at a later date than calcium. No comparable example exists in the rest of the GSMNP data or in the two highest elevation Vermont data where such a sustained increase in calcium concentrations occurs. Similar calcium trends do occur in four out of five of the Vermont cores from 550 m elevation [10].

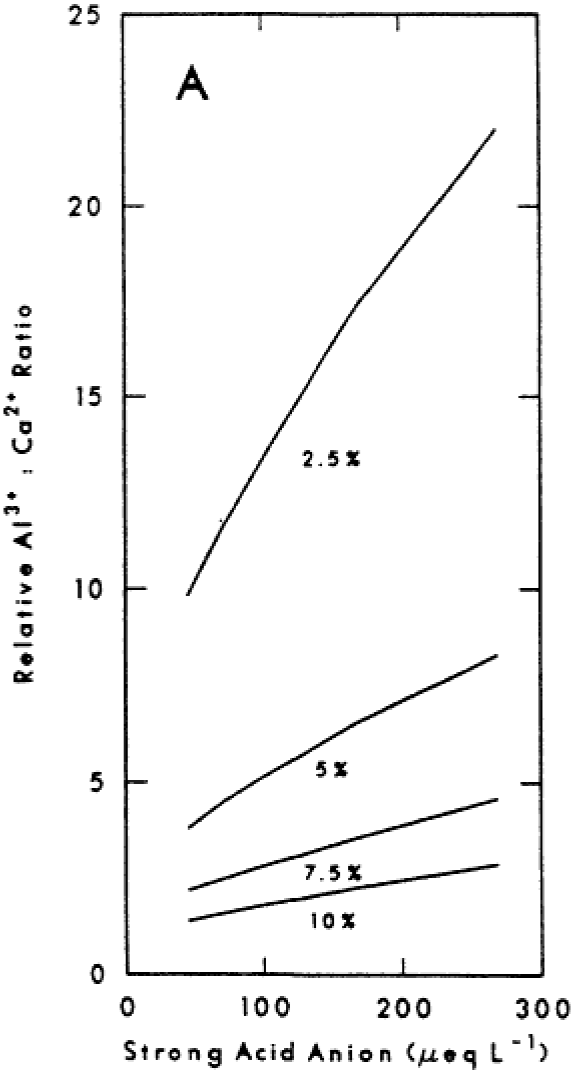


Figure 3a.

Potential interrelationships between atmospheric deposition and aluminum availability to trees: a generic model of the effect of increasing soil solution concentrations of mineral acid anions on the solution Al^{3+}:Ca^{2+} ratios in soils with 2.5 to 10% base saturation.

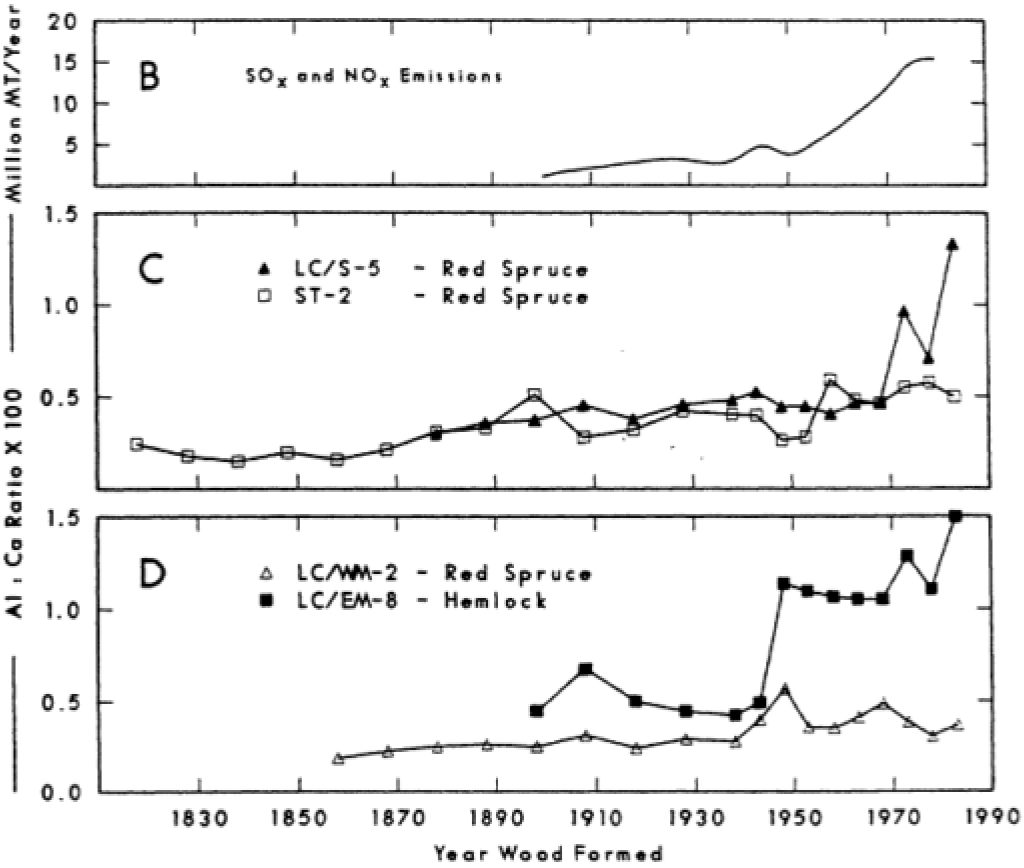


Figure 3b,c,d.

Potential interrelationships between atmospheric deposition and aluminum availability to trees: (B) historical emission trends of SO_x and NO_x upwind from the Great Smokey Mountains National Park; the trends in Al:Ca ratios in (C) individual red spruce sampled at Mt. LeConte summit (LC/S) and at Mt. Sterling (ST), in the Great Smokey Mountains National Park, and (D) in a red spruce sampled at Rainbow Falls (LC/WM) and an eastern hemlock sampled at Trillium Gap (LC/EM), also in the Great Smokey Mountains National Park.

Figure 4 presents both Al:Ca and Al:Mg ratios (4a) as well as concentration trends (4b) in a spruce core from Mr. LeConte in GSMNP. It illustrates that aluminum increases are responsible for most of the increases in aluminum ratios, and that calcium and magnesium concentrations generally decline regardless of how aluminum concentrations are changing. Another feature of the data set in Figure 4 is that radial growth declines frequently coincide with increases in aluminum or aluminum ratios. Using the Spearman Rank Order Correlation test [18], we have found that after the 1938 increment, over 50% of the spruce and hemlock cores have a significant (95% confidence level) inverse relationship between radial growth and the Al:Ca ratio. No such strong relationship was found before 1938. Increasing aluminum availability either coincides with or is linked to radial growth declines.

Another perspective on both the increase in Al:Ca ratios and the decreases in radial growth can be obtained by plotting Al:Ca ratios vs. radial growth for two sets of cores sampled at sites only 37 km apart and at similar elevations [9]. Figure 5 shows the four cores from Indian Gap, where the Al:Ca ratio increased over three-fold after 1938 (Fig. 5a), and the four cores from Mr. Sterling, where the ratio did not even double (Fig. 5b). The four cores from Indian Gap indicate that slower radial growth rates coincide with the high Al:Ca ratios.

Another difference between the two sets of cores illustrated in Figure 5 is that at Indian Gap an abrupt decrease in the amount of calcium stored in wood in proportion to magnesium (the Ca:Mg ratio) occurred in the 1940s at the same time that the Al:Ca ratio increased (5a). The Mt. Sterling cores (not illustrated) showed smaller changes in the historical Ca:Mg ratio trend in only two cores. The coincidence between the aluminum increases, the Ca:Mg decreases, and the radial growth declines at Indian Gap are probably not fortuitous. It has long been known that even adding neutral salts like $CaSO_4$ or KCl to very acid soils mobilizes aluminum and reduces plant growth [19,20] and that the ratio of aluminum or other cations to calcium is critical in the calcium nutrition of trees [e.g., reference 21].

CONCLUSIONS AND RECOMMENDATIONS

Current and Future Utility of Using Al:Ca and Other Ratios in Wood as Markers of Air Pollution Effects on Forests.

1. The trend in Al:Ca ratios in a tree growing on a relatively undisturbed site is a sensitive method of evaluating when aluminum mobilization by atmospheric deposition occurred. The measurement of Al:Ca ratios is a survey methodology now being used to compare sites where red spruce is in various stages of growth decline.
2. The use of Al:Ca ratios and radial growth rate correlations is a promising approach by which the linkage between aluminum mobilization (due to atmospheric deposition or any other cause) and growth rate declines can be evaluated. An expansion of its use is recommended. Similarly, the use of Al:Ca and Ca:Mg ratios appears to be a sensitive method for detecting altered calcium and magnesium availability during growth. An increase in aluminum availability or changes in alkaline earth behavior are not necessarily evidence of an adverse physiological effect, however.

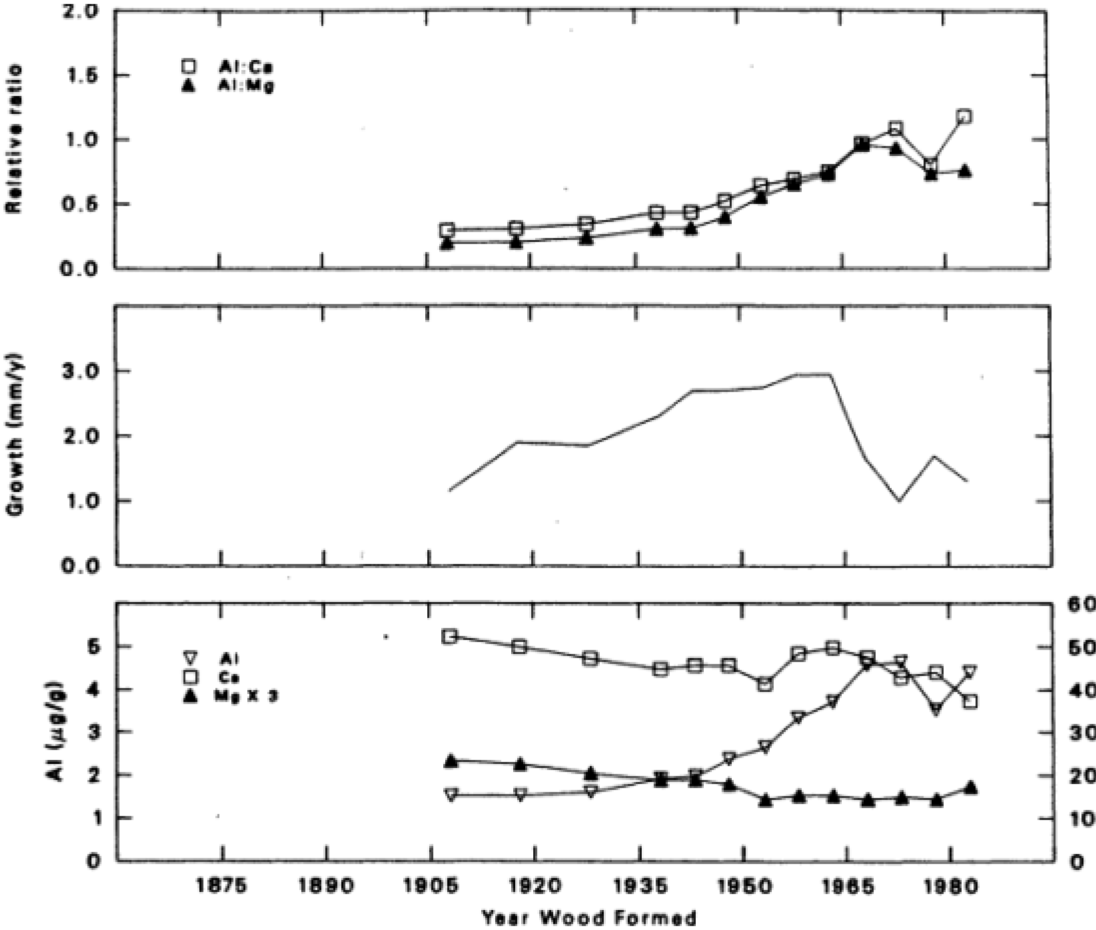


Figure 4.
The trends in Al:Ca and Al:Mg ratios (A), radial growth (B) and concentrations in a red spruce sampled at Mt. LeConte summit, Great Smoky Mountains National Park.

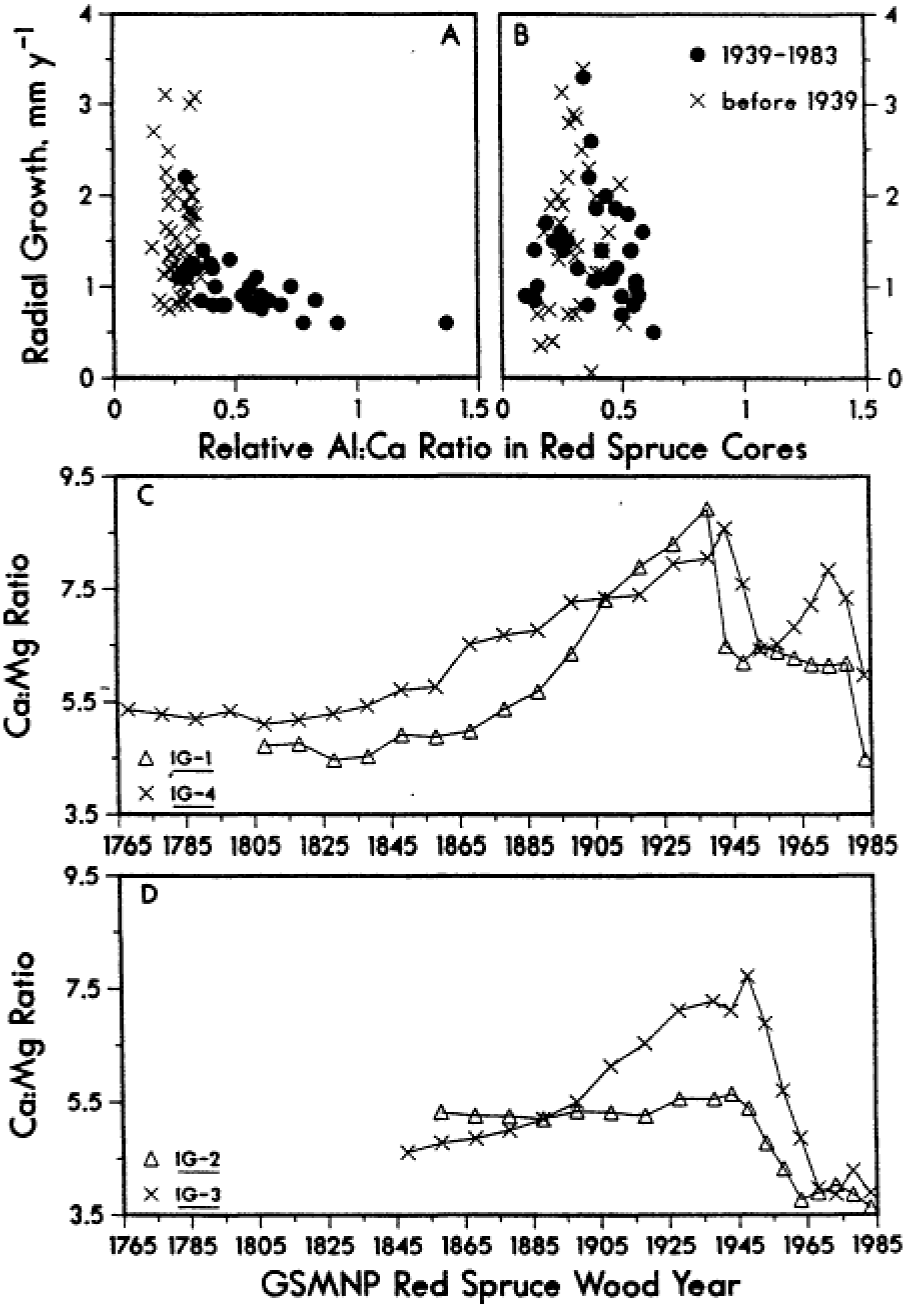


Figure 5.
A comparison of Great Smoky Mountain National Park red spruce cores from locations 37 km apart but at similar elevations: (A) radial growth vs. Al:Ca ratios (x 100) for four trees sampled at Indian Gap (1682 m elevation), and (B) for four trees sampled at Mt. Sterling (1781 m elevation). Subplots (C) and (D) show that an abrupt decrease in Ca:Mg ratios occurred in the four Indian Gap (IG) cores in the decade of the 1940s.

3. Although not discussed here, Mn:Ca ratios in wood generally do not change when Al:Ca ratios change, strongly suggesting that aluminum mobilization, not net base cation loss, is the cause of the increased aluminum availability to trees. This comparison has utility in evaluating watershed-scale effects of acidic deposition on forest soils.
4. To capitalize on the large inventory of existing increment cores and associated evaluation data, we suggest that research on analytical applications of (for example) laser ionization-mass spectrometry be supported. This is a promising technology which uses a laser to vaporize solids (in this case wood in mounted increment cores). The volatile elements are then fed into a mass spectrometer for analysis. With such a system, the Al:Ca trends in existing increment core sets could be evaluated without the necessity of additional field sampling.

ACKNOWLEDGMENTS

Publication No. 3126, Environmental Sciences Division. Research sponsored by the Oak Ridge National Laboratory and the Electric Power Research Institute (Integrated Forest Study on Effects of Atmospheric Deposition). Oak Ridge National Laboratory is operated by Martin Marietta Energy Systems, Inc., under Contract No. DE-AC05-84OR21400 with the U. S. Department of Energy.

References

1. J. O. Reuss and D. W. Johnson. 1986. Acid Deposition and the Acidification of Soils and Waters (Springer-Verlag, New York).
2. I. B. Ferguson and E. G. Bollard. 1976. The movement of calcium in woody stems. Ann. Bot., 40:1057-1065.
3. J. F. McGrath and A. D. Robson. 1984. The movement of zinc through excised stems of seedlings of *Pinus radiata* D. Don. Ann. Bot., 54:231-242.
4. S. Saka and D. A. I. Goring. 1983. The distribution of inorganic constituents in black spruce wood as determined by TEM-EDXA. Mokuzai Gakkaishi, 29:648-656.
5. H. Riekerk. 1967. The movement of phosphorus, potassium, and calcium in a Douglas-fir forest ecosystem. Ph.D. dissertation, University of Washington, Seattle.
6. H. R. Orman and G. M. Will. 1960. The nutrient content of *Pinus radiata* trees, New Zealand J. Sci., 3:510-22.
7. C. V. McMillan. 1970. Mineral content of loblolly pine wood as related to specific activity, growth rate, and distance from pith. Holzforschung, 24:152-157.
8. R. Tout, W. Gilboy, and N. Spyrou. 1977. Neutron activation studies of trace elements in tree rings. J. Radioanal. Chem., 37:705-709.
9. C. F. Baes III and S. B. McLaughlin. 1986. Multielemental Analysis of Tree Rings: A Survey of Coniferous Trees in the Great Smoky Mountains National Park. Report ORNL-6155 (National Technical Information Service, Springfield, Virginia).

10. E. A. Bondietti and C. F. Baes III. 1988. Multielemental Analysis of Tree Cores from the Great Smoky Mountains National Park, a Supplement. Report ORNL-6155/S, Oak Ridge National Laboratory. (in prep.)
11. M. A. S. Burton. 1985. Tree Rings. Pp. 175-202 in Historical Monitoring, MARC Report Number 31. Monitoring and Assessment Research Centre, University of London, U.K.
12. T. G. Siccama. 1974. Vegetation, soil and climate on the Green Mountains of Vermont. Ecol. Monographs, 44:325-349.
13. J. R. McClenahen, J. P. Vimmerstedt, and R. C. Lathrop. 1987. History of the chemical environment from elemental analysis of tree rings. Report CONF-8608144, Technical Information Center, U.S. Department of Energy, Oak Ridge, in Proceedings, International Symposium on Ecological Aspects of Tree-Ring Analysis, pp. 690-698 (August 17-21, 1986, Tarrytown, New York).
14. R. J. McCracken, R. E. Shanks, and E. E. C. Clebsch. 1962. Soil Morphology and Genesis at higher elevations of the Great Smoky Mountains. Soil Sci. Soc. Am. Proc., 26:384-388.
15. S. B. McLaughlin, D. J. Downing, T. J. Blasing, B. L. Jackson, D. J. Pack, L. K. Mann, and T. W. Doyle. FORAST Data Base Documentation. U.S. Environmental Protection Agency Report (in press).
16. C. F. Baes III and S. B. McLaughlin. 1984. Science, 224:494-497.
17. R. Guyette and E. A. McGinnes, Jr. 1987. Potential in using elemental concentrations in radial increments of old growth eastern red cedar to examine the chemical history of the environment. Report CONF-8608144, Technical Information Center, U.S. Department of Energy, Oak Ridge, Tennessee, in Proceedings, International Symposium on Ecological Aspects of Tree-Ring Analysis, pp. 671-680 (August 17-21, 1986, Tarrytown, New York).
18. SAS Users Guide. 1986. SAS Institute, Inc., Cary, North Carolina.
19. Fried, M. and M. Peech. 1946. The comparative effects of lime and gypsum upon plants grown on acid soils. J. Amer. Soc. Agron., 38:614-633.
20. Ragland, J. L., and N. T. Coleman. 1959. The effect of soil solution aluminum and calcium on root growth. Soil Sci. Soc. Am. Proc., 23:355-357.
21. Lyle, E. S. Jr., and F. Adams. 1971. Effect of available soil calcium on taproot elongation of loblolly pine (*Pinus taeda* L.) seedlings . Soil Sci. Soc. Am. Proc., 35:800-805.

Carbon Allocation Processes as Indicators of Pollutant Impacts on Forest Trees

McLaughlin, S. B.
Environmental Sciences Division Oak Ridge National Laboratory Oak Ridge, Tennessee 37831-6038.

ABSTRACT

The physiological processes linking carbon assimilation and net primary production in forest trees offer a broad spectrum of reference points for documenting, evaluating, and predicting the effects of atmospheric pollutants on forests. Measurements of photosynthesis, dark respiration, leaf maintenance costs, energy storage reserves, secondary metabolites associated with plant resistance to pathogens, dry matter partitioning, and patterns of annual radial growth of forest trees represent useful indicators of pollutant effects that encompass levels of detection ranging from short term mechanistic processes to longer term responses that integrate seasonal or multi-year effects. Productive utilization of measurements of these processes requires that particular emphasis be placed on (1) concurrent examination of multiple processes, (2) integration of information on these processes into a whole tree physiological context and (3) seasonal integration of temporal variations in the magnitude of measured responses. Collectively these processes can provide much-needed tools for evaluating qualitative and quantitative changes in growth and physiological resilience of forest trees in relationship to chronic air pollutant exposure regimes.

The effects of atmospheric pollutants on forests have been documented at scales of resolution ranging from biochemical and cytological alterations to changes in community dynamics and structure (Mudd and Kozlowski, 1975). Effects on carbon allocation are particularly important in understanding the causes and consequences of these effects because of the pivotal role that carbon plays not only in biomass accumulation, but also in nutrient and water use capacity of forest trees (Mclaughlin, 1988a). The balance of carbon assimilated from the atmosphere and distributed to the many sinks within forest trees plays the pivotal role not only in the amount of growth, but also in the many processes that determine the resistance and resilience of growth processes in the face of environmental stress (McLaughlin and Shriner, 1980).

The carbon allocation pathways and processes that link gross primary production and net primary production offer many reference points for evaluating pollutant effects at scales of resolution ranging from mechanistic to community level (Figure 1). Included among the effects of primary concern are reduced photosynthesis, increased respiration, reduced translocation, and changes in patterns of storage, mobilization, and utilization of energy storage reserves. These reference points collectively represent a powerful system

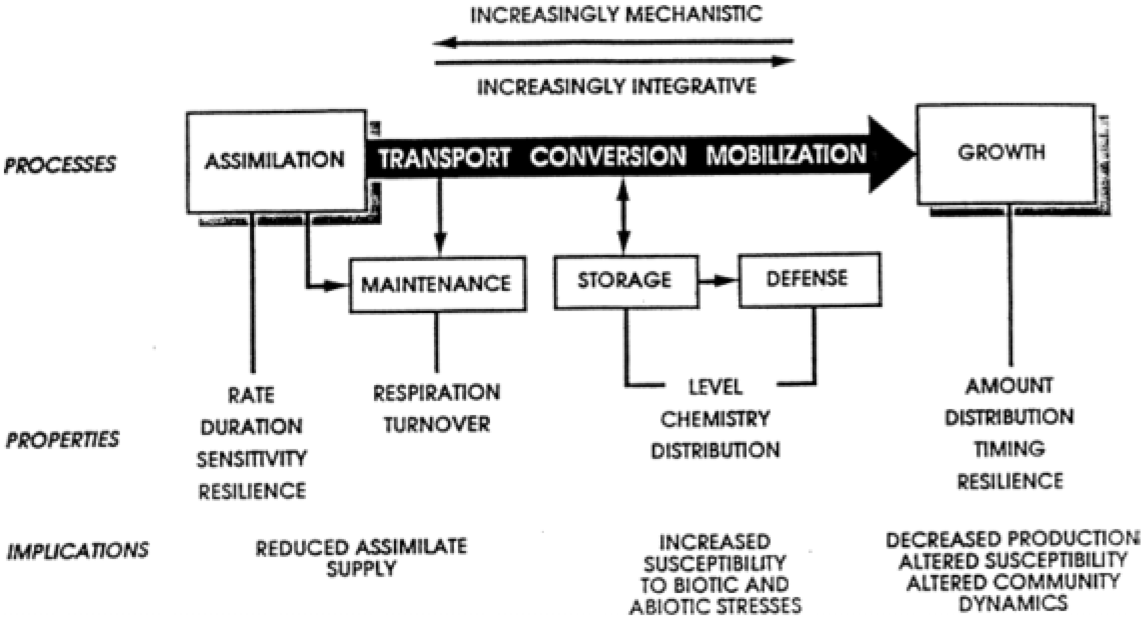


Figure 1.
Some components of carbon allocation pathways that provide useful endpoints in evaluating mechanisms of action and implications for impacts of air pollutants on growth potential of forest trees (after McLaughlin, 1988a).[1,2]

---

[1] Research sponsored by the USDA, National Acid Deposition Assessment Program under Interagency Agreement 40-1647-45 with the U.S. Department of Energy under contract DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc.

[2] Publication No. 3128, Environmental Sciences Division, ORNL.

of indicators that can be used both analytically to determine whether forests have been impaired physiologically and diagnostically to determine the principal points of impact and likely causes of those impacts.

In examining the effects of pollutants or stress in general on tree growth and physiology, it is important to view stress and response from a whole tree perspective (McLaughlin, 1988b). The concepts of whole plant allocation are particularly important to understanding the effects of pollutants on tree physiology since both sources and sinks for carbon may be influenced by pollutant deposition processes. This alteration may take the form of either a decrease or an increase in the activity of sources or sinks within the tree. Such changes may alter the patterns of tree growth as well as altering tree responses to other environmental stresses.

In general we know considerably more about the assimilation of carbon and associated energy and the net annual increment of biomass, which we measure as growth, than about the many important processes that link the two. As an example, the obvious pivitol role of photosynthesis as the source of carbon and energy for tree growth and maintenance processes is reflected in the numerous studies of tree photosynthesis (Shaedle, 1975). However, relatively little emphasis has been directed toward understanding subsequent allocation processes, which, in forest trees, may consume from 30 to approximately 80% of the energy captured in gross photosynthate (Kira, 1975). Thus, the processes of respiration, translocation, allocation, and biosynthesis may be equally as important as photosynthetic capacity in determining levels of productivity (Evans, 1975). Where the vigor of forest trees is reduced by stress, relatively small changes in efficiency of carbon allocation may have major consequences for the physiological integrity of trees. In spite of the potential importance of these processes, to date they have played a relatively minor role in efforts to quantify, characterize and predict the effects of pollutants or other stresses on forest physiology and growth. This paper briefly describes the basis of interest and information needs on four pivotal processes in the carbon allocation pathways: carbon assimilation, dark respiration, translocation and partitioning, and mobilization.

Carbon assimilation. The exchange of carbon, both photosynthetic uptake and respiratory losses, by foliage of forest trees has been an obvious focal point in many studies aimed at evaluating tree growth potential. With respect to air pollutant impacts, changes in photosynthesis particularly have figured prominantly in efforts to understand the concentration threshold for physiological responses (Botkin et al., 1972), characterize differences in sensitivity among genotypes of the same species (Boyer et al., 1986, Eckert and Houston 1980) or evaluate comparative sensitivity across a variety of different species (Oleksyn and Bialobok, 1986 and Reich and Amundson, 1985).

There are many dimensions of net photosynthesis (Pn) that can provide important insights into the impacts of pollutants on photosynthate production. These include both maximum capacity under saturating radiation levels, the light response curves (including the compensation point), kinetics of response to both pollutants and changes in radiation and the patterns of change in response over diurnal and seasonal cycles, and the distribution of Pn capacity within tree crowns both as a function of foliage age and position within the crown (McLaughlin, 1988c). In addition to measures of Pn capacity, Pn response surfaces to light (Hanson et al., 1987) and to CO_2 (Farquhar and Sharkey, 1982) offer possibilities for evaluating the efficiency of leaf photosynthetic processes.

Measurements of Pn at any point in time, while they may provide important information on the integration of exposure effects to that time, may not adequately describe the past or future kinetics of the photosynthetic system. Boyer et al. (1986)

indicate that Pn of white pine recovered following exposure to ozone at 0.05 ppm (6 h/d) but decreased more rapidly on each successive day of exposure, thus suggesting progressive impairment of the photosynthetic system. The manner in which response and recovery systems operate over time to determine seasonal influences on carbon assimilation capacity is an important issue. As an indicator of pollutant stress Pn has two dimensions: (1) the initial basal rate may be directly related to sensitivity to uptake of gaseous pollutants and hence the potential for Pn reduction. and (2) as an indicator of longer term capacity for the integration of pollutant and other stresses over the life of the foliage, decreases in Pn reflect sensitivity to deterioration of the integrity of photosynthetic or systems or (less likely) reductions in demand for assimilates.

It should be noted that compensatory factors may partially offset the effects of stress on photosynthetic systems. The capacity of foliage of some plants to respond to a decrease in source-to-sink ratio by increasing Pn efficiency may be an important characteristic determining tree resilience to foliar damage (McLaughlin and Shriner, 1980). Replacement of damaged foliage is, of course, another possibility for indeterminate species (Coleman, 1986). Because of such compensatory factors, reductions in growth can not be predicted on a 1:1 basis from reductions in Pn. Reich and Amundson (1985), for example, found that while reductions in growth were linearly related to reductions in photosynthesis, compensatory processes resulted in final growth reductions of plants exposed under laboratory conditions that ranged from 20 °/o for tree seedlings to about 60 % for crops.

In contrast to results from studies with O_3 (Reich, 1987), there is little indication to date that acid precipitation at ambient levels adversely affects carbon assimilation (Reich et al., 1986 and Hanson and McLaughlin, 1987). By contrast, Taylor et al. (1987) found that acid mist at pH 3.0 stimulated overall Pn capacity of red spruce seedlings due to increased foliar area produced at higher acidity and associated nitrogen levels.

Much additional work is needed to understand the likely effects of acid deposition on carbon assimilation processes. This task is made particularly complex and interesting by the likely influences on leaf physiology of nitrogen and other nutrients present in acid rain. The potential stimulation of foliage growth by deposition of nitrogen may increase plant sensitivity to moisture stress as observed with greenhouse grown red spruce seedlings by Norby et al. (1986). Results such as these emphasize the advantages of examining process responses to pollutants such as acid deposition from a whole plant perspective.

Dark respiration. To date relatively little emphasis has been placed on pollutant-induced effects on dark respiration (Rs). However, stimulation of dark respiration is an expected consequence of plant repair mechanisms (McLaughlin and Shriner, 1980) and may deplete as much or more carbon from available energy pools as reduced photosynthesis. Increased dark respiration may be particularly significant when coupled with reduced rates of Pn, and in fact, reduced Pn may be a consequence of increases in light respiration, a component of the assimilation process that has received little emphasis with respect to pollutant effects.

Barnes (1972) detected a reduction of photosynthesis (-10 °/o average) and stimulation of dark respiration (+33% average) in seedlings of three species of southern pines exposed to 0.15ppm O_3 under laboratory conditions. McLaughlin et a1. (1982) found that Rs was stimulated approximately 15% while photosynthesis was reduced only 6% in mature field grown white pine trees, thus showing high apparent sensitivity to ambient levels of ozone in east Tennessee.

Changes in the Pn:Rs over a range of temperature and light conditions could provide a more complete picture of alterations in assimilate supply. Obviously, increased emphasis on dark respiration is warranted in research aimed at accurately quantifying pollutant impacts on total assimilate available for export from foliage.

Translocation. The transport of assimilates away from production centers to points of utilization within the tree may be examined either at the leaf level or at the whole plant level. At the whole plant level there is good evidence that air pollutants may exert significant effects on plant productivity by altering partitioning of dry matter between plant parts (Manning, 1978, Oshima et al., 1979, Tingey, 1978, and Tingey et al., 1976).

At the leaf level, shifts in translocation may occur either as a consequence of interference of pollutants with the loading of the phloem with assimilates or as a consequence of increased assimilate demand by foliage. Internal demand in turn may be enhanced by either altered foliar nutrition (nitrogen assimilation directly by foliage from the atmosphere) or as a damage response requiring increased internal maintenance and repair. A review of studies with several plant species indicates that internal costs of maintaining leaf functions are high (McLaughlin and Shriner, 1980). These maintenance costs would be expected to be enhanced by exposure to pollutants at levels high enough to cause metabolic or cytologic injury. Several studies under both laboratory (Jones and Mansfield, 1982, Noyes, 1980, Teh and Swanson, 1982, and Tingey, 1978) and field conditions (McLaughlin et al., 1982) have indicated that carbohydrate translocation may be both sensitive to exposure to air pollutants and useful as a general indicator of pollution related stress.

At present the level of understanding of the aboveground system is far beyond that for the belowground system and the reasons for this are obvious. Yet belowground processes including both maintenance and turnover of fine roots may play a major role in whole tree energy budgets (Harris et al., 1974 and Persson, 1980) and are likely to be particularly sensitive to pollutant impacts (McLaughlin, 1988b). Measures of pollutant-induced impacts on transport of assimilates to support the belowground system are sorely needed as they relate directly to the functional integrity and to uptake of both water and nutrients by the root/rhizosphere system.

Storage and Mobilization. With respect to evaluating impacts of air pollutants on tree production potential, the storage reserves provide a potentially useful and temporally integrative indicator of the carbohydrate economy of the tree and its capacity both to meet the energy demands of annual growth cycles and to resist insects and disease. Resistance to disease may be a particularly important characteristic because allocation of resources to formation of protective chemicals appears to be of relatively low priority when energy reserves are in low supply (Mooney and Chu, 1974). Allocation of energy reserves to the production or maintenance of new foliage or roots represents a high priority that supercedes the demands for defensive strategies when trees are under stressed conditions (Waring and Pittman, 1985).

Plants have a wide variety of strategies for defense against diseases (Horsfall and Cowling, 1980), and many of those defenses could be weakened by the chronic exposure to atmospheric pollutants (Hain, 1987), particularly when alterations in carbon allocation occur. Phenolic compounds, tannins, and proteins are examples of metabolites that are considered important in host defenses against microbial attack (Schloesser 1980). Perhaps the best example of the importance of secondary metabolites to disease resistance of forest trees under pollutant stress has come from research in the San Bernardino Forest where increased susceptibility of oxidant-stressed ponderosa pine to attack from bark beetles was noted (Stark et al., 1968). Increased susceptibility to beetle attack was associated with both qualitative and quantitative changes in the oleoresin production of affected trees (Miller et al., 1968 and Cobb et al., 1968). Additional potentially useful situations in which the energy reserve status of forest trees may be playing an important role in host-pathogen relations include the southern pine bark beetle and balsam fir/woody aphid associations in the southeastern U.S. Both insect outbreaks have occurred in areas in which air pollutants are under investigation as contributing factors to forest growth decline.

Since trees store energy in many different biochemical forms, the question of which constituent to examine as an overall indicator of tree energy reserves is of obvious relevance. Kramer and Kozlowski (1960, 1979) have grouped forest tree species into those that store food reserves primarily as fats (principally diffuse-porous species), starch (primarily ring-porous species), and a third group that utilizes both forms. Regardless of the primary storage form, starch appears to be a significant constituent of energy storage in roots of most tree species (Ziegler, 1964). As an indicator of storage reserves, root starch offers apparent advantages over starch in foliage, which is more readily influenced by short-term climatic fluctuations (Adams et al., 1986). Studies with defoliating insects (Gregory and Wargo, 1986 and Wargo, 1981) indicate that starch content of xylem and roots provides a useful index of whether stress has occurred and of the vulnerability of trees to additional stress effects from either insects or other natural factors that induce mortality.

While starch may be a useful indicator of the vigor of root systems of pollution-stressed trees, it should be noted that interpretation of root starch data must be made within the context of the physiological status of the whole tree (Ericsson, 1980). Starch depletion may reflect either stimulated utilization of storage reserves during rapid growth or diminished capacity of the tree to assimilate carbohydrates rapidly enough to replace those utilized in normal growth and maintenance demands. Similarly short-term increases in starch accumulation may occur when utilization of assimilates is slowed by growth inhibition at the site of utilization.

The preceding discussion has focused on process level measurements as indicators of pollutant induced alteration of carbon allocation patterns. It should be noted in closing that useful evidence of altered carbon allocation patterns can also be derived from the observed patterns of growth itself (see Fig. 1). The timing, duration, response and recovery cycles following natural or anthropogenic stresses, and density and quality of wood formed all provide potentially useful indicators of the nature and causes of impaired tree vigor (McLaughlin, 1988b). Dendrochronology is an emerging discipline which has tremendous potential for examining the patterns and potential causes of annual growth patterns at annual and longer time scales (Cook, 1986). Observed response patterns at the stem level can provide testable hypotheses about basic physiological processes strongly influencing growth of a species as well as likely causal factors leading to disruption of the growth process (McLaughlin et al., 1987).

Several summary points can be made regarding the use of carbon allocation pathways to identify and diagnose responses of trees to air pollutants: (1) There are many reference points within plant carbon allocation pathways that respond to air pollutants and offer potentially useful diagnostic criteria for detecting pollution-induced damage. These occur at many levels of organization within the tree; (2) It is essential to recognize that allocation processes are tied to both the supply and demand of carbon, water, and nutrient resources and hence will be influenced by a wide variety of natural stresses. These stresses and the responses they induce must be considered concurrently with pollutant stresses. They may either be minimized by experimental design or used as modifiers to test the nature or consequences of pollutant-induced stresses; (3) The separation of natural phenological and diurnal patterns of response from those that may be induced by air pollutants requires that one consider these inherent response-recovery cycles in evaluating shifts in carbon allocation pathways; (4) Approaching the problems of detection and diagnosis of changes of carbon allocation processes can most effectively be accomplished from a whole-tree physiological perspective that recognizes and tests physiological interrelationships among both processes and plant parts.

Within this context an analytical framework can be suggested that first documents the magnitude and patterns of change in a biological indicator or indicator system and then tests both the physiological basis of the measured response as well as its consequences at successively higher levels of whole plant integration. The physiological basis must be understood to address adequately the causal relationships and the range of possible consequences, while the more integrative measures help document the range and probabilities of actual responses observed under field conditions. Consideration of both growth and process level responses within the carbon allocation system provides inferential cross references that can substantially improve our ability to address the inherent complexity and many uncertainties of forest decline issues.

ACKNOWLEDGMENTS

The author wishes to thank Drs. George Taylor, Chris Anderson, and Tim Tschaplinski of Oak Ridge National Laboratory for their review of this manuscript.

References

Adams, M.B., H.L. Allen, and C.B. Davey. 1986. Accumulation of starch in roots and foliage of loblolly pine (*Pinus taeda* L.): effects of season, site and fertilization. Pp. 35-46 in R.J. Luxmoore, J.J. Landsburg, and M.R. Kaufmann, Coupling of Carbon, Water, and Nutrient Interactions in Woody Plant Soil Systems. Tree Physiology, Vol. 2. 467 pp.

Barnes, R.L. 1972. Effects of chronic exposure to ozone on photosynthesis and respiration of pines. Environ. Pollut. 3: 133-138.

Botkin, D.B., W.H. Smith, R.W. Carlson, and T.L. Smith. 1972. Effects of ozone on white pine saplings: Variation in inhibition and recovery of net photosynthesis. Environ. Pollut. 3: 273-289.

Boyer, J.N., D.B. Houston, and K.F. Jensen. 1986. Impacts of chronic SO_2, O_3, and SO_2 and O_3 exposures on photosynthesis of *Pinus strobus* Clones. Eur. J. For. Path. 16: 293-299.

Cobb, F.W., Jr., D.L. Wood, R.W. Stark, and J.R. Parmeter, Jr. 1968. Theory on the relationships between oxidant injury and bark beetle infestation. Hilgardia 39: 141.

Coleman, J.S. 1986. Leaf development and leaf stress: increased susceptibility associated with sink-source transitions. Pp. 289-300 in R.J. Luxmoore, J.J. Landsberg, and M.R. Kaufmann, Coupling of Carbon, Water, and Nutrient Interactions in Woody Plant Soil Systems. Tree Physiology, Vol. 2. 467 pp.

Cook, E. R. 1986. The use and limitations of dendroecology in studying effects of air pollution on forests. Pp. 277-290 in Effects of Atmospheric Pollutants On Forests, Wetlands, and Agricultural Ecosystems. (T.C. Hutchinson and K.M. Meema, eds.). Springer Verlag, New York.

Eckert, R.T. and D.B. Houston. 1980. Photosynthesis and needle elongation responses of *Pinus strobus* clones to low level sulfur dioxide exposures. Can. J. For. Res. 10: 357-361.

Ericsson, A. 1980. Some aspects of carbohydrate dynamics in scots pine trees (*Pinus sylvestris* L.) Department of Plant Physiology, University of Umea, s-901 87 Umea, Sweden.

Evans, L. T. 1975. Beyond photosynthesis—the role of respiration, translocation, and growth potential in determining productivity. Pp. 695 in J.P. Cooper, (ed.). Photosynthesis and productivity in different environments. Cambridge University Press, New York.

Farquhar, G.D., and T.D. Sharkey. 1982. Stomatal conductance and photosynthesis. Ann. Rev. Plant Physiol. 33:317-345.

Gregory, R.A., and P.M. Wargo. 1986. Timing of defoliation and its effect on bud development, starch reserves, and sap sugar concentration in sugar maple. Can. J. For. Res. 16:10-17.

Hain, F.P. 1987. Interactions of insects, trees, and air pollutants. Tree Physiol. 3:93-102.

Hanson, P.J., R.E. McRoberts, J.G. Isebrands, and R.K. Dixon. 1987. An optimal sampling strategy for determining CO_2 exchange rate as a function of photon flux density. Photosynthetica 21(1):98-101.

Hanson, P. J. and S. B. McLaughlin. 1987. CO_2 exchange characteristics of *Pinus taeda* 1. shoots exposed to variable ozone levels and precipitation chemistries. Plant Physiol. Suppl. 83:81.

Harris,W.F., Sollins, P., Edwards, N.T., Dinger, B.E., and Shugart, H.H. 1975. Analysis of carbon flow and productivity in a temperate forest ecosystem. Pp. 116-122 in D.E. Reichle, J.F. Franklin, and D.W. Goodall (eds.), Productivity of World Ecosystems. National Academy of Sciences, Washington, D.C.

Horsfall, J.G. and E.G. Cowling. 1980. Plant Disease: An Advanced Treatise. Vol. 5 How Plants Defend Themselves. Academic Press, New York.

Jones, T., and T. A Mansfield. 1982. Studies on dry matter partitioning and distribution of ^{14}C-labelled assimilates in plants of *Phleum pratense* exposed to SO_2 pollution. Environ. Pollut. 28: 199-207.

Kira, T. 1975. Primary productivity of forests. Pp. 5-41 in J. P. Cooper , (ed.), Photosynthesis and productivity in different environments. Cambridge University Press, New York.

Kramer, P.J., and T.T. Kozlowski. 1960. Physiology of forest trees. McGraw-Hill, New York.

Kramer, P.J., and T.T. Kozlowski. 1979. Physiology of woody plants. Academic Press, New York.

Manning, W.J. 1978. Chronic foliar ozone injury: Effects on plant root development and possible consequences. Calif. Air Environ. 7: 3-4.

McLaughlin, S.B. 1988a. Carbon allocation as an indicator of pollutant impacts on forest trees. In M. Cannell and D. Lavender, (eds.), Proc. IUFRO symposium Plant Growth in a Changing Chemical and Physical Environment. Vancouver, BC. July, 1987. Invited presentation and paper (in press).

McLaughlin, S.B. 1985b. Whole tree physiology and forest responses to air pollutants. In Proc. Commission of European Communities Workshop Interrelationships between Above and Below Ground Influences of Air Pollutants on Forest Trees. Gennep, the Netherlands. Dec. 1987. Invited presentation and paper (in press).

McLaughlin, S.B. 1985c. The use of branch level measurements in evaluating whole plant responses to air pollutants. Pp. 165-185 in Proc. EPA/USDA Workshop on Response of Trees To Air Pollutants: The Role of Branch Chambers (W.E. Winner and L.B. Phelps, eds.). Boulder, CO. November, 1987. EPA Corvallis, OR.

McLaughlin, S.B., and D.S. Shriner. 1980. Allocation of resources to defense and repair. Pp. 407-431 in J.B. Horsfall, E.B. Cowling, eds. Plant Disease, Vol. 5, Academic Press, New York.

McLaughlin, S.B., R.K. McConathy, D. Duvick, and L.K. Mann. 1982. Effects of chronic air pollution stress on photosynthesis, carbon allocation, and growth of white pine trees. For. Sci. 28: 60-70.

McLaughlin, S.B., D.J. Downing, T.J. Blasing, E.R. Cook, and H.S. Adams 1987. An analysis of climate and competition as contributors to decline of red spruce in high elevation Appalachian forests of the eastern United States. Oecologia 72:487-501.

Miller, P.R., F.W. Cobb, Jr., and E. Zavarin. 1968. Effect of injury upon oleoresin composition, phloem carbohydrates, and phloem pH. Hilgardia 39: 135.

Mooney, H.A., and C. Chu. 1974. Seasonal carbon allocation in *Heteromales arbutifolia*, a California evergreen shrub. Oecologia 14: 295-306.

Mudd, J.B. and T.T. Kozlowski. 1975. Responses of Plants To Air Pollution. Academic Press, New York. 383 pp.

Norby R.J., G.E. Taylor, S.B. McLaughlin, C.A. Gunderson. 1986. Drought severity of red spruce seedlings affected by precipitation chemistry. Proc. Ninth North American Forest Biology Workshop, Stillwater, Oklahoma, June 15-18, 1986.

Noyes, R.D. 1980. The comparative effects of sulfur dioxide on photosynthesis and translocation in bean. Physiol. Plant Pathol. 16: 73-79.

Oleksyn, J., and S. Bialobok. 1986. Net photosynthesis, dark respiration and susceptibility to air pollution of 20 European provenances of scots pine *Pinus sylvestris* L. Env. Poll. (Series A) 40: 287-302.

Oshima, R.J., P.K. Braegelmann, R.B. Flagler, and R.R. Teso. 1979. The effects of ozone on the growth, yield, and partitioning of dry matter in cotton. J. Environ. Qual. 8: 474-479.

Persson, H. 1980. Spatial distribution of fine root growth, mortality and decomposition in young scots pine stands in Central Sweden. Oikos 34:77-87.

Reich, P.B. 1987. Quantifying plant response to ozone: a unifying theory. Tree Physiol. 3:63-92.

Reich, P.B., and R.G. Amundson 1985. Ambient levels of ozone reduce net photosynthesis in tree and crop species. Science 230: 566-570.

Reich, P.B., A.W. Schoettle, H.F. Stroo, and R.G. Amundson. 1986. Acid rain and ozone influence mycorrhizal infection in tree seedlings. J. Air Poll. Control Assoc. 36(6): 724-726.

Schloesser, E.W. 1980. Preformed chemical defenses. Pp. 161-174 in J.G. Horsfall and E.G. Cowling (eds.). Plant Disease: An Advanced Treatise. Vol. 5. How Plants Defend Themselves. Academic Press, New York.

Shaedle, M. 1975. Tree photosynthesis. Ann. Rev. Plant Physiol. 26: 101-115.

Stark, R.W., P.P. Miller, F.W. Cobb, Jr., D.L. Wood, and J.R. Parmeter, Jr. 1968. Incidence of bark beetle infestation on injured trees. Italgardia 39: 121.

Teh, K.H., and C.A. Swanson. 1982. Sulfur dioxide inhibition of translocation in bean plants. Plant Physiol. 69: 88-92.

Tingey, D.T. 1978. Effects of ozone on root processes. Calif. Air Environ. 7: 5.

Tingey, D.T., R.G. Wilhour, and C. Standley. 1976. The effect of chronic ozone exposure on the metabolite content of ponderosa pine seedling. For. Sci. 22: 234-241.

Wargo, P.M. 1981. Measuring response of trees to defoliation stress. Pp. 248-256 in The Gypsy Moth: Research Toward Integrated Pest Management. (C.C.Doane and M.L. McManus, (eds.). U.S. Forest Service Technical Bulletin 1584. U.S.D.A. Washington, D.C.

Waring, R.H., and G.B. Pitman. 1985. Modifying lodgepole pine stands to change in susceptibility to mountain pine bettle attack. Ecology 66:889-897.

Ziegler, H. 1964. Storage, mobilization and distribution of reserve material in trees. Pp. 304-320 in M.H. Zimmerman, ed. Formation of wood in forest trees. Academic Press, New York.

Photosynthesis and Transpiration Measurements as Biomarkers of Air Pollution Effects on Forests

Dr. William E. Winner
Department of General Science Weniger Hall 355 Oregon State University Corvallis, OR 97331

ABSTRACT

Gaseous air pollutants such as SO_2 and O_3 are known to alter rates of CO_2 fixation and water loss by leaves. In addition, acid deposition may increase the availability of nitrogen and other potential nutrients, thereby altering foliar nutrient content and metabolic capacity. Thus, many forms of air pollutants have the potential to affect photosynthesis, transpiration, and other important, derived features of leaves, such as stomatal conductance, CO_2 internal, and water use efficiency. Using these direct effects of air pollutants on leaf physiology as biomarkers of tree responses to air pollutants will be discussed. These physiological parameters may be useful as biomarkers because they change dynamically as pollutant exposures change, they are easy to measure with portable field equipment, they are related to long-term growth processes, and when taken together, they can provide an overview of how the leaf is linked to its environment. The fact that photosynthesis and transpiration change with season, leaf age, time of day, and with non-pollution environmental factors poses problems for using these parameters as biomarkers of air pollution. Also, the capacity for plants to compensate for stresses by reallocating resources between organs makes it difficult to extrapolate from leaf level measurements to an assessment of whole tree and canopy processes. Any attempt for such an application would require carefully established sampling protocols to define background rates of photosynthesis and transpiration, a method for defining control measurements, and an approach for putting single leaf measurements in perspective with the whole tree. Measurements of photosynthesis and transpiration may be most useful when included in a suite of parameters used in biomarker assessments.

INTRODUCTION

The idea of using physiological features of trees as markers of air pollution stress is attractive for a number of reasons. Most importantly, physiological features of trees are likely to respond in a dynamic way to air pollutants and, in so doing, indicate that air pollutants are causing important changes in the biological processes necessary for the tree to sustain itself. In addition, these physiological changes should be detectable before more overt symptoms of damage, such as foliar injury, are apparent. Another feature of physiological markers of air pollution response is that these markers can be viewed in the context of whole plant growth and survival, i.e., air pollution-caused changes in the physiological processes of plants can be linked to their productivity, longevity, and reproduction.

Many physiological parameters could be used as markers of tree responses to air pollutants. A few of these parameters, such as foliar gas emission, foliar enzyme activity levels, and cuticular integrity, are discussed in other chapters. Successful application of these leaf characteristics to monitor air pollution impact requires a detailed understanding of the way by which the leaf feature relates to environment (Fig. 1). For example, under optimal conditions, the status of a leaf parameter can be assessed. However, plants are almost always growing in the presence of naturally occurring environmental stresses, such as drought, inadequate water and nutrients, and low light. These natural stresses cause a deflection in the rate of the leaf process of interest and this deflection should be viewed as natural, normal, and with adaptive value over the longterm. Air pollutants typically cause a further deflection in the process of interest and represents the response which must be identified as the biomarker. Our capacity to see the marker depends upon our understanding of the process of interest, and the knowledge necessary to predict how the process will be influenced by naturally occurring stresses and by air pollutants.

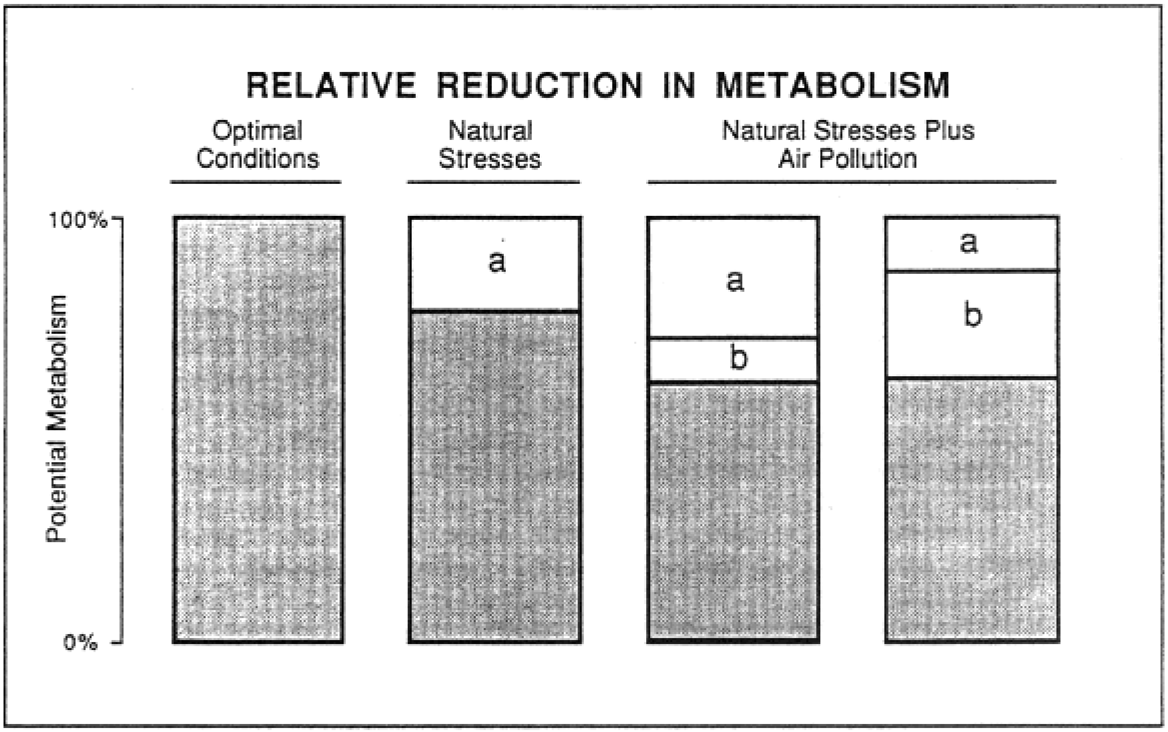


Figure 1.
The variable effects of natural stresses (a) combined with air pollutants (b) on depression of plant metabolism.

The purpose of this chapter is to highlight the use of photosynthesis, transpiration, and stomatal conductance measurements as indicators of air pollution stress. There are several reasons why assessments of photosynthesis and stomatal status should be important candidates in the attempt to identify markers of air pollution stress. For example,

1. The relationship between photosynthesis and conductance is under biological control and is related to productivity;
2. Photosynthesis and conductance are known to change quickly with the onset of air pollution exposures;
3. Stomatal conductance values can be used to calculate air pollution absorption by plants;
4. Photosynthesis and conductance can be easily measured with non-destructive techniques as well as by analysis of collected tissue.

Thus, one goal of this paper will be to expand on each of these points to clarify the unique advantages associated with the use of photosynthesis and conductance as markers of tree response to air pollutants.

There are a number of reasons why photosynthesis and conductance measurements are not currently used as biomarkers. Most importantly, it is difficult to associate changes in these leaf-level processes to specific exposures to air pollutants. In addition, calibrating photosynthetic and stomatal responses to air pollutants will prove to be difficult. Thus another goal of this paper will be to identify the barriers to the use of these measurements of leaf physiology as air pollution markers.

LINKS BETWEEN PHOTOSYNTHESIS, STOMATAL CONDUCTANCE, AND GROWTH

Stomata are small pores on leaf surfaces which can open and close. The importance of these pores is that they represent the main route of gas exchange between the leaf and air. The degree of stomatal opening is under biological control (Fig. 2) and represents one of the ways by which plants relate to environmental change (Jones 1983). The paradox for plants is that conductance should be as high as possible in order to maximize rates of carbon gain via photosynthesis, while at the same time, conductance should be such that transpiration rates are not so high that plants desiccate (Farquhar et al. 1980). Plants are thought to maximize carbon gain while minimizing water loss by continually sensing the environment and adjusting stomata to the appropriate pore size. For example, many plants close their stomata at night. This is adaptive because plants conserve water during dark periods when the light reactions of photosynthesis cannot occur. Thus, stomata respond to a CO_2 sensor. Stomata also respond to a sensor linked to plant water relations. If transpiration rates are high due to low relative humidity or limited soil water availability, many plants will close stomata. During these periods, CO_2 cannot diffuse from the air into the leaf, and plants sacrifice photosynthesis to conserve water. Although there are exceptions to the general rules above, stomata play a central role in regulating rates of carbon gain and water loss.

The rate of photosynthesis is intimately linked to growth. More than 90% of the dry weight of a plant is in the form of carbohydrate which originates from photosynthesis. Thus, the source of plant carbohydrate is known. However, the links between rates of CO_2 fixation and rates of growth are complex (Mooney, 1972). For example, one useful measure of photosynthesis is "photosynthetic capacity," a measure made on a leaf-area basis when environmental conditions are near optimal for metabolism. Plants which have high photosynthetic capacity can be small. In fact, desert annuals have among the highest photosynthetic rates but are typically small (Mooney et al., 1976). Crop cultivars which have the highest photosynthesis rates may not have the highest yield. Finally, the largest trees are often among the plants with the lowest photosynthetic capacities.

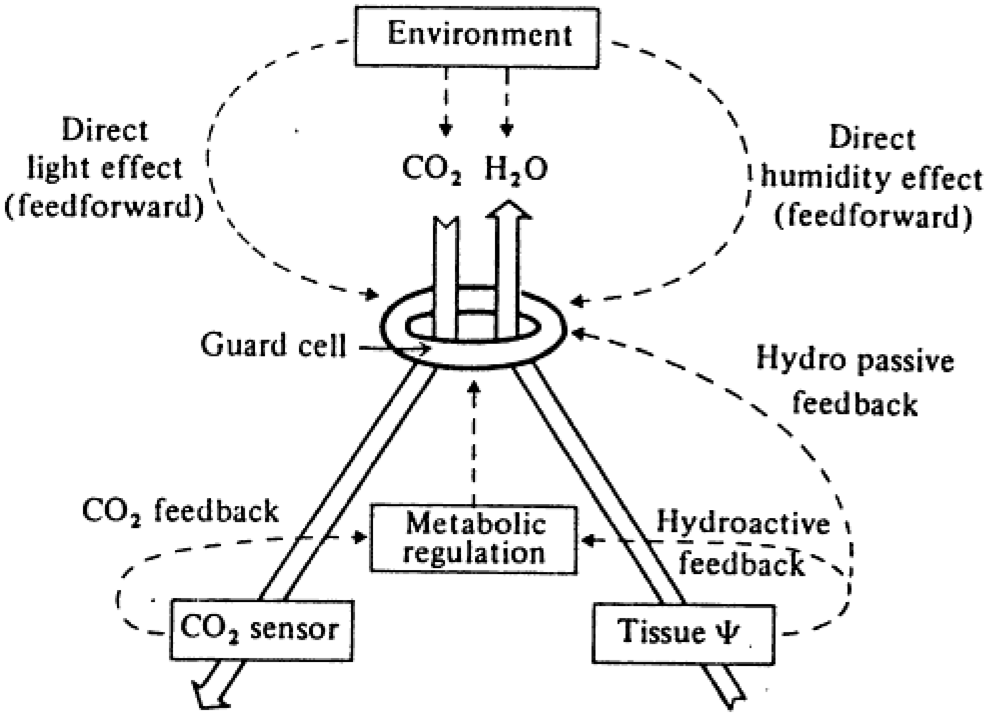


Figure 2.
Active and passive feedback control systems for regulation stomatal conductance involving CO_2. and water. Source: Reprinted with permission of Cambridge University Press from Plants and Microclimate 1983. Copyright 1983 by Cambridge University Press.

Photosynthesis is linked to growth via leaf longevity (Mooney 1972). Thus, leaves with low photosynthesis can contribute a great deal of carbohydrate to growth by remaining active for long periods. Conversely, leaves which are short-lived will not produce much carbohydrate regardless of their photosynthetic capacity.

Photosynthesis is also linked to growth via the process of carbon allocation (Monsi 1968; Mooney 1972) (Fig. 3). The product of leaf biomass and photosynthetic rate gives the carbon supply rate for the entire canopy. Some of this carbon is allocated to roots and some is allocated to shoots. The carbon allocated to shoots can be used to acquire more carbon for the plant whereas carbon allocated to roots, although essential for plant survival, does not acquire more carbon. Thus, plants with equal photosynthetic capacities, but different carbon allocation schemes will have different growth rates. Interestingly, many species differ in their patterns of carbon allocation between roots and shoots. In addition, environmental factors also cause changes in carbon allocation patterns. Atmospheric stresses which limit carbon gain shift allocation to favor the shoots whereas stresses which reduce uptake of nitrogen and water by roots shift allocation to roots. The potential for air pollutants to alter photosynthesis and growth of both roots and shoots increases the value of this physiological parameter as a biomarker of tree response to air pollution.

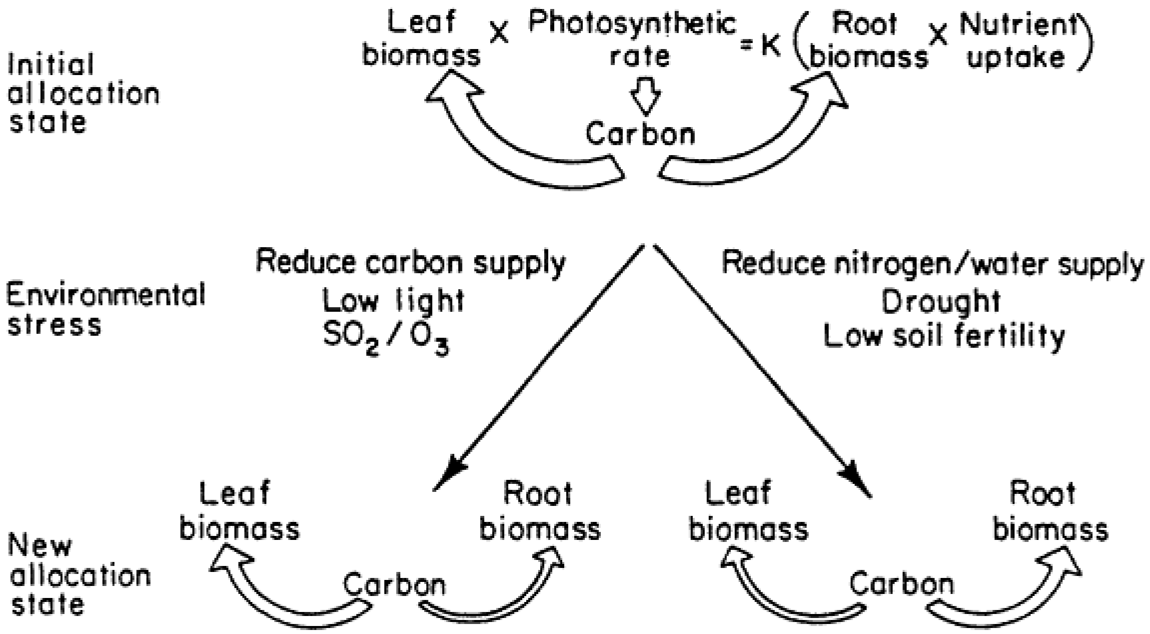


Figure 3.
Effects of environmental stresses on carbon allocation patterns of plants (Winner and Atkinson, 1986).

EFFECTS OF AIR POLLUTANTS ON PHOTOSYNTHESIS AND STOMATAL FUNCTION

Leaf-level Responses. Most forms of air pollutants have the potential to alter leaf metabolism. The effects of gaseous pollutants on CO_2 and water vapor exchange rates has been studied most thoroughly in experiments with SO_2 and O_3 (see reviews in Winner et al. 1985a; Guderian 1985). However, any gaseous pollutant known to alter plant growth will also alter both photosynthesis and conductance. The potential also exists for oxides of sulfur and nitrogen to affect leaves. More specifically, foliar nitrogen content should increase with deposition of nitrate from the atmosphere. Since photosynthetic capacity and conductance increase with foliar nitrogen content (Field and Mooney 1986), increased nutrient availability due to wet and dry deposition processes should be reflected in increased rates of leaf metabolism.

Studies with SO_2 and O_3 have shown that there are several mechanisms of stomatal response to gaseous pollutants (Fig. 4). These mechanisms may operate both at the leaf level and at the whole plant level. Leaf level responses to SO_2 and O_3 gas exchange studies have clearly shown that mechanisms of leaf level responses can be fast and extreme. For example, a radish leaf exposed to 0.4 ppm SO_2 decreased its photosynthetic rate by 75% within 10 minutes (Winner et al. 1988) (Fig. 5). In addition, stomata also responded to this treatment within 30 minutes.

MECHANISMS OF STOMATAL RESPONSES TO GASEOUS POLLUTANTS

* Leaf level

Direct effects on guard cells / epidermal cells

Indirect effects on photosynthesis

* Canopy / whole plant level

Premature leaf loss

Indirect effects on foliar N

Figure 4.
Some mechanisms of stomatal responses to gaseous air pollutants.

This simple experiment (Fig. 5) is important because it shows that a gaseous pollutant can cause stomatal closure. Many other studies have also shown that SO_2 (Winner and Mooney 1980a,b; Kimmerer and Kozlowski, 1981; Olszyk and Tingey, 1986) and O_3 (Reich and Lassoie, 1984; Olszyk and Tingey, 1986; Temple 1986,) can cause stomatal closure. Thus, the idea that severe and untimely stomatal closure might be a marker of tree response to air pollution stress seems attractive. However, other studies have shown that SO_2 (Mansfield and Majernik, 1970; Black and Unsworth, 1980) and O_3 (Evans and Ting, 1974) can also cause stomatal opening. Variation in stomatal response to SO_2 and O_3 has been recently reviewed (Winner et al. 1988). Thus, any attempts to use stomatal conductance as a marker of leaf level responses to gaseous pollutants must be framed within an understanding of factors which lead to either stomatal opening or closing. The radish fumigation experiment described above (Fig. 5) is also important because it provides a clue about why stomata can either open or close in response to air pollution exposure. SO_2 and O_3 have the potential of affecting stomata by acting directly on guard cells. In addition, it is possible for these pollutants to act first on photosynthesis which then activates the previously described CO_2 sensor (Fig. 2) and results in subsequent changes in conductance. The time sequence analysis of the radish experiment shows that SO_2 caused changes in photosynthesis before changes in conductance suggesting, that in this experiment, stomata may be responding to air pollutants via changes in carbon metabolism.

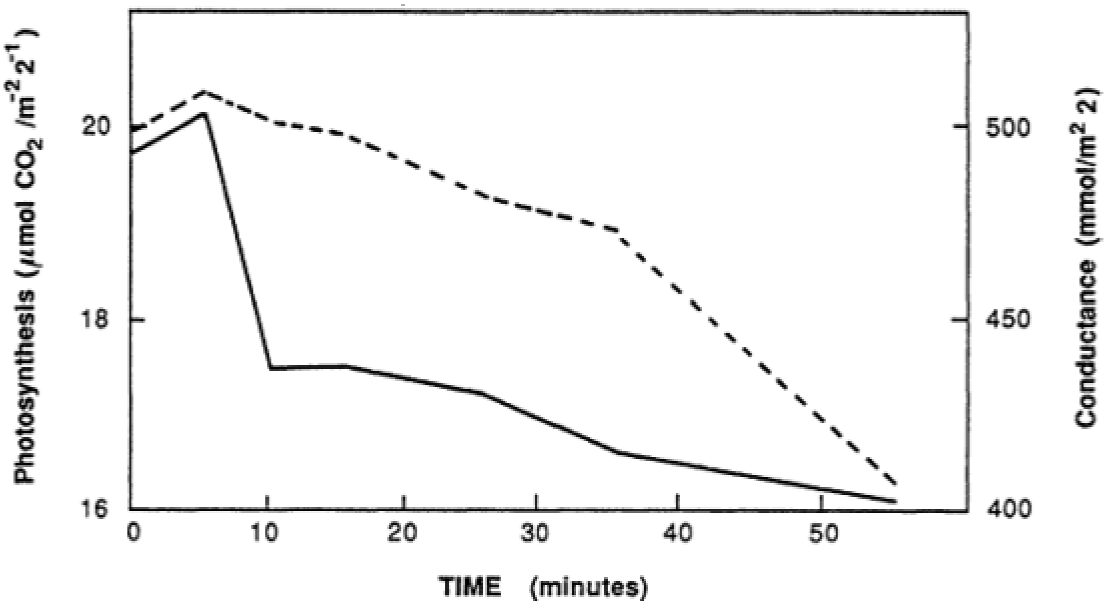


Figure 5.
Time course for changes in photosynthesis (———) and conductance (———) following the addition of SO_2 and 0.4 ppm at time 0. Ambient CO_2 concentration was 350 ppm (Winner et al., 1988).

A number of other studies have shown that SO_2 can cause changes in photosynthesis prior to changes in conductance (see summary in Winner et al., 1988). Data from many of these studies were not collected by investigators for the purpose of time series analysis, but the trends of response sequence can nonetheless be determined. Interestingly, reports indicating that stomatal responses to SO_2 precede photosynthetic responses were not found. In addition, there are no experiments with O_3 that allow such time course analyses.

If SO_2-caused decreases in photosynthesis can lead to stomatal closure, then SO_2-caused increases in photosynthesis may lead to stomatal opening. The CO_2 sensor operates by causing stomatal closure when CO_2 concentrations in the mesophyll increase. Such shifts in CO_2 would occur if SO_2 damaged the photosynthetic apparatus. Conversely, SO_2 may enhance electron transport processes associated with photosynthesis leading to increased photosynthesis and decreased CO_2 concentrations in leaves. Such a shift in CO_2 concentrations would result in stomatal opening.

Plant-level Responses. Air pollutants can also alter conductance of the entire canopy of trees by altering stomatal pore size. For example, O_3 is known to cause premature senescence and abscission of foliage (Guderian et al., 1985). One consequence of this leaf loss is a reduction in leaf surface area capable of exchanging gases between the canopy and air. Just as air pollution-caused stomatal closure is adaptive in that air pollution absorption rates are decreased, so too is the response of leaf loss a potentially adaptive response.

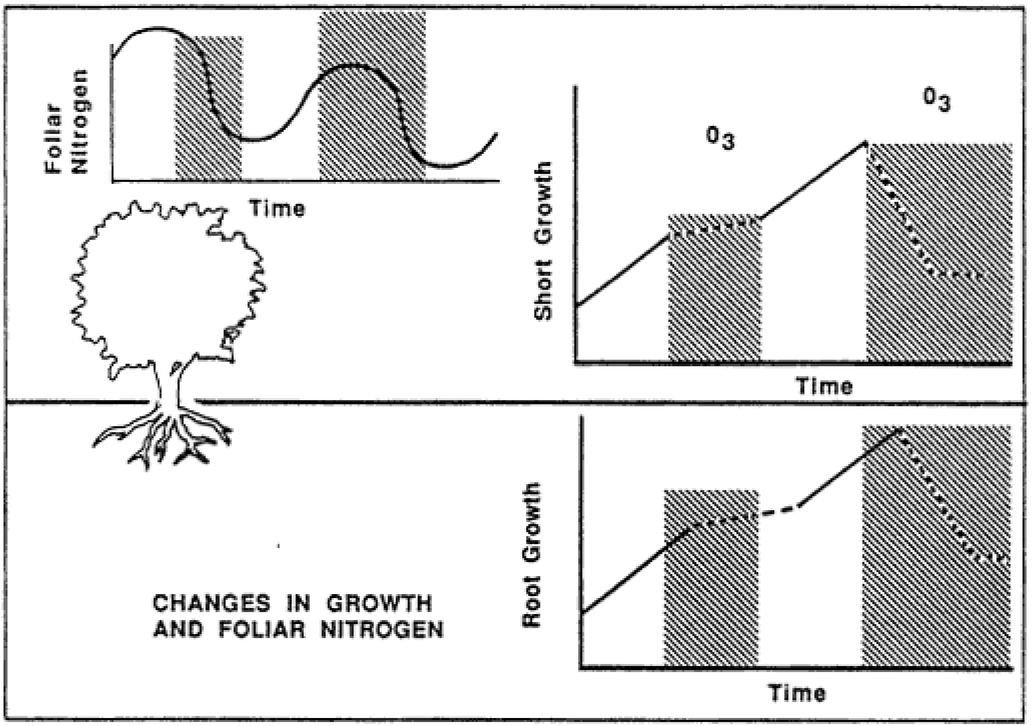


Figure 6.
Hypothetical time course of root and shoot growth responses to O_3 episodes.

Another mechanism by which gaseous pollutants can potentially alter whole plant canopy conductance involves the relative synchrony of suppression of shoot and root growth. Gaseous pollutants are known to shift root/shoot ratios (Winner and Atkinson, 1986) and many studies show that root growth is more sensitive to exposures than is shoot growth (Tingey and Reinert, 1971). These conclusions are typically based on studies in which plants are fumigated for periods ranging from 30 to 90 days and then harvested for whole-plant growth analysis. A strong possibility exists that shoot growth suppression and root growth suppression are not synchronous (Fig. 6). Similarly, recovery of shoot growth and recovery of root growth when air pollution exposures terminate are apt to be out of phase. The importance of root and shoot growth rates from the perspective of conductance is manifest through foliar nitrogen content. As discussed earlier, stomatal conductance and photosynthetic capacity increase with foliar nitrogen content (Field and Mooney, 1986). If shoot growth recovery precedes root growth recovery, then foliar nitrogen content may be reduced by dilution and photosynthetic capacity and conductance will also be lowered. However, if an air pollution episode results in a great deal of leaf loss, the recovery process might result in a large root system supplying nitrogen to relatively few leaves. In this case, the leaves may increase in N content resulting in higher levels of leaf metabolism. These patterns of whole-plant resource allocation might help explain why, in some studies, O_3 or SO_2 can bring apparent increases in leaf-level processes (Libera et al., 1973).

CALCULATIONS OF AIR POLLUTION ABSORPTION

Stomatal conductance to water vapor not only is important as it relates to fluxes of CO_2 and H_2O between the leaf and air but also as it relates to air pollution absorption (Winner and Mooney 1980 a, b; Winner and Atkinson, 1986). Calculating air pollution flux rates through stomata requires knowing leaf conductance values for H_2O, ambient concentrations of any gaseous pollutants, and the diffusion coefficient of that pollutant in air. Flux rates of pollutants can then be integrated over time to determine total quantity of pollutant absorbed during exposure. The strength of this approach is that physiological and growth responses of plants to air pollutants can be expressed on the basis of a quality of air pollution absorbed rather than on the basis of atmospheric concentrations.

Calculating flux rates of air pollutants into foliage on the basis of stomatal conductance assumes that intracellular concentrations of the pollutant are zero and that stomatal conductance is lower than all other components of leaf level conductance (Winner et al., 1985b). The extent to which these assumptions are untrue represents error in the method. Indications are that error from these assumptions are hard to measure but nonetheless small (Winner et al., 1985b). The error for estimating air pollution uptake by foliage by chemical analysis of foliar samples before and after exposure is known to be large, is not dynamic, requires destructive sampling, and is not useful for pollutants such as O_3 because oxygen is present in foliage in high concentrations. Thus, estimation of gaseous air pollution absorption by foliage is best done by calculating flux rates of pollutants through stomata.

MEASUREMENTS OF PHOTOSYNTHESIS AND CONDUCTANCE

Non-destructive Techniques. A number of scientific equipment manufacturing firms produce portable gas exchange systems useful for screening leaf populations for photosynthesis and conductance. For example, systems manufactured by LiCor, Inc. (Lincoln, NE) and by Analytical Design Corporation, Ltd. (Hodgested, England) are commonly available and are favorably compared (Parkinson et al., 1988). Also, Data Design Group, Inc. (La Jolla, LA) makes a comparable system. These systems are typically battery powered and easily carried into the field. Another important feature of these systems is their capacity to acquire and store data. The systems can be operated to survey numbers of leaves quickly with each measurement taking about 1 minute, and can be used to measure photosynthesis and conductance for many (20-200) leaves, that remain attached to trees, within a single day. This capacity to measure gas exchange rates of numerous leaves in a short time is essential if photosynthesis and conductance measurements are to play a useful role as air pollution markers.

Sample Collections. Leaves can also be collected from tree tops or other inaccessible places and then used in gas exchange measurements. This technique has been applied in a number of remote field sites (Lange et al., 1986). Use of this method requires measuring gas exchange rates of leaves attached to a tree, followed by remeasurement at intervals after the leaf is detached. This protocol is followed to characterize the effects of leaf removal, handling, and storage on gas exchange parameters. Handling techniques that minimize artifacts include storing the leaf in cool places, retrimming the collected branch or petiole under water, and leaving the cut surface in water, and keeping the time period between leaf collection and gas exchange measurements as short as possible.

The radio isotope ^{14}C has been used to generate $^{14}CO_2$ which is then used to assess the effects of air pollutants on photosynthesis (e.g., McLaughlin et al., 1982). In these studies, leaves are enclosed in a chamber and exposed to $^{14}CO_2$ for specific periods of time. The $^{14}CO_2$ provides a tag which can be used to document photosynthesis rate and the metabolic fate of photosynthate. Air pollution effects are determined by comparing the $^{14}CO_2$ fixation rates and fates for air pollution treated plants and controls.

Photosynthesis and conductance can also be indirectly assessed by collecting leaf samples for analysis of stable carbonisotope ratios, or more specifically, their ^{12}C/^{13}C ratios. The ö^{13}C values of C_3 plants, typically ranges from-26 to 32 (Fig. 7) (Troughton, 1979). This is because the CO_2 fixing enzyme of C_3 plants (RUBISCO) preferentially fixes the ^{12}C-containing CO_2 over the heavier form. Thus, carbohydrate of C_3 plants is depleted in ^{13}C relative to the atmosphere.

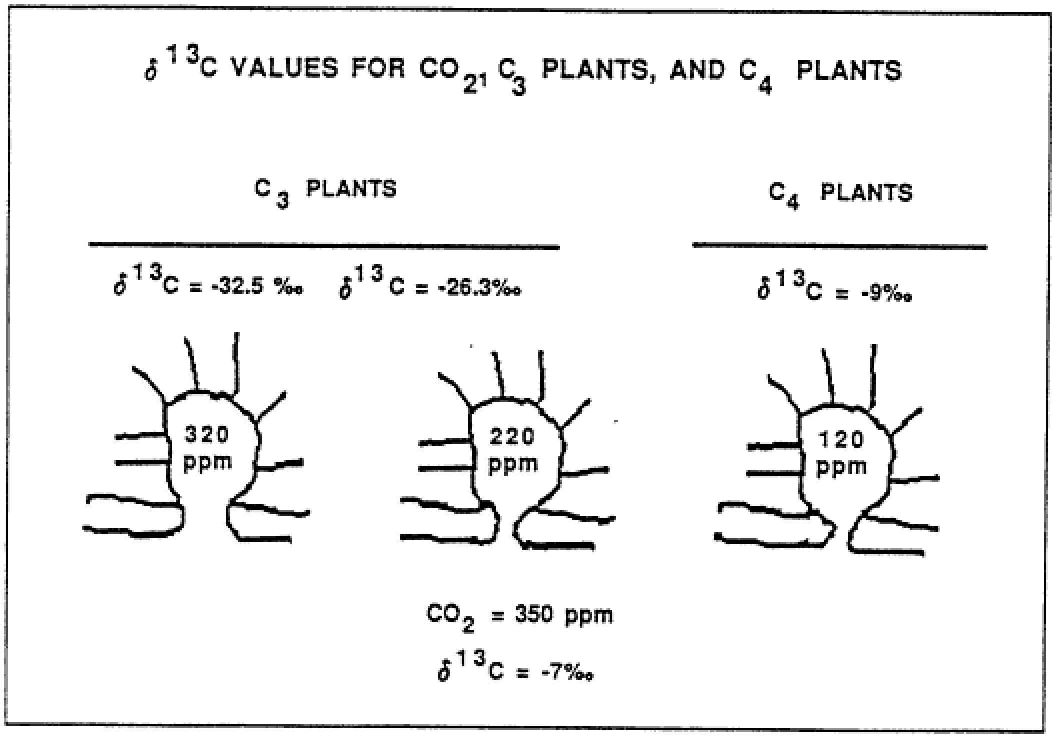


Figure 7.
ö^{13}C values for C in atmospheric CO_2 and in foliage.

The range of ö^{13}C values for C_3 plants is in large part dependent upon the CO_2 concentrations in the leaf mesophyll (Farquhar et al., 1982) (Fig. 7). These CO_2 concentrations reflect both the rate of photosynthesis and the degree of stomatal opening. Under some conditions, CO_2 internal concentrations for C_3 plants can be as low as 220 ppm. This would be under high light with closed stomata. Under these circumstances, RUBISCO is less descriminating and less fractionation of the two stable C isotopes occurs. C stable isotope fractionation is highest when CO_2 internal concentrations are high which occurs when stomata are open.

The idea that any environmental factor which changes CO_2 internally also changes the ^{ö}C ratio has been shown for drought and other stresses (Guy et al. 1980; Winter et al., 1982). Recent studies show that ambient O_3 levels can also alter ^{ö13}C values for leaves, stems and roots (Greitner and Winner, 1988) (Fig. 8). This is not surprising since this O_3 treatment was also found to deplete CO_2 internal concentrations. This study raises the possibility that ^{ö13}C values of plants in the field may be useful in identifying plants growing with air pollution stress. However, this will require developing the capacity for partitioning ^{ö13}C shifts due to air pollutants from shifts due to other environmental stresses.

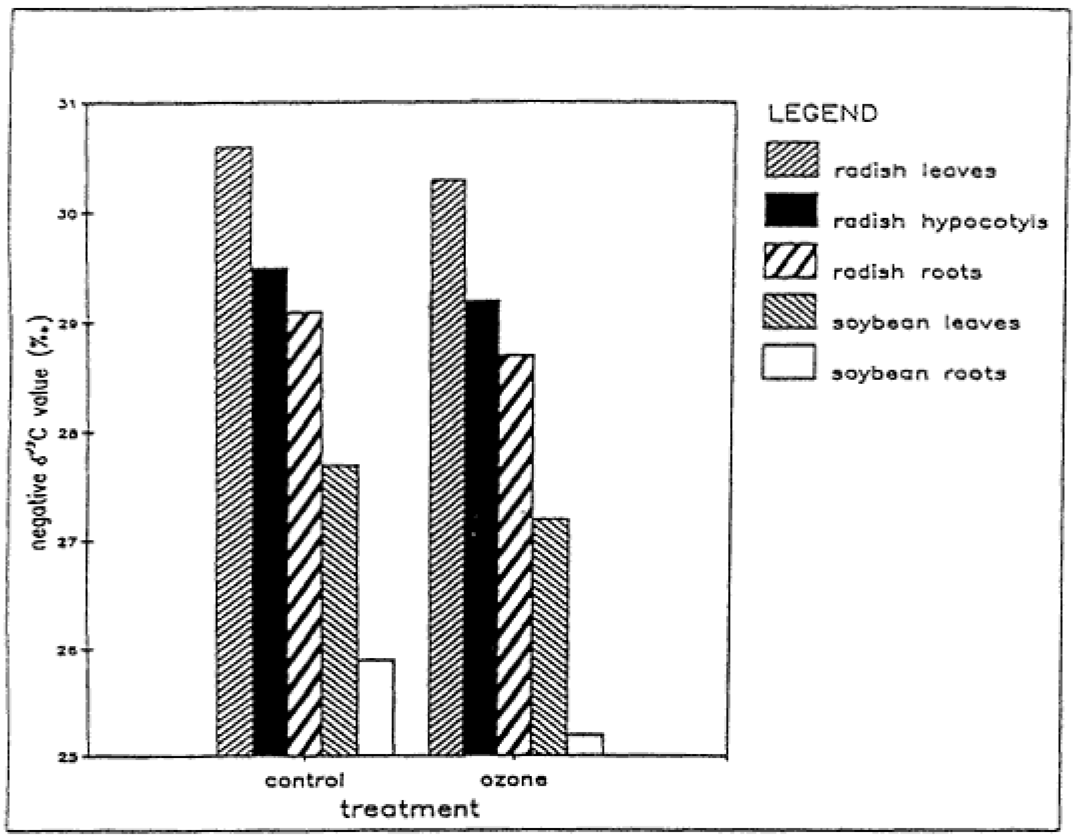


Figure 8.
O_3-caused shifts in ^{ö13}C values of leaves, stems, and roots (Greitner and Winner, 1988).

PROBLEMS USING PHOTOSYNTHESIS AND CONDUCTANCE AS AIR POLLUTION MARKERS

Pollutant Specificity. One difficulty with using physiological properties of leaves as markers of air pollution is that responses may not be specific to pollution. As mentioned above, most physiological processes of leaves, including photosynthesis and conductance, change with many environmental parameters. Identifying changes in metabolism due to air pollutants will be a difficult task.

Another problem is that physiological responses may not be specific for specific pollutants. For example, O_3 and SO_2 may both cause stomatal closure and decreases in photosynthesis. If an air pollution-caused decrease in leaf metabolism were identified, how would the causal agent be identified? Thus, the marker would reveal the presence of an air pollutant but the air pollutant which caused the effect would remain unknown.

Photosynthesis and conductance changes in response to air pollutants will be difficult to use as air pollution markers because even once the pollution response is known, it will not be quantified. The degree of stomatal response to SO_2 or O_3 is apt to change with temperature, humidity, age, season, and the presence of other air pollutants. Current understanding is not sufficient to predict the degree of physiological responses to well defined air pollution exposures in controlled environments. Therefore, it will be some time before that capacity is sufficiently developed for application to field sites.

One of the challenges to those intent on using photosynthesis and conductance measurements as air pollution markers for trees will be to use these data with other carefully selected data. Using only gas exchange measurements will be insufficient for assessing trees in controlled studies or in uncontrolled field surveys. Gas exchange measurements in both mechanistic experiments and surveys are most useful when combined with other data, including biochemical analysis, growth analysis, and community analysis, and thereby put into a broad biological perspective.

SUMMARY

- Photosynthesis, transpiration, and conductance have potential air pollution biomarker applications.
- Leaf-level metabolism, alone, is insufficient as an air pollution biomarker.
- Leaf-level metabolism, used with other measurements, might be used to develop an air pollution biomarker index.

References

Black, V.J., and M.H. Unsworth. 1980. Stomatal responses to sulfur dioxide and vapor pressure deficit. Exp. Bot., 31:667-677.

Evans, L.S., and I.P. Ting. 1974. Ozone sensitivity of leaves: relationship to leaf water potential, gas transfer resistance, and anatomical characteristics. Am. J. Bot., 61:592-597.

Farquhar, G., E.-D. Schulze, and M. Kuppers. 1980. Responses to humidity by stomata of *Nicotiana Glauca* L. and *Corylus avellana* L. are consistent with the optimisation of carbon dioxide uptake with respect to water loss. Australian J. Plant Physiol., 7:315-327.

Farquhar, G.D., M.H. O'Leary, and J.A. Berry. 1982. On the relationship between carbon isotope discrimination and the intracellular carbon dioxide concentration in leaves. Australian J. Plant Physiol., 9:121-137.

Field, C. and H.A. Mooney. 1986. The photosynthesis-nitrogen relationship in wild plants. Pp. 25-55 in On the Economy of Plant Form and Function, Givnish, T. (ed.). Cambridge University Press, Cambridge, England.

Greitner, C.S. and W.E. Winner. 1988. Increases in$^{\delta 13}$C values of radish and soybean plants caused by ozone. New Phytologist, 108:489-494.

Guderian, R. (ed.). 1985. Air Pollution by Photochemical Oxidents: Formation, Transport, Control and Effects on Plants. Springer Verlag, Berlin. 346 p.

Guderian, R., D.T. Tingey, and R. Rabe. 1985. Effects of photochemical oxidants on plants. P. 346 in Air Pollution by Photochemical Oxidants: Formation, Transport, Control and Effects on Plants. Guderian, R. (ed.). Springer Verlag, Berlin. 346 p.

Guy, R.D., D.M. Reid, and H.R. Krouse. 1980. Shifts in carbon isotope ratios of two C_3 halophytes under natural and artificial conditions. Oecologia, 44:241-247.

Jones, H. 1983. Plants and Microclimate. Cambridge University Press, Cambridge, England. 323 p.

Kimmerer, T.W., and T.T. Kozlowski. 1981. Stomatal conductance and sulfur uptake of five clones of *Populus tremuloides* exposed to sulfur dioxide. Plant Physiol., 67:990-995.

Lange, O.L., G. Fahrar, and J. Gaebel. 1986. Rapid field determination of photosynthetic capacity of cut spruce twigs (*Picea abies*) at saturating ambient CO_2. Trees, 1:70-77.

Libera, W., H. Ziegler, and I. Ziegler. 1973. Forderung der Hill-reaktion und der CO_2 Fixierung in isolierten spinatchchloroplasten durch niedere sulfitkonzentraionen . Planta, 190:269-279.

Mansfield, T.A., and O. Majernik. 1970. Can stomata play a part in protecting plants against air pollutants. Environ. Pollution, 1:149-154.

McLaughlin, S.B., R.K. McConathy, D. Duvick, and K.L. Mann. 1982. Effects of chronic air-pollution stress on photosynthesis, carbon allocation, and growth of white pine. Forest Sci., 28:60-70.

Monsi, M. 1968. Mathematical models of plant communities. Pp. 131-150 in Functioning of Terrestrial Ecosystems at the Primary Production Level, Eckardt, F. (ed.). UNESCO, Paris.

Mooney, H.A. 1972. The carbon balance of plants. Ann. Rev. Ecol. Syst., 3: 315-346.

Mooney, H.A., Ehleringer, J., and J. A. Berry. 1976. High photosynthetic capacity of a winter annual in Death Valley. Science, 194:322-324.

Olszyk, D.M., and D.T. Tingey. 1986. Joint action of O_3 and SO_2 in modifying plant gas exchange. Plant Physiol., 82:401-405.

Parkinson, K.J., D. McDermitt, S.C., Roemer, S.P. Cline, and W.E. Winner. 1988. Measurement and comparison of gas-exchange of *Pinus halepensis* and *Flaveria brownii* using commercial photosynthesis systems. Pp. 130-164 in Responses of Trees to Air Pollution, Winner, W.E. and L.B. Phelps (eds.). Proc. West. Conifer Res. Coop. Workshop, Nov., 1987, Boulder, CO.

Reich, P.B., and J.P. Lassoie. 1984. Effects of low level O_3 exposure on leaf diffusive conductance and water-use efficiency in hybrid poplar. Plant, Cell, and Environ., 7:661-668.

Temple, P.J. 1986. Stomatal conductance and transpirational responses of field-grown cotton to ozone. Plant, Cell, and Environ., 9:315-321.

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

Troughton, J.H. 1979.$^{\text{ö}13}$C as an indicator of carboxylation reactions. Pp. 140-149 in Photosynthesis II. Gibbs, M. and E. Latzko (eds.). Encyclopedia of Plant Physiology, New Series, Vol. 6, Springer Verlog, Berlin. pp. 140-149.

Winner, W.E., and C.J. Atkinson. 1986. Absorption of air pollutants by plants and consequences for growth. Trends in Ecol. and Evol., 1:15-18.

Winner, W.E., C. Gillespie, and W. Sheng. 1988. Stomatal responses to SO_2 and O_3. In: Physiological and Biochemical Responses of Plants to Air Pollutants. Welburn, A., and Darrall, N. (eds.). DeGruyter, Inc., Berlin. In Press.

Winner, W.E., and H.A. Mooney. 1980a. Ecology of SO_2 resistance: I. Effects of fumigations on gas exchange of deciduous and evergreen shrubs. Oecologia, 44: 290-295.

Winner, W.E., and H.A. Mooney. 1980b. Ecology of SO_2 resistance: II. Photosynthetic changes of shrubs in relation to SO_2 absorption and stomatal behavior. Oecologia, 44:296-302.

Winner, W.E., H.A. Mooney, and R.A. Goldstein. (eds.). 1985a. Sulfur Dioxide and Vegetation: Physiology, Ecology, and Policy Issues. Stanford University Press, Stanford, California. 593 p.

Winner, W.E., H.A. Mooney, K. Williams, and S. Von Caemmerer. 1985b. Measuring and Assessing SO_2 effects on photosynthesis and plant growth. Pp. 118-132 in Sulfur Dioxide and Vegetation: Physiology, Ecology, and Policy Issues. Winner, W.E., H.A. Mooney, and R.A. Goldstein (eds.). Stanford University Press, Stanford, CA.

Winter, K., J.A.M. Holtum, G.E. Edwards, and M. O'Leary. 1982. Effect of low relative humidity on $^{\text{ö}13}$C values of radish and soybean plants caused by ozone. New Phytologist, 108:489-494.

Nutrient-Use Efficiency as an Indicator of Stress Effects in Forest Trees

R. J. Luxmoore
Environmental Sciences Division Oak Ridge National Laboratory Oak Ridge, Tennessee 37831-6038

ABSTRACT

The ratio of plant dry weight gain to total nutrient uptake provides a lumped whole plant estimate of nutrient use efficiency (NUE), the net carbon fixed per unit of nutrient uptake. This is not a practical indicator for whole trees even if the measurement could be interpreted mechanistically. NUE is difficult to interpret since the seasonality of nutrient uptake and carbon gain may not directly coincide, and the internal supply and demand for carbon and nutrients is buffered by internal storage and matched in growth through mobilization and internal transport. Sampling of plant tissues (leaf, fine root) and solutions (phloem, xylem) for determination of the carbon and nutrient relationships is feasible but provides fragmented information. Frequent tissue or solution sampling may nevertheless lead to a reliable basis for interpreting stress effects on trees, but this approach requires intimate knowledge of the diurnal, wetting and drying, and annual cycles of tree physiology and phenology. The use of nutrient use efficiency as an indicator of stress is not practical on a whole plant basis and is not definitive on a tissue basis at our present state of knowledge.

The ratio of dry weight gain to net nutrient uptake is one definition of nutrient-use efficiency (NUE). Another definition is given by the ratio of dry weight gain to nutrient content and for some tissues such as leaves, NUE is the reciprocal of nutrient concentration since leaf dry weight is the dry weight gain, and the nutrient content is the net nutrient uptake. Consideration of the utility of nutrient-use efficiency as an indicator of stress effects requires evaluation of stress effects on the relative dynamics of carbon gain and nutrient acquisition. A hyperbolic function (Fig. 1) describes the expected relationship between carbon gain and nutrient uptake up to the maximum carbon gain (M), where the increment in carbon gain (ΔC) per increment of nutrient uptake (ΔN) is zero. The optimum carbon gain (O) is defined as the point at which the second derivative of the function is zero. In field situations, plants typically operate at about half the maximum rate of carbon gain (T). An approximately linear section of the relationship between L and the typical point (T) has one NUE value ($\Delta C/\Delta N$). Approaching the origin, NUE reaches the highest value (H); however, this condition is associated with abnormally weak or dying plants! Although high NUE may be an indicator of extreme stress, it does not provide an early warning.

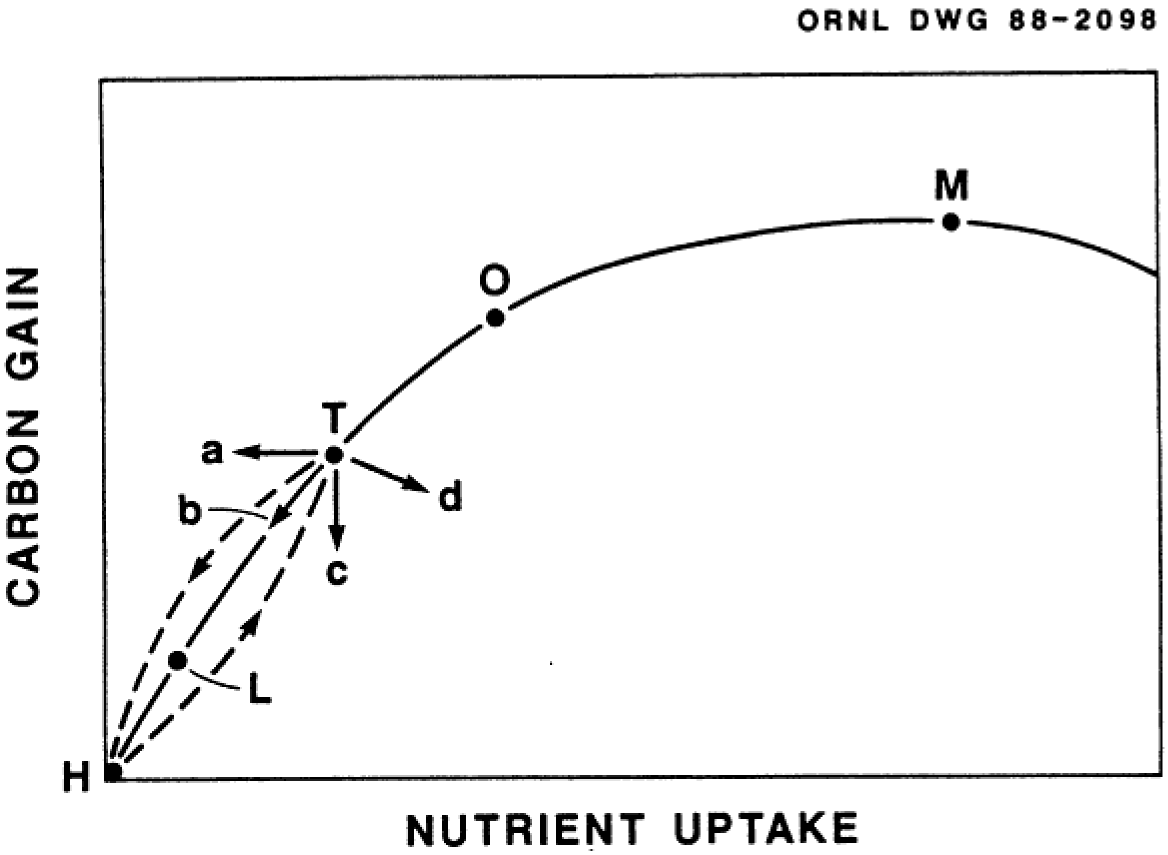


Figure 1.
Relationship between carbon gain and nutrient uptake where M and O are the maximum and optimum carbon gain, TL is a linear range, and H is the highest nutrient-use efficiency. See text for discussion of a, b, c, and d. A hypothesized natural progression with increasing stand age is indicated by- - where canopy closure occurs at point T.

Nutrient-use efficiency can be a useful indicator of stress if the stress causes a shift, such as Td, in the relationship (Fig. 1). Perhaps such an effect could occur with increases in ozone exposure (reducing carbon gain) and nitrogen deposition (increasing N uptake). If other shifts in the relationship occur, such as Ta, Tb, and Tc, then NUE would be a poor indicator of stress. In two cases, change in nutrient uptake or carbon gain (Ta or Tc, respectively) would be a better indicator of stress than NUE, whereas in the case of Tb, NUE does not change and is completely insensitive as an indicator.

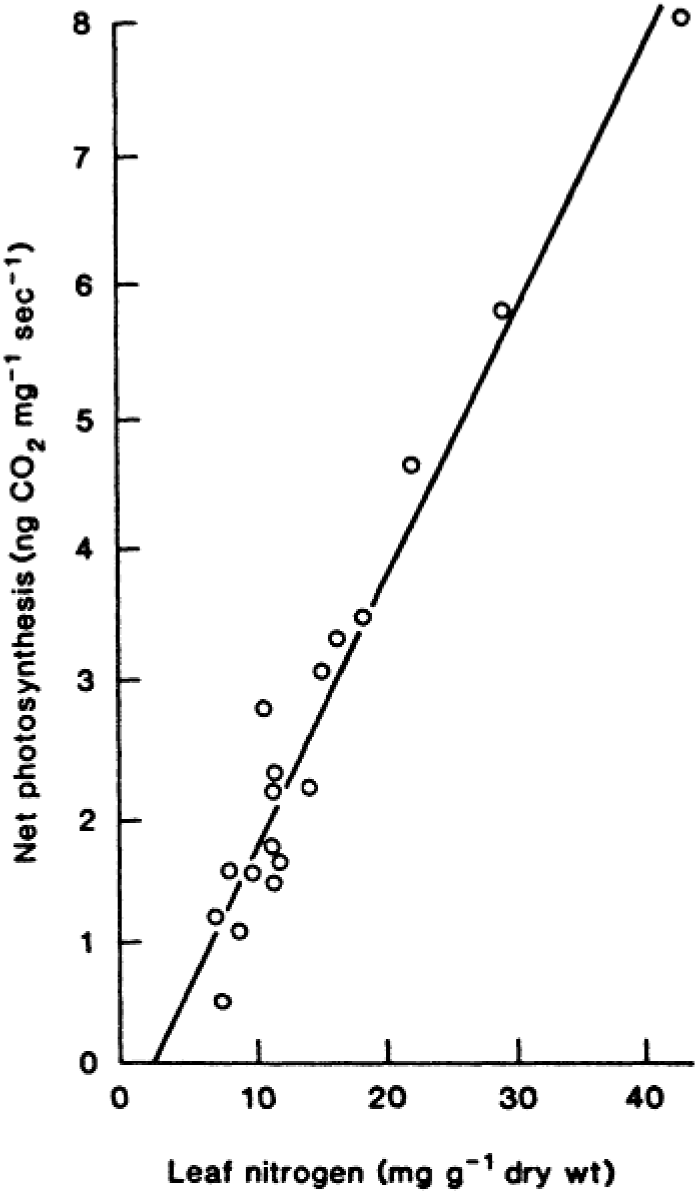


Figure 2.

Light-saturated net photosynthesis rates of leaves of Eucalyptus species with differing nitrogen concentration. Source: Reprinted with permission of Springer-Verlag N.Y., Inc. from Oecologia, 1978. Copyright 1978 by Springer-Verlag.

EXAMPLES OF NUTRIENT-USE EFFICIENCY

Positive relationships have been demonstrated between light-saturated net photosynthesis and foliar nitrogen concentration (Fig. 2) with occasional exceptions (Fig. 3). In the latter case, the relationship was positive between full-sun net photosynthesis of *Pinus radiata* and leaf phosphorus but was negative for leaf nitrogen with the oldest needle age class generally having the lowest NUE for both nitrogen and phosphorus. Short-term phytotoxic air pollutant exposure can reduce photosynthesis (Reich and Amundson 1985), presumably reducing NUE since foliar nutrient levels change at slower rates (Sheriff et al. 1986). Monitoring of light-saturated leaf photosynthesis and nutrient concentrations will provide valuable information on foliar functioning. In a scenario of increasing ozone exposure and nitrogen deposition, a significant shift from the nonstressed relationship between leaf photosynthesis and leaf nitrogen concentration may occur.

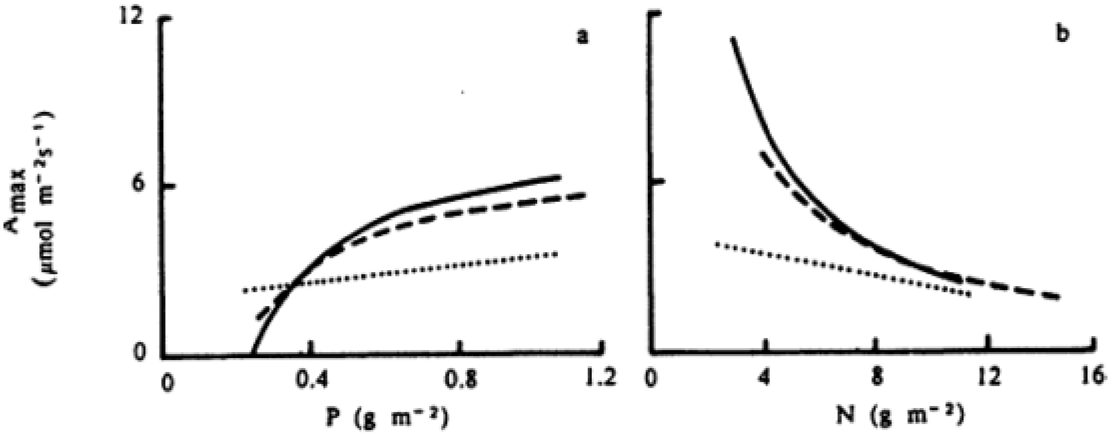


Figure 3.
Full-sun photosynthesis (A_{max}) of Pinus radiata needles in relation to leaf phosphorus (P) and leaf nitrogen (N) for current needles _____, current + 1 year _____, and current + 2 year ..... (from Sheriff et al. 1986).

Changes in NUE at the biochemical level are inferred for shifts to more energy-efficient nitrogen nutrition. Pate (1986) theoretically calculated the costs of nitrogen (N) assimilation in terms of adenosine triphosphate (ATP) required for various forms of N *relative* to ATP needed for assimilation of ammonium. Symbiotic nitrogen fixation required 16-29 mol ATP/mol NH_4^+, decreasing for NO_3^- assimilation (3-15 mol ATP/mol NH_4^+) with the relative energy requirements for NH_4^+ assimilation being 1 tool ATP in the Pate (1986) analysis. Nitrate assimilation in leaves was more efficient than root assimilation due to linkage of nitrate reduction with photosynthesis. Foliar uptake of nitric acid vapor is a more efficient N source (3 mol ATP/NH_4^+) than nitrate uptake into roots (15 mol ATP/NH_4^+).

The nitrogen-use efficiency of birch (*Betula verrucosa*) seedlings was shown to decrease with increase in plant nitrogen concentration in the investigations of Ingestad (1979). Highest efficiency was obtained for the plants with lowest nitrogen concentration (Fig. 4). The relationship between net primary productivity (NPP) of aboveground components and annual nitrogen uptake was shown by Miller (1984) to be linear (log-log plot) for 39 stands (r^2 = 0.90) that included coniferous and broadleaf forests from regions that ranged from boreal to tropical (Fig. 5). Since these data come from a wide range of natural stress conditions, it is apparent that NUE is an insensitive measure of plant response to stress. The nitrogen-use efficiency for aboveground tissues was about 170. A linear relationship-was also shown between NPP and annual phosphorus uptake but with higher variability (r^2 = 0.74). The phosphorus-use efficiency for aboveground components was about 17. Again, this value applied to a wide range of natural stress conditions, suggesting that the aboveground response of NUE to stress follows the trajectory Tb of Fig. 1.

Vitousek (1982) has suggested that for perennial vegetation, NUE is more appropriately defined in terms of the organic matter loss (or storage) per unit of nutrient loss (or storage). Monitoring of annual litterfall may thus provide an index of aboveground NUE. The relationship between the inverse of litter nitrogen concentration (NUE) and total nitrogen in litterfall (Fig. 6) derived for a wide range of forest communities follows the pattern of Fig. 4, in which low abscissa values are associated with high NUE. Given the rather wide scatter in the observations of Fig. 6, it may be difficult to associate any change in litter NUE with a particular stress in a monitoring program. There is much less variability if data for one species are considered, as shown by the line segments in Fig. 6 for the fertilization study of Miller et al. (1976) with Corsican pine (*Pinus nigra*). Determination of NUE on annual time scales restricts the identification of particular stresses with the response since many stresses impact trees during a year. Determination of NUE at shorter time steps is, however, not without problems.

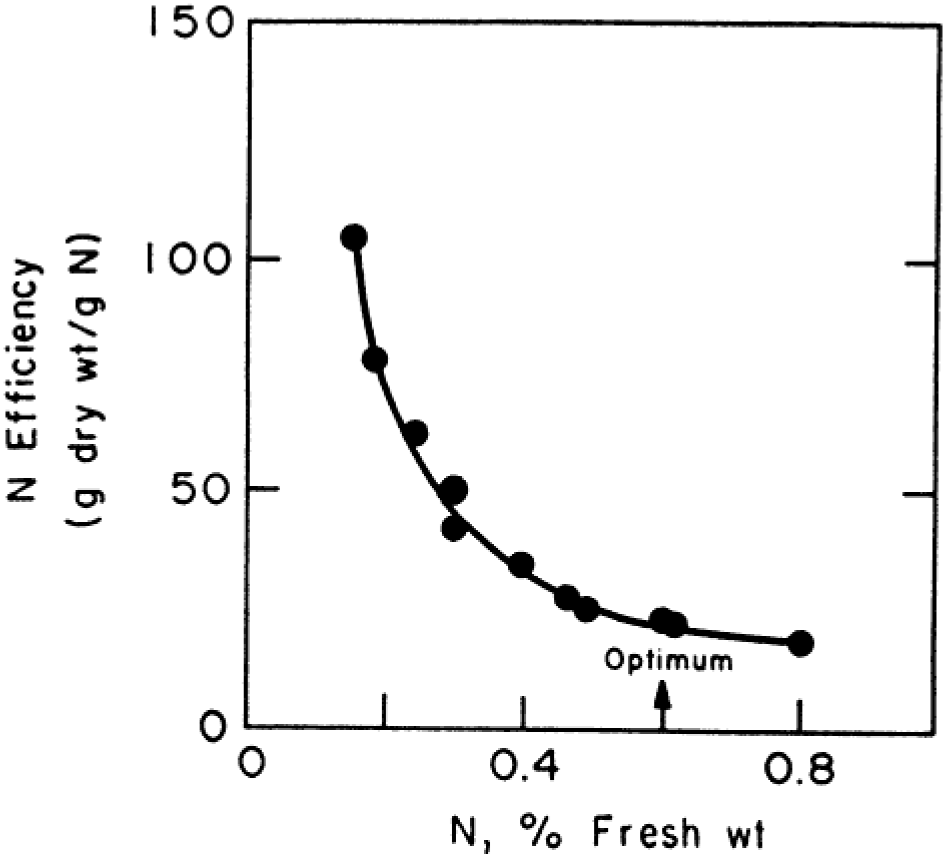


Figure 4.
Relationship of N-use efficiency to N status of birch (Betula verrucosa) seedlings. Nitrogen status is measured as N percentage of seedling fresh weight. The range in N status results from differing experimental N regimes (modified from Ingestad 1979).

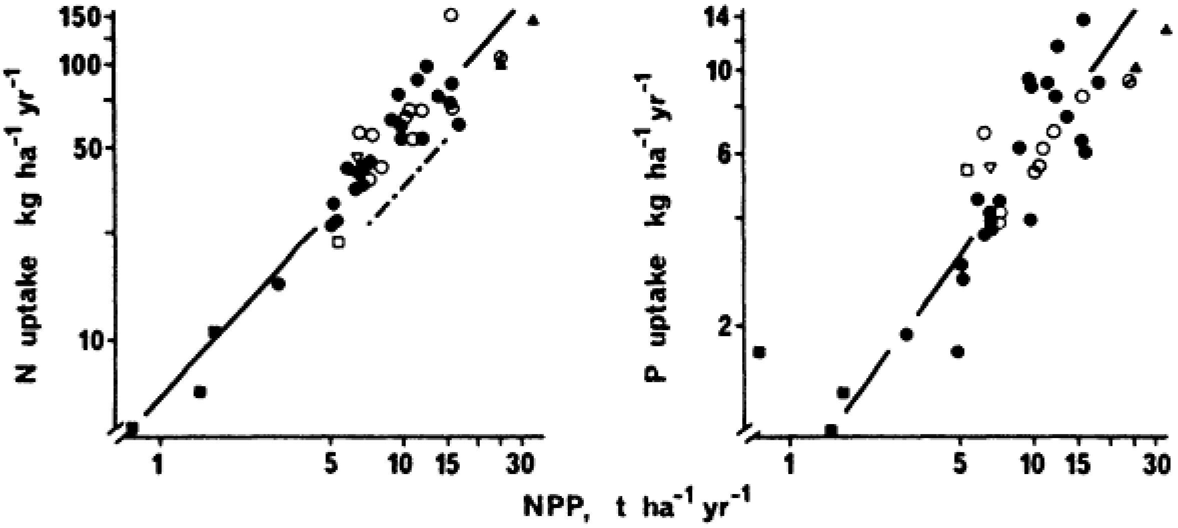


Figure 5.
Uptake of nitrogen and phosphorus into aboveground components as a function of net primary production (NPP) using data reported for coniferous forests from boreal ( ), temperate ( ), and tropical ( ) regions, and for broad-leaved forests from boreal ( ), temperate (ø, Alnus rubra), and mediterranean (Δ) regions. Uptake is calculated as change in accumulation over time plus release in litterfall. Both regressions are significant at $P < 0.001$, r^2 values being 0.90 and 0.74 for N and P, respectively (from Miller 1984).

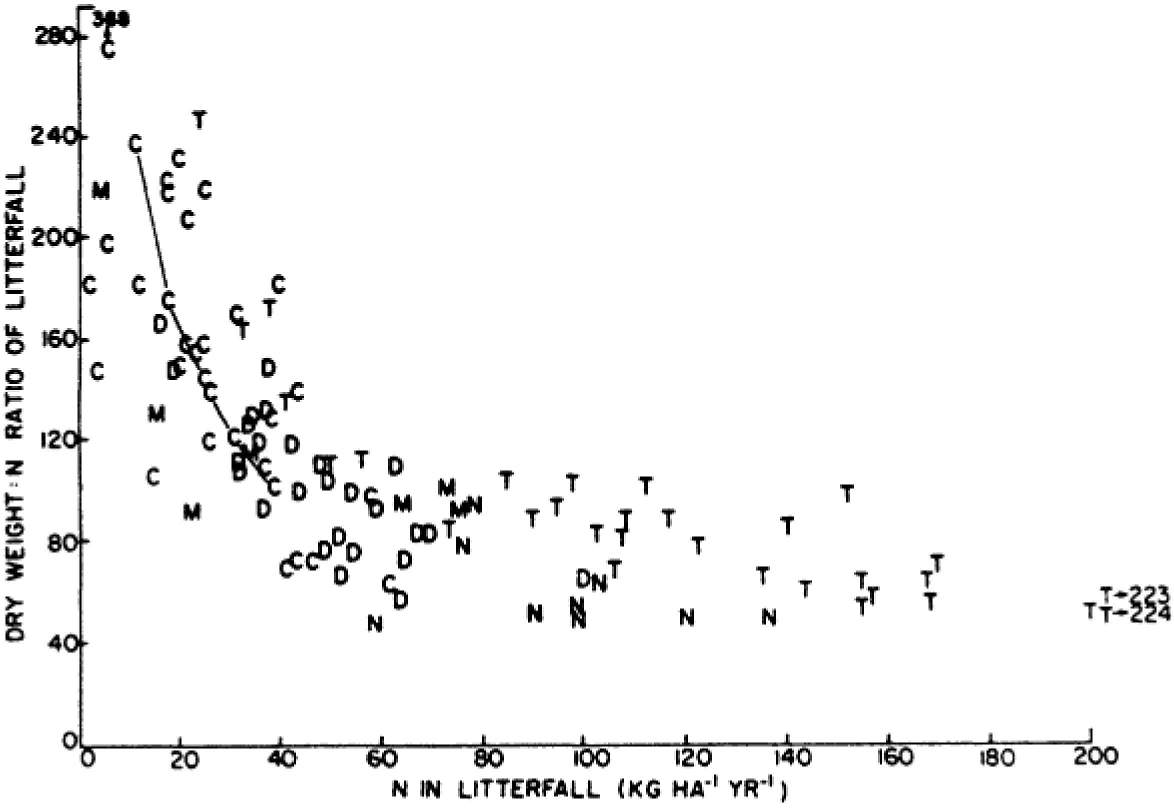


Figure 6.
The relationship between the amount of nitrogen in litterfall and the dry mass to nitrogen ratio of that litterfall. Symbols are as follows: C = coniferous, D = temperate deciduous, T = evergreen tropical, M = mediterranean, N = temperate nitrogen fixers. The line segments link data from a long-term fertilization study by Miller et al. (1976) (from Vitousek 1982).

NUE DURING THE ANNUAL CYCLE

Determination of ΔC/ΔN (NUE) during part of the growing season is a straightforward harvesting and chemical analysis exercise involving substantial effort when roots are included in a whole-plant measurement. Interpretation of the results as NUE, however, is problematic due to phenological controls on growth, utilization of internal carbon and nutrient storages, remobilization of nutrients, and the seasonality of nutrient uptake (van den Driessche 1984). Utilization of carbon and nutrient storage in springtime foliar growth is initially "negative growth" because respiration exceeds carbon gain. Shoot growth induces nutrient remobilization from adjacent leaf and twig tissues and is essentially independent of nutrient uptake from soil (Titus and Kang 1982); thus, NUE of shoot growth is apparently infinite.

Many temperate forest species have deterministic leaf, twig, and flower growth (bud control) and indeterminant stem thickening. Root growth tends to be indeterminant but with periodic behavior due to reductions in root growth occurring during periods of shoot growth (Bevington and Castle 1985). Due to the asynchrony of shoot and root growth, the same nutrient reserves can serve several functions during the growing season (Chapin 1980). Fine-root (<1 mm diam) growth and mortality can be a large component of the plant-carbon budget (Santantonio and Grace 1987), and the neglect of carbon allocation to belowground processes in an aboveground determination of NUE greatly limits the interpretation of this index as a stress indicator.

It is difficult to identify a time frame within the annual cycle in which measurement of $\Delta C/\Delta N$ can provide an unambiguous NUE indicator of plant response to stress. Alternatively useful indicators based on carbon and nutrient dynamics may come from consideration of these components in a whole-plant physiological framework that is less constrained than the NUE concept.

CARBON-NUTRIENT RELATIONSHIPS

Foliar nutrient analysis (also soil chemical analysis) has been well established as a means for identifying and monitoring nutrient deficiencies in plants. Diagnostic criteria of nutrient stress (deficiency, toxicity) have been developed for a wide range of plants (Chapman 1966, van den Driessche 1974), and these can provide useful guidance, although retranslocation can mask deficiency in new foliage. Ozone exposure can result in increases in foliar nutrient concentration in some cases. Skeffington and Roberts (1984) showed increased Mg, K, and P concentrations in *Pinus sylvestris* needles exposed to 300 mg O_3/m^3 for 56 days but no effects from acid mist treatments. Decline in conifer growth in southwestern West Germany has been associated with Mg and Ca deficiency (Hüttl and Wisniewski 1987) in needles and apparently rectified with fertilizer application. Foliar analysis will continue to be a useful indicator of plant response to stress, and the approach may be usefully extended by consideration of element ratios for elements with differing retranslocation and storage dynamics (e.g., Ca immobile, N, P, K mobile in leaves).

Cotrufo (1985) has evaluated several tissue analysis approaches as predictors of loblolly pine response to nitrogen fertilization. Total N in xylem, total soluble N, and arginine N of twigs and needles were greater in fertilized than in unfertilized trees. Total needle N is not always a good indicator of tree N status (Ballard 1980, Sheriff et al. 1986); however, the reliability of alternative indicators is not well established. Cotrufo (1985) noted that the interpretation of arginine N assays was complicated by phosphorus nutrition and soil-water status, and Xylem N of loblolly pine did not show a strong relationship to N fertilization.

The relationship between starch and nitrogen content has been determined in birch (*Betula pendula*) seedlings (McDonald et al. 1986) and in 30-year-old loblolly pine (*Pinus taeda*) needles (Birk and Matson 1986); both showed a similar pattern during the growth period (Fig. 7, Fig. 8b), but the pine study conducted under field conditions showed much more variability. Variability is likely to be a problem in interpretation of carbon-nutrient relationships of tissues sampled from the field. During the dormant period (February), the pattern of starch accumulation in relation to needle nitrogen was reversed, with high starch levels being associated with high nitrogen levels (Fig. 8a).

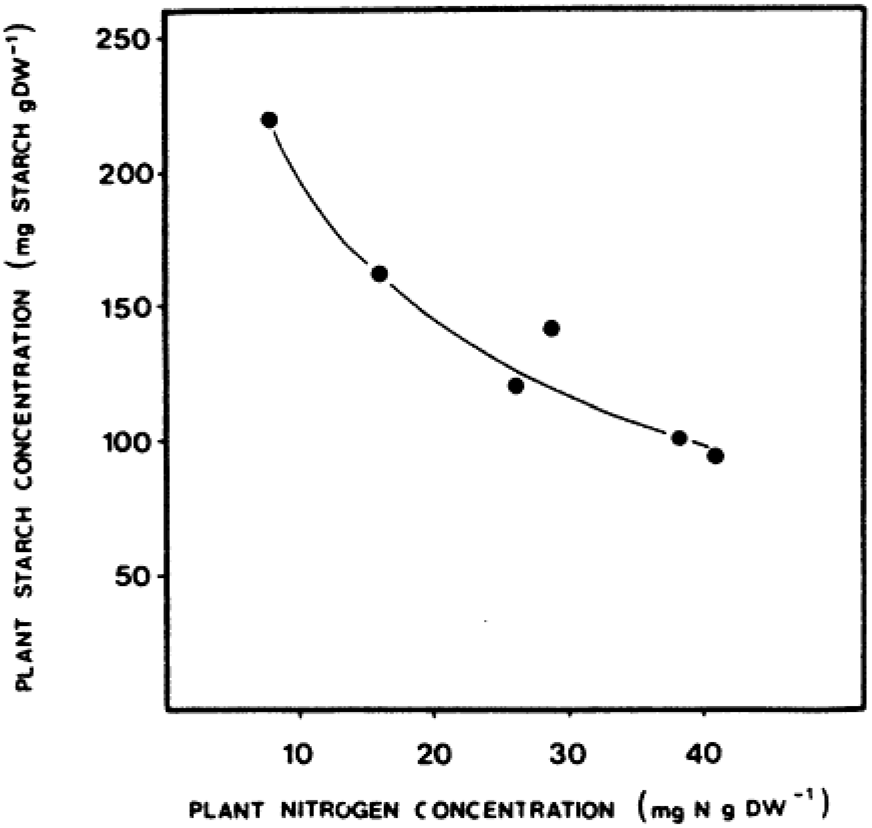


Figure 7.

Steady-state dependence of plant starch concentration (mg starch g^{-1} dry weight) on plant nitrogen concentration (mg N g^{-1} dry weight) in seedling birch (from McDonald et al. 1986).

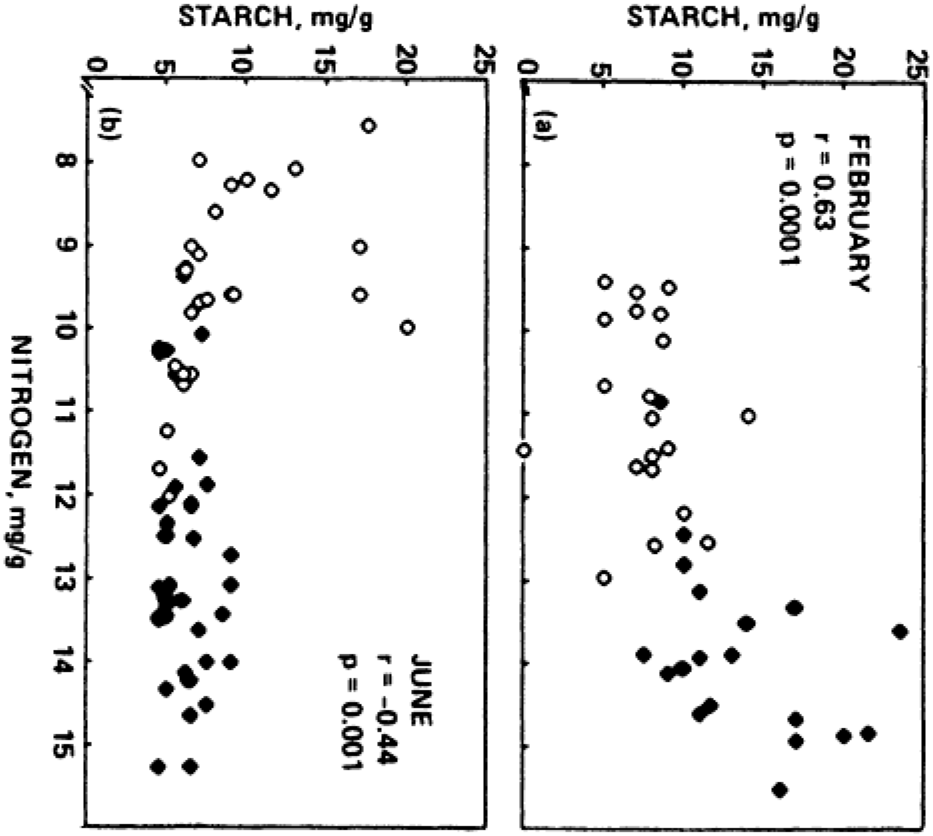


Figure 8.
Relationship between starch and nitrogen concentrations in loblolly pine needles in control (open symbols) and fertilized (solid symbols) sites on the coastal plain of South Carolina with a 30-year old pine stand for February (a) and June (b) (from Birk and Matson 1986).

CARBON AND NUTRIENT STORAGE

The principal soluble forms of nitrogen storage in woody plants are arginine, which has a high N:C ratio, and asparagine (Titus and Kang 1982) with glutamine being significant in some conifer species. Proteins are probably the most significant insoluble nitrogen reserves in trees (van den Driessche 1984). Phospholipids and "nonhydrolyzable esters" were suggested as the main overwintering forms of phosphorus in cold-hardy species (Chapin 1980, Chapin and Kedrowski 1983). Sulfur can be stored in proteins, amino acids, and sulfate forms (Linzon 1978). Potassium and inorganic phosphate accumulate in vacuoles, and calcium is probably held in exchangeable form on cell walls, particularly of xylem vessels. Starch is the dominant form of carbon storage, although secondary compounds (terpenes, latex) can be significant carbon forms in some species. Determination of the stored quantities of carbon and nutrients at the end of the growing season could become a useful indicator of plant response to cumulative environmental effects as well as of plant internal resources available for the next growing season. Titus and Kang (1982) cite several examples where high levels of storage N are highly correlated with new shoot growth of fruit trees during the following spring. Greater knowledge of how to measure internal storage and how to interpret the values for forest trees is needed before storage can be used as a definitive indicator. It is likely that not all storage locations and forms are equally accessible. Perhaps leaf starch can be more readily utilized by stem cambium than starch in ray cells of the stem. Further, the concept of capacity defined as the change in storage per unit of chemical potential energy (i.e., per unit biochemical driving force) may usefully distinguish between a range of plant storage forms.

CHANGES IN CARBON-NUTRIENT RELATIONSHIPS WITH STAND AGE

Nutrient retranslocation from mature leaves to new leaves (Sheriff et al. 1986), from twigs to new shoot growth, and from old roots to new roots (Titus and Kang 1982) becomes increasingly important with time as a tree increases internal nutrient storage relative to the annual growth demands. Miller (1984) noted that the relative demands made by forests for soil minerals decrease markedly after canopy closure; trees increasingly depend on internal retranslocation to meet nutrient requirements for annual growth. Nitrogen retranslocation in a pine plantation was calculated to increase from 11 to 69 kg ha^{-1} $year^{-1}$ with increase in age from 10 to 40 years. Similar stand age changes were estimated for phosphorus and potassium. Meier et al. (1985) also estimated an increased contribution of retranslocation in meeting the nutritional requirements of aging *Abies amabilis* stands. Following canopy closure additional phytomass accumulates largely as low-nutrient wood. This implies that with increasing stand age, NUE before canopy closure is less than after canopy closure. A hypothesized progression of carbon gain in relation to nutrient uptake with increasing stand age (Fig. 1) would give a range of NUE values that may explain some of the variance in the data summarized by Miller in Fig. 5.

COMMENTS

Plants tend to operate within narrow ranges in the ratios of carbon to nutrients and of nutrients to other nutrients (Chapin 1980, Garten 1976). In the short term, plants respond to stress with a change in physiological activity per unit tissue (leaf, root); however, in the longer term, changes in leaf area and needle age classes (and, by inference, fine-root turnover) are often significant signals of plant response to stress. Continued stress, such as with high nitrogen deposition, can lead to physiological imbalances. Mohren et al. (1986) identified large increases in N:P ratios in Douglas fir needles in the Netherlands in recent times that were associated with phosphorus deficiency. Nambiar (1987) evaluated the ratios of calcium (generally not retranslocated) to other mobile nutrients (e.g., Ca:N, Ca:P, Ca:K) in fine-root and leaf tissues and showed the relatively greater retranslocation of mobile nutrients from senescing leaves than from old roots. It is possible that nutrient retranslocation from fine-roots may only be significant in the zone close to the root tip with the effect being masked by analysis of long lengths of root tissue. He also noted that prolonged drought had very little effect on nutrient concentrations in fine-roots. Monitoring of tissue-nutrient relationships can provide indications of stress impacts, although normal dynamics of nutrient retranslocation and storage must be understood before abnormal signals can be interpreted.

SUMMARY

—NUE determined on annual time steps seems to be an insensitive indicator of stress since changes in carbon gain and nutrient uptake tend to occur in the same direction.
—Determination of NUE within a growing period is complicated by deterministic controls on growth, internal storage and retranslocation, and allocation to root processes.
—Light-saturated net photosynthesis generally increases with foliar nutrient (N, P) concentration, and changes in this relationship may be a sensitive indicator of short-term stress.
—Identification of imbalances in the relationships between carbon and nutrient dynamics of forest trees may lead to useful early warning indicators of stress impacts, but this needs to be developed within a whole-plant physiological context.

ACKNOWLEDGMENT

Research sponsored by the Carbon Dioxide Research Division, U.S. Department of Energy under Contract No. DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc. Publication Number 3123, Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831.

References

Ballard, R. 1980. Nitrogen fertilization of established loblolly pine stands: A flexible silvicultural technique. Pp. 223-229 in Gen. Tech. Rept. SO-34, USDA Forest Service, New Orleans.
Bevington, K. B., and W. S. Castle. 1985. Annual root-growth pattern of young citrus trees in relation to shoot growth, soil temperatures, and soil-water content. J. Am. Soc. Hort. Sci. 110:840-845.
Birk, E. M., and P. A. Matson. 1986. Site fertility affects seasonal carbon reserves in loblolly pine . Tree Physiol. 2:17-27.
Chapin, F.S. 1980. The mineral nutrition of wild plants. Ann. Rev. Ecol. Syst. 11:233-260.
Chapin, F. S., and R. A. Kedrowski. 1983. Seasonal changes in nitrogen and phosphorus fractions and autumn retranslocation in evergreen and deciduous Taiga trees. Ecology 64:376-391.

Chapman, H. D. (ed.). 1966. Diagnostic criteria for plants and soils. University of California, Riverside.

Cotrufo, C. 1985. Progress in tissue analysis to determine the response of loblolly pine to nitrogen fertilization. Pp. 385-389 in Gen. Tech. Rept. SO-54, USDA Forest Service, New Orleans.

Garten, C. T. 1976. Correlations between concentrations of elements in plants. Nature 261:686-688.

Hüttl, R. F., and J. Wisniewski. 1987. Fertilization as a tool to mitigate forest decline associated with nutrient deficiencies. Water Air Soil Poll. 33:265-276.

Ingestad, T. 1979. Nitrogen stress in birch seedlings. II. N, K, P, Ca, and Mg nutrition. Physiol. Plant. 45:149-157.

Linzon, S. N. 1978. Effects of airborne sulfur pollutants on plants. Pp. 109-162 in J. O. Nriagu (ed.), Sulfur in the Environment. II. Ecological Impacts. John Wiley and Sons, New York.

McDonald, A. J. S., T. Lohammar, and A. Ericsson. 1986. Uptake of carbon and nitrogen at decreased nutrient availability in small birch (Betula pendula Roth.) plants. Tree Physiol. 2:61-71.

Meier, C. E., C. C. Grier, and D. W. Cole. 1985. Below-and aboveground N and P use by *Abies amabilis* stands. Ecology 66:1928-1942.

Miller, H. G. 1984. Dynamics of nutrient cycling in plantation ecosystems. Pp. 53-78 in G. D. Bowen and E. K. S. Nambiar (eds.), Nutrition of Plantation Forests. Academic Press, New York.

Miller, H. G., J. M. Cooper, and J. D. Miller. 1976. Effect of nitrogen supply on nutrients in litterfall and crown leaching in a stand of Corsican pine. J. Appl. Ecol. 13:233-248.

Mohren, G. M. J., J. van den Burg, and F. W. Burger. 1986. Phosphorus deficiency induced by nitrogen input in Douglas fir in the Netherlands. Plant Soil 95:191-200.

Mooney, H. A., P. J. Ferrar, and R. O. Slatyer. 1978. Photosynthetic capacity and carbon allocation patterns in diverse growth forms of Eucalyptus. Oecologia 36:103-111.

Nambiar, E. K. S. 1987. Do nutrients retranslocate from fine roots? Can. J. For. Res. 17:913-918.

Pate, J. S. 1986. Economy of symbiotic nitrogen fixation. Pp. 299-325 in T. J. Givnish (ed.), On the Economy of Plant Form and Function. Cambridge University Press, New York.

Reich, P. B., and R. G. Amundson. 1985. Ambient levels of ozone reduce net photosynthesis in tree and crop species. Science 230:566-570.

Santantonio, D., and J. C. Grace. 1987. Estimating fine-root production and turnover from biomass and decomposition data: A compartment-flow model. Can. J. For. Res. 17:900-908.

Sheriff, D. W., E. K. S. Nambiar, and D. N. Fife. 1986. Relationships between nutrient status, carbon assimilation, and water-use efficiency in *Pinus radiata* (D. Don) needles. Tree Physiol. 2:73-88.

Skeffington, R. A., and T. M. Roberts. 1984. The effects of ozone and acid mist on Scots pine saplings. Report TPRD/L/2695/N84, Central Electricity Generating Board, Surrey, United Kingdom.

Titus, J. S., and S. M. Kang. 1982. Nitrogen metabolism, translocation, and recycling in apple trees. Hort. Rev. 4:204-246.

van den Driessche, R. 1974. Prediction of mineral nutrient status of trees by foliar analysis. Bot. Rev. 40:347-394.

van den Driessche, R. 1954. Nutrient storage, retranslocation, and relationship of stress to nutrition. Pp. 181-209 in G. D. Bowen and E. K. S. Nambiar (eds.), Nutrition of Plantation Forests. Academic Press, New York.

Vitousek, P. 1982. Nutrient cycling and nutrient-use efficiency. Am. Nat. 119:553-572.

Leaf Cuticles as Potential Markers of Air Pollutant Exposure in Trees

Virginia Seymour Berg
Biology Department University of Northern Iowa Cedar Falls, IA 50614

ABSTRACT

Because leaf cuticle covers a large area and is continually exposed to the environment, it might serve as a sensitive and early indicator of exposure to air pollutants. Some investigations have shown alterations of the ultrastructure of surface waxes with field or laboratory exposure to air pollutants, while others have not detected significant differences. It is possible that the changes in the surface structure are largely a product of pollutant-induced stress, rather than a direct consequence of exposure of the cuticle itself. The healthiest trees in polluted zones may show less alteration of cuticle than the least healthy trees of less polluted zones. Laboratory studies have demonstrated that prolonged exposure of isolated cuticle to acid can increase the permeability of the cuticle to the acid, but such studies have not yet been carried out on intact leaves. Because the changes seen for cuticles are nonspecific, resembling an acceleration of natural aging processes or exposure to other stresses, it is currently difficult to use them as specific markers of air pollution exposure, especially if the object is to distinguish air pollution from other sources of stress.

THE CUTICLE AS A BARRIER

Plant cuticles present the main barrier between the interior of the leaf and the external environment. They are in constant contact with air pollutants, including gaseous pollutants, particulates and acid precipitation. If the cuticle is altered in a specific and predictable manner by exposure to air pollutants, it could serve as a diagnostic marker of air pollution exposure. Here we will look first at the structure of the cuticle, then at changes in the outer surface associated with air pollution exposure, and finally at some information on the movement of acid through cuticles and changes in permeability of isolated cuticles caused by acid exposure.

CUTICLE STRUCTURE

The innermost part of the cuticle is characterized by interspersed pectin, cellulose and lipids (Martin and Juniper 1970). The portion of the cuticle exterior to this layer can typically be enzymatically separated from the outer walls of the epidermal cells using a pectinase/cellulase solution (Schoenherr 1976). The next layer (moving toward the outside) consists primarily of cutin and waxes. The cutin, which provides much of the mechanical integrity of the cuticle, consists of esterified fatty acids which may be further crosslinked by ether bridges (Kolattukudy 1980). There are some free acidic (dissociable) groups on the cutin polyester matrix, and these may be important in transport of substances through the cuticle. The term "waxes" is used loosely here, indicating members of a large collection of saturated straight-chain hydrocarbons and their derivatives, chiefly primary and secondary alcohols, ketones and aldehydes, fatty acids and wax esters (Kolattukudy 1976). The waxes are embedded in the cutin matrix and form a continuous layer on top of it. It is this final, uninterrupted, wax layer that forms the primary barrier between the internal and external environments of the leaf (Schoenherr 1976). On top of this wax layer may be crystalline deposits of epicuticular wax; these play an important role in interactions between acid precipitation and leaf surfaces, and may serve to collect or retain pollutants deposited in dry form, as they can substantially increase the area of the wax surface.

EPICUTICULAR WAX: AN INDICATOR OF POLLUTANT EXPOSURE?

The structure of epicuticular waxes changes with age (Reicosky and Hannover 1976, Franich et al. 1977, Crossley and Fowler 1986, Grill et al. 1987). Although they are normally crystalline, the material is rather soft and can be altered or removed by the impact of rain or other precipitation (Baker and Hunt 1986, Mayeux and Jordan 1987), by abrasion from wind-blown particulates (Rotem 1965), or by contact with other leaves or adjacent surfaces (Wilson 1984). In addition, the crystals appear to degrade slowly over time, fusing into amorphous masses and eventually into a largely continuous layer (Reicosky and Hannover 1976, Crossley and Fowler 1986, Grill et al. 1987). These changes are accompanied by a modification of the appearance of the leaf surface (blue spruce, for instance, turns green; Reicosky and Hannover 1978) and by alteration of the wettability of the surface. The wettability determines how readily a drop of liquid is retained by the leaf, and how well it contacts the surface, and, therefore, it indicates the potential degree of interaction between precipitation (including acid precipitation) and the leaf. This contact may be important in the process of leaching of substances from leaves, and is necessary for the movement of acid into leaves through the cuticle.

There are many reports of differences in the structure and wettability of epicuticular wax between polluted and unpolluted sites (Percy and Riding 1978, Huttunen and Laine 1983, Crossley and Fowler 1986, Grill et al. 1987). Typically the symptoms are similar to those seen with increased leaf age: fusion of wax crystals eventually to form continuous layers of wax on top of the normal uninterrupted wax layer. This wax may completely cover stomates, or may largely occlude antestomatal chambers. Other differences in the waxes over stomates have been observed (Trimble et al. 1982). There is considerable uncertainty as to the mechanism by which the normal aging process takes place, and more still as to how pollutants might accelerate it. It is possible that there is some direct chemical effect of pollutants on the waxes themselves, or that there is a mechanical effect associated with wetting and drying of surface. The wetting and drying of the surface could be altered by the presence of particulate matter on the cuticle, a feature of many field sites with high pollution levels. There are two reports which further complicate this issue. Jean Fincher of the Boyce Thompson Institute (pets. comm.) has examined cuticles of outdoor-grown red spruce saplings which have repeatedly received experimental treatments of acid rains of nitrate or sulfate, along with cuticles of ozone-treated saplings. She has seen no evidence of direct effects of these treatments on the cuticle, and no clear indication of accelerated aging. (Ozone-treated plants did sometimes show increased insect damage to the cuticle, however.) Grill and colleagues (1987) examined the cuticles of relatively vigorous trees and declining trees from polluted and relatively clean sites in Germany. The epicuticular wax on vigorous trees (selected visually, based on quantity and quality of foliage) appeared to be in good condition, while the trees showing symptoms of decline had needle surfaces which appeared very much older than their chronological age. The authors suggest that these results show that the condition of the cuticle is an indicator of the general vigor of the tree, rather than specifically of exposure of the outside surface of the leaf to pollutants.

This leads to the question of whether changes in the cuticle are an indication of direct effects of air pollutants on the cuticle waxes, or an indication of changes in the metabolism of the leaf, eventually leading to alteration of the surface. A change in the rate or duration of wax production could have such an effect, as could changes in the chemical composition of the waxes. There is some evidence that deficiencies of mineral nutrients also lead to changes in the cuticle and its wettability (Seymour 1976). Preliminary studies of acid exposure of waxes isolated from cuticles do not show obvious changes in the compound classes (Berg, unpublished). Nonetheless, some untested effects, such as that of SO_2, which, being quite soluble in the wax, might disrupt its crystal structure, could provide a mechanism for accelerated aging. The compounds that make up the epicuticular waxes are chemically stable, but the crystal structures they form are not.

With the current state of knowledge, it is impossible to use the condition of epicuticular waxes as an accurate predictor of tree decline due to pollution exposure, although the two are clearly correlated at many field sites. The state of the epicuticular waxes is important because these crystals act to reduce contact between precipitation and the leaf surface. An increase in wettability has been observed for leaves of plants artificially exposed to acid precipitation (Percy and Baker 1988), and for needles from plants in polluted sites (Cape 1983, McIlveen, Ontario Department of the Environment, pers. comm.), but the increased wettability of needles in the more polluted sites is of the same general magnitude as that observed for increased needle age. An additional difficulty in using the condition of epicuticular wax as an indication of pollution exposure is the variation seen from plant to plant due to environmental factors such as light level (Baker 1974), temperature (Armstrong and Whitecross 1976, Haas 1977) and humidity. This may mean that the cuticle can serve as a general marker of plant stress, but not a specific one.

MOVEMENT THROUGH CUTICLES

While the epicuticular waxes are important, the final barrier to nonstomatal entry of air pollutants is the continuous wax layer upon which the epicuticular wax lies. When the wax components are removed from isolated cuticles, the cutin matrix that supports and anchors the wax has a greatly increased permeability to water, indicating that at least for water, the wax layer is the principal limiting structure (Schoenherr 1976). The wax layer appears to be less important for the movement of ions (Schoenherr 1976). There are two alternative views of the movement of polar or dissociated compounds through the cuticle, one involving relatively polar pores and the other involving relatively polar areas throughout the wax. There is support for both of these models, in different species (Schoenherr 1976, Seymour 1980). The polar pores or areas are thought to be aggregations (continuous for polar pores and discontinuous for polar areas) of the more polar parts of the molecules that form the wax, possibly including acids and alcohols. This model does not envision pores as "holes" to be filled, but rather an area of the cuticle into which water molecules may pass, forming a channel. In either case, a material moving through the cuticle might pass either through the hydrophobic, nonpolar portion of the wax, as would be the case for SO_2, which has a substantial solubility in wax (Lendzian 1984), or through the pores or other polar sites, as would be the case for ions, such as sulfate, nitrate or cations.

PERMEABILITY OF CUTICLES

Pollutants in acid precipitation can pass through intact cuticles. Damage to the tissue is first apparent in the epidermal cells, which can collapse, along with mesophyll cells, in the absence of any obvious damage to the cuticle (Smith and Davis 1978, Paparozzi and Tukey 1983, Adams et al. 1984). This has been observed frequently in experimental systems (Evans et al. 1977a,b, Paparozzi and Tukey 1983, Adams et al. 1984, Musselman 1988), but seldom in the field. In part the difference may be due to the frequent use of greenhouse-grown plants in experimental systems. The wax of these plants typically is different in quantity (and possible in quality) from that of outdoor-grown plants. Damage of leaf tissue indicates the failure of the cuticle as a barrier, not necessarily damage to the cuticle. The importance of the quantity of wax can be seen from the sensitivity of cabbage cotyledons to acidity, contrasted with the resistance of cabbage true leaves to acid-induced damage (Caporn and Hutchinson 1986). The quantity of wax on the cotyledons is orders of magnitude less than that on the true leaves (Overholtzer and Berg 1987).

The permeability of isolated cuticles of *Berberis aquifolium* to a variety of acids has been measured (Dreyer et al. 1981). Acids do pass through cuticles, but many hours are required for substantial changes in the pH on the "inside" of the cuticle. Acids that were less completely dissociated passed through the cuticle more readily, indicating that they were probably moving through the wax itself, rather than through pores in the wax. Recent experiments involving HCl with grapefruit leaf cuticles (Berg and Overholtzer 1987), and H_2SO_4 with pear and lemon leaf cuticles (Berg and Overholtzer 1987), showed a more complicated pattern of permeability (Hauser and Berg 1988). After an initial very low permeability lasting from several hours to a day or more, the permeability increased substantially. The original permeability could not be restored by removing the acid, soaking the cuticle in water overnight, or soaking it in base (KOH) overnight. The presence of Ca^{2+} at physiological levels (1 to 10 mM) on the "inside" of the cuticle makes it highly impermeable to acid. This may account for the lack of visible acid precipitation damage in the field, despite rain and fog at pH 3 or below. The Ca^{2+} effect described here may be due to the ion bridging between negatively charged sites in the cutin, perhaps creating a bottleneck where the wax meets the cutin. Because it is impossible to isolate cuticles and measure permeability for most plants, it is not possible to measure these properties for many of the plants involved in forest decline. At this time we cannot make general statements about changes in the properties of isolated, intact cuticles due to the action of air pollutants. Studies of neutralization of acid droplets have shown that much, but not all, of the neutralization of acids is due to particulates on the leaf surfaces (Adams and Hutchinson 1984). Calcium nutrition has not been shown to be important in determining acid drop neutralization.

The current state of knowledge concerning the interactions between plant cuticles and air pollutants does not suggest the use of cuticles as a sensitive marker of air pollutant exposure. At present, the cuticle best serves as an indicator of, rather than a predictor of, forest decline.

In the future, however, it may be possible to use subtle changes in cuticle properties, including wettability, to indicate exposure to certain pollutants. If we wish to use and understand such techniques, we must have a better understanding of the natural changes that occur in all cuticles in the field.

References

Adams, C.M., N.G. Dengler and T.C. Hutchinson. 1984. Acid rain effects on foliar histology of *Artemesia tilesii*. Canadian Journal of Botany 62, 463-474.

Adams, C.M. and T.C. Hutchinson. 1984. A comparison of the ability of leaf surfaces to neutralize acid rain drops. New Phytologist 97, 463-478.

Armstrong, D.J. and M.I. Whitecross. 1976. Temperature effects on formation and fine structure of *Brassica napus* leaf waxes. Australian Journal of Botany 224, 309-318.

Baker, E.A. 1974. The influence of environment on leaf wax development in *Brassica oleracea* vat. *Gemmifera*. New Phytologist 73, 955-966.

Baker, E.A. and G.M. Hunt. 1986. Erosion of waxes from leaf surfaces by simulated acid rain. New Phytologist 102, 161-173.

Berg, V. and K. Overholtzer. 1987. Movement of acidity through grapefruit leaf cuticles. Plant Physiology, 83 (suppl.), 85.

Cape, J.N. 1983. Contact angles of water droplets on needles of Scots pine (*Pinus sylvestris*) growing in polluted atmospheres. New Phytologist, 93, 293-299.

Caporn, S.J.M. and T.C. Hutchinson. 1986. The contrasting response to simulated acid rain of leaves and cotyledons of cabbage (*Brassica oleracea* L.) New Phytologist 103, 311-324.

Crossley, A. and D. Fowler. 1986. The weathering of Scots pine epicuticular wax in polluted and clean air. New Phytologist 103, 207-218.

Dreyer, S.A., V. Berg Seymour and R.E. Cleland. 1981. Low proton conductance of plant cuticles and its relevance to the acid-growth theory. Plant Physiology 68, 664-667.

Evans, L.S., N.F. Gmur, F. Da Costa. 1977a. Leaf surface and histological perturbations of leaves of *Phaseolus vulgaris* and *Helianthus annuus* after exposure to simulated acid rain . American Journal of Botany 64, 903-913.

Evans, L.S., N.F. Gmur and J.J. Kelsch. 1977b. Perturbations of upper leaf surface structures by simulated acid rain. Environmental and Experimental Botany 17, 145-149.

Franich, R.A., L.G. Wells and J.R. Barnett. 1977. Variation with tree age of needle cuticle topography and stomatal structure in *Pinus radiata* needles. Phytochemistry 17, 1617-1623.

Grill, D., H. Pfeifhofer, G. Halbwachs and H. Waltinger. 1987. Investigations on epicuticular waxes of differently damaged spruce needles. European Journal of Forest Pathology 17, 246-255.

Haas, K. 1977. Einfluss von Temperatur und Blattaelter auf das Cuticularwachs von *Hedera helix*. Biochemie und Physiologie der Pflanzen 171, 26-31.

Hauser, H.D. and V.S. Berg. 1985. Effect of repeated acid exposure on acid permeability of pear leaf cuticle. Plant Physiology, 86 (suppl.), 59.

Huttunen, S. and K. Laine. 1953. Effects of air-borne pollutants on the surface wax structure of *Pinus sylvestris* needles. Annales Botanici Fennici 20, 79-86.

Kolattukudy, P.E. 1976. Introduction to natural waxes. Pp. 1-15 in Kolattukudy, P.E. (ed.), *Chemistry and biochemistry of natural waxes* . Elsevier Sci. Pub. Co., Amsterdam and New York.

Kolattukudy, P.E. 1980. Biopolyester membranes of plants: cutin and suberin. Science 208, 990-1000.

Lendzian, K.J. 1984. Permeability of plant cuticles to gaseous air pollutants. In Kohoil, M.J. & F.R. Whatley, (eds.) Gaseous air pollutants and plant metabolism. Butterworths, London.

Martin, J.T. and B.E. Juniper. 1970. The cuticle of plants. St. Martin's Press, New York.

Mayeux, Jr., H. and W. Jordan. 1987. Rainfall removes epicuticular waxes from *Isocoma* leaves. Botanical Gazette 148, 420-425.

Musselman, R.C. 1988. Acid neutralizing capacity of leaves exposed to acid fog. Environmental and Experimental Botany 28, 27-32.

Overholtzer, K.D. and V.S. Berg. 1987. Surface waxes and sensitivity to acid precipitation: cabbage cotyledons and leaves. Proceedings of the Iowa Academy of Science 94, abstract 62.

Paparozzi, E.T. and H.B. Tukey, Jr. 1983. Developmental and anatomical changes in leaves of yellow birch and red kidney bean exposed to simulated acid precipitation. Journal of the American Society of Horticultural Science 108, 890-898.

Percy, K.E. and R.T. Riding. 1978. The epicuticular waxes of *Pinus strobus* subjected to air pollutants. Canadian Journal of Forest Resources g, 474-477.

Percy, K.E. and E.A. Baker. 1988. Effects of simulated acid rain on leaf wettability, rain retention and uptake of some inorganic ions. New Phytologist 108, 75-82.

Reicosky, D.A. and J.W. Hannover. 1976. Seasonal changes in leaf surface waxes of *Picea pungens*. American Journal of Botany 63, 449-456.

Reicosky, D.A. and J.W. Hannover. 1978. Physiological effects of surface waxes. I. Light reflectance of glaucous and non-glaucous *Picea pungens*. Plant Physiology 62, 101-104.

Rotem, J. 1965. Sand and dust storms as factors leading to *Alternaria* blighta epidemics on potatoes and tomatoes. Agricultural Meterology 2, 281-288.

Schoenherr, J. 1976. Water permeability of isolated cuticular membranes: the effect of cuticular waxes on diffusion of water. Planta 131, 159-164.

Seymour, V. Berg. 1976. Leaf wettability—a mechanism for the increased foliar leaching of nutritionally stressed plants. M.S. Thesis, University of Washington, Seattle, WA.

Seymour, V. Berg. 1980. A study of water movement through plant cuticles. Ph.D. Dissertation, University of Washington, Seattle, WA.

Smith, H.J. and D.D. Davis. 1978. Histological changes induced in Scotch pine needles by sulfur dioxide. Phytopathology 68, 1711-1716.

Trimble, J.L., J.M. Skelly, S.A. Tolin and D.M. Orcutt. 1982. Chemical and structural characterization of the needle epicuticular wax of two clones of *Pinus strobus* differing in sensitivity to ozone. Phytopathology 72, 652-656.

Wilson, J. 1984. Microscopic features of wind damage to leaves of *Acer pseudoplatanus* L. Annals of Botany, 53, 73-82.

Air Pollutant-Low Temperature Interactions In Trees

R.G. Alscher
Virginia Polytechnic Institute and State University Blacksburg, VA 24061
J.R. Cumming
Boyce Thompson Institute Ithaca, NY 14853
J. Fincher
Boyce Thompson Institute Ithaca, NY 14853

ABSTRACT

Evidence is accumulating to suggest a causative role for ozone in winter injury of red spruce. This could be due to an impairment of the winter hardening process brought about by ozone. Hardening in conifers involves a complex series of physiological and ultrastructural adaptations. Ozone is known to affect both photosynthesis and carbohydrate allocation and to stimulate antioxidant production in actively growing crop species, but its metabolic effects on winter hardening in conifers have not been studied. Results from a dose-response study carried out on red spruce seedlings at Boyce Thompson Institute suggest that ozone exposure during the summer and fall leads to changes in carbohydrate metabolism associated with winter hardening and to cell damage during the late fall and early winter.

Forest decline at high elevations in both Europe and North America and the lack of a uniform causal agent have led to the suggestion that several interacting stresses combine to cause loss of tree vigor (Johnson and Siccama, 1983; Blank, 1985). Trees growing at high elevations are often functioning at their physiological limits such that any additional stress, such as pollutant exposure, may lead to tree mortality. In Western Europe, data accumulated over more than a decade in Finland indicate that conifers growing around industrial areas are more susceptible to winter injury in comparison to trees from relatively unpolluted environments (Huttenen et al., 198 I; Huttenen and Soikkeli, 1984). This pattern suggests that industrial pollutants are in some way altering cold tolerance of trees. Winter injury of declining trees has repeatedly been reported in the case of the German forests, and ozone has explicitly been proposed to play a central role in the decline phenomenon (Rehfuess, 1987). In the United States, extensive evidence has been assembled for a change in the response of red spruce to temperature at high elevations over the past 20 years at Whiteface Mountain, NY, in the Adirondack Mountain Range. This change was found to be correlated in time with increased incidence of tree decline (Johnson et al., 1986, 1987).

Recent data directly implicate ozone as a causative agent in decreased winter hardiness of spruce. An interaction between ozone and cold temperatures leading to visible injury has been reported in Norway spruce (Brown et al., 1987; Barnes and Davison, 1987). Several clones that had experienced high ozone concentrations during the summer exhibited uniform browning and abscission of older year class needles. This pattern contrasts with decline symptoms in North America but is consistent with observed damage in Europe. In addition, clonal variation in response to ozone and cold temperature exposure suggests that there is a strong genetic component to resistance, the physiological basis of which is unknown.

The mechanism(s) by which ozone caused this pattern of injury are not understood. Some hypotheses may be formulated, however, through the synthesis of what is known concerning metabolic effects of ozone, changes that underlie the winter hardening process, and physiological responses to cold temperatures.

Ozone may impair plant metabolism through the formation of toxic free radicals *in vivo*. Free radicals are metabolically neutralized through the action of a variety of antioxidants, which remove free radicals and their toxic by-products. Antioxidant compounds such as glutathione and SOD are produced in greater quantities by plant cells in response to oxidative stress (Lee and Bennett, 1982) and represent one possible resistance mechanism which may vary both within and among tree species. Mehlhorn et al. (1986) demonstrated increases in the levels of GSH and alpha-tocopherol in needles of spruce and fir as a consequence of long-term, low-level exposures to ozone. Only when the protective capacities of these mechanisms are overwhelmed will injury, such as lipid peroxidation, occur (Halliwell, 1981; Heath, 1987).

The winter hardening process in conifers involves a series of orchestrated physiological, histological, and biochemical events that prepare cells for exposure to cold temperatures. Among these are reductions in photosynthesis, increased hydrolysis of starch to form soluble sugars (Aronsson et al., 1975), an accumulation of antioxidants (Esterbauer and Grill, 1978), and vast ultrastructural changes (Soikkeli, 1978) as trees enter dormancy. The conversion of starch to soluble sugars in leaf tissue represents one mechanism by which plant cells increase their tolerance to freezing temperatures. Oligosaccharides such as sucrose and raffinose are known to protect membranes against the dehydrative effects of freezing (Quinn and Williams, 1985). Raffinose in particular has been implicated as a cryoprotectant in Norway spruce.

In spite of these changes in the cellular environment, damage due to cold temperatures does occur. Under conditions of relatively high light and low temperature, excess light is absorbed by antennae pigments. Reduced electron transport capacity of dormant tissue impairs the transfer of reductant to acceptors such as NADP (Oquist, 1982, 1983, 1985). Instead, molecular oxygen will serve as an electron acceptor and consequently will be reduced to superoxide in the Mehler reaction. The photoscavenging cycle will act to remove the superoxide and attendant molecular species, and antioxidant compounds will be consumed in the course of this process. If it continues beyond the limit of antioxidant capacity, the oxidation of labile components such as photosynthetic pigments will follow. Oxidation of this kind does in fact occur. For example, photobleaching of up to half of the total chlorophyll content of Scots pine has been reported to occur under natural conditions in northern Sweden in early spring (Oquist, 1985). Shade needles show less bleaching than do needles in full sun, as would be expected from the mechanism of injury involved.

Increased photo-oxidation of chlorophyll, as well as other free radical injury that occurs at low temperatures and high light in conifers, may be observed under conditions where free radicals are being, or have been, generated by an air pollutant such as ozone. This damage may be due to the exhaustion of antioxidant mechanisms of plant cells brought about by the joint stress of ozone during the growing and/or hardening season and by low temperatures, which occur during the winter months. During the winter, when *de novo* production of antioxidants may be low, plants that have been previously depleted of antioxidant reserves by ozone may incur damage when protective and repair mechanisms are insufficient to protect tissue further from cold injury.

We have biochemical and histological evidence that ozone exposure both altered winter hardening processes and increased cellular damage during early winter frosts of red spruce seedlings. Four-year-old seedlings were exposed to charcoal filtered air or to doses of ozone ranging from ambient to 4X ambient in Ithaca, NY during the summer and fall of 1987. In December there were no visible symptoms on any of the current year foliage. However, histological examination of these needles showed that there was damage to mesophyll cells in needles exposed to ozone. This damage included vesiculation, and at its most extreme involved total disruption of cells with breakage of cell walls and leakage of contents into intercellular spaces. In needles with patches of damage, nearby cells appeared to be undergoing the normal transition, characterized by alterations of chloroplasts, lack of starch, and increased tannin in vacuoles. Disruption of this nature was not seen earlier in the season prior to freezing temperatures.

An examination of the patterns of seasonal changes in carbohydrate levels in the seedlings revealed that exposure to ozone also resulted in a shift in the time course for starch mobilization in the late summer and fall. Early frost susceptibility and winter dieback of ozone-treated spruce foliage was observed by Amundson and Cumming (unpublished). Interacting influences on cold tolerance in conifers of growth regulating substances, oxidative stress, and seasonal cycling in response to environmental cues remain to be understood. The combined influences on winter hardening in conifers of growth regulating substances and oxidative stress remain to be elucidated.

SUMMARY AND DISCUSSION: METABOLIC "THRESHOLDS" FOR STRESS

We have accumulated biochemical, physiological, and histological data suggesting that exposure to ozone increases the susceptibility of red spruce foliage to cold temperatures. The patterns can be fit into a scenario where prior exposure to ozone overloads the cell's antioxidant resistance mechanisms past some critical level or "threshold," beyond which the cells can no longer tolerate the processes associated with exposure to low temperatures, e.g., free radical production. Were this threshold to be established experimentally, it might be possible to use it as an indicator or "marker" of air pollution stress on coniferous forest tress. It is important to keep in mind that a concept such as this is useful only when implemented with discretion. A tree that is severely stressed with respect to nutrition or water will most probably have a lower threshold for the additional stress of oxidizing air pollutants than one which is not. As a consequence, the need for further research to establish the impact of interacting stresses on the physiology and metabolism of forest trees should be apparent.

Oxidative pollutant and cold temperature stresses may further interact if changes in the timing of physiological events associated with the hardening process occur because of pollutant exposure and such alterations increase the susceptibility of foliage directly. Alternatively, these changes could influence the period of time during which the foliage is vulnerable to cold temperatures. Again, further experimentation is required before any evaluation of these various possibilities can be made.

References

Aronsson, A., T. Ingestad, and L. Lars-Gorau. 1976. Carbohydrate metabolism and frost hardiness in pine and spruce seedlings at different photoperiods and thermoperiods. Physiol. Plant. 36:127-132.

Barnes, J.D., and A.W. Davison. 1987. The influence of ozone on the winter hardiness of Norway spruce (*Picea abies* L.). New Phytol. 108: 159-166.

Blank, L.W. 1985. A new type of forest decline in Germany. Nature. 314: 311-314.

Brown, K.A., T.M. Roberts, and L.W. Blank. 1987. Interaction between ozone and cold sensitivity in Norway spruce: a factor contributing to the forest decline in Central Europe? New Phytol. 105: 149-155.

Esterbauer, H., and D. Grill. 1978. Seasonal variation of glutathione and glutathione reductase in needles of *Picea abies*. Plant Physiol. 61:119-121.

Heath, R.L. 1987. Biochemistry of ozone attack on the plasma membrane of plant cells. Rec. Adv. in Phytochem. 21: 29-54.

Huttunen, S., L. Karenlampi, and K. Kolari. 1981. Changes in osmotic potential and some related physiological variables in needles of polluted Norway spruce (*Picea abies*). Ann. Bot. Fennici. 18: 63-71.

Huttunen, S., and S. Soikkeli. 1984. Pp. 117-128 in Gaseous Air Pollutants and Plant Metabolism (Koziol, M.J., Whatley, F.R., eds.) Botany School, University of Oxford, Oxford, UK.

Johnson, A.H., and T.G. Siccama. 1983. Acid deposition and forest decline. Environ. Sci. Technology 17: 294a-305a.

Johnson, A.H., A.J. Friedland, and J. Dushoff. 1986. Recent and historic red spruce mortality: Evidence of climatic influence. Water Air Soil Pollut. 30: 319-330.

Johnson, A.H., E.R. Cook, and T.G. Siccama. 1988. Relationships between climate and red spruce growth and decline. Proc. Nat. Acad. Sci. (in press).

Lee, E.H., and J.H. Bennett. 1982. Superoxide dismutase. A possible protective enzyme against ozone injury in snap beans (*Phaseolus vulgaris* L.). Plant Physiol. 69: 1444-1449.

Mehlhorn, H., G. Seufert, A. Schmidt, and K.J. Kunert. 1986. Effect of SO_2 and O_3 on production of antioxidants in conifers. Plant Physiol. 82: 336-338.

Oquist, G. 1982. Seasonally induced changes in Acyl lipids and fatty acids of chloroplast thylakoids of *Pinus silvestris*. Plant Physiol. 69: 869-875.

Oquist, G. 1983. Effects of low temperature on photosynthesis. Plant, Cell and Environ. 6: 281-300.

Oquist, G. 1986. Effects of winter stress on chlorophyll organization and function in Scots pine. J. Plant Physiol. 122: 169-179.

Quinn, P.J., and W.P. Williams. 1985. Pp. 1-48 in Photosynthetic Mechanisms and the Environment (Barber, J. and Baker, N.R. eds.) Vol. 6. Amsterdam, New York and Oxford.

Rehfuess, K.E. 1987. Perceptions on forest diseases in Central Europe. Forestry. 60(I): 1-11.

Soikkeli, S. 1978. Seasonal changes in mesophyll ultrastructure of needles of Norway spruce (*Picea abies*). Can. J. Bot. 56: 1932-1940.

Alteration of Chlorophyll in Plants Upon Air Pollutant Exposure

Robert L. Heath
Department of Botany and Plant Sciences University of California Riverside, CA 92521

ABSTRACT

The loss of chlorophyll from plants (chlorosis) has been long used to assess injury induced by varied air pollutants, and is believed to signal a decline in photosynthetic capacity and plant productivity. Unfortunately, chlorosis is not specific for air pollutant stress; other stresses and, even, normal development can alter pigment content. In fact, it is not even clear that a loss of chlorophyll consistently mirrors a loss of photosynthetic capacity. This paper will briefly review some concepts of pigment loss, including photosynthetically-linked mechanisms, and the development of pollutant injury. Recently, chlorophyll fluorescence has been touted as a useful monitor of injury and so, some discussion on the methods and instrumentation of chlorophyll measurements, including the use of chlorophyll fluorescence as a monitor, will be presented.

CHLOROPHYLL CONTENT

Often in stress literature, the terms necrosis and chlorosis are intermixed due to their joint use in monitoring *in vivo* alterations in the leaf. Necrosis often means chlorosis in essence, since chlorophyll molecules in dead cells, when exposed to both light and oxygen in the absence of protectants, are chemically modified and lose visible light absorbance, and so bleach. Chlorosis, however, only means "yellowing caused by loss of or *reduced development* of chlorophyll... (emphasis added)"[19]. In many cases, it is difficult to know to which term the research is referring. For the most part, only visible injury, which includes both chlorosis and necrosis, is reported. Only a portion of the research reported actually measures chlorophyll loss by extracting the pigments and measuring the decline in total extractable pigments.

Chlorophyll is porphyrin derivative (a flattened ring of four pyrrole rings) with a fifth ring (the pentanone ring) attached to pyrrole ring III and the τ-methylene bridge, with a central Mg ion (Fig. 1). The chlorophyll molecule is made hydrophobic by the attachment of a phytol chain (tetramethyl hexadecenol) to ring IV (at the 7b carbon) [2]. Chlorophyll a and b differ by only one group, a methyl or formyl group on the 3b carbon of ring II (Fig.1). Chlorophyll can be easily transformed by the loss of the central Mg ion, caused by acidification, to pheophytin (a distinctly yellow-green pigment), while the loss of phytol, caused by chlorophyllase, produces chlorophyllide with a slightly different spectra The 6d carbon in the cyclopentone ring can easily isomerize in alcoholic solutions to form allo-chlorophyll, whose spectra is virtually identical to chlorophyll. In the presence of oxygen, various carbons in the pentone ring can be oxidized to form several poorly defined oxidation products.

Chlorophylls, of course, have distinctive spectral characteristics. The blue band of the spectra (the Soret Band with an absorbance about 400 rim) overlaps with carotenoid spectra; however, the red band (alpha-band) can be used to separate chlorophyll a from chlorophyll b [2,3,7]. Generally, the ratio of chl a/chl b is about 2.5 and so the separation of individual chlorophyll peaks can be accomplished by a good spectrophotometer. Yet the determination of ratios greater than 3 are generally inaccurate. Also some of the oxidative species possess spectra which are slightly shifted and so can confound the determinations of pure chlorophylls by spectrophotometric methods (see ref. [3,4,12,26,27,30] for some of the peaks and extinction coefficients of chlorophylls and their products in varied solvents). The extraction of chlorophylls by organic solvents can be accomplished to a varied degree. Acetone (80%) is easy and traditional [12,29], ethanol is adequate and often used [4,30], but a series of organic solvents (chloroform methanol), used for general lipid extraction, perform the best in fully extracting the pigments and their products [4,21,26]. Some chemical alterations of chlorophylls can be observed by spectral analysis (however, one should use a relatively sophisticated spectrophotometer); others require chromatography techniques to discover. Examples of the oxidative conversions is shown in Figure 2, in which pigments are extracted through lipid extraction techniques (by the use of chloroform and methanol) and subjected to thin layer chromatography (TLC). After separation, the pigments are rapidly scraped from the plate and extracted from the silica by chloroform and separated by high pressure liquid chromatograph (HPLC). Clearly, other chlorophyll-like compounds have been induced by air exposure through TLC. Some of these extra bands are allomerized chlorophyll [27] and others are oxidized species (see ref [4,21,26] for a more complete discussion of oxidations, spectra and HPLC separations).

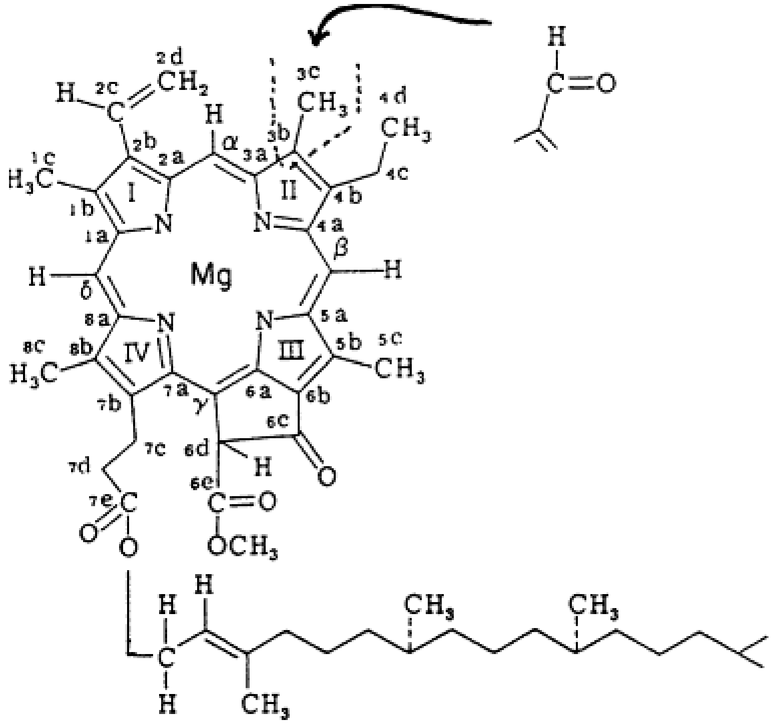


Figure 1.
Structure of Chlorophyll Molecule. Adapted from Aronoff [2]. The structure shown is chlorophyll A; the replacement of the methyl group on carbon 3b, with a formyl group (as shown by the insert and arrow) changes the molecule into chlorophyll B. The hydrophobicity of the molecule is provided by the phytol chain shown on the bottom and attached through carbon 7e.

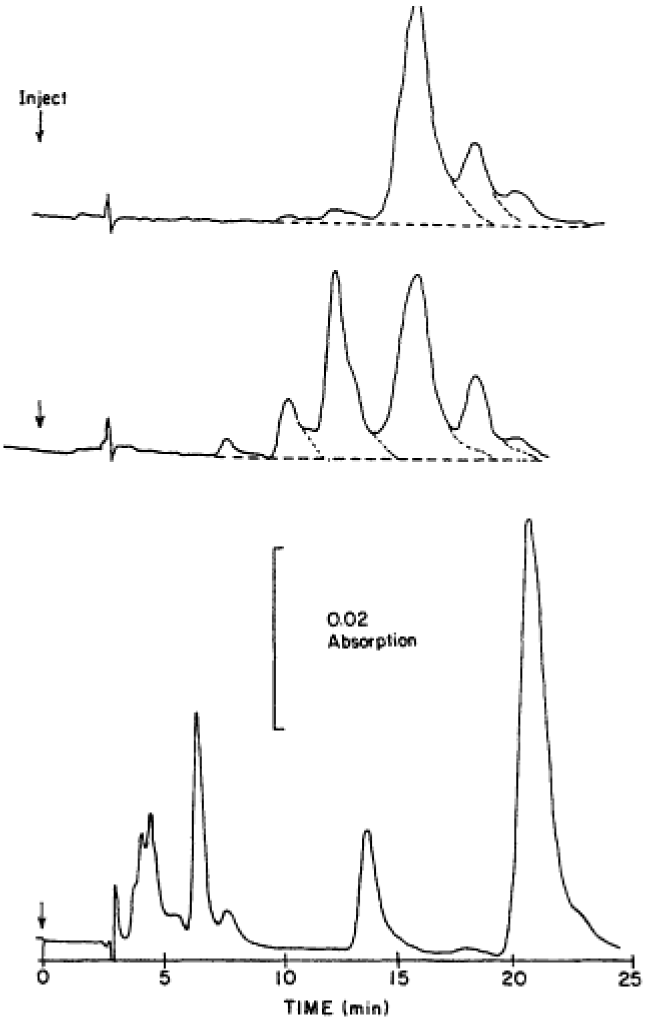


Figure 2.

The Modification of Chlorophyll Pigments upon Exposure to Air During Thin Layer Chromography Separation. Pigments from spinach were isolated by standard lipid extractions using chloroform:methanol (1:1, v/v) and the Folch phase separation. The pigment fraction was separated from the polar lipids by a Sep-pak (Silica, from Waters, Inc.) removing β-carotene by chloroform wash and the pigments by a chloroform:ethyl acetate (4:6, v/v) wash. The resulting fraction was concentrated by flash evaporation and boiling down under nitrogen and was resuspended in a small volume of chloroform. The concentrated fraction was either run on a C-8 HPLC column (5 μm pore size, 4.5 × 150 ram, Beckman; solvent of 80:10:10, methanol/chloroform/water at a flow rate of 1.5 ml/min) or spotted on a TLC plate (Silica HF plate, 100 m thickness) and developed by a solvent of diethyl ether. The figure shows HPLC runs (injected volume, 20μl two regions of the TLC plate (top, chlorophyll A; middle, chlorophyll B) after scrapping, eluting with chloroform, and filtering through a 0.2μm filter. The bottom run shows the pigments fraction that was not run on the TLC plate.

This figure also demonstrates the power of HPLC for quantifying pigments. In one determination using an isocratic solvent which is complete in 25 minutes, carotenoids and chlorophylls can be quantitated, although the polar carotenoids are not well separated. To separate these and to allow *β*-carotene to be determined in a single trial, a solvent gradient or several trials with differing solvents of varying hydrophobic characteristics must be used, thus lengthening each analyses. Not shown in this diagram but tabulated in Table 1, tocopherol and its quinone can also be separated on this HPLC system.

Table 1 Polarity of Solvent System Used on HPLC and the Relative Retention Times of Several Lipid Components.

		RETENTION TIME (RELATIVE) Solvent (methanol/chloroform + 10 water)			
Component	**60/25**	**65/15**	**70/20**	**80/10**	**90/10**
β-carotene	3.35				
Chloro A	1.94	2.54	3.22	5.25	17.0
Chloro B	1.42	1.73	2.10	3.25	9.95
Tocopherol	1.08			2.85	
Tocoquinone	0.86			1.92	
Lutein		0.47	0.70	1.18	3.63
Neoxanthin		0.26	0.35	0.55	1.50
Vioxanthin		0.12	0.21	0.44	1.24

The retention times of the indicated components were made relative to the retention time of the void volume of the column (see legend in Figure 2). Solvent for development of HPLC was made as indicated + 10 volumes water, as v/v.

Chlorophyll can also be quantified *in vivo* to a certain extent, by the use of an integrating sphere [7] (such a system can be purchased through LiCor, model LI-1800). When light passes through a leaf, it is highly scattered by the material within the leaf. If the leaf-scattered light is collected after multiple re-scattering, the loss of light by only true absorbance can be determined [7]. This system is not perfect and spectra thus derived still possess a large spectral broadening which confounds the actual chlorophyll absorbance. Yet the chlorophyll peak can be observed and quantified. More importantly, this determination is not destructive. However, the ratio of chl a/chl b cannot be determined.

Thus, three methods can be used to determine chlorophyll: (1) *in vivo* re-scattering absorbance determination; or organic solvent extraction followed by (2) spectrophotometric analysis; or (3) HPLC analysis. Number 1 is the fastest and non-destructive but is the most inaccurate. Both numbers 2 and 3 destroy the plant material. Number 2 is relatively fast but cannot determine all types of pigments accurately. With automatic equipment (which is expensive), number 3 is the most accurate and inclusive. As previously stated, chlorophyll loss is generally related to damage by air pollutants. Lendzian and Unsworth [18] believe that sulfur dioxide leads to lysis of chloroplasts and then destruction of the chlorophyll, but only in the "marginal and intercostal areas". Hällgren [9] suggested that the loss of pigments for *Pinus contorta* (using sulfur dioxide in solution) was caused by the pollutant inducing a solubilization of enzymes from the membrane (in particular, chlorophyllase) which formed chlorophyllids, leading to a loss of chlorophyll from the grana membranes. Ziegler [31] suggested sulfur dioxide fumigation of lichen caused the formation of pheophytin (presumably by acidification and monitored by both bleaching and a spectral shift) but stated that chlorophyll loss was too slow to cause the measured decline in photosynthesis. Hällgren

[9] suggests that much of the chlorophyll loss is initiated by pollutant-induced free radicals [6]. Certainly, this is plausible in chemical systems where fatty acid hydroperoxides (which break down and form free radicals) can initiate, and tocopherol and carotene (which are antioxidants and free radical chain interruptors) can inhibit chlorophyll bleaching.

However, rather than a direct cause of pollutant or its products upon chlorophyll, Davison and Barnes [5] suggested that stress may "pre-dispose the leaf to photooxidation" and "swamp" the photoprotective mechanisms. Many of their experiments involved the combined action of cold and air pollutants in conifers. Frost inhibited photosynthesis and the secondary pollutant stress induced a photooxidation of the structure of the chloroplast.

Knudson et al. [15] exposed young pinto bean seedlings to 1 hour of 0.9μ/L v/v) of ozone and followed to the chlorophyll loss by extraction in ethanol for 2, 4, and 6 days post-fumigation. They used visual examination to determine "chlorosis" or "visible injury" before extraction. They found a close 1:1 relationship between a visually scored plant injury and a loss of extractable chlorophyll. A typical range of visual scoring for a 10% reduction of chlorophyll was about 6 ± 6 % while for 50% chlorophyll reduction, the range was 55 ± 18%. There was no indication in this experiment that chlorophyll a was more or less sensitive that chlorophyll b. Thus, the visual scoring monitored general chlorophyll loss and it can be carried out in the field with a minimum of effort. In this experiment, the chlorophyll extraction was likewise easily done by allowing the leaves to sit in ethanol for several days in the dark, but long term ethanolic extraction can have problems with multiple product formation.

Lower levels of pollutants may cause a premature senescence rather than a direct chlorophyll loss. Adedipe et al. [1] found in several varieties of tobacco that exposure to 0.3μl/L ozone (for 2 hours) induced chlorophyll, RNA, and protein loss which followed normal senescence patterns. On the other hand, Leffler and Cherry [17] suggested that the site of the predisposition of injury was the chloroplasts, since reduction of the activity of nitrate reductase (found within the chloroplast) and of the amount of chlorophyll were identical upon ozone fumigation of soybean. On the basis of chemical studies and the results of Sakaki et al. [28], it has been suggested that chlorophyll a is less stable than chlorophyll b and so the increase in the ratio of a/b is a good indication of free-radical-induced pollution effects. Rabe and Kreeb [22] do not believe this, however, based upon their study with ambient air in Stuttgart; they did observe a large increase in the levels of pheophytin within the injured plants, especially spruce and turnip.

Several conclusions can be made:

(1) Chlorophyll loss is found to develop in leaves exposed to oxidants or other air pollutants (such as sulfur dioxide and oxides of nitrogen), yet the loss of chlorophyll takes several days to be observed, and presumably requires light.
(2) Chlorophyll loss can be measured with accuracy only when the episode is somewhat severe. Mild exposure may cause chlorophyll changes, but they may be more connected with abnormal states of development (e.g., early senescence or partially closed stomates).
(3) While one would expect other products of chlorophyll oxidations to appear, only pheophytin has been observed. Yet a concerted effort to measure products has not yet been undertaken, even in chemical systems.
(4) While changes in (chlorophyll a)/(chlorophyll b) ratios are suggested by some to be a monitor of injury, the results are not yet compelling and difficult to observe accurately.
(5) Chlorophyll loss is a relatively simple monitor of leaf changes and should be

used for monitoring air pollution injury, but only in relation to other parameters (for example, protein levels or total cell mass) if realistic conclusions wish to be drawn.

CHLOROPHYLL FLUORESCENCE

Schreiber et al. [25] used chlorophyll fluorescence *in vivo* to monitor ozone-induced stress. Chlorophyll upon absorption of light will naturally fluoresce if a polar solvent is present. The fluorescence has a life time of about 15 nano-seconds with a yield of emitted light to absorbed light of about 30%. In dry solvents there is no fluorescence; the light energy is dissipated by non-radiative pathways. Light energy absorbed by both the Soret and red bands is reduced to the level of the red band through non-radiative pathway and, when released by fluorescence, is emitted as a band centering about 682 nm (for solvents in which the red band peaks at 663 nm).

In plants, the chlorophyll molecules are found within a slightly polar molecular domain and so they fluoresce with a yield of about 2 to 8% efficiency (and a 5-to 10-fold faster life-time). Furthermore, there are at least several molecular domains in which the chlorophylls are located, which shifts the emitted fluorescence wavelength peaks to 684 and approximately 720 nm. At room temperature the 720 nm emission is much less (by a factor of 20 or so) than the 684 nm emission. At liquid nitrogen temperature (77 K), where enzymic mechanisms cannot operate, three bands are observed (684, 693, and ?30 rim). Researchers have used the 77 K emission to ascertain electron transport properties in leaves, but this is not practical in field experiments.

Under normal conditions fluorescence is not constant but varies with post-illumination time [10,16]. The initial level of fluorescence is measured (Fo) under a minimum of amount of illumination time. In general, Fo is the smallest level of fluorescence that can be measured regardless of conditions and must be done within milliseconds of the beginning of illumination. The level then begins to rise immediately after illumination up to a new level (Fm = ca. 1.15 x Fo), within about 20 to 40 milliseconds, depending upon the light intensity. These changes are thought to be due to only primary photochemistry of Photosystem II of the electron transport pathways [16,25,28] and can only be measured with rapidly responding equipment (certainly not pen recorders). A typical slow kinetic trace is shown in Figure 3. The Fo can not be detected under these conditions. However, after a slight, rapidly-occurring hesitation (or sometimes a slight dip) the fluorescence rises again to a new, maximum observable level (called Fp = ca. 3 × Fo) in about 1 second. This level is very sensitive to the illumination history of the leaf and to stress. As one of the first observations of pollutant injury [8,11,25], it tends to be reduced. After several seconds of illumination, the peak lowers with the possibility of a shoulder on the decline side occurring at about 5 to 10 seconds. The final steady-state level occurs at about 50 to 100 seconds of illumination ("T" level), and is slightly above Fo.

Herbicides affect this fluorescence kinetic curve dramatically and help understand what the kinetics mean. Methyl viologen (paraquat, MV) can be infiltrated into leaf discs and by accepting electron at the reducing side of Photosystem I (before NADP reduction), inhibits carbon dioxide fixation. By taking electrons rapidly from the electron transport pathway, MV keeps the photosystem largely oxidized and, as is seen in the figure, keeps the kinetics of fluorescence at a low level (near Fo). Dichlorophenyl dimethyl urea (DCMU) blocks the exit of electrons out of photosystem II and keeps photosystem I largely oxidized and Photosystem II largely reduced. DCMU also inhibits carbon dioxide fixation and keeps the fluorescence level very high with little kinetic change. The DCMU-induced level is nearly the highest fluorescence level that can be seen under any conditions. Thus, MV and DCMU can induce the minimum level (Fo) and the maximum level (Fmax) of fluorescence, respectively. The range between these two levels is often denoted as the variable fluorescence (Fv).

A water stress can be applied to leaf discs by floating them on a concentration solution of Polyethylene Glycol 6000 (PEG), which osmotically removes water from the tissues. The fluorescence pattern is modified by this treatment [8], as shown in the figure. The peak is lowered, which accentuates the shoulder at about 8 seconds.

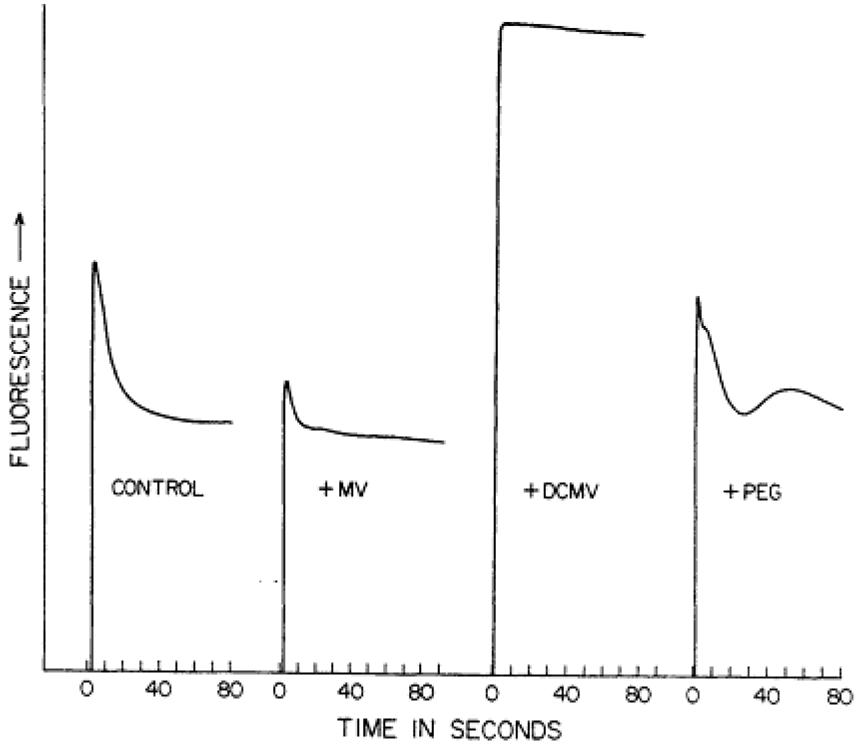


Figure 3.
In Vivo Fluorescence Kinetics of Spinach Leaf Disc. Discs from spinach leaves were cut and floated on water solutions for 1 hour in the dark, as pre-conditioning. The fluorescence data (excited by 400-450 nm light and observed at 680-690 nm) were obtained from a Hansatech Oxygen-/fluorometer [28 and see text]. The vertical scale is relative but constant. The water solutions were (a) water alone (control), (b) methyl viologen (100µm, mv), (c) Dichlorophenyl dimethyl urea (30 m, DC), and (b) polyethylene glycol 6000 (12%, PEG).

Furthermore, a new peak can be seen at 60 seconds (the M peak), which is thought to be a modification of the light energy flow within the chloroplast (see [13,16,28]). Ozone injury resembles this pattern (see Schreiber et al., 25). A lowering of Fo is only observed when gross injury to the system has occurred and most probably great chlorophyll loss is likewise occurring [11,25].

The immense body of literature on chlorophyll fluorescence and its interpretation (for some reviews, see [8,10,11,13,14,16,20,24,28]), cannot be summarized here. It is possible to monitor fluorescence relatively easily now, even in the field. The interpretation of fluorescence kinetics alterations due to stress, however, is much more difficult.

The above description of the kinetics represents only the direct illumination and emission type of measurements [10]. Another type of instrument involves the direct measurement of yield and is independent of illumination [20]. A constant, weak measuring beam of light (which is modulated for ease of detection) illuminates the leaf and the detector is "locked" onto the modulated, emitted fluorescences, which is in phase with the illumination. Other direct, non-modulated light will not be detected by the instruments, but will excite the photochemistry of the leaf. Thus, the measurement is one of a base level (near Fo), and its rise to a higher level (Fp) due to extra-illumination. When the extra light is turned off, the yield declines to the base level again (see Figure 4). Again the Fp is sensitive to stress, as shown in the figure for a preliminary experiment with Jeff cry Pine (from G. Goldstein, Argentine, and P. Randel, UCLA).

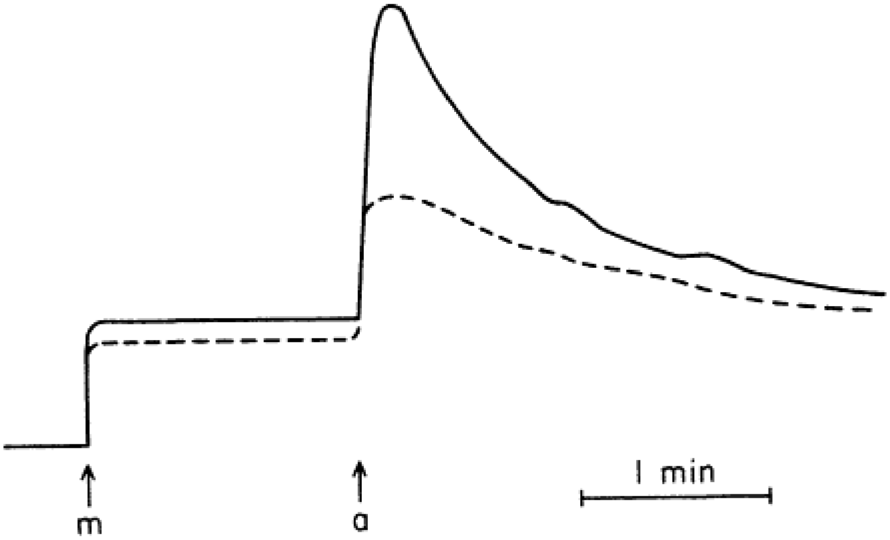


Figure 4.
The Fluorescence Yield of Pine Needles. Data provided by G. Goldstein and P. Randel. Needles from Jeffrey Pine trees exposed to ambient conditions in the Los Angeles basis were measured on the yield fluorometer [13 and see text]. The measuring light is turned on at m and the actinic white light (intensity of 2000µm-teins/cm^2 sec) is turned on at a. The bottom panel shows four year old needles from non-injured (solid line) and severely injuried (dotted line) trees.

Thus, fluorescence can monitor the reactions occurring within the chloroplast. The changes of the pattern can be easily detected currently but the understanding of what is occurring is less easy to come by, regardless of what some believe. Certainly, under mild stress, fluorescence may turn out to be a reasonable monitor. However, under very mild stress (0.24 zone for 4 hours under low humidity conditions), reproducible changes in Fp, in spinach and bean, have not been observed, even when the yield of dry matter was reduced 10-20% (unpublished data of D. Olszyk and R.L. Heath). It is clear that more basic work needs to be done before chlorophyll fluorescence is a simple monitor of photosynthetic alterations by pollutants.

CONCLUSION

While visible injury still dominates the literature as a marker of pollutant injury, the technology is available to measure chlorophyll loss *in vivo* and to monitor some alterations of the primary events of the chloroplasts through chlorophyll fluorescence kinetics. However, it is too soon to be able to interpret these changes biochemically and these alterations may not be specific to only air pollutant injury. Indeed, it is doubtful that a specific monitor of air pollutant injury will ever be discovered. More probably, several markers must be correlated before it is certain that only air pollutants are the cause of a plant's stressful state.

References

1. Adedipe, N.O., R.A. Fletcher, and D.P. Ormrod. 1973. Ozone Lesions in Relation to Senescence of Attached and Detached Leaves of Tobacco. Atmosph. Environ. 7.' 357-361.
2. Aronoff, S. 1966. The Chlorophylls—An Introductory Survey. In.' The Chlorophylls. (Eds., L.P. Vernon and G.R. Seely) Academic Press, New York, Pp. 3-21.
3. Bruinsma, J. 1961. A Comment on the Spectrophotometric Determination of Chlorophyll. Biochim. Biophys. Acta 52: 576-578.
4. Braumann, T., and L. H. Grimme. 1981. Reversed-Phase High Performance Liquid Chromatography of Chlorophylls and Carotenoids. Biochim. Biophys. Acta 637: 8-17.
5. Davison, A.W., and J.D. Barnes. 1986. Effects of Winter Stress on Pollutant Responses. In: How are the Effects of Air Pollutants on Agricultural Crops Influenced by the interaction with Other Limiting Factors? Proc. of Workshop, Commission of European Communities and Natl. Agency of Environ. Protect., Denmark, pp.16-32.
6. Saran, M., C. Michel, and W. Bors. 1988. Reactivities of Free Radical In: Air Pollution and Plant Metabolism (Eds., S. Schulte-Hostede, N. Darrall, L. Blank, A.R. Wellburn), Elsevier Publishers, London Pp. 76-93.
7. French, C.S. 1960. The Chlorophylls in Vivo and in Vitro. In: Encycl. of Plant Physiology. (Ed. W. Ruhland) Vol. V/I, Springer-Verlag, Berlin, pp. 252-297.
8. Govindjee, W.J.S. Downton, D.C. Fork, and P.A. Armond. 1981. Chlorophyll A Fluorescence Transients as an Indicator of Water Potential of Leaves. Plant Sciences Lett. 20:191-194.
9. Hällgren, J.E. 1979. Physiological and Biochemical Effects of Sulfur Dioxide on Plants. In: Sulfur in the Environment Part II. Ecological Impacts. (Ed. J. O. Nriagn). John Wiley and Sons, New York. Pp. 164-209.
10. Heath, R.L. 1973. The Energy State and Structure of the Isolated Chloroplast: The Oxidative Reactions Involving the Water Splitting Step of Photosynthesis. Intl. Rev. Cytol. 34: 49-101.
11. Heath, R.L., P.E. Frederick, and P.E. Chimiklis. 1982. Ozone Inhibition of Photosynthesis in Chlorella Sorokiniana. Plant Physiol. 69: 229-233.
12. Inskeep, W.P., and P.R. Bloom. 1985. Extinction Coefficients of Chlorophyll a and b in N,N-Dimethylformamide and 80% Acetone. Plant Physiol. 77:483-485.
13. Jenkins, G.I., N.R. Baker, M.Bradbury, and H. W. Woolhouse. 1981. Photosynthetic Electron Transport During Senescence of the Primary Leaves of Phaseolus vulgaris L. III. Kinetics of Chlorophyll Fluorescence Emission from Intact Leaves. J. Expt. Bot. 32: 999-1008.
14. Krause, G.H., and E. Weis. 1984. Chlorophyll Fluorescence as a Tool in Plant Physiology. II. Interpretation of Fluorescence Signals. Photosynth. Res. 5: 139-157.
15. Knudson, L.L., T.W. Tibbitts, and G.E. Edwards. 1977. Measurement of Ozone Injury by Determination of Leaf Chlorophyll Concentration. Plant Physiol. 60: 606-608.
16. Lavorel, J., and A.-L. Etienne. 1977. In Vivo Chlorophyll Fluorescence. In: Primary Processes of Photosynthesis (Ed., J. Barber) Elsevier/ No. Holland, London. Pp.206-268.

17. Leffler, H.R., and J.H. Cherry. 1974. Destruction of Enzymatic Activities of Corn and Soybean Leaves Exposed to Ozone. Can. J. Botany 52: 1233-1238.
18. Lendzian, K.J., and M.H. Unsworth. 1983. Ecophysiological Effects of Atmospheric Pollutants. In: Encycl. of Plant Physiol. New Series. (Eds., D.L. Lange, P.S. Nobel, C.B. Osmond, H. Ziegler) Vol. IV. Springer-Verlag, Berlin. Pp.466-502.
19. Little, R.J., and C.E. Jones. 1980. A Dictionary of Botany. Van Nostrand/Reinhold Co., New York. Pp. 80.
20. Ogren, E., and N.R. Baker. 1985. Evaluation of a Technique for the Measurement of Chlorophyll Fluorescence from Leaves Exposed to Continuous White Light. Plant, Cell, Environ. 8: 539-547.
21. Prenzel, U., and H.K. Lichtenthaler. 1979. Separation of Prenyllipids by High Performance Liquid Chromotography. In: Adv. in Biochem. and Physiol. of Plant Lipids. (Eds., L.-A. Appelqvist and C. Lijenberg), Elsevier/No. Holland, New York. Pp. 391-325.
22. Rabe, R., and K.H. Kreeb. 1980. Bioindication of Air Pollution by Chlorophyll Destruction in Plant Leaves. Oikos 34: 163-167.
23. Sakaki, T., N. Kondon, and K. Sugahara. 1983. Breakdown of Photosynthetic Pigments and Lipids in Spinach Leaves with Ozone Fumigation: Role of Active Oxygens. Physiol. Plant. 59: 28-34.
24. Schreiber, U. 1983. Chlorophyll Fluorescence as a Tool in Plant Physiology. I . The Measuring System. Photosynth. Res. 4: 361-373.
25. Schreiber, U., W.Vidaver, V.C. Runeckles, and P. Rosen. 1978. Chlorophyll Fluorescence Assay for Ozone Injury in Intact Plants. Plant Physiol. 61:80-84.
26. Shioi, Y., R. Fukae, and T. Sasa. 1983. Chlorophyll Analysis by High-Performance Liquid Chromatography. Biochim. Biophys. Acta 722:72-79.
27. Vernon, L.P. 1960. Spectrophotometric Determination of Chlorophylls and Pheophytins in Plant Extracts. Analyt. Chem. 32:1144-1150.
28. Walker, D.A. 1987. The Use of the Oxygen Electrode and Fluorescence Probes in Simple Measurements of Photosynthesis. Oxygraphics Limited Publishing, Chichester, and Hanstech Limited, Norfolk, England. 144 Pp.
29. Wellburn, A.R., and H. Lichtenthaler. 1984. Formulae and Program to Determine Total Carotenoids and Chlorophyll A and B of Leaf Extract in Different Solvents. In: Advances in Photosynthesis Research. (Ed. C. Sybesma) Vol. II, Martinus Nijhoff/ Junk Publishers, the Hague. Pp. 1.9-1.12.
30. Wintermarts, J.F.G.M., and A. DeMots. 1965. Spectrophotometric Characteristics of Chlorophyll a and b and their Pheophytins in Ethanol. Biochim. Biophys. Acta 109: 448-453.
31. Ziegler, I. 1975. The Effect of Sulfur Dioxide Pollution on Plant Metabolism. Residue Reviews 56: 79-105.

Co-Occurring Stress: Drought

Mel Tyree
Department of Botany University of Vermont Burlington, VT 05405

ABSTRACT

Drought stress always restricts the growth of trees. The immediate factor most influencing growth response to drought is turgor pressure which is the force causing plastic enlargement of cells, leaves and stems. Reduced shoot and leaf growth in one dry season can reduce the vigor and growth potential of trees for several subsequent years. Net assimilation of carbohydrates is also reduced by drought through its effect on stomatal closure, increased diffusional resistance to CO_2 transport in mesophyll cells, reduction of electron flow in photosystem I and II and disruption of enzyme activity, thereby reducing the dark reactions of photosynthesis (1). Reduction in carbohydrate reserves can cause loss of frost hardiness and subsequent stem die-back (2).

Winter dehydration can also be a very important cause of stem die-back, a symptomatology normally associated with forest decline. Winter dehydration and consequent stem embolism can reduce the capacity of small stems to conduct water by more than 80% (3). Models indicate that during a growth season a loss of water conductivity greater than 20% can cause mid-day stomatal closure or catastrophic xylem dysfunction (4). Although mechanisms are normally in place to reverse embolism prior to bud break, anthropogenic stresses could interfere with these mechanisms, thus leading to crown die-back.

Water deficits influence all types and phases of tree growth, and always in a restrictive way. Kramer (1980) estimates that water deficits probably limit tree growth more than the effects of all other causes combined. In arid areas up to 90% of the annual variation in xylem production of conifers has been attributed to water deficits (Zahner, 1968); in humid areas drought accounts for up to 80%. However, in temperate climates a broad mix of microenvironmental factors can determine the growth pattern of individual trees (Cook, 1987). Drought stress alone or in combination with other environmental stresses can contribute to forest decline. This paper is intended to provide a brief introduction to some of the relevant literature regarding drought stress, and is specifically designed to point out decline syndromes caused by drought that may be confused with causes derived from atmospheric pollutants.

The mediating factor most often influencing the growth response of trees to drought is turgor pressure, because it is the force causing plastic enlargement of cell walls and vegetative growth (Hsiao et al., 1976; Bradford and Hsiao, 1982; Tyree and Jarvis, 1982). High turgor pressure is needed to cause sufficient enlargement for cells to reach the critical size for division (Doley and Leyton, 1968). It is presumed that a minimum turgor pressure is required to permit such enlargement; low turgor will also reduce growth of developing tissues after cell division. Drought may affect both primary and secondary growth and the effects of a brief drought period can retard growth for years to come as outlined below.

During primary growth, cell division at the apical meristem and the subsequent elongation of newly formed cells results in the formation of leaf bud primordia. Water deficits may affect the formation of leaf buds and/or the subsequent elongation of preformed leaf buds in conifers. Clements (1969) showed that the number of needle fascicles on new shoots, shoot length and fascicle spacing were correlated with the size of the bud formed the previous year. The bud size in turn depended on the level of moisture stress at the time it was formed (Kramer and Kozlowski, 1960; Lotan and Zahner, 1963). Drought reduces both shoot growth and needle production and the resulting reduction in leaf area may have significant effects on photosynthesis and growth for years to come. Needles persist for many years in most conifers, so poor growth in one year will have a lasting depressive impact on growth and vigor for the life of the needles.

Secondary growth involves three stages: (a) cambial division, (b) cell enlargement and (c) maturation where walls increase in thickness. Five requirements for cambial activity and wood formation have been identified by Kramer (1964) and all but the first are influenced by drought: (1) a temperature appropriate to a high level of metabolic activity, (2) a supply of growth regulators, (3) a supply of carbohydrates and nitrogen containing substances, (4) a supply of mineral nutrients and (5) sufficient water to maintain high turgor in the cells.

Kennedy (1961) related high production of earlywood in Douglas fir to years of high rainfall and low temperatures in spring. Earlywood production in irrigated trees will not cease until September (Zahner et al., 1964). The development of the small, thick-walled latewood cells appears to require moisture deficits (Zahner, 1963, 1968). False growth rings (i.e., two or more alternating bands [earlywood-latewood] occurring within one growing season) are caused by drought early in the growth season followed by adequate moisture. At times of low moisture availability, cells spend longer times in the zone of maturation and less time in the zone of enlargement than earlier in the spring, and this accounts for the characteristic form of latewood (Whitmore and Zahner, 1966).

Water availability can also directly affect the rate of carbon fixation in two ways: (1) via stomatal limitation of photosynthesis and (2) biochemical limitations of photosynthesis. Stomatal closure in response to decreasing water availability has commonly been thought to be the major contributor to reductions in photosynthesis through its restriction on carbon dioxide uptake. But photosynthesis may also decline independently of stomatal closure in response to the same factors that cause stomatal closure (Farquhar and Sharkey, 1982).

Water stress may act to reduce photosynthesis by (1) causing stomatal closure and thereby increasing the diffusional resistance to carbon dioxide uptake, (2) reducing chloroplast activity and electron flow in the light reactions of photosynthesis or (3) disrupting enzyme activity and thereby reducing the dark reactions of photosynthesis. Correlations among photosynthesis, transpiration and leaf conductance have often been interpreted to mean that the effect of water stress on photosynthesis is controlled mainly by stomatal closure (Brix, 1962; Boyer, 1976; Beadle and Jarvis, 1977). But it is now known that drought can directly reduce electron transport in photosystems I and II (Boyer, 1976; Keck and Boyer, 1974). Kaiser (1982) has recently demonstrated that reductions in photosynthesis during water stress were correlated with changes in total protoplast volume of leaf tissue in a variety of species. A possible mechanism for this correlation is apparently tied to the effect of volume changes on concentration changes of K^+ and H^+, which affect activities of key enzymes like bisphosphatase (FBP) and RUBP carboxylase (see Berkowitz et al., 1983 and related work from his lab).

All of the above effects of drought, e.g., reduced stem growth, reduced leaf production and reduced photosynthesis, can lead to reduction in carbohydrate reserves and general tree vigor. These effects can be compounded to result in loss of frost hardiness in winter and a common syndrome associated with tree dieback, i.e., death of minor branches due to frost damage (Gregory et al., 1987). Drought in some conifers (e.g., spruce and some firs) can also cause immediate needle loss (the Christmas tree syndrome).

An important mechanism by which stem dieback and general decline can occur is by stem embolism during the growth season and from winter dehydration. Since these mechanisms have not been reported until very recently, the rest of this paper will be devoted to explaining the events that lead to embolism and the consequences of it.

Trees are hydraulically designed to confine most of the water stress to minor branches. This is the basis of the "segmentation" hypothesis (see below) first proposed by Zimmermann. He formulated this hypothesis during his classical study of the "hydraulic architecture" of trees (Zimmermann, 1978). Generally the minor branches of all trees are 10 to 1000 times less capable of supplying water to their leaves than the boles of trees. In minor branches the water potential gradients are 10 to 1000 times greater than in the bole to overcome the larger hydraulic resistance per unit area of leaves fed by the stem (Zimmermann, 1978; Tyree et al., 1983; Ewers and Zimmermann, 1984a,b; Sperry, 1986). An important consequence of this hydraulic architecture is that the hydraulic resistance to water flow from the ground level to all minor branches is approximately the same for all twigs whether the twig is located near the base of a crown and at the end of a short hydraulic path or at the top of a crown and at the end of a long hydraulic path. All shoots are approximately equally capable of competing for the water resources of the tree (Tyree et al., 1988).

The segmentation hypothesis proposes that trees are hydraulically designed to confine embolism to minor branches. Embolism is the presence of air-filled (embolized) tracheids and vessels, and it can represent a substantial impairment of xylem transport. Environmental causes of embolism include water stress and winter freezing; potential consequences include reduction of growth and dieback.

Water stress leads to embolism via the process of "cavitation," the breaking of water continuity in xylem conduits subject to negative pressures arising from static effects (dry or frozen soil) and dynamic effects (transpiration). The immediate result of a cavitation is a lumen filled with water vapor and some air. Eventually the lumen becomes fully embolized when more air comes out of solution to fill the void left by the cavitation event. Winter freezing causes embolism by a variety of mechanisms including sublimation, expansion (after thaw) of air bubbles formed by freezing xylem sap, and cavitation in water-stressed flees rooted in frozen soil.

We have recently obtained support for the segmentation hypothesis from modeling studies based on the water relations and hydraulic architecture of a number of woody plants from diverse taxa and environments (Tyree, 1988; Tyree and Sperry, 1988). In these papers we discussed the relationship between dynamically changing tension gradients required to move water rapidly through the xylem conduits of plants and the proportion of conduits lost through embolism as a result of water tension. Tyree and Sperry (1988) compiled quantitative data on the water relations, hydraulic architecture and vulnerability to embolism of four widely different species: *Rhizophora mangle*, a salt excluding tropical mangrove, Rhizophoraceae, *Cassipourea elliptica*, a tropical moist-forest Rhizophoraceae, *Acer saccharum*, a temperate dicot, and *Thuja occidentalis*, a temperate gymnosperm. Using these data, we modeled the dynamics of water flow and xylem blockage for these species. The model is specifically focused on the conditions required to generate "runaway embolism," whereby the blockage of xylem conduits through embolism leads to reduced hydraulic conductance causing increased tension in the remaining vessels and generating more tension in a vicious circle. For this reason we specified the transpiration rate rather than building stomatal regulation into the model.

There were great differences among these species in hydraulic architectures, maximum transpiration rates, specific hydraulic conductances of stem tissue, and water relations. Despite these differences the model predicted for all species that: (1) embolism occurs more in minor than in major branches (thus supporting the segmentation hypothesis); (2) xylem tensions could lead to 5 to 30% loss of hydraulic conductance in minor shoots (depending on species) and still maintain a stable state; (3) if embolism causes more than 5 to 30% loss of transport capacity, then runaway embolism occurs leading to catastrophic xylem dysfunction (blockage) in a patch-work fashion throughout the crown; (4) after catastrophic failure of selected minor branches, there is an improved water balance (less negative Ψ) of surviving minor branches due to leaf loss from dead shoots. The model predicted that all species operate near the point of catastrophic xylem failure due to dynamic water stress.

The implication of these results and models is that xylem structure and vulnerability to embolism places important constraints on the water relations, morphology, and physiology of trees. Specifically the model shows that stomatal regulation and xylem physiology must function and evolve as an integrated unit in order to prevent catastrophic dysfunction. Trees must also evolve mechanisms to keep an appropriate balance for carbon allocation between leaves which increase evaporative demand and stems which supply the demand for water evaporated from leaves.

It is rare to find individual trees that suffer significant leaf loss duo to drought. This is presumably because stomates close and reduce evaporative flux before catastrophic xylem dysfunction and leaf loss. But progressive minor mounts of embolism presumably cannot be reversed during the growth season in trees. And as embolism increases, then minor branches will maintain lower stomatal conductances for longer periods of the day especially during times of high evaporative demands. Since embolisms are not easily reversible, the effects of drought can be more or less permanent during any one growth season. But summer embolism and embolism from winter dehydration may be reversible in spring.

To our knowledge, our study of sugar maple (Sperry et al., 1988) is the only one in which the natural incidence of xylem embolism has been systematically quantified over the longterm. We studied 5-8-year-old saplings as well as forest trees from May 1986 to July 1987. During the growth season embolism in minor branches grew enough to reduce the hydraulic conductance of stems by 10 to 30%. According to our model this is enough to begin causing partial stomatal closure during periods of high evaporative demand. In winter, however, very extensive embolism occurred. In February embolism was enough to reduce hydraulic conductance by 69% in stems 1 to 3 cm diameter and by 84 to 100% in minor branches. Perfusion of 0.1% safranin dye though main-axis segments revealed that embolism was localized on the southern sides of the trunk. Partly embolized twigs also showed one-sided embolism.

This embolism was primarily due to dehydration on sunny cold days in January and February. Beginning with the March measurement, embolism gradually declined until June 1987, when it was not significantly higher than the previous year. In some trees this recovery occurred over a very short period in response to positive stem pressures that dissolved air bubbles to make the vessels functional again.

We do not believe that maple could be unusual in its susceptibility to winter dehydration. Presumably conifers will dehydrate just as much if not more and mechanisms must be in place to reverse this presumed embolism. Our models show that without embolism reversal the stems would be hydraulically incapable of supplying water to the foliage attached to it. Without reversal there would be stem and leaf dieback—a syndrome that would be difficult to distinguish from other causes of dieback.

We have begun studies to look at these underlying biological processes. We have not ruled out the possibility that the mechanism of damage by anthropogenic stresses might be that it inhibits or otherwise damages mechanisms in place to reverse natural winter embolism.

Measurement of water potential parameters or embolism alone could not be used as markers for air pollution effects. However, many of the symptoms of drought stress might be confused with pollution induced decline syndromes, e.g., reduced leaf size, reduced leaf area, reduced bole diameter growth, reduced shoot elongation and stem dieback. For this reason it may be advisable to measure water relations parameters *before* ascribing a cause-and-effect relationship between decline and air pollution levels.

References

Beadle, C.L., and P.G. Jarvis. 1977. Effects of shoot water status on some photosynthetic partial processes in Sitka Spruce. Physiol. Plant 41: 7-13.

Berkowitz, G.A., C. Chen, and M. Gibbs. 1983. Stromal acidification mediates *in vivo* water stress inhibition of nonstomatal-controlled photosynthesis. Plant Physiol. 72: 1123-1126.

Boyer, J.S. 1976. Water deficits and photosynthesis. Pp. 154-191 in Kozlowski, T.T. (ed.), Water deficits and plant growth. Vol. 4, Academic Press, New York.

Bradford, K.J., and T.S. Hsiao. 1982. Physiological responses to moderate water stress. P. 263 in Encyclopedia of Plant Physiology NS, Vol. 12b, Springer-Verlag, Berlin, Heidelberg, New York.

Brix, H. 1962. Effects of water stress on the rates of photosynthesis and respiration in tomato plants and loblolly pine seedlings. Physiol. Plant 15:10-20.

Clements, J.R. 1969. Shoot responses of young red pine to watering applied over two seasons . Can. J. Bot. 48:75-86.

Cook, E.R. 1987. The use and limitations of dendrochronology in studying effects of air pollution on forests. Pp. 277-240 in Hutchinson, T.C., and Meema, K.M. (eds), Effects of Atmospheric Pollutants on Forests, Wetlands and Agricultural Ecosystems. Springer-Verlag, New York.

Doley, D., and L. Leyton. 1968. Effects of growth regulating substances and water potential on the development of secondary xylem in *Fraxinus* . New Phytologist 67: 579-594.

Ewers, F.W., and M.H. Zimmermann. 1984a. The hydraulic architecture of balsam fir (*Abies balsamea*). Physiol. Plant. 60: 453-458.

Ewers, F.W., and M.H. Zimmermann. 1984b. The hydraulic architecture of eastern hemlock (*Tsuga canadensis*). Can. J. Bot. 62:940-946.

Farquhar, G.D., and T.D. Sharkey. 1982. Stomatal conductance and photosynthesis. Ann. Rev. Plant Physiol. 33:317-345.

Gregory, R.A., M.W. Williams, J. Donnelly, and M.T. Tyree. 1987. The effects of stress factors on carbohydrate reserves, cold acclimation, and dieback in sugar maple. Pp. 186-191 in NAPAP Terrestrial Effects Task Group V. Session C, Tree Physiology. NAPAP, Washington, DC.

Hsiao, T.C., E. Acevedo, E. Fereres, and D.W. Henderson. 1976. Water stress, growth, and osmotic adjustment. Phil. Trans. R. Soc. Lond B 273: 479-500.

Kaiser, W.M. 1982. Correlation between changes in photosynthetic activity and changes in total protoplast volume in leaf tissue from hygro-, meso-, and xerophytes under osmotic stress. Planta 154: 538-545.

Keck, R.W., and J.S. Boyer. 1974. Chloroplast response to low leaf water potentials. III. Differing inhibition of electron transport and photophosphorylation. Plant Physiol. 53: 474-479.

Kennedy, R.W. 1961. Variation and periodicity of summer wood in some second growth Douglas fir. TAPPI 44: 161-166.

Kramer, P.J. 1964. The role of water in wood formation. Pp. 519-532 in Zimmermann, M.H. (ed.). The formation of wood in forest trees. Academic Press, New York.

Kramer, P.J. 1980. Drought, stress and the origin of adaptations. P. 7 in Turner, N.C., and Kramer, P.J. (eds.), Adaptations of plants to water and high temperature stress. Wiley & Sons, New York.

Kramer, P.J., and T.T. Kozlowski. 1960. Physiology of Trees. McGraw-Hill, New York.

Lotan, J.E., and R. Zahner. 1963. Shoot and needle responses of 20-year-old red pine to current soil moisture regimes. Forest Sci. 9:497-506.

Sperry, J.S. 1986. Relationship of xylem pressure potential, stomatal closure and shoot morphology in the palm *Rhapis excelsa*. Plant Physiol. 80:110-116.

Sperry, J.S., J. Donnelly, and M.T. Tyree. 1988. Seasonal occurrence of xylem embolism in sugar maple (*Acer saccharum*). Amer. J. Bot. In press.

Tyree, M.T. 1988. A dynamic model for water flow in a single tree. Tree Physiol. In press.

Tyree, M.T., and P.G. Jarvis. 1982. Water in tissues and cells. P. 35 in Encyclopedia of Plant Physiology NS. Vol. 12b, Springer-Verlag, Berlin, Heidelberg, New York.

Tyree, M.T., L.B. Flanagan, and N. Adamson. 1988. Response of trees to drought. Pp. 201-218 in Hutchinson, T.C. and Meema, K.M. (eds.), Effects of Atmospheric Pollutants on Forests, Wetlands and Agricultural Ecosystems. Springer-Verlag, New York.

Tyree, M.T., M.E.D. Graham, K.E. Cooper, and L.B. Bazos. 1983. The hydraulic architecture of *Thuja occidentalis* L. Can. J. Bot. 61:2105-2111.

Tyree, M.T., and J.S. Sperry. 1988. Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Answers from a model. Plant Physiol. In press.

Whitmore, F.W., and R. Zahner. 1966. Development of the xylem ring in stems of young red pine trees. Forest Sci. 12:198-210.

Zahner, R. 1963. Internal moisture stress and wood formation in conifers. Forest Prod. J. 13:240-247

Zahner, R. 1968. Water deficits and growth of trees. Pp. 191-254 in Kozlowski, T.T. (ed), Water deficits and plant growth, Vol. 2. Academic Press, New York.

Zahner, R., J.E. Lotan, W.D. Baughmann. 1964. Earlywood-latewood features of red pine grown under simulated drought and irrigation. Forest Sci. 10:361-370.

Zimmermann, M.H. 1978. Hydraulic architecture of some diffuse-porous trees. Can. J. Bot 56:2286-2295.