1998

Acidic deposition and heavy metal effects on seedling development of Northern Rocky Mountain Engelmann spruce

Linda L. Gilliland

The University of Montana

Acidic Deposition and Heavy Metal Effects on Seedling Development of Northern Rocky Mountain Engelmann Spruce

Submitted by

Linda L. Gilliland

School of Forestry

B.S. Biology, University of South Dakota, 1984
M.A. Biology, University of South Dakota, 1986

Presented in partial fulfillment of the requirements
for the degree of
Doctor of Philosophy
University of Montana
1997

Approved by:

Chairman, Board Examiners

Dean, Graduate School

4/28/98
Date

UMI Number: 9833498

UMI
300 North Zeeb Road
Ann Arbor, MI 48103

ABSTRACT

Gilliland, Linda L., Ph.D.,
1993
Forestry

Acidic Deposition and Heavy Metal Effects on Seedling Development of Northern Rocky Mountain Engelmann Spruce

Director: Donald J. Bedunah DJB

High-elevation western forests are potential sites of pollutant damage resulting from the amount of fog and the documented acidic and heavy metal levels in fog occurrences. Research is lacking on the physiological stresses caused by air pollution to Engelmann spruce (*Picea Engelmannii* Parry), which is significant because spruce species tend to be highly sensitive to air pollution in other ecosystems. This species is an important component of higher altitude ecosystems and an important timber source. Furthermore, the threat to newly established and naturally regenerating seedlings becomes a concern in pollution threatened forests.

The objectives of this study were to determine: (1) effects of foliar applied acidic deposition and heavy metals on the growth of two-year-old seedlings and (2) fog and immersion deposition of acid and heavy metals in two-year-old seedlings. A comparison of the differences between two types of acidic deposition, total saturation and fog, was accomplished by analyzing three pH and three heavy metal levels. Root lengths and shoot heights, root and shoot biomass, and foliar damage were determined. A two-year posttreatment monitoring of treatment effects was also conducted.

Increase in shoot biomass at documented western United States urban metal and acid level averages was considered the threshold level for both depositions and was attributed to a nitrogen fertilization effect. The fog treatment at pH 3.5 and ambient metal level was 272% greater than the immersion treatment at the same level. Possible fertilization due to the 2:5 nitrate-to-sulfate ratio was further supported by a large increase to the root:shoot ratio. No immersion seedlings survived past the second posttreatment bud break. The greatest number of surviving fog treated seedlings was at the ambient metal level.

PREFACE

"It is too bad", cried Eduard, "that we can no longer learn anything for our entire lives. Our predecessors could rely on the formal instruction of their youth; but now we have to revise our ideas every five years if we are not to become completely out of date." van Goethe (1809)

Using the key words "acid deposition" in a computerized literature search of the *Biological Abstracts* yields some 45,000 references on the subject over the last 20 years. Effects of acidic substances on forest ecosystems, particularly physiological, are being investigated. In 1986 researchers began to investigate the interactions among acidity, other pollutants and natural stresses on forests. The widespread mortality of high-elevation red spruce (*Picea rubens Sarg.*) in the northern Appalachians led to speculation that heavy metal and acidic deposition rates could be a predisposing or concurrent stress with climatic factors on high-elevation forests. At what pH and metal level significant physiological damage to forest ecosystems occurred was not certain.

The chemical composition of acidic deposition is a central unknown to the debate. This is especially important since the form of precipitation is now being used as an indicator of atmospheric quality. Deposition chemical analyses indicate large variation in types of acidic components and background chemicals over a region. Nitric acid is becoming increasingly predominant in chemical analyses as well as ammonia.

How atmospheric pollutants reach the Earth's surface is an additional area of debate. Differentiation between four types of deposition are being recognized. Wet deposition requires precipitation while dry deposition includes sedimentation, turbulence and molecular diffusion. Cloud and fog impaction, dew, and frost compose occult deposition. The total of wet, dry and occult deposition is termed bulk deposition. Measurements of deposition were historically of wet precipitation only, and more recently of bulk deposition.

At the time of this study, measurements of cloud and fog's contribution to forest throughfall as a nutrient, moisture and pollutant input were just being reported. Weather data for fog occurrence was almost nonexistent. In high-elevation ecosystems, input from cloud and fog water may exceed that of any other type of deposition because these types of deposition predominantly occur at higher elevations. Aerosol particle scientists believe the unique properties of a fog particle were due to both wet and dry deposition. The fog particle will come to rest on the conifer needle surface and eventually evaporate, leaving a dry deposition which can possibly damage the needle surface and affect the nutrient balance of the plant. In areas of repeated pollutant occurrences, disruption of the forest ecosystem could eventually occur. However, no proof existed as to the degree of damage caused by an acidified fog particle on the needle surface of high-elevation vegetation such as conifers.

A central focus of this dissertation was to determine how acidic fog particles related to wet precipitation from a physiological damage perspective. I decided to compare the fog treatment with a worst case scenario, total coverage of the stem. Heavy metal uptake by needle surface was also considered. Since the possibility of heavy metal uptake solely by the needle surface was being studied, no contamination of the soil from the acid treatment by drip or throughfall could take place. Therefore, the submersion technique and associated platform design evolved as described in the Methods sections of Chapter II and III.

A toxicological approach was employed to determine a significant damage level due to pH and metal level. A preliminary examination to determine treatment levels was necessary and is described in Chapter II. Significant damage level has not only a toxicological implication, but also a parallel to reality. If damage occurs only at a pH of 2 and high metal level, conducting the study is pointless.

Chapter III describes the formal portion of the study. The chapter was originally written as a journal submission; the format has been modified for the dissertation due to the length of the introduction, statistical description and results section. The introduction to Chapter III gives a different perspective to the study than the introduction to the dissertation.

Another question addressed by the author was the verification of foliar uptake of heavy metals. Various German scientists have researched the disruption of soil chemistry and nutrient uptake due to acidic input and heavy metal levels in the soil. Little research is available on metal uptake by leaf surface. This aspect of the study is described in Chapter IV. Problems using the inductively coupled plasma emission spectroscopy analytical technique (ICP) represent the most frustrating part of this dissertation. The results, indicate a possible increased metal uptake at recorded metal pollution levels.

Each chapter is a self contained section with a specific purpose. Chapter II describes the methodology to determine useful treatment combinations for the formal experiment described in Chapter III. Both of these chapters contain an introduction, objectives, hypotheses, methods, results and conclusions section. Chapter IV gives a perspective on choosing an analytical technique and solving technical difficulties. Chapter I, the Introduction, is lengthy, but it serves an important purpose in its entirety. A large quantity of references have been composited and reflect current research perspectives on the physiological implications of acidic deposition to forest ecosystems. The threat of acid deposition as an environmental problem is very real. I want the Introduction to serve as a possible quick reference for the reader to the acid deposition problem from a physiological perspective. Thus, the Introduction describes how conifer physiological processes are disrupted by the imbalances wrought by abnormal amounts of acid and heavy metals; levels which are documented to occur in urban areas of the western United States. The Introduction was originally written as a Comprehensive Question in 1990 and has been updated.

TABLE OF CONTENTS

	PAGE
ABSTRACT	ii
PREFACE	iii
TABLE OF CONTENTS	vi
LIST OF TABLES	viii
LIST OF ILLUSTRATIONS	ix
ACKNOWLEDGEMENTS	x

CHAPTER

1 INTRODUCTION 1
- Background 1
- Effects of Acids and Metals on Leaves 3
- Effects of Acids plus Metals on Mineral Nutrition 8
- Acid and Metal Stress - Conifer Physiological Interaction 13
- Conclusion 15
- References 17

2 DETERMINATION OF EFFECTIVE DOSES AND DAMAGE ASSESSMENT CRITERIA 23
- Introduction 23
- Methods and Materials 23
- Results and Discussion 27
- References 30

CHAPTER PAGE

3 DETERMINING THE EFFECTS OF ACIDIC DEPOSITION AND HEAVY METALS ON SPRUCE SEEDLING GROWTH BY COMPARING FOG AND IMMERSION APPLICATIONS 32
Abstract 32
Introduction 32
Materials and Methods 37
Results 53
Discussion 57
Conclusions 59
References 61

4 HEAVY METAL FOLIAR UPTAKE BY ENGELMANN SPRUCE FROM ACIDIC FOG 64
Introduction 64
Methods and Materials 65
Results and Discussion 68
Recommendations 70
References 72

5 A Perspective 73
References 79

LIST OF TABLES

TABLE PAGE

2.1 Preliminary treatment levels of pH and heavy metal concentrations 24

3.1 Ionic composition of acidic fog and immersion solution 37

3.2 ANOVA table and underlying statistical model for root and shoot length 46

3.3 ANOVA table and underlying statistical model for old and new needle damage rating 47

3.4 ANOVA table and underlying statistical model for shoot biomass 48

3.5 Statistical Method Summary 49

3.6 Comparing two–year old Engelmann spruce seedling shoot dry biomass treated by immersion and fog deposition with three pH and three metal levels 51

3.7 Mean two-year-old Engelmann spruce root and shoot lengths, their standard deviations, and sample sizes by deposition type 54

3.8 Two-year posttreatment tally of fog deposition treated Engelmann spruce seedlings 56

4.1 Heavy metal foliar uptake analyses 69

LIST OF ILLUSTRATIONS

FIGURE PAGE

2.1 Immersion platform used in preliminary testing.. 26

3.1 Fog treatment apparatus.. 42

3.1a Fog treatment apparatus - pressure gauges

3.1b Fog treatment apparatus - nozzle body

3.2 Immersion apparatus...45

3.2a Immersion apparatus - preliminary testing platform

3.2b Immersion apparatus - normal experiment

3.3 Needle damage rating graphs grouped by pH level, needle level, deposition type, and seed source.. 52

ACKNOWLEDGEMENTS

In Defense of My Mother

I have often been asked during the last six years why a woman at forty would want to start a Ph.D. in Forestry. The key words in the inquiry were: woman, forty, Ph.D. and forestry. This correlation of facts did not occur to me during my decision making process.

The attitude may be considered naive. I would offer another explanation. We are all products of our individual genetic make-up, upbringing and experiences. My mother's grandparents left Munich, came by sea to New York and arrived in Illinois by train; my father's grandparents also left Munich, came by way of the Mississippi by barge and then oxen to Iowa. What greeted them on their arrival were Sioux Indians, wild animals, a sea of prairie grass – and absolutely no civilization. They faced the bitter cold of winter, the searing heat of summer, winds that drove minds to the brink of sanity, and killing tornadoes. There were no special privileges for the women of this land. They worked the fields, built the barns, and slaughtered, side by side with the men. Not only did they survive - they flourished. I am a daughter of that prairie family - a member of a fourth generation from Otter Valley in a land now best described as "Gods Garden".

I am the product of a working mother. More importantly, of a mother who questioned the validity of every institution, political figure, or person of authority touching her life. My mother has one infallible method of judgement: how self serving is the individual or institution in question.

Education is a priority in the thinking of the state of Iowa - certainly in the funding; and my mother has insisted on the education of her children being a priority in her life. Her other values have been very simple: 1) do the best you can 2) realize your goals and 3) never, never, never give up. A guiding caveat has been not to let outside influences set your expectations or limit your horizons.

My educational career has been influenced in turn by three men who have each had to deal with the daughter of such a mother: Dr. George Hoffman, Biology Department Head at University of Northern Iowa, Cedar Rapids; Dr. Donald Dunlap, retired Professor of Biology at the University of South Dakota, Vermillion; and Dr. Donald Bedunah, Professor of Range Management, School of Forestry, University of Montana, Missoula. Each gentleman has a unique approach to the mentoring process. I especially thank Dr.

Donald Bedunah for realizing my mother's third value during the process of producing his first Ph.D. student.

I would like to extend a very special thanks to Dr. Raymond Hunt for the encouragement and advice; also, for teaching the best graduate physiology course. Huzzah to Dr. Hans Zuuring, not only as an instructor and statistician, but for his bluntness and honesty. A thank you to Dr. Paul Hansen for his professionalism these twelve years of our acquaintance. To Dr. Robert Pfister – blessings for your special mediation abilities. Dr. Johnny Moore and Don Essig are to be thanked for their technical support. A salute to the committee - Clint Carlson, Ed Waali and Ed Burke for their critical reviews. And a special thank you to my editors Karin Stouton and Jane Taylor, and computer support specialist Robert E. Slattery. I did not do this alone.

Chapter I

INTRODUCTION

Section I
Background

In the mid 1970s, in a high-elevation Bavarian forest called Fichtelgebirge, a spring yellowing of Norway spruce (*Picea abies* [L.] Karst.) needles was observed. In 1982, German scientists noted falling tree stands, thinning canopies, and increasing tree mortality affecting 8% of West German trees; by 1987, roughly 52% of stands were affected. Currently, more than one-third of stands are significantly damaged, with increased tree mortality. German scientists believe that atmospheric acidic pollution has played a decisive role in the forest's downward spiral (Raloff 1989).

The slower decline of high-elevation U.S. forests may be explained by the differences in genotypic susceptibility of European silver fir (*Abies alba* Mill.) and the two spruce species, Norway and red spruce (*Picea rubens* Sarg.) (Larsen *et al.* 1988), differences in European forest plantation management practices, or pollution load. Given the widespread mortality of high-elevation red spruce in the northern Appalachians, it is logical to ask if the western United States spruce forests are possibly being damaged by heavy metal and acidic deposition.

Both the European and United States spruce forests are above cloud base for considerable portions of the year. Composition of cloud acidity has changed over the past century and cloud moisture tends to be much more acidic than precipitation (Johnson and Siccama 1984).

Events leading to forest decline involve three phases of deposition in Europe. During the first phase (1870 - 1900), sulfate was the major pollutant in the atmosphere. Soil acidification did not reach the currently observed rates because it was probably immobilized as aluminum-hydroxosulfate at the lower end of the exchange-buffering range. The second phase (1900 - 1960) involved a nitrate deposition increase. A third phase (1960 - present) demonstrates an exponential increase of aerial deposition of nitrate and ammonium (Schulze 1989).

Major population and industrial growth in western United States cities during the past two decades have increased motor vehicle, fossil-fueled power plants, and industrial furnace output of acids and heavy metals. Increasing acidic fog on the Colorado Rockies (Nagamoto *et al.* 1983; Miller and Borys 1988), episodic pH readings in the mid 3 range on Mt. Rainier in the Cascades and western Washington (Hegg and Hobbs 1981; Larson *et al.* 1987), and high pH 2's in California's Coastal Range (Munger *et al.* 1983) have focused concern on western United States high-elevation forests.

Current hypotheses regarding cloud water mechanisms fall in four categories (Shriner and Henderson 1990; Joslin and Wolfe 1992; Joslin *et al.* 1992):

1. High concentrations of hydrogen, sulfate, and nitrate ions in cloud water causing direct damage to foliar tissues (Jacobson *et al.* 1990) reduce photosynthetic capacity, increase respiratory carbon losses (McLaughlin *et al.* 1990), and possibly increase susceptibility to winter damage (Fowler *et al.* 1989; DeHayes 1992; DeHayes *et al.* 1991; Johnson *et al.* 1991).

2. Foliar cation deficiencies from foliar leaching primarily result from high depositional loading (Joslin and Wolfe 1988; Jacobsen *et al.* 1990; Schulze 1989).

3. High concentrations of hydrogen and aluminum ions in soil solution cause root mortality, root growth reduction, and interferences in cation uptake (Raynal *et al.* 1990).

4. High depositional loading cause below ground effects, such as nutrient deficiencies (Robarge *et al.* 1989; Shortle and Smith 1988; Schulze 1989).

A chemically altered ecosystem results with both direct and indirect effects on established and reproducing plants. The effects of acids and metals on the leaf and root with resultant growth and reproduction consequences will be emphasized in the following discussion. Of particular importance are: (1) the photosynthetic and respiration relationship hypothesized in Sections II through IV below and (2) the aberrant root-shoot relationship discussed in Sections III and IV.

In Section II, the interacting effects of acidic and metal interactions on leaf structure are reviewed. Damage caused by hydrogen ions contacting the leaf surface results in an imbalanced buffering system possibly affecting internal cell pH range, and leaching of ions critical to plant nutrition. In addition, acid-caused surface necrotic lesions result in cell tissue and organelle damage affecting photosynthetic-respiration relations and, ultimately, carbon allocation. Concurrent possible foliar absorption of heavy metals and resultant vacuolar accumulation may affect needle senescence.

Simultaneous soil absorption of sulfates, nitrates, hydrogen ions, heavy metals, mineral nutrition, and ammonium occurs. Section III (Effects of Acids and Metals on Mineral Nutrition) documents the resultant soil pH decline causing ionic toxicity by aluminum and manganese with implications to root physiology. The root system's inability to perform normal growth and stress responses is possibly explained.

In Section IV (Acid and Metal Stress-Conifer Physiological Interaction) physiological interactions between the canopy and soil structure are explained with resultant implications to growth and reproduction. Section IV should be regarded as the thrust of the chapter. Dealing with the leaf and root interactions with the acids and metals separately aids in understanding the validity of the hypothesized relationship between photosynthesis, respiration, and carbon allocation to root growth.

Effects of weather, climate, and animal and pathogen interactions will be extremely limited or not discussed. Such secondary and tertiary interactions are not central to my research problem: How do acids and metals applied as foliar deposition predispose a tree to die?

Section II
Effects of Acids and Metals on Leaves

Susceptibility of Leaf Surfaces to Acidic Deposition

Some deciduous species have throughfall pH and cation measurements higher than incoming rain (Eaton *et al.* 1973; Cole and Johnson 1977; Hoffman *et al.* 1980; Scherbatskoy and Klein 1983); whereas coniferous species tend to acidify throughfall (Johannes *et al.* 1981; Cronan and Reiners 1983). (Throughfall will be discussed further in the section after leaching.) An individual species leaf acidic sensitivity appears to be influenced by structural vegetational features, foliage wettability, increasing foliage injury, and/or retention of rainwater or fog droplet (Shriner and Johnston 1985).

Epicuticular waxes provide the main resistance to movement of ions and water across the cuticle and reduce the area of contact between the leaf and rain droplets (Schonherr 1976; Juniper and Jeffree 1982); however, young Norway spruce exposed to acidic fog (pH 3) had a disintegration of the epicuticular waxes of the current year's needles (Mengel *et al.* 1989). Exposure of red spruce needles to pH 3 fog, whose current year needles have thin wax layers relative to other conifers, resulted in wax deposits within epistomatal chambers (Percy 1987; Percy *et al.* 1990; Rinallo *et al.*

1986). Upright irregular wax plates appeared in nonstomatal areas. Damage resulted from direct pollutant interaction with the wax formation process on elongating needles. The ability of the epistomatal wax deposits to interfere with stomatal action has not been determined.

Leaf surfaces are also believed to be involved in buffering the effects of acid precipitation. If leaf surfaces are buffering the acidity of precipitation, a physiological response in the plant is expected. An input of hydrogen ions affects the acid-base-equilibria within the leaf. As buffering systems are weakened, hydrogen ion concentration in plant tissues will rise and lead to increased leaf acidity and development of foliar injury symptoms. The intercellular pH is important in the uptake of nutrients, cell-wall growth, membrane carrier transport, and enzyme activity (Linskens *et al.* 1989).

With an imbalanced buffering system and damage to the epicuticular waxes, leaching of cations potassium, calcium, and magnesium will occur. Potassium exists in mobile forms (Tukey 1970; Lindberg and Lovett 1986), and a large internal pool may be capable of providing a continuous supply to the leaf surface. The reason for the depletion of calcium and magnesium by acidic incident is probably because of their strong association with cell walls, which makes them available for exchange processes with acidic raindrops on the leaf surface. Internal replacement of calcium and magnesium are slower than potassium (Demarty et al. 1984). There are a number of reports of increased leaching of cations from leaves due to acid rain treatments (Adams and Hutchinson 1984; Joslin and Wolfe 1988; Jacobsen et al. 1990; Schulze 1989). In addition to loss of inorganic ions, plants exposed to strongly acidic precipitation will lose organic compounds, including amino acids, proteins, and hydrocarbons (Hoffman et al. 1980; Scherbatskoy and Klein 1983).

Precipitation is chemically altered as it passes through a canopy and interacts with stems and boles. The change is a result of washing off of leached deposits and dry deposition. Neither is easily quantified (Lovett and Lindberg 1984). The problem is further complicated by degrees of alteration because of types of wet interaction, that is, rain vs. fog. Throughfall and stemflow are greatly influenced by type of canopy, (deciduous or conifer), time of year, type of deposition, and location of canopy, to name a few.

Several studies have shown leaf canopy uptake of nitrate from rainwater. In a deciduous canopy, mean dry-deposition rates were sulfate>calcium>potassium>nitrate (Lovett and Lindberg 1984). Dry deposition accounted for 30, 30, and 71% of the total atmospheric deposition (wet plus dry) for sulfate, nitrate, and calcium. Klemmedson *et al.* (1983) reported ponderosa pine (*Pinus ponderosa* Laws.) throughfall containing one third more nitrate than either bulk precipitation or stemflow, with throughfall containing higher calcium, magnesium, and potassium. Obviously, throughfall and stemflow have consequences for canopy soil interactions. Further additions of sulfate and nitrate to the soil, in addition to bulk precipitation, are indicated. The enrichment of the soil by further additions of macronutrients calcium, magnesium, and potassium does not necessarily indicate that the effects of soil acidification will be ameliorated, as a discussion in Section III will suggest.

Responses in Conifer Needle Ultrastructure to Acid Rain

The following discussion of cell tissue, organelle damage, and foliar uptake of heavy metals precedes photosynthetic and respiration interactions.

Tissue Damage

Acid mist is capable of eroding cuticular waxes (Baker and Hunt 1986) and causing necrotic lesions, which lead to a loss of turgor and collapse of epidermal and mesophyll cells (Bytnerowicz *et al.* 1986; Crang and McQuattie 1986). In most forest decline studies, injuries, both in the stelar and mesophyll tissues, have been found (Schmitt and Grosch 1985; Sutinen 1987). Damage to the epidermis and underlying palisade and mesophyll cells has been shown to arise from exposure to simulated rain with a pH below 3.5 (Evans and Curry 1979; Adams *et al.* 1984).

Organelle Damage

Nygren and Hari (1987) present the hypothesis that the acidity of the mesophyll solution is primarily regulated by exchange reactions between hydrogen and potassium ions on leaf surfaces, or in the stomata. Holopainen and Pekka (1988) demonstrated potassium deficiency, increased vacuolar space, vesiculation of the tonoplast, and increased irregularly shaped cytoplasmic lipid structures in pine needles exposed to simulated acid rain, potassium deficiency, or the combination. The even distribution of the chloroplast protrusions in the mesophyll suggests a general chemical injury mechanism. The symptoms in pine needles may be a consequence of increased hydrogen ion concentration at the cell surface, resulting mainly from cation exchange reactions on the needle surface.

The increase of lipids in the cytoplasm and vacuoles, observed both in the potassium-deficient and acid-rain treated needles, apparently is a general stress symptom in conifer needles. This phenomenon has been described for industrial environments and after experimental exposures to sulfate, drought, and fluorides (Soikkeli 1980; Karenlampi and Houpis 1986). Lipid accumulation can be related to membrane degradation caused by these stresses, but translocation also may be inhibited (Huber *et al.* 1985).

Damage to red spruce at Camels Hump, Vermont, has been attributed to acid precipitation deposited as rain, cloud moisture, and snow (Scherbatskoy and Klein 1983), along with high levels of trace metals such as lead, zinc, copper, and aluminum ions (Friedland *et al.*1984; Johnson and Siccama 1984). Cellular damage evidenced from field studies (Vogelmann and Rock 1988) included dramatic mesophyll damage. Protoplasmic clumping is attributed to tannins, which are possibly involved in plant defense reactions. Cranulations were extensive in the cell wall.

Foliar Uptake of Heavy Metals

Little (1973) demonstrated the possibility of foliar absorption of heavy metals in *Ulmus* (elm), *Crataegus* (hawthorn), *Salix* (willow), and *Quercus* (oak) using zinc, lead, and cadmium ions. Of these, zinc and cadmium were incorporated into the leaf structure. Increased cadmium affects water relations, photosynthesis, and transpiration, and increases dark respiration in *Acer* (maple) species (Lamoreaux and Chaney 1977, 1978). Sugarcane's leaf callus absorb zinc, copper, and molybdenum (Kannan 1986). Leaves accumulating heavy metals become inefficient due to waste accumulation and must therefore be replaced. Such leaves typically have low transpiration rates and thus low photosynthetic rates, which tend to minimize waste accumulation (Chapin *et al.* 1990).

Photosynthesis and Related Processes

The general stress hypothesis regards the initial air pollutant plant stress to be impaired photosynthetic capacity. Subsequent symptoms include production of secondary metabolites and growth hormones, reduced translocation of carbohydrates to roots, and impaired root development and function. All are results of general disruption of wholeplant carbohydrate economy (McLaughlin 1985; McLaughlin *et al.* 1992).

Vogelmann and Rock (1988), studying red spruce at Camels Hump Mountain, demonstrated stress affecting chlorophyll b > chlorophyll a > carotenoid pigments.

CO_2 Uptake

Another hindrance may be factors affecting the performance of photosynthetic enzyme Rubisco (ribulose-1,5-biphosphate carboxylase-oxygenase). Nitrogen plays an important role via its contribution to photosynthetic enzymes, albeit less than a 25% increase in conifer photosynthetic rates upon fertilization (Waring and Schlesinger 1985). Carboxylation (the Calvin Cycle, the dark reactions) rate is limited by the amount of Rubisco present. During carboxylation, Rubisco is strongly activated by magnesium, whose uptake is highly inhibited by aluminum and manganese toxicity, as discussed in Section III (Effects of Acids and Metals on Mineral Nutrition). Carboxylation can further be limited by low levels of carbon dioxide due to stomatal closure, which is affected by transpiration. Transpiration and water relations are greatly inhibited by heavy metals and will be further discussed in Section III.

Furthermore, the export rate of carbon compounds from the chloroplasts is controlled by the concentration of inorganic phosphate in the cytoplasm; inorganic phosphate strongly affects the ratio of starch accumulation to sugar release (Marschner 1986). A sugar-starch ratio is implied. The concentration of inorganic phosphate is inhibited by aluminum toxicity in the soil; it is precipitated out by aluminum, as will be explained in Section III.

In a discussion of starch storage, Chapin *et al.* (1990) alludes to damaged leaves unable to produce starch which then accumulate sugars in the leaves during the day. The higher sugar concentration, in turn, leads to a higher leaf respiration rate, causing an overall decline in plant growth.

Respiration and Carbon Demands

Dark respiration, or nongreen tissue respiration, is an expensive pathway in trees, requiring a large amount of carbon for construction and maintenance of conducting and storage tissue in the sapwood. For conifers, estimations of construction vs. maintenance respiration costs are few. Construction costs involve an estimate of annual growth and storage reserves; maintenance costs involve an estimation of living cells in various tissues and organs of the tree (Waring and Schlesinger 1985).

For a tree under stress, respiration maintenance costs should increase to correct damage caused by natural and anthropogenic stresses. Growth or construction costs would be expected to decline because of the lack of reserves available for the growth process due to declining photosynthetic rates and carbon reserves being used for maintenance demands. Indeed, such results have been reported by McLaughlin *et al.*

(1990, 1991, 1992). A significant reduction in the ratio of net photosynthesis to dark respiration was found at high-elevation sites exposed to acidic deposition in southern Appalachian Mountain sites.

Summary

A nutrient deficiency is expected with acidic precipitation's damage to the leaf surface epicuticular waxes and buffering mechanism, resulting in an altered acid-base equilibria, leaching of potassium, calcium, and magnesium, amino acids, proteins, and hydrocarbons. Tissue and organelle damage would result in an altered photosynthetic rate. Light harvesting pigment damage would affect the amount of NADPH (nicotinamide adenine dinuclotide phosphate hydrogenase) and ATP (adenosine triphosphate) available for assimilation of carbon dioxide.

1. Assimilation of carbon dioxide is further mediated by concentrations of the enzyme Rubisco:
    a. Rubisco requires large amounts of nitrogen, and
    b. the Rubisco carboxylase pathway is affected by levels of magnesium which are affected by aluminum and manganese toxicity, and the interference of ammonium in the soil.

2. Release of sugars from photosynthesis is controlled by concentrations of inorganic phosphate which are affected by aluminum toxicity in the soil.

3. Maintenance respiration rates would be expected to increase in a stressed tree. Growth respiration would be expected to decline.

4. Carbon allocation would be greatly altered due to stress demands.

Section III

Effects of Acids and Heavy Metals on Mineral Nutrition

Buffering Processes in Forest Soil

Most regions of the world receiving acidic deposition are covered with north temperate forests, whose soils are somewhat acidic. Soils most susceptible to further acidification are noncalcareous, sandy, and well drained, and not strongly acidic. These soils are almost saturated by basic cations, such as calcium, magnesium, potassium, and sodium, which are leached away as they are replaced by acid cations, i.e., hydrogen, aluminum, or hydroxyl-aluminum ions. When the soil becomes dominated by acidic actions, the pH declines. Fine-textured soils, i.e., high clay content and a high cation exchange capacity, have a better buffering capacity than the coarse-textured, sandier soils. More acidic podzolic soils are not highly susceptible to further acidification because the exchange sites are dominated by hydrogen ions. Regions with granitic, highly siliceous bedrock exposed at the surface are sensitive to acid deposition. Such areas have no calcium carbonate in the sedimentary rocks, which supplies a buffering action, neutralizing acids in solution (Gates 1981).

Soil acidification has several phases. Calcium-carbonate dissolves and acts as a buffer in the pH 8.6 to 6.2 range. The 6.2 to 4.2 range is the exchange-buffering range, where clay exchanges cations for hydrogen ions. The dominant reactive species are aluminum ions originating from silicates. Aluminum-hydroxosulfate will immobilize the action of sulfate unless sufficient amounts of organic or nitric acids are present. If the latter are available, pH will drop below 4.2, releasing aluminum ions into the soil.

Ionic Toxicity

The acidification of a soil stresses a plant by reducing nutrient availability or producing toxic levels of ions in the soil solution, both resulting in decreased root growth and ion uptake. It is caused by the rate of proton input being higher than the rate of proton consumption via the soil buffering processes. The element of most concern is aluminum. Excessive levels of manganese and deficiencies of phosphorus, calcium, and magnesium are also involved. Less frequently, levels of sulfur, potassium, molybdenum, zinc, and copper are important.

The presence of aluminum in the soil solution inhibits the uptake of calcium and of magnesium. Where a soil pH <5.5 exists, an increasing proportion of the cation exchange sites is occupied by aluminum, which replaces polyvalent cations (calcium and magnesium) and acts as a strong adsorber of phosphate. Thus a strong cause-effect relationship exists between the proportion of exchangeable aluminum in soils, pH, and inhibition of conifer growth. Toxic levels of lead, manganese, copper, and nickel also occur in soil.

Ionic toxicity of aluminum and manganese results in fine-root damage. Addition of heavy metals compounds the effect. Membrane activity is affected by high nitrate levels. Ionic competition results in cell leakiness. High nitrate levels damage mycorrhiza giving rise to preferential uptake of ammonium. Signs of aluminum toxicity first appear in the root system, which becomes stubby as a result of elongation inhibition of the main axis and lateral roots. The physiological mechanisms of the toxic effects of aluminum on root growth are not fully understood. The primary effect of aluminum is inhibition of cell division in root apical meristems. Cell division ceases within a few hours after the exposure of roots to aluminum because it binds directly to DNA. Accumulation of aluminum is high in the nuclei of root cap cells (Marschner 1986). Root elongation inhibition might be the result of injury to root cap cells, which act as sensors for environmental stress. The uptake of phosphorus may be lessened due to the precipitation of aluminum phosphate at the root surface and/or the free space. This could further limit the formation of ATP. Inhibition of magnesium and calcium uptake is often observed and is mainly the result of cation competition or blocking of binding sites.

The aluminum and calcium-magnesium relationship has been the subject of major publications in the past two years. Research is being conducted through the Integrated Forest Study at Oak Ridge National Laboratory, Oak Ridge, Tennessee. For the southeastern United States, evidence has been presented for nutrient deficiencies and aluminum toxicity as factors in red spruce decline (Joslin *et al.* 1992; McLaughlin *et al.* 1990, 1991). Joslin and Wolfe (1992) in studies at Whitetop Mountain, Virginia, noted differences in forest floor deposition of water, sulfate, nitrate, and ammonium between high and low cloud sites; a strong positive correlation between soil solution concentrations of hydrogen and aluminum and concentrations of nitrogen; and a lower fine-root biomass at the high cloud site, corresponding with low calcium and aluminum concentrations; and aluminum exceeding the toxicity concentrations for red spruce growth. Johnson *et al.* (1991) described the nutrient cycles of two red spruce areas in Great Smoky Mountains National Park, fairly representative of the southern Appalachians. The red spruce ecosystems were affected by atmospheric deposition by being dominated by throughfall and stemflow rather than litter-fall fluxes, which is unusual for nitrogen, phosphorous, calcium, and magnesium; also, by high rates of nitrogen and sulfate deposition, which increased soil acidity and increased aluminum concentrations.

Whiteface Mountain, New York, has been the site of documented red spruce decline since the mid 1970s. Miller *et al.* (1992) notes that while the concentration of aluminum has probably increased in high-elevation red spruce forests of the northeastern United States due to increasing rates of acidic deposition, levels of aluminum have not reached those demonstrated in reducing growth of red spruce.

However, Schlegel *et al.* (1992) studying aluminum and calcium or magnesium interference, also at Whiteface Mountain, suggested that utilizing ratios of calcium:aluminum and magnesium:aluminum, more useful than concentrations. Average values of these ratios in the soil solution at Whiteface Mountain suggest that uptake of both calcium and magnesium by red spruce should be affected. Low foliar content of calcium and magnesium has been demonstrated at Whiteface Mountain (Amundson *et al.* 1992). McLaughlin *et al.* (1990, 1991) presented documented increases in dark respiration under low calcium levels for the importance to red spruce physiology of foliar aluminum-to-cation ratios. Low nutrient status is driven by low ratios of magnesium and calcium to aluminum (Schlegel *et al.* 1992) in the soil solution.

Solubility of manganese in the soil solution is less complicated; the pH and redox potential are the dominant factors. High concentrations of manganese in the soil solution are to be expected only in acid soils with high levels of readily reducible manganese in combination with a large content of organic matter, high microbial activity, and anaerobiosis. The root is not directly affected by manganese; rather, it affects the shoot. Of particular importance for plant growth in acid soils is the inhibition of calcium and magnesium uptake by high manganese concentrations.

Bauch and Schroder (1982) have demonstrated by cellular analysis of fine roots of damaged and healthy fir and spruce trees that magnesium and calcium deficiency in roots is always associated with fine-root damage. Acid deposition on the needles results in an ongoing leaching of calcium and magnesium from needles. The leaching becomes a serious problem when uptake of elements is limited by aluminum saturation in the apoplast of the root or root injury in acid soil. Deposition of copper, lead, and nickel leads to additional root physiological damage.

In plants, copper is associated with enzymes involved in oxidation-reduction. Toxicity results in chlorosis and stunting, with a corresponding iron deficiency. The first symptom is root reduction, which is caused by faulty nutrient absorption leading to inhibition of growth.

Nickel, as a divalent cation, is readily absorbed and competes with other cations, including calcium, magnesium, iron, and zinc in root uptake mechanisms, leading to characteristic symptoms of chlorosis. Inhibition of root elongation is the most sensitive parameter of heavy metal toxicity.

It would appear that when organic matter and other mineral nutrients are in abundant supply, lead toxicity does not occur. A major factor in the precipitation and hence detoxification of lead in the soil is phosphorus; but other factors such as levels of sulfate, pH, and organic matter content may also influence the level of available lead in the soil. A wide range of toxicity symptoms in plants is attributable to lead: root elongation is inhibited, roots form adventitiously, and stem elongation and leaf expansion are inhibited.

Ionic Competition During Root Uptake

The influence of valency on the uptake rate of nutrients is obvious when two ionic species of the same mineral element occur together in the external solution. Considering the pH range of 8.5-5.5, a positive correlation between the proportion of phosphate (H_3PO_4) available as $H_2PO_4^-$ in the external solution and the uptake rate of phosphate is obvious. In contrast, there is a smaller effect on the uptake rate of sulfate, because in this pH range only the divalent anion SO_4^{2-} occurs. The increase in phosphate uptake by lowering the pH to below 8.5-5.5, with a corresponding change in valence, however, is an oversimplification. At the lower pH, the sulfate uptake increases. Changes induced by pH in membrane potential and in proton-anion cotransport also contribute to this enhanced anion uptake (Marschner 1986).

Alterations in the lipid composition of root membranes is a typical response to fluxes in the mineral nutrient supply. Monocarbonic acids, such as acetic acid (CH_3COOH) and butyric acid ($CH_3[CH]_2COOH$) induce membrane injury. The undissociated species of these acids are rapidly taken up by the membrane and cause leakiness, as measured by the amounts of potassium and nitrate emitted from root tissue. Monocarbonic acid's ability to induce membrane leakiness increases with a more acidic pH, and with an increase in chain length of the acids, an increase in lipophilicity that changes the fatty acid composition of the membranes (Marschner 1986).

Ammonia, Nitrate, and Mycorrhizal Interaction

Soil leaching of nitrate and the resulting soil acidification is exasperated by the preferential root uptake of ammonium. Ammonium also interferes with the uptake of magnesium. Atmospheric input of ammonium and nitrate are higher under acidic conditions, resulting in elevated soil levels of both elements. Microbial nitrification appears to be lowered under acidic conditions (Mancinelli 1986). Higher concentrations of phosphorus have been shown in needles at study sites measuring increased soil ammonium and nitrate levels; such levels indicate potassium is used as a counter ion (Schulze 1989).

Summary

Much of North America is subject to significant acid and heavy metal deposition as a result of air pollutants. Acid deposition affects the buffering mechanisms in soils with low reserves of organic matter, resulting in aluminum and magnesium toxicity and reduced phosphorus, calcium, and magnesium uptake. Changes in lipid composition of the cell membrane result in cell leakiness. Rhizospheres tend to be stressed due to concentrations of acids and metals affecting nitrification, water, and nutrient uptake mechanisms.

Current metal depositions are resulting in soil levels whose metal residence time is measured in decades or centuries. Their presence affects litter decomposition rates, nutrient cycling, and soil solutions for incalculable time periods. Physiological effects of heavy metals include reduced growth, blackening, and abnormal branching patterns, and death. Further interactions of acids' and metals' influence on the root and forest canopy will be discussed in the following section.

Section IV
Acid and Metal Stress - Conifer Physiological Interaction

Simultaneous nutrient interference is occurring both to canopy and root processes with the deposition of acids and metals. Interference to normal root growth affects nutrient and carbon allocation. Water uptake and transpiration are interrupted and altered. The compounding effects take their toll on growth and reproduction. The end result is a starving, thirsty tree.

A leaching of nutrients due to damaged needles and aluminum and manganese toxicity plus ammonium's interference results in a chronic deficiency of calcium and magnesium. Fichtelgebirge's trees developed serious deficiencies both of calcium and magnesium, and of potassium, manganese, and iron, particularly in their stems and needles (Raloff 1989). Nutrient deficiency normally stunts growth in conifers. However, with the current atmospheric deposition rates of nitrogen (as reflected in the current research project's nitrogen-sulfate ratio of 2.5:1) on foliage and soil, a fertilization effect takes place spurring nutrient deficient conifers to attempt increased growth. A highly stressed state results.

Compounding the aluminum-induced calcium-magnesium deficiency resulting from excessive acidity and nitrogen is a higher level of soil ammonium, interfering with a tree's magnesium uptake. Norway and red spruce roots preferentially absorb ammonium. Below a pH of 5, soil acidification will cause death of microbes normally converting ammonium to nitrate in the soil.

Since calcium is not recycled from woody tissues, a calcium imbalance results; the functional sapwood (food storage zone) area becomes smaller, resulting in a less vital tree. The older the tree, the less the ability to adjust. Suppression of new wood results as the crown thins and the tree rings narrow (Raloff 1989). Thinning crowns allow more light to the forest floor, increasing radiation, raising floor temperatures, and potentially increasing the need for water uptake.

Water Relations

A normal conifer response to a decreased nutrient level is an increased proportion of carbohydrates allocated for growth of fine roots, with an associated reduction in foliage and stem growth (Waring 1987). However, with the nutrient deficiency described in the previous section, aluminum has a detrimental effect on root growth, which is further amplified by the presence of heavy metals. Death of fine roots results. Larger roots may also be damaged due to increasing acidity (Raloff 1989; Klein 1985). In addition to an impaired ability to respond to nutrient demand, reduction of root growth and death of fine roots affects water uptake negatively.

Changes in epicuticular waxes and in stomatal function by acid rain/fog may also alter plant capacity to regulate transpirational water loss. For example, water stress will occur if the stomates have an impaired ability to close due to epicuticular wax deposits; or, if water loss is occurring through damaged cuticles when the roots are unable to respond to transpiration demands with increased water uptake.

The Bavarian and Appalachian forests have been subjected to drought years in the past several decades. Root excavation of more mature trees in Germany shows the deeper roots have died and been replaced by shallower roots, which may account for the significant decline of European forests after dry years in the early 1980s and for a degree of recovery in recent wet years (Raloff 1989). Narrower tree rings due to reduced growth result in decreased transfer of water from the roots to the leaves, thereby compounding the problem of water stress.

Effects on Growth and Reproduction

Reproduction. Reproductive potential is an important measure of plant health. In severely polluted regions natural regeneration is limited. For example, very few red spruce seedlings were found on Camels Hump Mountain polluted sites. Remaining seed source trees are few, cone and seed sizes are small, and capacity for germination is reduced (Klein and Perkins 1988). Seedling establishment is generally more sensitive to soil acidity, decreasing rapidly below a soil pH of 4.4 (McLaughlin 1985).

Little information is available on the effects of acidity and heavy metals on the flowering physiology of forest trees. Bazzaz *et al.* (1979) found substantial variation in the photosynthetic rates of flowers and fruits among tree species. An obvious problem estimating reproductive photosynthesis arises. But an interesting estimation of respiration importance is emphasized. Carbon allocation tends to reflect the distribution of other nutrients, but it also tends to be biased toward the most limiting resources. Also, a plant with a low cost of reproduction may be able to allocate a larger proportion of its resources to reproduction. Bazzaz *et al.* (1987) has shown reproduction to even enhance vegetative growth in some cultivated crops. Whether a nutrient-stressed conifer can afford a long-term investment in resource allocation depends on how stressed the tree is regarding nutrients and carbon reserves. A tree with high reproductive costs and minimal nutrient resources would probably bypass the reproductive efforts.

Growth. Large trees make increased demands for calcium and magnesium from fine roots. Reduced cambial growth is probable as fine roots become less able to absorb calcium and magnesium because of interference from increased aluminum, manganese, and ammonium. Cambial growth suppression over a period of time results in reduced sapwood basal area. Reduced sapwood equates to a reduced ability to conduct water and store food. When a tree has less than 25% sapwood in a cross-sectional area, vulnerability to death from secondary stresses, i.e., cold, drought, pathogens, and insects, increases (Shortle and Smith 1988; DeHayes *et al.* 1991).

Section V
Conclusion: The Starving, Thirsty Tree Scenario

The concentrations of sulfate, nitrate, and ammonia and other ions in rain and fog affect nutrient and water uptake and soil-buffering processes. The effects are cumulative. Major soil acidic changes appear to take place in well-drained soils with a high pH rather than soils already acidified. Interactions among the aluminum ions and possible additional manganese toxicity in the soil profile interfere with magnesium, calcium, and potassium uptake. With the presence of aluminum, phosphorus is also precipitated out. Compounding the problem is the presence of excess ammonia, which spruce seedlings will preferentially absorb over magnesium in acidified soils. In addition, microbial nitrification is probably inhibited under acidifying soil conditions. Ammonium seems to counteract the effect of aluminum precipitation of phosphorus by using it as a counter ion. The interaction has implications on net effects of formation of ATP via respiration.

Changes in root allocation patterns result as a consequence of soil acidification. Deeper roots die in the more acidified layers, resulting in shallower root depth. In addition, the presence of aluminum and other metals causes further root death and growth stunting affecting water relations and rendering the tree susceptible to long- and short-term drought situations.

Uptake of atmospheric nitrogen promotes canopy growth but interferes with general nutrient uptake by inhibiting the tonoplast membrane pump. The nutrient imbalance and water deficiency are further exacerbated by leaching of cations and organic compounds through damaged needles. The canopy will not translocate remaining carbon and nutrients, but preserve the carbon and nutrients for growth, defense, and possible reproduction; further demands will be made on a damaged root system, unable to adequately respond, for nutrients and water.

Research Implications

Preliminary literature research indicated no work had been conducted on the foliar uptake of heavy metals in the context of acidic deposition. Additionally, no work had been done on translocation processes of heavy metals within the seedling. A central research question was whether documented toxic effects of heavy metals on plant species would have a coinciding effect on Engelmann spruce, a western United States spruce species.

Additionally, given the effects of acid rain, how would the application of an aerosol particle, such as fog bearing potentially toxic pollution loads, affect the physiology of a western United States spruce species? The fog particle was believed to be more damaging than a raindrop because of its ability to dehydrate on the leaf surface, leaving behind concentrated pollution loads. How these questions were addressed is the subject of Chapters II through V.

References

Adams, C.M., Dengler, N.G., and Hutchinson, T.C. 1984. Acid rain effects on foliar histology of *Artmeisia tilesii.* Can. J. Bot. 62:463-478.

Amundson, R.G., Hadley, J.L., Fincher, J.F., Fellows, S., and Alscher, R.G. 1992. Comparison of seasonal changes in photosynthetic capacity, pigments, and carbohydrates of healthy sapling and mature red spruce and of declining and healthy red spruce. Can. J. For. Res. 22:1605-1616.

Baker, E.A., and Hunt, G.M. 1986. Erosion of waxes from leaf surfaces by acid rain. New Phytol. 102:161-73.

Bauch, J., and Schroder, W. 1982. Evidence of chemical elements in the cells of fine roots of healthy and diseased firs (*Abies alba* Mill.) and diseased spruce trees (*Picea abies* [L.] Karst.). Zellularer Nachweis einiger. Elements in den Feinwurzein gesunder und erkrankter Tannen (*Abies alba* Mill.) und Fichten (*Picea abies* [L.] Karst.).

Bazzaz, F.A., Carlson, R.W., and Harper, J.L. 1979. Contribution to the reproductive effort by photosynthesis of flowers and fruits. Nature (Lond.). 279:554-555.

Bazzaz, F.A., Chiariello, N.R., Coley, P.D., and Pitelka, L.F. 1987. Allocating resources to reproduction and defense. BioScience. 37:1:58-67.

Bytnerowicz, A., Temple, P.J., and Taylor, O.C. 1986. Effects of simulated acid fog on leaf acidification and injury development of pinto beans. Can. J. Bot. 64:918-22.

Chapin, F.S. III, Schulze, E.D., and Mooneu, H.A. 1990. The ecology and economics of storage in plants. Annu. Rev. Ecol. Syst. 21:423-447.

Cole, C.W., and Johnson, D.W. 1977. Atmospheric sulphate additions and cation leaching in a Douglas Fir ecosystem. Water Resources Research. 13:313-317.

Crang, R.E., and McQuattie, C.J. 1986. Qualitative and quantitative effects of acid misting and two air pollutants on foliar structure of *Liriodendron tulipifera.* Can. J. Bot. 64:6:1237-1243.

Cronan, C.S., and Reiners, W.A. 1983. Canopy processing of acidic precipitation by coniferous and hardwood forests in New England. Oecologia. 59:216-223.

DeHayes, D.H. 1992. Winter injury and developmental cold tolerance of red spruce and fir. p. 295-337. In: (E. Eagar and M.B. Adams, eds.) Ecology and decline of red spruce in the eastern United States. New York, Springer-Verlag.

DeHayes, D.H., Thornton, F.C., Waite, C.E., and Ingle, M.A. 1991. Ambient cloud deposition reduces cold tolerance of red spruce seedlings. Can. J. For. Res. 21:1292-1295.

Demarty, M., Morvan, C., and Thellier, M. 1984. Calcium and the cell wall. Plant, Cell and Environment. 7:441-448.

Eaton, J.S., Likens, D.E., and Bormann, F.H. 1973. Throughfall and stemflow chemistry in a northern hardwoods forest. Ecology. 61:495-508.

Evans, L.S., and Curry, T.M. 1979. Differential responses of plant foliage to simulated acid rain. Amer. J. Bot. 66:953-962.

Fowler, D., Cape, J.N., Deans, J.D., Leith, I.D., Murray, M.B., Smith, R.I., Sheppard, L.J., and Unsworth, M.H. 1989. Effects of acid mist on the frost hardiness of red spruce seedlings. New Phytol. 113:321-335.

Friedland, A.J., Johnson, A.H., and Siccama, T.G. 1984. Trace metal content of the forest floor in the Green Mountains, New Hampshire. Bull. Torrey Bot. Club. 110:141-153.

Gates, D. 1981. Energy and Ecology. Sunderland, Mass., Sinauer Associates, Inc.

Hegg, D.A., and Hobbs, P.V. 1981. Cloud water chemistry and the production of sulfates in clouds. Atmospheric Environment.15:9:1597-1604.

Hoffman, W.A., Jr., Lindberg, S.E., and Turner, R.R. 1980. Precipitation acidity: the role of the forest canopy in acid exchange. Environmental Quality. 9:95-100.

Holopainen, T., and Pekka N. 1988. Responses in Scots pine needle ultrastructure to simulated acid rain and potassium deficiency. p.430-432. In: (J.B. Bucher and I. Bucher-Wallin, Eds.) Air Pollution and Forest Decline. Proc. 14th Int. Meeting for Specialists in Air Pollution Effects on Forests Ecosystems. IUFRO P2.05. Interlaken, Switzerland, Oct.2-8, 1988. Birmensdorf, 1989.

Huber, V.K., Esterbauer, H., and Grill, D. 1985. Qualitative and quantitative pattern of fatty acids in spruce needles. Qualitative und quantitative Untersuchungen uber Fettsauren in Lipden der Fichtennadein. Beitr. Biol. Pflanz. Berlin, W. Germany. Duncker & Humblot. 60:3:413-425.

Jacobson, J.S., Lassoie, J.P., Osmeloski, J.F., and Yamada, K. 1990. Changes in foliar elements in red spruce seedlings after exposure to sulfate and nitric acid mist. Water Air Soil Pollut. 48:141-160.

Johannes, A.H., Altwicker, E.R., and Clesceri, N.L. 1981. Characterization of Acidic Precipitation in the Adirondack Region. EPRI Report EA-1826. Research Project 1155-1. Troy, N.Y. Rensselaer Polytechnic Institute.

Johnson, A.H., and T.G. Siccama. 1984. Decline of red spruce in the northern Appalachians: assessing the possible role of acid deposition. Tappi Journal. 67:1:68-72.

Johnson, D.W., van Miegroet, H., Lindberg, S.E., Todd, D.E. and Harrison, R.B. 1991. Nutrient cycling in red spruce forests of the Great Smoky Mountains. Can. J. For. Res. 18:1614-1623.

Joslin, J.D., Kelly, J.M., and van Miegroet, H. 1992. Soil chemistry and nutrition of North American spruce-fir stands: evidence for recent change. J. Environ. Qual. 21:1:12-30.

Joslin, J.D., and Wolfe, M.H. 1988. Responses of red spruce seedlings to changes in soil aluminum availability in six amended forest soil horizons. Can. J. For. Res. 18:1614-1623.

Joslin, J.D., and Wolfe, M.H. 1992. Red spruce soil solution chemistry and root distribution across a cloud water deposition gradient. Can. J. For. Res. 22:893-904.

Juniper, B.E., and Jeffree, C.E. 1982. Plant Surfaces. London, Edward Arnold.

Kannan, S. 1986. Foliar absorption and transport of inorganic nutrients. CRC. Critical reviews in Plant Sciences. 4:4:341-375.

Karenlampi, L., and Houpis, J.L.J. 1986. Structural conditions of mesophyll cells of *Pinus ponderosa* var. *scopulorum* after SO_2 fumigation. Can. J. For. Res. 16:1381-1385.

Klein, R.M. 1985. Effect of acidity and metal ions on water movement through red spruce. pp. 275-282. In: (D.D. Adams and W.P. Page, Eds.). Acid Deposition New York: Plenum.

Klein, R.M., and Perkins, T.D. 1988. Primary and secondary causes and consequences of contemporary forest decline. The Botanical Review. 54:1:2-43.

Klemmedson, J.O., Meier, C.E., Campbell, R.E., and Marx, D.D. 1983. Effect of stand composition and season on chemistry of throughfall and stemflow of Ponderosa pine forests. For. Sci. 29:871-887.

Lamoreaux, R.J., and Chaney, W.R. 1977. Sulfur dixoide and plant response: an overview (Pollution injury). Biologist-Denver. 59:2:61-69.

Lamoreaux, R.J., and Chaney, W.R. 1978. The effect of cadmium on net photosynthesis, transpiration, and dark respiration of excised silver maple leaves. Physiol. Plant. 43:231-236.

Larsen, J.B., Qian, X.M., Scholz, F., and Wagner, I. 1988. Ecophysiological reactions of different provenances of European Silver fir (*Abies alba* Mill.) to SO_2 exposure during winter. Eur. J. For. Path. 18:44-50.

Larson, T.V., Edmonds, R., and Basabe, T. 1987. Ionic composition of cloudwater at Mt. Rainier. Paper presented at Forest Cloud Chemistry Workshop. Corvallis, Oregon.

Lindberg, S.E., and Lovett, G.M. 1986. Field measurements of particle dry deposition rates to inert surfaces and leaves in a forest canopy. Environmental Science and Technology. 19:238-244.

Linskens, H.F., Derks, F.H.M., and Schonenberg-Linders, G.F.J. 1989. The influence of artificial acid rain on the physiology and morphology of *Phaeolus vulgaris*, *vicia faba* and *Spinacia oleracea* after separate spraying of the shoots and soil. Angew. Botanik. 63:67-80.

Little, P. 1973. A study of heavy metal contamination of leaf surfaces. Environ. Pollut. 5:159-172.

Lovett, G.M., and Lindberg, S.E. 1984. Dry deposition and canopy exchange in a mixed oak forest as determined by analysis of throughfall. J. Applied Ecology. 21:1013-1027.

Mancinelli, R.L. 1986. Alpine tundra soil bacterial responses to increased soil loading rates of acid precipitation, nitrate, and sulfate, front range, Colorado, U.S.A. Arctic and Alpine Research. 18:3:269-275.

Marschner, H. 1986. Mineral Nutrition of Higher Plants. Academic Press. London.

McLaughlin, S.B. 1985. Effects of air pollution of forests. A critical review. JAPCA. 35:512-533.

McLaughlin, S.B., Anderson, C.P., Edwards, N.T., Roy, W.K., and Layton, P.A. 1990. Seasonal patterns of photosynthesis and respiration of red spruce saplings from two elevations in declining southern Appalachian stands. Can. J. For. Res. 20:485-495.

McLaughlin, S.B., Anderson, C.P., Hanson, P.J., Tjoelku, M.G., and Roy, W.K. 1991. Increased dark respiration and calcium deficiency of red spruce in relation to acidic deposition at high-elevation southern Appalachian Mountain sites. Can. J. For. Res. 21:1234-1244.

McLaughlin, S.B., Adams, M.B., Cook, E.R., DeHayes, D.H., Eager, C., Fernandez, I.J., Johnson, D.W., Kohut, R.J., Mohnen, V.A., Nicholas, N.S., Peart, D.R., Schier, G.A., and White, P.S. 1992. A synthesis of epidemiological and mechanistic studies. p. 385-411. In: (C. Eager and M.B. Adams, Eds.) Ecology and decline of red spruce in the eastern United States. New York: Springer-Verlag.

Mengel, K.A., Hogrebe, M.R., and Esch, A. 1989. Effect of acidic fog on needle surface and water relations of *Picea abies*. Physiol. Plant. 75:201-207.

Miller, D.F. and Borys, R.D. 1988. Characterization of cloud chemistry and frequency of canopy exposure to clouds in the Rocky Mountains. Desert Research Institute, University of Nevada at Reno. Prepared for the U.S. Environmental Protection Research Laboratory. 200 SW 35th Street, Corvallis, Oregon.

Miller, E.K., Huntington, T. G., Johnson, A.H., and Friedland, A.J. 1992. Aluminum in soil solutions from a subalpine spruce-fir forest at Whiteface Mountain, New York. J. Environ. Qual. 21:345-352.

Munger, J.W., Jacob, D.J., Waldman, J.M., and Hoffmann, M.R. 1983. Fogwater Chemistry in an Urban Atmosphere. J. of Geophysical Research. 88:9:5109-5121.

Nagamoto, C.T., Parungo, F., Reinking, R., Pueschel, R., and Gerish, T. 1983. Acid clouds and precipitation in eastern Colorado. Atmospheric Environment. 17:6:1073-1082.

Nygren, P., and Hari, P. 1987. The interactive effect of potassium deficit and acid rain on photosynthesis of Scots pine seedlings. Aquilo. Ser. Botanica. 25:2:129-135.

Percy, K.E. 1987. Effects of simulated acid rain on leaf cuticular characteristics and surface properties. Ph.D. thesis, University of Bristol, Bristol, England.

Percy, K.E., Krause, C.R., and Jensen, K.F. 1990. Effects of ozone and acidic fog on red spruce needle epicuticular wax ultrastructure. Can. J. For. Res. 20:117-120.

Raloff, J. 1989. Where acid reigns. Science new. 136:56-58.

Raynal, D.J., Joslin, J.D., Thornton, F.C., Schaedle, M., and Henderson, G.S. 1990. Sensitivity of tree species to Al:III. Red spruce and loblolly pine. J. Environ. Qual. 19:180-187.

Rinallo, C., Raddi, P., Gellini, R., and DiLonardo, V. 1986. Effects of simulated acid deposition on the surface structure of Norway spruce and silver fir needles. Eur. J. For. Path. 16:440-446.

Robarge, W.P., Pye, J.M., and Bruck, R.I. 1989. Foliar elemental composition of spruce-fir in the southern Blue Ridge province. Plant Soil. 114:19-34.

Scherbatskoy, T., and Klein, R.M. 1983. Response of spruce and birch foliage to leaching by acidic mists. J. Env. Qual. 12:189-195.

Schlegel, H., Amundson, R.G., and Huttermann, A. 1992. Element distribution in red spruce (*Picea rubens*) fine roots: evidence for aluminum toxicity at Whiteface Mountain. Can. J. For. Res. 22:1132-1138.

Schmitt, G., and Grosch, S. 1985. Measurement of atmospheric damage of trace elements in forests. Messungendes atmospharischen Eintrags von Spurenelementen in Waldgebieten. p.313-332. Waldschaden: Einflussflactoren ihre Bewertung: Kolloquium Goslar, 18. bis 20. Juni 1985. CDI-Kommission Reinhalktung der Luft.Dusseldorf. VDI Verlag.

Schonherr, J. 1976. Water permeability of isolated cuticular membranes: the effect of cuticular waxes on diffusion of water. Planta. 128:113-126.

Schulze, E.D. 1989. Air Pollution and forest decline in a spruce (*Picea abies*) forest. Science. 244:776-783.

Shortle, W.C., and Smith, K.T. 1988. Aluminum-induced calcium deficiency syndrome in declining red spruce. Science. 240:1017-1022.

Shriner, D.S., and Johnston, J.W., Jr. 1985. Acid rain interactions with leaf surfaces: a review. pp. 177-185. In: [D. D. Adams and W. Page, eds.]. New York: Plenum.

Shriner, D.S., and Henderson, G.S. 1990. Sulfur distribution and cycling in a deciduous forest watershed. J. Environ. Qual. 7:3:392-400.

Soikkeli, S. 1980. Ultrastructure of the mesophyll in Scots pine and Norway spruce: seasonal variation and molarity of the fixative buffer. Protoplasma. 103:241-242.

Sutinen, S. 1987. Cytology of Norway spruce needles II. Changes in yellowing spruces from the Taunus Mountains, West Germany. Eur. J. For. Path. 17:74-85.

Tukey, H.B., Jr. 1970. Leaching of metabolites from foliage and its implication in the tropical rain forest. In: (H.T. Odum, ed.) A tropical rainforest p.155-160. U.S. Atomic Energy Commission, Washington, D.C.

Vogelmann, A.F., and Rock, B.N. 1988. Anatomy of red spruce needles from forest decline sites in Vermont. Environ. Exp. Bot. 28:19-26.

Waring, R.H. 1987. Characteristics of trees predisposed to die. BioScience. 37:8:569-574.

Waring, R.H. and Schlesinger, W.H. 1985. Forest Ecosystems. Concepts and Management. Orlando: Academic Press.

Chapter II

DETERMINATION OF EFFECTIVE DOSES AND DAMAGE CRITERIA

Introduction

A toxicological testing protocol has been developed for animal experimentation, and the botanical community has tried to mimic this testing protocol, but with considerable problems. No clear definitions or guidelines agreed and adhered to by the plant research community are available. The first problem arises with the definition of a testing period. With the life span of a spruce tree at about 400 years, subacute and chronic testing are impractical during human life spans. Sub-acute testing would involve a minimum of 16 years duration-chronic, 200 years.

This preliminary study was conducted to determine the effective dose-range of pH and heavy metal concentrations for two-year-old Engelmann spruce. An effective treatment length and damage assessment criteria were also determined.

For practicality, an acute study involving the administration of an effective dose response present in 50% of the plants (ED 50) was used (Connell and Miller 1984). When such visual symptoms as necrosis, chlorosis (an indication of metal and general oxide effects), brown banding on the needles, needle tip color change, needle drop (an indication of nitric oxide effects in spruce), or lesions appeared, the preliminary study was terminated. *A priori* if no responses occurred, then the study was to be terminated after two months.

Objectives

The objectives of the preliminary study were:

1. to determine effective dose-range
2. to ascertain correctness of treatment length
3. to identify and evaluate visual damage criteria.

Methods and Materials

Seedling Source

Bare-root, two-year-old (2/0) Engelmann spruce were obtained from the Champion Nursery, Plains, Montana in February 1989. The seed source for the 2/0 seedlings was from Warland Creek near Libby, Montana (sections 25, 32, and 29 at approximately 1433m), and planted at the Champion Nursery at Plains, Montana in 1987. Seedlings were transplanted to a peat-vermiculite 1:1 medium in 15cm pots. Plants were watered as needed. No fertilizer was applied at any time during the treatment because of a possible fertilizer-by-treatment interaction.

Growth Chamber

Seedlings were grown and treated in a Conviron PBW36 walk-in growth chamber. A combination of florescent, incandescent, and grow lights maintained a light intensity of 107 klux. Light duration was from 0600 to 2200 hours. The 3023 Conviron microprocesser was programmed on a ramp mode to maintain 7°C between 2430 and 0730 hours, escalating to 24°C by 1500 hours. A gradual decline to 7°C began at 1800 hours.

Treatment Levels

Treatments applied to Engelmann spruce were solutions with combinations of different pH levels and concentrations of the heavy metals: manganese, nickel, copper, and lead according to Table 2.1:

Table 2.1 Preliminary treatment levels of pH and heavy metals.

pH	Metal Increase in Mn^{2+}, Ni^{2+}, Cu^{2+}, Pb^{2+}		
2	2x[1]	3x	4x
3	2x	3x	4x
4	2x	3x	4x
5.4	0	0	0

1. x indicates metal level above solution discussed under Chemistry Section.
n = 6 per pH and metal level.

Hypotheses

(H_0): No visual damage would be apparent in any treatment group after two months.

(H_1): Visual symptoms of stress, i.e., necrosis, chlorosis, brown banding on the needles, needle tip color change, needle drop, or lesions would appear within the two month treatment period to all the treatment groups other than the control.

Chemistry

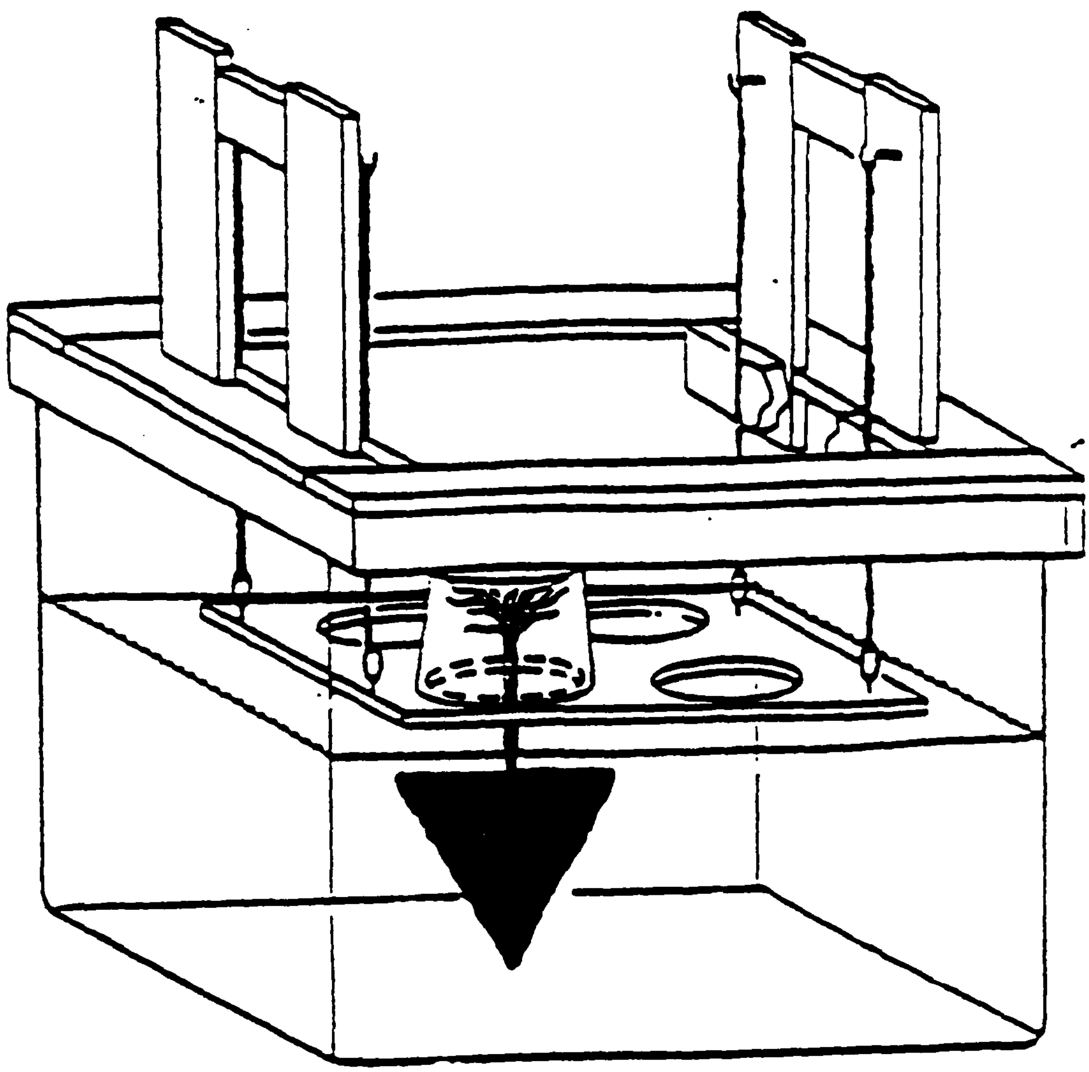
Solutions used for immersion background ions were derived from the averages of chemical analyses for southern California fog (Table 3.1). Background ions were added to deionized water and titrated to treatment pH levels. A 2.5:1 ratio of nitric-sulfuric acids was used based on the acid ratios reported in southern California fog and international analyses (Brewer *et al.* 1983; Munger *et al.* 1983; Galloway *et al.* 1982; Jacob *et al.* 1985; Waldman *et al.* 1982).

Immersion Design

Seedlings were immersed in 58 liter plastic containers. To control chemical and water costs, yet allow for seedling growth, adjustable platforms were designed (Fig. 2.1). Prior to immersion, the seedling pot was wrapped in plastic and a cotton band surrounded the stem base, sealing the root area from contact with the immersion solution.

Duration of immersion was four hours; frequency of immersion was three times weekly for two months; six seedlings were treated per metal and pH level. Thus pH 2 at a twice ambient metal level was used to test six seedlings. The pH 5.4 and no metal level was used to treat a group of six seedlings (Table 2.1). This treatment period was derived by studying length of recorded fog events both from western United States coastal and from Rocky Mountain weather records. Also, cost of treatment, availability of the Conviron growth chamber, and other published treatment protocol were taken into consideration.

Fig. 2.1 Immersion platform used in preliminary testing



Preliminary Testing Platform

Results and Discussion

The preliminary study revealed significant acute treatment effects. At pH 2, all heavy metal concentration treatment combinations were terminated after 30 days. "Firing" of new and old growth appeared. Damaged needles were either a light brown or light brown to orange coloration. Both types of damage gradually increased, giving a dark orange to red coloration after about 30 days of treatment. In some instances, entire browning of new shoot growth was apparent. Leith *et al.* (1989) found similar needle responses using acid mist containing equimolar ammonium sulfate and nitric acid. Skeffington and Roberts (1985) also reported "firing" symptoms after 3-year-old Norway spruce were exposed to acid mist at pH 3. In a study of red spruce growing in an Appalachian spruce decline region at Newfound Gap, North Carolina (elevation 1548m), needle browning, similar to the reported damage in all laboratory studies noted, was described by Sheppard *et al.* (1988).

Stress shoots were observed at pH 2. These usually appeared as a whorl of new growth around the lead shoot. Apical tips browned and then bent over in both metal concentration level 2 (ambient) and 3 (2x ambient) within the first month. At the time of establishing damage criteria for the preliminary experiment, neither symptom had appeared in the literature. Both were included as part of the damage assessment criteria in the formal study.

At pH 2, root damage was also prevalent. In some instances, root growth was less than 20% of the control. The majority of previous air pollution research had focused on aboveground biomass. However, several studies in the 1980s indicated roots could be a more sensitive indicator of air pollution than needles (Chappelka *et al.* 1985; Hogsett *et al.* 1985; Chappelka and Chevone 1986; Cooley and Manning 1987). Root growth was shown to be sensitive to acidic precipitation (Chevone 1985) and heavy metals (Tyler *et al.* 1989). Root biomass was therefore included along with shoot biomass in the formal portion of the study.

At pH 3 and for all metal concentrations, loss of new needle growth and browning similar to firing appeared in the second month. Extensive needle loss was observed for both old and new growth affected by "firing" in both pH 2 and 3 after the two-month treatment period. Similar needle loss was also noted by Leith *et al.* (1989).

At pH 4 and all metal concentrations, an increase in root, stem, and needle growth, needle chlorosis, and stress shoots within the two-month time frame was indicated. This was attributed to the additional nitrogen provided by the treatment; Seiler and Paganelli (1987) found acidic mists and rain to often stimulate growth and photosynthesis.

At all metal concentrations, aside from the control: (1) banding, (2) needle breakage due to tip necrosis, (3) chlorotic and necrotic tips, and (4) stress shoots were observed. Banding, tip discoloration, and breakage are usually considered indications of SO_2 damage in a gaseous form (Evans and Miller 1975; Carlson and Gilligan 1983).

Since all pH levels below 5.4 and all metal concentrations other than 0 showed effects within the prescribed time period, pH 2 and 3 were retained for the formal experiment. The pH 3 treatment level was changed to pH 3.5 to represent a pH level of greater concern as an episodic event in documented fog occurrences. Metal concentrations for the formal study were set at 0 (as a control), ambient (concentrations indicated in the chemistry solution Table 3.1), and a twice ambient concentration (worst case parameter).

At the strong suggestions of Bill Hoggsett[1] and Paul Miller[2] in 1989, seedlings in both the preliminary and formal portion of the experiment were retained two years following termination of treatment combinations to study aftereffects. In this study, all seedlings at pH 2 and all seedlings at the 3x and 4x metal concentrations died within a year. At all metal concentrations, seedlings exposed to pH 3 and pH 4 seemed the least affected. However, all seedlings at the 2x metal concentrations died within the two year period. In the preliminary experiment, controls pH 5.4 and 0 metal concentrations remained relatively unaffected visually for the two-year period.

In addition, the question of confounding effects due to fertilization of seedlings by using a nutrient solution were addressed. The pollutant pH and metal concentration of concern in this study is the documented pH 3.5 and ambient metal level. One aspect of this study was to determine whether responses to treatments were adverse or adaptive. Such determinations are critical in toxicology studies involving sublethal effects (Connell and Miller 1984). In most polluted systems, a diverse range of toxicants rather than a single agent is the usual scenario. Such is the case in this study. Not only is a 2.5:1 ratio of nitrogen-sulfate present, raising the question of a possible fertilization effect, but also the heavy metals copper, nickel, manganese, and lead are present. The possibility of interactions between these pollutants is present, which may

---

[1] 1989. Personal communication. U.S. Environmental Protection Agency. Western Conifers Research Cooperative. Corvallis Environmental Research Laboratory. 200 SW 35th Street, Corvallis, Oregon.

[2] 1989. Personal communication. U.S.D.A., Forest Service, Pacific Southwest Forest and Range Experiment Station, 4955 Canyon Crest Drive, Riverside, California.

enhance or inhibit toxic responses. Additionally, at times, copper and manganese are applied to plants as part of a nutrient program. Because of these considerations, no fertilizer was applied during the preliminary or formal study.

As a result of the preliminary study, an effective dose range and duration of treatments and observation period were determined. For the formal study, pH levels would be 5.4, 3.5, and 2, with 5.4 representing normal atmospheric pH; 3.5 representing repeated episodic fog occurrences of questionable damage potential; and pH 2 representing a documented damage event, but of rare occurrence. Metal levels would be 0, ambient (1x) and double the ambient (2x) with 0 acting as the control; ambient (1x) representing the level of concern; and 2x representing damage levels recorded in the preliminary study. Based on the preliminary study, an ED 50 had been determined. Damage should occur to a portion of the formal study seedlings at the pH 2, 2x metal level. This treatment level and the pH 5.4 metal level 0 would act as outside treatment parameters.

Damage criteria were also established. These included old and new needle damage as evidenced by firing, banding, stress shoots, apical tip damage, chlorotic and necrotic needle tips, and needle breakage. New root and shoot growth by length and dry biomass would also be used as indicators of any damage.

The preliminary study successfully ended with method of treatment developed, and the establishment of dose-range, treatment duration, and damage criteria. Whether damage response in 50% of the seedlings after two months of treatment and at what treatment level it would occur was determined in the formal portion of the study.

References

Brewer, R.L., Gordon, R.J., and Shepard, L.S. 1983. Chemistry of mist and fog from the Los Angeles urban area. Atmospheric Environment. 17:11:2267-2270.

Carlson, C.E. and Gilligan, C.J. 1983. Histological differentiation among abiotic causes of conifer needle necrosis. Res. Pap. INT-298. Ogden, UT: U.S. Department of Agriculture, Forest Service, Intermountain Forest and Range Experiment Station. 17p.

Chappelka, A.H., III, Chevone, B.I. and Burk, T.E. 1985. Growth response of yellow poplar (Liriodendron tulipifera L.) seedlings to ozone, sulfur dioxide, and simulated acidic precipitation, alone and in combination. Environ. Exp. Bot. 25:233-234.

Chappelka and Chevone, B.I. 1986. White ash seedling growth in response to ozone and simulated acid rain. Can. J. For. Res. 16:786-790.

Chevone, B.I. 1985. Gaseous and wet atmospheric pollutant effects on forest tree seedling growth. p.87-94. In: Air pollutants effects on forest ecosystems. Acid Rain Foundation, St. Paul, Minn.

Cooley, D.R. and Manning, W.J. 1987. The impact on assimilate partitioning in plants: a review. Environ. Pollut. 47:95-113.

Connell, D.W. and Miller, G.J. 1984. Chemistry and ecotoxicology of pollution. John Wiley and Sons, Inc. New York.

Evans, L.S. and Miller, P.R. 1975. Histological comparison of single and additive O_3 and SO_2 injuries to elongating Ponderosa pine needles. Amer. J. Bot. 62:4:416-421.

Galloway, J.N., Likens, G.E., Keene, W.C. and Miller, J.M. 1982. The composition of precipitation in remote areas of the world. J. of Geophysical Research. 87:11:8771-8786.

Hogsett, W.E., Plocher, M. and Wildman, V. 1985. Growth response of two varieties of slash pine seedlings to chronic ozone exposures. Can. J. Bot. 63:2369-2376.

Jacob, D.J., Waldman, J.M., Munger, J.W. and Hoffmann, M.R. 1985. Chemical composition of fogwater collected along the California coast. Environ. Sci. Technol. 19:8:730-736.

Leith, I.D., Murray, M.B., Sheppard, L.J., Cape, J.N., Deans, J.D., Smith, R.I. and Fowler, D. 1989. Visible foliar injury of red spruce seedlings subjected to simulated acid mist. New Phytol. 113:313-320.

Munger, J.W., Jacob, D.J., Waldman, J.M. and Hoffmann, M.R. 1983. Fogwater chemistry in an urban atmosphere. J. of Geophysical Research. 88:C9:5109-5121.

Seiler, J.R. and D.J. Paganelli. 1987. Photosynthesis and growth response of red spruce and loblolly pine to soil-applied lead and simulated acid rain. For. Sci. 33:668-675.

Sheppard, L.J., Smith, R.I. and Cannell, M.G.R. 1988. Frost hardiness of Picea rubens growing in spruce decline regions of the Appalachians. J. of Tree Physiology. 5:25-37.

Skeffington, R., and Roberts, M. 1985. Effects of ozone and acid mist on Scots pine and Norway spruce. An experimental study. Forest dieback: influencing factors and their evaluation. pp. 747-760. In: Proceedings of a Symposium, Goslar, W. Germany, June 1985. VDI-Berichte no 560.

Tyler, G., Balsberg Pahlsson, A.M., Bengtsson, G. *et al.* 1989. Heavy-metal ecology of terrestrial plants, microorganisms and invertebrates: a review. Water Air Soil Pollut. 47:189-215.

Waldman, J.M., Munger, J.W., Jacob, D.J., Flagan, R.C. Morgan, J.J. and Hoffmann, M.R. 1982. Chemical composition of acid fog. Science. 218:677-680.

Chapter III

DETERMINING THE EFFECTS OF ACIDIC DEPOSITION AND HEAVY METALS ON SPRUCE SEEDLING GROWTH BY COMPARING FOG AND IMMERSION APPLICATIONS

Abstract

The contribution of fog to forest nutrient cycles, the amount of tree coverage by fog particles, and the degree of tree damage caused by pollutant fog particles are areas of current research interest. Western United States high-elevation and coastal locations are of prime concern because of the amount of fog; incidences of greater acidity and increased metal content of western fog; and pollution damage demonstrated in Europe and eastern United States spruce-fir forests. Engelmann spruce (*Picea Engelmannii* Parry) is a dominant species of the subalpine forests in the Rocky Mountain west. An assessment of the response of this species to heavy metal and acid fog is important because other *Picea* species have been shown to be sensitive to pollution. The objectives of this study were to determine the effects of pH concentration, deposition type (fog or immersion), heavy metal concentration, seed source, and their interactions on:

1. foliar injury of old and new spruce seedling needle growth,
2. root and shoot length and root and shoot dry biomass of two-year-old spruce seedlings, and
3. spruce seedling survival two years after treatment.

Needle necrosis and cuticle damage increased in the fog deposition experiment when both metal and pH treatment levels became more toxic. Abscission of the older needles increased with increasing acidic pH and metal concentrations in the immersion treatments; cuticle damage was also apparent at pH 2. Shoot length and biomass increased at intermediate pH and metal concentrations. Death occurred post-bud break in the immersion group, whereas seedlings of the fog treated group continued to live after 20 months of a two-year post treatment monitoring. The increase in shoot dry biomass at the moderate pH and metal concentrations was attributed to a fertilization effect.

Introduction

Approximately 15 years ago, in a high-elevation Bavarian forest called

Fichtelgebirge, a spring yellowing of Norway spruce (*Picea abies* [L.] Karst.) was observed. In 1982, German scientists noted falling needles, thinning canopies and an 8% increase in tree mortality. By 1987, roughly 52% of the stands were affected. Currently, more than one-third of the stands show heavy damage and increased tree mortality. German forest scientists believe that atmospheric acidic pollution has played a decisive role in the forests' downward spiral (Raloff 1989).

The decline in forest health in certain high-elevation forest areas of the United States has apparently been less severely affected by air pollutants, which may possibly be explained by three factors: (1) differences in genotypic susceptibility of European silver fir (*Abies alba* Mill.) and the two spruce species (Norway and red spruce [*Picea rubens* Sarg.]); (2) European forest plantation management practices; and/or (3) pollution load (Larsen *et al.* 1988). Nevertheless, the widespread mortality of high-elevation red spruce in the northeastern United States has occurred and has been attributed to heavy metal and acidic deposition.

It is known that both red and Norway spruce areas are above cloud base for considerable portions of the year. Cloud moisture tends to be much more acidic than precipitation, and the composition and acidity of cloud moisture has changed over the past century. Events leading to forest decline in Europe involve three phases of deposition. During the first phase (1870 - 1900), sulfate was the major pollutant in the atmosphere. The second phase (1900 - 1960) was characterized by an increase in nitrate deposition. In the third phase (1960 - present), there has been an exponential increase in the aerial deposition of nitrate and ammonium (Schulze 1989).

During the past two decades, the population growth in the western United States has coincided with increased numbers of motor vehicles, fossil-fueled power plants, and industrial furnaces expelling acids and heavy metals. With the declining pH readings along the Colorado Rockies eastern front, episodic pH readings in the mid 3 range on Mt. Rainier in the Cascades and high pH 2 range in southern California's Sierra Nevada mountains, public concern has now also focused on western United States high-elevation forests. A major species of the high-elevation Rocky Mountain forests is Engelmann spruce (*Picea Engelmannii* Parry), which typically grows with subalpine fir *(Abies lasiocarpa)* to form the extensive Engelmann Spruce-Subalpine fir forest cover type (Alexander and Shepperd 1990). The Engelmann Spruce-Subalpine Fir habitat is an important component of the Northern Rocky Mountains. Moist Pacific air masses exert strong climatic influences in the western and northern portions of these northern mountains, while the southern and eastern areas are drier, with more continental climates. Cloud moisture is an important weather feature to many of these areas. Montana alone has more than 25 separate mountain ranges rising above the tree line (Arno and Hammerly 1984).

Acid deposition researchers are in the process of defining types of damage to plant species caused by acid fog in cloud moisture events (Evans 1986; Masuch *et al.* 1986; Musselman 1988; Joslin *et al.* 1988; Mengel *et al.* 1989; Geballe *et al.* 1990; Jacobson *et al.* 1990; Percy *et al.* 1990; Ashenden *et al.* 1991). One of the major problems is determining and quantifying the extent and type of damage caused by acid fog on foliage. An important question is whether acidic fog manifests damage symptoms similar to acid rain. Also, can these symptoms be differentiated from damage caused by natural causes, such as climate or disease? Furthermore, can these symptoms be defined when more than one pollutant such as heavy metals is present in a pollutant load?

Heavy metals have been shown to be accumulating in forest ecosystems in Europe (Mayer 1983) and North America (Friedland *et al.* 1984). Heavy metals have been shown to decrease plant biomass and lateral root initiation (Marschner 1986). Lead, cadmium, and zinc reduce spruce seedling root growth in nutrient solution (Godbold and Huttermann 1985), affect water relations (Schlegel *et al.* 1987), and reduce transpirational water flow in red spruce (Klein and Perkins 1988). Leaves accumulating heavy metals are damaged, become inefficient and must therefore be replaced. Such leaves typically have low transpiration rates and thus low photosynthetic rates that tend to minimize waste accumulation (Chapin *et al.* 1990) but also require nutrients for replacement that could be used for growth. Such leaves do not photosynthesize at capacity levels near the potential of leaves without heavy metal accumulation.

Heavy metals are of concern because of their increasing content in western United States air pollution, particularly from automobile exhaust. The documented air pollution chemistry used for this study contained lead, nickel, copper, and manganese. These heavy metals have many potential effects on plants. Of particular importance for plant growth in acid soils is the inhibition of calcium and magnesium uptake by high manganese concentrations. Plant roots are not injured directly by manganese; rather, it affects the shoot (Marschner 1986). Copper is associated with enzymes involved in oxidation-reduction in plants. Toxicity results in chlorosis and stunting, with a corresponding iron deficiency. Root system reduction appears first and implies faulty nutrient absorption and inhibition of growth. Nickel is readily absorbed. As a divalent cation, nickel competes with other cations, including calcium, magnesium, iron, and zinc in root uptake mechanisms leading to characteristic symptoms of chlorosis. The effects of lead on forest trees have been documented (Seiler and Paganelli 1987) and include: root elongation inhibition; roots forming adventitiously; and stem elongation and leaf expansion inhibition. Overall, the inhibition of root elongation is the most sensitive parameter of heavy metal toxicity.

Objectives

To determine the effects of pH concentrations, deposition type, heavy metal concentration, seed source, and their interactions on:

1. foliar injury of old and new spruce seedling needle growth,
2. root and shoot length and root and shoot dry biomass of two-year-old spruce seedlings, and
3. spruce seedling survival two years after treatment.

Hypotheses

The general null hypotheses for the stated objectives are:

1. H_0: There is no difference in mean foliar injury of old and new spruce seedling needle growth due to pH, deposition type, heavy metal, seed source, or their interaction.

2. H_0: There is no difference in mean root and shoot length and root and shoot dry biomass of spruce seedlings due to pH, deposition type, heavy metal concentrations, seed source, or their interactions.

3. H_0: There is no difference in spruce seedling survival two years after treatment due to pH, deposition type, heavy metal, seed source, or their interactions.

However, there is an individual hypothesis for each 1 degree of freedom orthogonal comparison that was constructed for each dependent variable that was tested (Table 3.2, 3.3, and 3.4). Thus following the model statement for old and new needle damage (Table 3.2), a partial list of hypotheses statements would be as below:

4. H_0: There is no difference in mean foliar injury of new spruce seedling needle growth due to pH 3.5 or pH 2 compared to pH 5.4.

5. H_0: There is no difference in mean root and shoot length and root and shoot dry biomass of spruce seedlings due to pH 3.5 or pH 2 compared to pH 5.4.

6. H_0: There is no difference in spruce seedling survivability two years after treatment due to pH 3.5 or pH 2 compared to pH 5.4.

7. H_0: There is no difference in mean foliar injury of new spruce seedling needle growth due to ambient or twice ambient heavy metal concentration compared to 0 heavy metal concentration.

8. H_0: There is no difference in mean root and shoot length and root and shoot dry biomass of spruce seedlings due to ambient or twice ambient heavy metal concentration compared to 0 heavy metal concentration.

9. H_0: There is no difference in spruce seedling survival two years after treatment due to ambient or twice ambient heavy metal concentration compared to 0 heavy metal concentration.

10. H_0: There is no difference in mean foliar injury of new spruce seedling needle growth due to pH 3.5 or pH 2 interacting with the ambient or twice ambient heavy metal concentration compared to pH 5.4 interacting with the 0 heavy metal concentration.

11. H_0: There is no difference in mean root and shoot length and root and shoot dry biomass of spruce seedlings due to pH 3.5 or pH 2 interacting with the ambient or twice ambient heavy metal concentration compared to pH 5.4 interacting with the 0 heavy metal concentration.

12. H_0: There is no difference in spruce seedling survival two years after treatment due to pH 3.5 or pH 2 interacting with the ambient or twice ambient heavy

metal concentration compared to pH 5.4 interacting with the 0 heavy metal concentration.

The above hypotheses should clarify the extent of interactions involved in the model.

Materials and Methods

Treatment Solution

The treatment solutions used for immersion and fogging contained background ions based on the average chemical analyses of ambient southern California fog (Table 3.1). These averages represent actual anthropogenic source contamination, including heavy metals, ammonia, and a 2.5:1 nitrate-to-sulfate ratio. The possibility of nutrient interactions with pH levels is thus more accurately explored. In replicating the fog chemical analyses, background ions were added to deionized water and titrated to specific treatment pH and heavy metal concentrations. Treatment pH levels were 7.0, 5.4, 3.5, and 2.0. The heavy metals lead, nickel, copper, and manganese were not introduced into treatment solutions at the control level (0).

Table 3.1. Ionic composition of acidic fog and immersion solution (from Musselman and Sterrett, 1988).

Ion	$\mu eq/m^3$	Ion	$\mu eq/m^3$
NO_3	4.787	F-	.384
NH_4+	4.735	K+	.165
SO_4	2.256	Fe++	.024
Na+	1.357	Pb++	.01
Ca++	1.038	Cu++	.007
Mg++	.500	Mn++	.005
Cl-	.4163	Ni++	.002

These heavy metals were at the ambient levels (1x) indicated in Table 3.l and were double the levels (2x) in Table 3.1 for the remaining treatment levels. A 2.5:1 ratio of nitric-to-sulfuric acid represented ratios reported in southern California fog and European analyses (Munger *et al.* 1983; Waldman *et al.* 1982). Ammonia remained at the constant level indicated in Table 3.1 throughout the treatments. Immersion treatment solutions were prepared prior to each treatment period. Thus any seedling absorption of additional ions could not take place from material leached into the solution from previous treatments. Fog solution was prepared as needed.

Greenhouse Procedures

In March 1989, two-year-old Engelmann spruce seedlings from four seed sources were obtained from United States Forest Service nursery facilities at Coeur d'Alene, Idaho. Seed lots and elevations were: Bitterroot National Forest - lot 4097, elevation 1,828m; Clearwater National Forest - lot 3300, elevation 1,737m; Kootenai National Forest - lot 0878, elevation 1,828m; and Flathead National Forest - lot 1645, elevation 1,707m.

Four spruce seedling sources were also obtained from the United States Forest Service nursery facilities at Lucky Peak Nursery, Boise, Idaho. These included elevations between 2,666m and 3,000m. The seedlings were planted, but soon showed signs of root rot, which was confirmed in the pathology laboratory at the United States Forest Service Regional Office in Missoula, Montana. Root stripping occurred during extremely wet conditions at the spring seedling lift in Boise, leaving the seedlings susceptible to a pathogenic infection. The seedlings were treated for root rot, but did not recover and were not utilized in the study.

The selected seedlings for planting were from a wide population based on habitat type, elevation, and area. Seedling uniformity was maintained by a selection based on vigor, intact apical bud, stem diameter, and height. Selected seedlings were randomly assigned to treatment blocks. Each seedling was labeled so its treatment reponse could be identified or traced. Seedlings were planted in a 1:1 mix of peat moss and vermiculite in approximately 900ml waxed paper dairy milk containers (1 quart).

The School of Forestry greenhouse on the University of Montana campus, Missoula, Montana, served as the site for the formal experiment beginning in March 1989. Relative humidity and air temperature were recorded by six hygrothermographs continuously during the course of the experiment to monitor correlations between humidity and temperature, fog droplet formation, and droplet duration; the measurements also indicated temperature extremes.

Beginning in the summer of 1989, the conifer seedlings were exposed to an immersion or fog treatment for four hours, three times weekly (Monday, Wednesday, and Friday) for two months shortly after sunrise. The treatment time frame was determined in a preliminary study in February and March 1989. Seedlings were removed from the immersion containers or the fog framework was lifted off the seedlings after each treatment exposure period. With varying greenhouse humidity levels, droplets would remain intact up to six hours after termination of fogging. Little drip from leaf surfaces occurred. To avoid growth response differences between frame enclosure and immersion container placement in the greenhouse, each enclosure and immersion container was rotated around the greenhouse, where treatments took place, and plants were rotated within frame enclosures after each treatment exposure.

Height measurements were taken to ± 1mm prior to bud break and one week prior to the beginning of treatments because height variation between seed sources was apparent at the time of seedling planting in the greenhouse. Measurements were taken from the root crown along the length of the shoot to the tip.

Visual inspection of the seedlings occurred just before treatment to verify presence of damage or disease. Seedlings were examined weekly for treatment damage criteria during the experiment. A visual rating system of 0 - 10 was employed, 0 being the least damaged and representing 10% or smaller damage effects. Each seedling was rated for such visual symptoms as necrosis, chlorosis (an indication of metal and general oxide effects), brown banding on the needles, needle tip necrosis, needle abscission, and cuticle damage. Two people did the visual recording during the experiment, one rating the fog and another the immersion portion of the treatments. Periodic examination of the ratings were done by myself to ensure ratings were consistent and represented the visible damage for that week's reading. Although only the foliar injury measurements of the last week of the two-month treatment period were used in damage assessment, the weekly measurements documented a pattern of developing damage response.

At the end of the two-month treatment period, the plants were divided into two groups: (1) the posttreatment monitoring group held for two years; and (2) the plants used for growth measurements following the treatments. Half the plants of the four seed sources, four acid levels, three heavy metal concentrations, two deposition types, and two repetitions, equaling 384 plants, were maintained for the two-year monitoring (which was not part of the original experimental design). The remaining 384 plants were used for the growth and ICP measurements.

The plants used for the growth and ICP measurements were removed from the cardboard containers, washed free of soil, and separated into roots and shoots for root, shoot measurements and dry weight determinations. Lengths were measured to ±1mm and biomass was recorded to ±.01g. Shoots were measured from the root crown the length of the old shoot growth; then, from the base of the new shoot growth to the top of the shoot. Old root growth was taken from the root crown following the center length of the suberized growth; new root growth was taken from the center length of the unsuberized new root growth to the bottom of the new growth. Plant material was dried at 112°C for 72 hours and weighed with an electronic balance. Unequal subclass numbers resulted because of mortality, additional plant samples needed for ICP analyses, and lab processing errors.

Application by Fog

Design Phase

Generating a true floating fog particle size presented three major problems: (1) duplicating and testing a canister design; (2) finding an atomizer nozzle capable of producing a particle size under 20 microns in size; and (3) finding a canister size that could withstand the pressure necessary to deliver a 20-micron particle size. A canister design by Musselman and Sterrett (1988) was used to deliver the chemical solution in Table 3.1. Bill Hogsett[3] was using canisters based on the Musselman Sterrett design in preliminary testings with Paul Miller, United States Forest Service, Riverside, California. The canisters had delivered a mist-size particle, not a true fog particle. Two prototypes of the canister were built and tested by Missoula Tank Testing during the summer of 1988. Based on these tests, the canister design was abandoned because of the size of canister necessary to generate the pressure required for a 20-micron or less particle. Also, the EPA group in Riverside, California, had a series of mishaps using their canisters during the same summer, including a near decapitation of a prominent visiting researcher when one of the canisters exploded.

While I was researching alternative fog application methodology, Dr. Hogsett suggested talking with Dr. Susan Bechneil, Department of Forestry, Humboldt State College, Arcata, California. Dr. Bechneil was testing industrial atomizers, also trying to generate a true floating fog particle in her research with coastal conifers. She was kind enough to send two nozzles for my personal use during the preliminary testing. I contacted Spraying Systems Co., Wheaton, Illinois, designers of the nozzle Dr. Bechneil

[3] Personal conversation. Bill Hogsett, 1987, Environmental Protection Agency, Western Conifers Research Cooperative, Environmental Research Laboratory, 200 SW 35th St., Corvallis, Oregon.

had sent.

The air atomizers produced by this company are used in such applications as storage bin fumigations, lubricant applications, moisturizing corrugated paperboard, and spraying protective coatings on tires during production. However, one outstanding feature of these units is the ability to operate on a siphon or gravity-fed liquid principle. The setup draws liquid through the feed line into the air flow where it is then atomized.

The fog apparatus required an air compressor, an air atomizer setup, a solution canister, a frame enclosing plant species under treatment, and an appropriate hose and other hardware (Fig. 3.1). The frame enclosure for each fog treatment was 90cm x 55cm x 90cm (length x width x height) built with 2.5cm x 5.0cm pine lengths and covered with polythene sheeting. The dimensions of the fog enclosures were determined by trial and error. Working with the sample atomizers sent by Dr. Bechneil, the size of the frame was determined by the number of seedlings to be treated at one time, the height necessary to achieve a true floating fog from the atomizers, platform space in the greenhouse, and total area available for the experiment. Each frame enclosure was fitted with an air atomizer. Compressed air delivered to the atomizers ultrasonically shattered the siphoned treatment solution into aerosol particles dispersed into the frame enclosure at a low velocity. Individual pressure gauges allowed even pressure maintenance and monitoring to each fog cage (Fig. 3.1 A). During application of the fog regime, the buckets containing the solution were raised to maintain siphon height, which, along with constant pressure, assured a constant flow of fog particles under 20 microns in size. We used a 60-liter Ingersoll Rand Model IR536VA, 5-hp electric air compressor with a maximum pressure of 860kPa as an air pressure source, in addition to an existing compressor in the greenhouse.

Air pressure and siphon height are adjustable for individual spraying situations. The 1/4J SU2A nozzle body spray setup with an air cap 70 (Fig. 3.1 B), used in the experiment at a siphon height of 45cm and air pressure of 104kPa, delivered a median particle diameter in the range of 17 microns. By volume, 68% of the total spray is under 21 microns. Working with Rudolf Schick, research scientist for Spraying Systems, the percentage was derived by spraying water through the system at room temperature under Spraying Systems' laboratory conditions. Aerosol particles were collected using Spraying Systems' Malvern Analyzer (a light scattering/lazer type device) and fitted to a distribution function to determine the percentage of particles under 20 microns. Initially, the spray pattern is cone shaped, matching the general

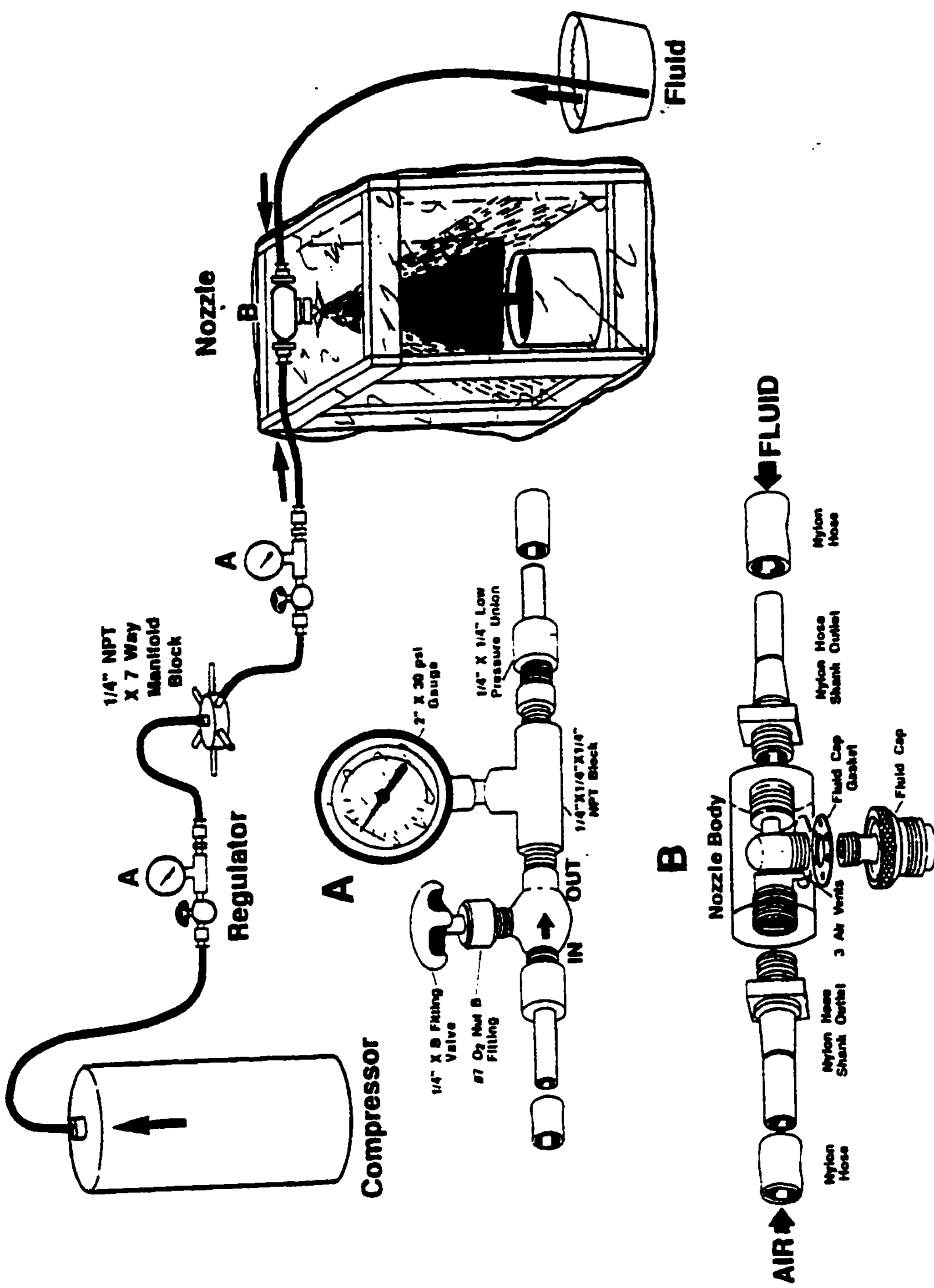


Figure 3.1 Fog treatment apparatus

shape of the seedling, but after the floating fog particle is achieved, usually within 10 - 15 minutes, even distribution of the seedling is assured.

The connection procedure for one fog cage is diagramed in Figure 3.1. Twentyfour fog cages were constructed for the experiment. A block fitting was added after the air compressor regulator to permit additional lines. A pressure regulator (Fig. 3.1 A) was required for each fog cage to control the appropriate pressure to the air atomizer because lower pressures result in larger particle sizes. Nontoxic 0.63cm-diameter tubing was used throughout.

Line connections between the air compressors and individual fog cages proved to be one of the most difficult portions of the design phase. The effort was successful because principles of the gas law and fluid dynamics were utilized. Consistent air flow to the twenty-four cages could not be determined until all of the fittings were airtight, which took hours of testing. Hundreds of feet of nalgene tubing were used in the hookup, some footage with varying thickness; the tubing had to be of one thickness in order to maintain uniform gas flow. If tubing were not of equal distance or height from the air compressors or regulators, resistance in the form of distance and temperature differences caused pressure differences, blowing clamp systems from individual regulators. Consistency in distance, whether between cages, regulators, or compressors, proved to be the key to even air flow.

A humorous note was provided in the siphon design. Eighteen-gallon glass containers were originally used in the preliminary testing. These containers proved cumbersome and unsafe in transport from the solution preparation laboratory to the greenhouse. Five-gallon buckets were cleaned and rinsed with a 10% HCL solution prior to use as containers for the acidic and heavy metal solution. Siphon tubing from the solution buckets to the atomizers proved to be expensive because of acidic damage and diameter considerations. Excess catheters from the local university chemical supply were free and worthy substitutes; the catheter tubing proved resistant to chemical damage, thus did not contaminate treatment solutions.

Application by Immersion

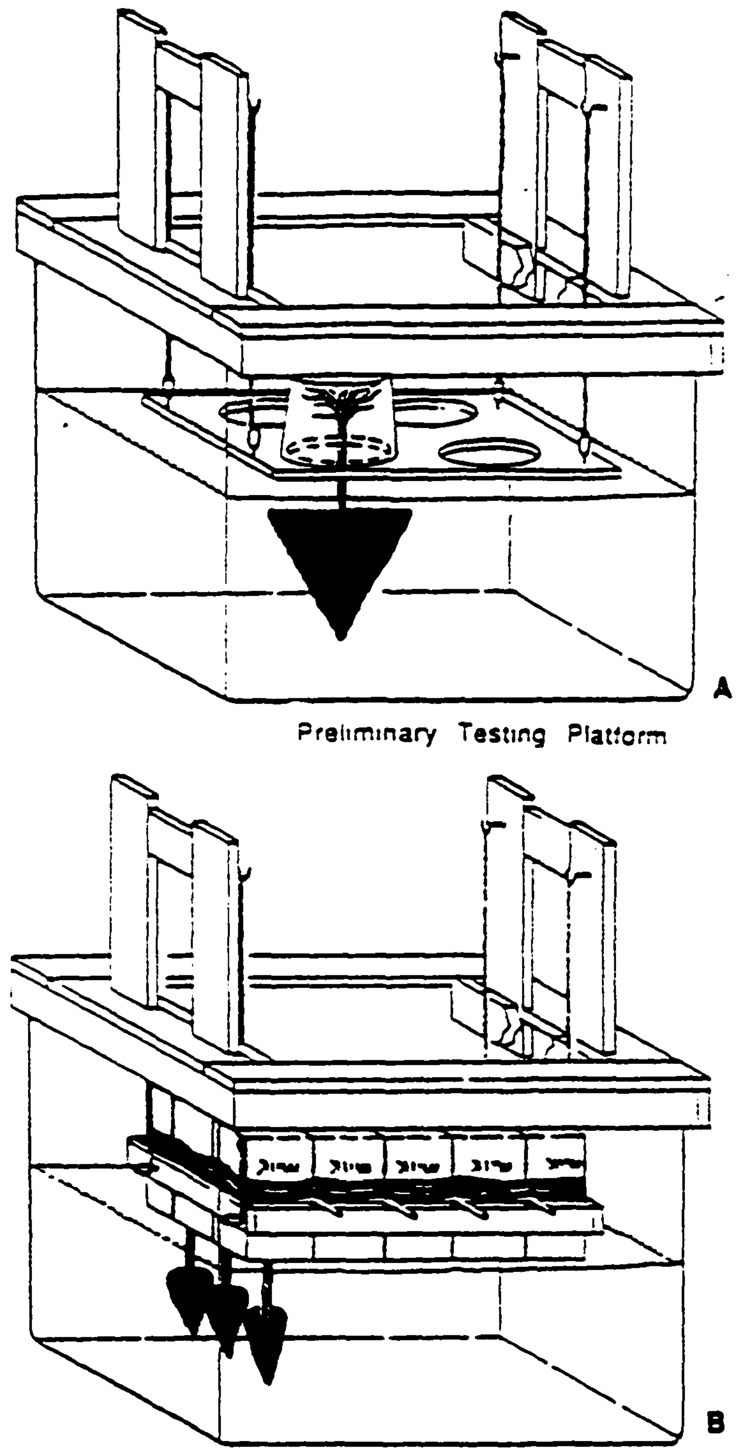
Since foliar absorption of the acidic solution and heavy metals is the hypothesized mechanism of uptake of an airborne pollutant, isolation of the roots from the treatment solution by direct contact is important. Furthermore, complete coverage of the needle surface was considered important as a worst-case scenario to be contrasted with the fog treatment. Therefore, seedling shoots were immersed upside down in 58-liter plastic containers measuring 60cm x 40cm x 45cm high, which contained the acid-heavy metal solution used for the fog treatments. Adjustable platforms were designed to reduce chemical and distilled water costs, and to allow for seedling growth (Fig. 3.2 A). The bracing portion of the platform was constructed of 2.5cm x 5.0cm wood lengths and was designed to rest on the upper edges of the 58-liter plastic containers. In Chapter II, which was designed to determine an effective pH and heavy metal dose-range, 15cm pots rested on the base, where circular 10cm diagonal cuts allowed the shoot portion of the seedlings to immerse in the liquid. Prior to immersion, a cotton band was wrapped around the seedling stem, and the seedling pot was then sealed in plastic, to protect the root area from contamination with the immersion solution (Fig. 3.2 A).

For this chapter (the formal study), a large number of seedlings required immersion. Groups of 16 two-year-old Engelmann spruce seedlings were taped together as a group using duct tape (Fig. 3.2 B). Two 7.5 x 20cm diameter dowels were placed between two interior rows of containers. The seedlings were lowered onto the frame as a block, resting on the frame via the dowels, while the seedlings were immersed in the treatment liquid.

Statistics and Experimental Design

The treatments were a factorial combination of four seed sources (Kootenai, Clearwater, Bitterroot, and Flathead), four pH's (7.0, 5.4, 3.5, and 2), three heavy metal concentrations (0, ambient, and twice ambient), two repetitions for temperature in the greenhouse (cooler and warmer), two deposition types (fog and immersion), a 4 x 4 x 3 x 2 x 2 factorial, (seed source, metal level, pH level, deposition, and temperature gradient), - in a completely randomized design with 16 replicates. The ANOVA tables and statistical models are illustrated in Tables 3.2, 3.3, and 3.4. A summary of statistical methodology and significance is available in Table 3.5. Data were analyzed using analysis of variance employing Systat software (Systat, Inc. 1988). Treatment variances for seed source, metal and pH level, deposition type, and temperature gradient were partitioned into one degree of freedom linear components using orthogonal polynomial contrasts for the new and old needle damage rating analyses.

Several problems had to be solved during the analyses. The first was the inability of either Systat version 4.1 or 5.0 to analyze the number of treatment combinations (192) present in the factorial design. The maximum allowable number was 87. Therefore, pH level 7.0 was deleted from the subsequent analyses, and



IMMERSION APPARATUS

Figure 3.2 Immersion apparatus diagrams for preliminary tests (A) and formal experiment (B).

Table 3.2 ANOVA table and underlying statistical model for root and shoot length.

Source		DF
pH	(A)	2
metal	(B)	2
A x B		4
deposition	(C)	1
A x C		2
B x C		2
seed source	(D)	3
A x D		6
B x D		6
C x D		3
A x B x C x D		12
Error		22
Total		65

Underlying Statistical Model for Dry Shoot Biomass

$$Y_{ijklm} = u + A_i + B_j + (AB)_{ij} + C_k + (AC)_{ik} + (BC)_{jk} + D_l + (AD)_{il} + (BD)_{jl} + (CD)_{kl} + (ABCD)_{ijkl} + e_{ijklm}$$

where:

Y_{ijklm} = root and shoot length

A_i = pH concentration $i = 1,\ldots, 3$

B_j = heavy metal concentration $j = 1,\ldots, 3$

$(AB)_{ij}$ = pH by metal concentration

C_k = deposition type $k = 1,2$

$(AC)_{ik}$ = pH by deposition

$(BC)_{jk}$ = heavy metal concentration by deposition

D_l = seed source $l = 1,\ldots, 4$

$(AD)_{il}$ = pH by seed source

$(BD)_{jl}$ = heavy metal concentration by seed source

$(CD)_{kl}$ = deposition by seed source

$(ABCD)_{ijkl}$ = pH by heavy metal concentration by deposition by seed source +

e_{ijklm} = error term = $(ABC)_{ijk}+(ABD)_{ijl}+(ACD)_{ikl}+(BCD)_{jkl}$

Notes:

1) Due to unequal subclass numbers, treatment combination means were analyzed, and higher order interaction terms judged to be nonsignificant were forced into the error term.

2) Due to missing observations only 66 out of 72 treatment combination means were analyzed.

Table 3.3 ANOVA table and underlying statistical model for old and new needle damage rating.

Source		DF
pH	(A)	2
metal	(B)	2
A x B		4
deposition	(C)	1
A x C		2
B x C		2
A x B x C		4
seed source	(D)	3
A x D		6
B x D		6
A x B x D		12
C x D		3
A x C x D		6
B x C x D		6
A x B x C x D		12
Error		504
Total		575

Underlying Statistical Model for Old and New Needle Damage Rating

$$Y_{ijklm} = u + A_i + B_j + (AB)_{ij} + C_k + (AC)_{ik} + (BC)_{jk} + (ABC)_{ijk} + D_l + (AD)_{il} + (BD)_{il} + (ABD)_{ijl} + (CD)_{kl} + (ACD)_{ikl} + (BCD)_{ijk} + (ABCD)_{ijkl} + e_{ijklm}$$

where:

Y_{ijklm} = new or old needle damage rating

A_i = pH concentration

B_j = heavy metal concentration

$(AB)_{ij}$ = pH by metal concentration

C_k = deposition type

$(AC)_{jk}$ = pH by deposition type

$(BC)_{jk}$ = heavy metal concentration by deposition type

$(ABC)_{ijk}$ = pH by heavy metal concentration by deposition

D_l = seed source

$(AD)_{il}$ = pH by seed source

$(BD)_{jl}$ = heavy metal concentration by seed source

$(ABD)_{ijl}$ = pH by heavy metal concentration by seed source

$(CD)_{kl}$ = deposition by seed source

$(ACD)_{ikl}$ = pH by deposition by seed source

$(BCD)_{ijk}$ = heavy metal concentration by deposition by seed source

$(ABCD)_{ijkl}$ = pH by heavy metal concentration by deposition by seed source

e_{ijklm} = error

Table 3.4 ANOVA table and underlying statistical model for shoot biomass.

Source		DF
pH	(A)	2
metal	(B)	2
A x B		4
deposition	(C)	1
A x C		2
B x C		2
seed source	(D)	3
A x D		6
B x D		6
C x D		3
A x B x C x D		12
Error		26
Total		69

Underlying Statistical Model for Dry Shoot Biomass

$$Y_{ijklm} = u + A_i + B_j + (AB)_{ij} + C_k + (AC)_{ik} + (BC)_{jk} + D_l + (AD)_{il} + (BD)_{jl} + (CD)_{kl} + (ABCD)_{ijkl} + e_{ijklm}$$

where:

Y_{ijklm} = dry shoot biomass

A_i = pH concentration $\quad i = 1,\ldots, 3$

B_j = heavy metal concentration $\quad j = 1,\ldots, 3$

$(AB)_{ij}$ = pH by metal concentration

C_k = deposition type $\quad k = 1, 2$

$(AC)_{ik}$ = pH by deposition

$(BC)_{jk}$ = heavy metal concentration by deposition

D_l = seed source $\quad l = 1,\ldots, 4$

$(AD)_{il}$ = pH by seed source

$(BD)_{jl}$ = heavy metal concentration by seed source

$(CD)_{kl}$ = deposition by seed source

$(ABCD)_{ijkl}$ = pH by heavy metal concentration by deposition by seed source

$+ e_{ijklm}$ = error term = $(ABC)_{ijk} + (ABD)_{ijl} + (ACD)_{ikl} + (BCD)_{jkl}$

Notes:

1) Due to unequal subclass numbers, treatment combination means were analyzed, and higher order interaction terms judged to be nonsignificant were focused in the error term.

2) Due to missing observations, only 70 of 72 treatment combinations means were analyzed.

Table 3.5. Statistical method summary with significant mean comparisons of foliar damage, root and shoot length, and shoot dry biomass plus posttreatment survival of Englemann spruce seedlings treated with acid and heavy metal concentrations.

Statistical design

	Anova design	Factorial components	# of treatment combinations
1.	4 x 4 x 3 x 2 x 2 factorial - 192 cells 16 replicates	4pHs, 4 seed sources, 3 metal levels, 2 temps. 2 depositions	192 @
2.	4 x 4 x 3 x 2 factorial	4 pHs, 4 seed sources, 3 metal levels, 2 deps.	96 @
3.	4 x 3 x 3 x 2 factorial	4 seed sources, 3pHs, 3 metal levels, 2 deps.	72 @

Statistical analyses

	Response Variable	Factor	Significance *
1.	new foliar damage	main effects	pH 2, 2x metal and Flathead seed source
		1st order interactions	pH 2 by 2x metal, pH 2 by fog deposition, 2x metal by Flathead seed source, 2x metal by fog deposition
		2nd order interactions	pH 2 by 2x metal by fog deposition
2.	old foliar damage	main effects	pH 2, 2x metal
		1st order interactions	ph 2 by 2x metal, pH 2 by fog deposition, 2x metal by fog deposition
		2nd order interactions	pH 2 by 2x metal by fog deposition
3.	root dry biomass		none
	shoot dry biomass	main effects	pH 3.5, 1x metal, fog deposition
4.	new root length	main effects	ph 3.5, 2
		1st order interactions	pH 2 by 2x metal, pH 2 by immersion pH 3.5 by immersion
5.	new shoot length+	main effect	Clearwater seed source

Posttreatment survival

1.	fog treatment	acid level x heavy metal concentration treatment combinations	proportion surviving is different

* *All items are 1 degree of freedom orthogonal comparisons and significance is based on contrasts.*
\+ *Origional shoot length prior to treatment used as covariate-not significant; ANOVA results presented.*
@ *SYSTAT maximum of 87 treatment combinations exceeded.*

replicates were collapsed so that 72 treatment combination means remained. The pH 7.0 served as a neutral pH control; however, normal or unpolluted cloud water chemistry is ~pH 5.4, and served as the ambient pH control. At the end of the treatment period no distinct differences could be evaluated in the foliar damage between the two experiment repetitions designated for temperature difference analysis in the greenhouse. Therefore, the decision was made to combine the two repetitions by designating half of each repetition for the posttreatment monitoring. Collapse of means was done after consulting with the Systat statistical staff and Dr. Hans Zuuring, statistician at the School of Forestry, University of Montana, Missoula. This also reduced the cells to 72 by reducing the number of independent factors to four. Thus a 4 x 3 x 3 x 2 factorial resulted. Orthogonal polynomial contrasts were constructed for the new and old needle damage because cell replicate size was equal (Table 3.2).

An analysis of new shoot length was performed by an analysis of covariance using pretreatment old shoot length as the covariate. There were no significant differences between means. Therefore an analysis of variance was employed.

Because unequal cell replicates resulted for the root and shoot length and dry biomass measurements, the following statistical procedure was performed for root and shoot length and biomass analyses: standard analysis of variance procedures were followed with the exception that nonsignificant second order interaction degrees of freedom were forced into the error term (Tables 3.3 and 3.4). This procedure was considered legitimate because no second order interactions were significant for the root and shoot new growth or shoot dry biomass. This allowed for a valid error term. Contrasts were performed to test at which treatment concentration differences existed. Significance was tested for the main effects using one-way statistical contrasts. The one degree of freedom orthogonal comparisons were then constructed and tested using oneway statistical contrasts that determined at which concentration level of the treatment factor a significant difference existed. All posthoc contrasts were designed to make comparisons between two treatment concentration levels.

A further complication occurred when measuring the shoot dry biomass analysis. Needle abscission occurred during the experiment and increased after the experiment. While pulling the seedlings for measurements, certain seedlings in advanced stages of stress would lose needles on the tables in the greenhouse or when moved from the greenhouse. Needle loss presented problems in accurate measurements of shoot biomass. Therefore, where abscission exceeded 15% of the needles at the time of removing seedlings from the greenhouse, a zero was recorded for seedling biomass and an analysis of variance was performed on the full data set. A truncated data set presenting the means of the unabscized seedlings is presented as a comparison to the full data set (Table 3.6).

Treatment effects are not always readily apparent during the experimental phase or immediately after treatment. Thus, half of the immersed and fogged groups of seedlings were retained for observation over a two-year period. The seedlings were watered as needed. No fertilizer was applied to any of the seedlings after planting, during the experimental phase, or for the two-year posttreatment observation period.

Table 3.6. Comparing two-year old Engelmann spruce seedling shoot dry biomass (g) treated by immersion and fog deposition with three pH and three metal levels. Full and truncated data set means are presented.

Deposition Type		Metal Level								
	pH	0			1x			2x		
FOG		$\bar{x}$	(sd)	n	$\bar{x}$	(sd)	n	$\bar{x}$	(sd)	n
Full	5.4	2.6	(1.9)	15	2.1	(1.8)	11	2.7	(1.8)	12
Truncated	5.4	3.1	(1.5)	13	3.1	(1.2)	8	3.3	(1.6)	8
Full	3.5	2.3	(2.1)	12	4.9	(3.8)	16	2.3	(2.3)	15
Truncated	3.5	3.4	(1.6)	8	9.8	(2.4)	8	3.8	(1.6)	9
Full	2.0	1.8	(2.1)	16	1.8	(1.9)	16	1.8	(1.3)	12
Truncated	2.0	3.5	(1.6)	8	3.2	(1.9)	9	2.7	(0.8)	8
IMMERSION										
Full	5.4	1.9	(1.2)	16	2.9	(2.3)	16	1.7	(1.7)	16
Truncated	5.4	2.3	(0.8)	13	3.8	(1.9)	9	2.7	(1.4)	10
Full	3.5	2.2	(2.3)	16	4.6	(2.9)	7*	2.6	(1.1)	7*
Truncated	3.5	3.6	(1.9)	10	4.6	(2.9)	7*	2.6	(1.1)	7*
Full	2.0	2.1	(2.4)	12	2.2	(2.0)	16	2.0	(1.7)	13
Truncated	2.0	3.5	(2.1)	7	3.5	(1.1)	10	2.5	(1.3)	9

* values for full and truncated data set are the same, because less than 15% of the replicates suffered needle loss.

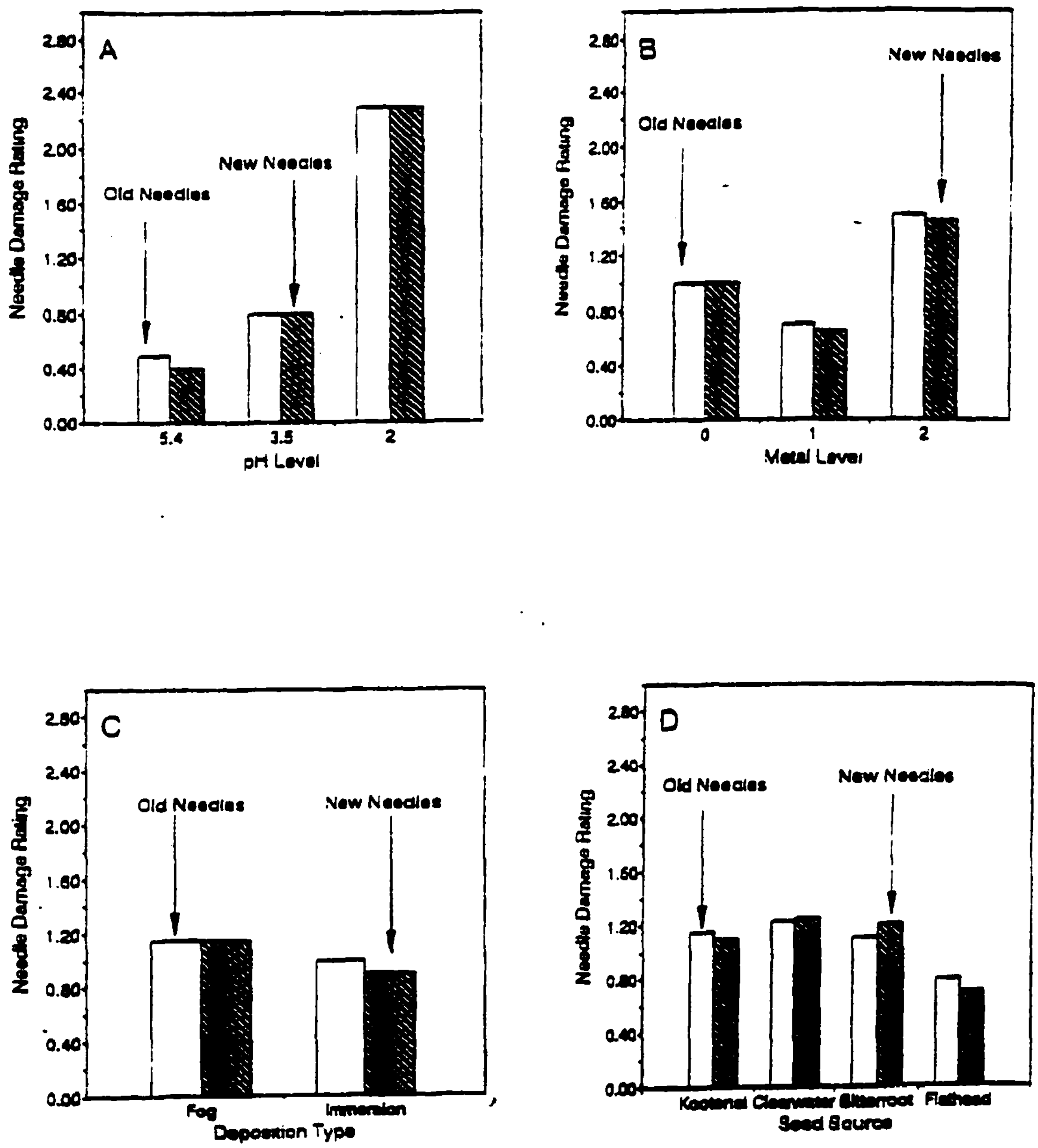


Figure 3.3 Needle damage rating graphs grouped by pH level, metal level, deposition type and seed source.

Results

New Root and Shoot Length

Main treatment effects on new root length were significant (P=0.03) for pH at the 3.5 and 2x metal concentrations (F- ratio 3.986). Two-way interactions were significant (P=0.05) for pH 2, 3.5 and the immersion deposition (F-ratio 3.337). Although there was no significant (P≥0.05) pH and metal interaction, root growth declined from the controls at all metal levels at pH 3.5 and pH 2, with the greatest decline at pH 3.5 (Table 3.6). A decline of ~30% occurred at the ambient (10.8 vs. 7.6) and twice ambient (10.2 vs. 7.0) metal levels at pH 3.5 from the same metal levels at pH 5.4 in the immersion treatment.

In the fog treatment, although not significant (P≥0.05), there was a greater mean root length at pH 5.4 with increasing heavy metal concentration. In contrast, a decrease in root length occurred at pH 5.4 and pH 3.5 at the ambient and twice ambient metal concentration.

There was a significant difference (P=0.00) in new seedling shoot length due to seed source, (F-ratio 12.294). New seedling shoot length was significantly less for the Clearwater Forest seed source compared to other seed sources. An analysis of covariance adjusting for pretreatment height variation between seed sources was not significant (P≥0.05).

Differences between treatments in shoot length were evident (Table 3.7). The fog and immersion treatment had an increase in mean shoot length with increasing metal concentrations at pH 2. The lowest mean shoot length occurred with the fog treatment at pH 3.5 and the ambient metal concentration at pH 2.0 and the 0 metal level. The lowest mean shoot length occurred with the immersion treatment at pH 3.5 and the twice ambient metal concentration (Table 3.7).

Foliar Injury

Mean needle damage ratings for new foliage were significantly different due to pH, heavy metal concentration, and seed source. At pH 2 there was greater foliar damage (P=0.00) than at pH 3.5 or 5.4 (F-ratio 70.183) (Fig. 3.3); the twice ambient heavy metal level (P=0.00, F-ratio 14.423) (Fig. 3.3); the Flathead Forest seed source had the least susceptibility (P=0.02) to foliar injury (F-ratio 3.194) **(Fig. 3.3D)**. There was a significant two-way interaction for pH and heavy metal concentrations, pH and treatment administration, and pH and seed source. Two-way interactions proved significant (P=0.00) for pH 2 and the twice ambient heavy metal

Table 3.7. Mean two-year-old Engelmann spruce root and shoot lengths, their standard deviations (sd), and sample sizes by deposition type: pH and heavy metal concentration treatment combinations.

Deposition	pH	Heavy Metal Concentrations								
		None			Ambient			Twice Ambient		
Root Length (cm)		$\bar{x}$	(sd)	n	$\bar{x}$	(sd)	n	$\bar{x}$	(sd)	n
Immersion	5.4	9.0	(2.86)	6	10.8	(2.74)	12	10.2	(6.68)	16
	3.5	6.8	(5.08)	16	7.6	(2.01)	2	7.0	(1.52)	3
	2.0	7.3	(2.70)	11	8.0	(5.90)	16	7.2	(2.94)	12
Fog	5.4	4.3	(2.82)	16	10.4	(4.17)	12	11.1	(8.48)	12
	3.5	8.1	(2.21)	8	8.1	(5.32)	8	4.2	(1.98)	15
	2.0	10.2	(5.32)	11	1.5	(6.60)	16	10.0	(6.96)	11

Deposition	pH	Heavy Metal Concentrations								
		None			Ambient			Twice Ambient		
Shoot Length (cm)		$\bar{x}$	(sd)	n	$\bar{x}$	(sd)	n	$\bar{x}$	(sd)	n
Immersion	5.4	8.4	(0.54)	6	9.3	(1.53)	12	8.4	(2.36)	16
	3.5	8.7	(3.09)	16	8.5	(2.76)	2	7.3	(0.87)	3
	2.0	7.6	(2.47)	11	7.6	(1.44)	16	9.8	(6.17)	12
Fog	5.4	7.8	(3.70)	16	7.8	(2.66)	12	8.1	(1.91)	12
	3.5	9.1	(2.78)	8	7.3	(1.68)	8	7.8	(2.39)	15
	2.0	7.3	(2.66)	16	8.3	(3.75)	16	8.7	(3.79)	11

level (F-ratio 4.37); pH 2 and fog deposition (P=0.00, F-ratio 9.425); pH 2 and the Flathead seed source, (P=0.038, F-ratio 2.2); and the twice ambient metal level and fog deposition (P=0.01, F-ratio 4.72). The three way interaction between pH, metal and deposition, were significant (P=0.00, F-ratio 6.339) at pH 2, the twice ambient heavy metal level and the fog deposition.

There was a significant difference in mean needle damage rating of old foliage associated with pH and heavy metal levels. Mean needle damage ratings for old foliage were significantly different (P=0.46) for pH (F-ratio 67.01), and heavy metal concentrations (P=0.00, F-ratio 15.33) also at pH 2 and twice the ambient heavy metal level. Two-way interactions proved significant (P=0.00) for pH 2 and the twice ambient heavy metal level (F-ratio 4.25); pH 2 and the fog deposition (P=0.00, F-ratio 9.7); pH 2 and the Flathead seed source (P=0.012, F-ratio 2.8); and the twice ambient metal level and fog deposition (P=0.01, F-ratio 5.2). The three-way interaction was significant (P=0.00) for pH, metal and deposition (F-ratio 6.43) at pH 2, the twice ambient heavy metal level and fog deposition.

Overall, the fog treatment had more needle damage both to the old and new foliage than the immersion treatment (Fig. 3.3 C). However, on a scale of 0 to 10, none of the damage ratings exceeded ~2.2.

Several foliar damage patterns were observed. Needle necrosis and cuticle damage increased for the fog deposition with increasing heavy metal concentrations and decreasing pH. Needle abscission on the older needles increased with more acidic pH and increasing metal concentrations in the immersion treatment. Cuticle damage predominated in the immersion treatment at the more caustic pH levels. Needle tip necrosis and necrotic banding are conifer foliar symptoms exposed to sulfate (Skelly *et al.* 1987). Premature needle loss has been documented with Engelmann spruce seedlings treated with sulfate fog (Leininger *et al.* 1991).

Root and Shoot Dry Biomass

Mean root dry biomass was not significantly (P≥0.05) different due to any main effect. However, mean shoot dry biomass was significant (P=0.00) at pH 3.5 from the remaining pH levels (F-ratio 6.93); at the ambient metal level from the remaining metal levels (P=0.033, F-ratio 3.89); and fog deposition (P=0.030, F-ratio 5.23). At pH 3.5 and ambient metal level in the fog treatment, mean shoot dry biomass increased 233% (2.1g to 4.9g) from the pH 5.4 and ambient metal level using the full data set means and 316% (3.1g to 9.8g) using the truncated data set means (Table 3.6). Similarly, in the immersion treatment shoot mean dry biomass increased from pH 5.4 to 3.5 at all metal levels. An increase of 157% (2.9g to 4.6g) using the full data set means is noted between pH 5.4 and pH 3.5 at the ambient metal level.

Posttreatment Observations

The remaining seedlings both for the immersion and fog depositions (16 seedlings per treatment level) were monitored through the summer of 1991, two years after treatment. Seedlings exhibited increasing mortality for both depositions as time passed. Water was applied as needed but no fertilization was given. In the late spring of 1991, after bud break, all seedlings of the total immersion treatment died. Some seedlings of the fog treatment continued to live through the summer of 1991. In September a tally of remaining treatment seedlings was taken (Table 3.8). A chi-square test of homogeneity was done for the hypotheses:

H_0: The proportion of seedlings alive at the end of the two-year posttreatment monitoring would be equal for all pH and heavy metal concentration treatment combinations of the fog deposition.

H_1: The proportion of seedlings alive at the end of the two-year posttreatment monitoring would not be equal in all pH and heavy metal concentration treatment combinations of the fog deposition.

At a probability level of 0.05, and 8 degrees of freedom, the tabular chi-square (X^2) equals 15.5. Since the calculated X^2 equals 22.09, and is > X^2 (8,.05)=15.5, the H_0 is rejected and the H_1 is accepted. The conclusion is survivability varies between the pH and heavy metal concentration treatment combinations of the fog deposition. The number of seedlings alive in the fog treatment at the ambient and twice ambient heavy metal concentrations, regardless of pH, is evident.

Table 3.8. Two year posttreatment tally of fog deposition treated Engelmann spruce seedlings.

Treatment Combinations										
Metal	None			Ambient			2x Ambient			
pH	5.4	3.5	2.0	5.4	3.5	2.0	5.4	3.5	2.0	Subtotal
Alive	0	4	2	8	9	7	8	3	5	46
	(5.15)	(5.15)	(5.15)	(5.15)	(5.15)	(5.15)	(5.15)	(4.83)	(5.15)	
Dead	16	12	14	8	7	9	8	12	11	97
	(10.85)	(10.85)	(10.85)	(10.85)	(10.85)	(10.85)	(10.85)	(10.17)	(10.85)	
Stotal	16	16	16	16	16	16	16	15	16	143

H_0: $P_i = P_j$ $x^2_c = 22.09$ reject H_0 if $x^2_c > x^2(8, .05) = 15.5$

H_I: $P_i \neq P_j$ therefore reject H_0

Discussion

Several correlations between foliar damage, root and shoot length, and dry shoot biomass are evident. At pH 2, foliar damage for old and new needles was evident for the fog and immersion treatments. This damage was similar to the effects obtained in the preliminary trials, using the immersion treatment, at this pH. The effects were greater at pH 2 than at pH 3.5 or 5.4 for both treatments. The most significant effect was at pH 2, the twice ambient metal level and fog deposition. When a comparison was made between the two deposition types, fog showed greater needle damage. Damage at this pH is to be expected, and was proven in the preliminary work. Of greater interest was the 3.5 pH levels, since these are rather commonly recorded pH levels in the western United States at air quality and fog monitoring stations.

At the intermediate pH and heavy metal concentrations, the fog deposition exhibited greater cuticle damage and needle necrosis, while the immersion treatment yielded the greater amount of needles lost. The ability of the aerosol particle to sustain needle contact after treatment for longer periods of time may account for the effects noted in the fog treated needles. However, the fog particles do not totally cover the needle surface; the total immersion treatment results in complete needle surface coverage, thus possibly accounting for loss of needles in this treatment.

Needle necrosis and cuticle damage increased for the fog deposition in higher metal concentrations and less caustic pH levels, while needle abscission of the older needles increased with the more acidic pH and increasing metal concentrations in the immersion treatment. Cuticle damage was more apparent for the immersion treatment at the more caustic pH levels. Such damage in the immersion treatment is to be expected. However, the damage exhibited by the fog deposition raises questions concerning the interactions of pH and metal level. Does the presence of heavy metal toxic levels provide the additional stress factor for needle damage or is pH sufficient at this level. Turner *et al.* (1990) used a 2:1 nitrate-to-sulfate ratio in an atmospheric chemical solution reported for Henninger Flats, California. The research group treated with fog at a mean mass particle size of 33 microns. Acid fog induced visible foliar injury in western hemlock *(Tsuga hetorophylla)* at pH 2.1 and 3.1 and western red cedar *(Thuja plicata)* at pH 2.1. The necrosis was dark bronze in hemlock and light orange in redcedar. The injury was either a tip necrosis or proceeded to completely necrotic needles.

Both fog and immersion treatments had increased shoot length at pH 2 with increasing heavy metal concentrations, but there was not a corresponding increase in shoot dry biomass. Rather, mean shoot dry biomass decreased, particularly in the fog treatment (Table 3.6). The immersion treatment shoot length and shoot biomass means at pH 5.4 indicate a peaked growth at the ambient metal concentration and a decline in growth at the twice ambient metal concentration. The increase in dry biomass at the ambient metal concentration is also evident both in the immersion and fog dry biomass means at pH 3.5. A disparity is apparent in the fog treatment's mean shoot dry biomass increase at pH 3.5 and the ambient heavy metal concentration compared to the decline in mean shoot length at the same level.

Turner *et al.* (1990), while examining the effect of acidic fog on plant mineral nutrition, researched four coniferous species of the western United States other than spruce for the effects of acidic fog on growth characterisitics. The highest foliar and root tissue dry weights occurred with the pH 3.1 fog treatment. The root dry weight of western hemlock significantly decreased with the lower fog pH at 2.1 and 3.1.

The dry mass increase at pH 3.5 both for the fog and immersion treatments is even more significant when comparing root length decreases at pH 3.5, particularly for the immersion deposition. In the immersion treatment, a decrease in growth at pH 3.5 at the 0, 1x, and 2x from either the pH 5.4 or 2 is evident. However, with the fog deposition, an increase in mean root length with increasing pH at the 0 metal concentration occurred–237% between the 5.4 and 2.0 level; a corresponding mean length increase occurred at the 5.4 pH level with increasing heavy metal concentration–a 258% increase. Mean new root length fluctuations in response to both deposition occurred consistently at pH 3.5.

Engelmann spruce may be susceptible to foliar injury from acidic fog due to needle characteristics. The needles are square in cross section, much like a wooden match, and tightly grouped, thus aiding contact angle of the aerosol particle (Shriner and Johnston 1985; Haines *et al.* 1985). Needle shape and grouping may be another variable accounting for the variances observed in western conifer species exposed to acid fog.

Conclusions

The study was designed to observe types of damage caused by acidic fog and heavy metal concentrations to Engelmann spruce. Recorded averages of actual anthropogenic sources of contamination-heavy metals, nitrate and sulfate levels and background ion contamination, such as ammonia, were addressed within the context of an aerosol particle delivery.

The increase in mean shoot dry biomass at the ambient heavy metal concentration and pH 3.5 is considered the threshold level for both depositions and is attributed to a fertilization effect from the nitrate and heavy metal concentrations. The mean shoot dry biomass of the fog and immersion treatments at pH 3.5 and ambient metal show an elevated biomass gain compared to any other treatment mean in corresponding depositions. These dry biomass gains are associated with significant declines in root lengths at the same treatment concentrations and appear to be a clear pH and metal response. This is particularly true in the immersion treatment, with a clear decrease in root length at 0, 1x, and 2x metal concentrations.

Possible fertilization due to the nitrate and heavy metal concentrations is further suggested by the posttreatment seedling tally (Table 3.8). No immersion seedlings survived past the second posttreatment bud break. Such lack of survival indicates a response to the type of deposition treatment. In addition, response of the surviving seedlings to the fog deposition were at the ambient and twice ambient heavy metal concentrations. The 2.5 nitrate-to-sulfate ratio remained constant throughout the 0, ambient, and twice ambient heavy metal concentrations.

This conclusion of a fertilization response becomes more evident by noting root and shoot mean length response in the fog deposition group. In both responses, length increases were observed with increasing acidity at the 0 heavy metal concentration and with increasing heavy metal concentrations at pH 5.4. This suggests a response to the available nitrate concentrations. Declining root growth is a classic heavy metal response. Between pH 2 and pH 5.4 at the ambient heavy metal concentration, mean root length declined 26% for the immersion treatment. Similarily, it declined 32% at the twice ambient heavy metal concentrations between pH 3.5 and pH 5.4 for the immersion treatment. A decrease in mean root length at pH 3.5 between the ambient and twice ambient heavy metal concentrations in the fog treatment group was also evident.

The immersion treatment was designed to serve as a "worst case" comparison to the fog treatment. The two-year posttreatment death response of the immersion treatment group suggests these seedlings sustained more damage than those receiving the fog treatment; such a conclusion is further supported by root and shoot length responses taken immediately after the treatments. Contrarily, given the combination of wet and dry deposition believed available for needle surface interaction via fog aerosol particle delivery, an argument can be made that the fertilization effect exhibited by the dry biomass responses was due to greater physiological damage to the needle surface. Such damage would possibly facilitate a greater uptake of nitrate, heavy metals, and other background ions present in the acidic fog treatment solution.

References

Alexander, R. R. and Shepperd, W.D. 1990. Engelmann spruce in Silvics of North America. Vol. 1. Agriculture Handbook 654. Forest Service. United States Department of Agriculture. Washington, D.C.

Arno, S.F. and Hammerly, R.P. 1984. Timberline, Mountain and Arctic Forest Frontiers. Seattle: The Mountaineers.

Ashenden, T.W., Rafarel, C.R. and Bell, S.A. 1991. Exposure of two upland plant species to acidic fogs. Environ. Pollut. 74:217 - 225.

Chapin, F.S. III, Schulze, E.D., and Mooneu, H.A. 1990. The ecology and economics of storage in plants. Annu. Rev. Ecol. Syst. 21:423 - 427.

Evans, L.S. 1986. Proposed mechanisms of initial injury-causing apical dieback in red spruce at high elevation in eastern North America. Can. J. for. Res. F. 16:1113 - 1116.

Friedland, A.J., Johnson, A.H. and Siccama, T.G. 1984. Trace metal content of the forest floor in the Green Mountains, New Hampshire. Bull. Torrey Bot. Club 110:141 - 153.

Geballe, G.T., Smith, W.H., and Wargo, P.M. 1990. Red spruce seedling health: an assessment of acid fog deposition and heavy metal soil contamination as interactive stress factors. Can. J. For. Res.20:1680–1683.

Godbold, D.L., and Huttermann, A. 1985. Effect of zinc, cadmium and mercury on root elongation of *Picea abies* (Karst.) seedlings, and the significance of these metals to forest die-back. Environ. Pollut. (Series A) 38:375 - 381.

Haines, B.L., Jernstedt, J.A. and Newfeld, H.S. 1985. Direct foliar effects of simulated acid rain. pt. II. Leaf surface characteristics. New Phytol. 99:407 - 416.

Jacobson, J.S., Heller, L.I., Yamada, K.E., Osmeloski, J.F., Bethard, T., and Flassoie, J.P. 1990. Foliar injury and growth response of red spruce to sulfate and nitrate acidic mist. Can. J. For. Res. 20:58 - 65.

Joslin, J.D., McDuffie, C. and Brewer, P.F. 1988. Acidic cloud water and cation loss from red spruce foliage. Water Air and Soil Pollut. 39:355 - 363.

Klein, R.M., and Perkins, T.D. 1988. Primary and secondary causes and consequences of contemporary forest decline. Botanical Review. 54:1:2 - 43.

Larsen, J.B., Qian, X.M., Scholz, F., and Wagner, W. 1988. Ecophysiological reactions of different provenances of European silver fir (*Abies alba* Mill.) to SO_2 exposure during winter. Eur. J. For. Path. 18:44–50.

Leininger, T.D., Miller, P.R., Schillings, S.L., and Dunn, P.H. 1991. Seedling responses of five species of Western conifers to simulated ambient sulfur dioxide exposures. Forest Science. 37:6:1538 - 1549.

Marschner, H. 1986. Mineral Nutrition of Plants. London. Academic Press.

Masuch, G., Kettrup, A., Mallant, R.K.A.M., and Slanina, J. 1986. Effects of H_2O_2 containing acidic fog on young trees. Intern. J. Environ. Anal. Chem. 27:183 - 213.

Mayer, R. 1983. Interaction of forest canopies with atmospheric constituents: aluminum and heavy metals. pp. 47-56. In: (B. Ulrich and J. Pankrath, eds.) Accumulating air pollutants in forest ecosystems. Dortrecht: DeReidel.

Mengel, K., Hogrebe, A.M.R. and Esch, A. 1989. Effect of acidic fog on needle surface and water relations of Picea abies . Physiol. Plant.75: 201 - 207.

Munger, J.W., Jacob, D.J. Waldman, J.M. and Hoffman, M.R. 1983. Fogwater chemistry in an urban atmosphere. Journal of Geophysical Research. 88:5109 - 5121.

Musselman, R.C. 1988. Acid neutralizing capacity of leaves exposed to acidic fog. Environ. Exper. Bot. 28:1:27-32.

Musselman, R.C., and Sterrett, J.L. 1988. Sensitivity of plants to acidic fog. J. Environ. Qual.17:329.

Percy, K.E., Krause, C.R. and Jensen, K.F. 1990. Effects of ozone and acidic fog on red spruce needle epicuticular wax ultrastructure. Can. J. For. Res. 20:117 - 120.

Raloff, J. 1989. Where Acids Reign. Science News.136:56-58.

Schlegel, H., Godbold, D.L. and Huttermann, A.1987. Whole plant aspects of heavy metal induced changes in CO_2 uptake and water relations of spruce (Picea abies) seedlings. Physiologia Plantarium.69:265-70.

Shriner, D.S. and Johnston, J.W., Jr. 1985. Acid rain interactions with leaf surfaces: a review. pp. 77-85. In: (D.D. Adams and W. Page, eds.) Acid Deposition. New York: Plenum.

Schulze, E.D. 1989. Air pollution and forest decline in a spruce (Picea abies) forest. Science.244:776-783.

Seiler, J.R. and Pagnelli, D.J. 1987. Photosynthesis and growth response of red spruce and loblolly pine to soil-applied lead and simulated acid rain. Forest Science. 33:3:668-675.

Skelly, J.M., *et al.* (eds.) 1987. Diagnosing injury to Eastern forest trees. Agricultural Information Services, The Pennsylvania state University and USDA Forest Service, 122p.

Turner, D.P., Tingey, D.T. and Hogsett, W.E. 1990. Air Pollution and Forest Decline. p. 125-129. In: (J.B. Bucher and I. Bucher-Wallin, eds.). Proc. 14th Int. Meeting for Specialists in Air Pollution Effects on Forest Ecosystems, IUFRO P2.05, Interlaken, Switzerland, Oct. 2-8, 1988. Birmensdorf, 1989.

Waldman, J.M., Munger, J.W., Jacob, D.J., Flagan, R.C. and Morgan, J.J. 1982. Chemical composition of acid fog. Science.218:677-680.

Chapter IV

HEAVY METAL FOLIAR UPTAKE BY ENGELMANN SPRUCE FROM ACIDIC FOG

Introduction

Acid deposition may alter the buffering processes of forest soils leading to increased soil acidity. Nitrogen and sulfates initiate the chemical imbalance of the soil. Several studies (Schlegel *et al.* 1992; Joslin and Wolfe 1992; Raynal *et al.* 1990) suggest this is followed by ionic toxicity of aluminum, resulting in fine-root damage. As acidity increases, aluminum and heavy metals are freed from soil exchange sites in large quantities, forming a toxic situation for root growth. Additionally, aluminum competes with calcium and magnesium for absorption by the roots. Both of the latter elements are crucial for normal conifer development. Ammonium levels present in documented atmospheric pollution (Chapter III, Gilliland 1997) further impact calcium and magnesium root absorption. Norway and red spruce roots preferentially absorb ammonium. Soil acidification below a pH 5 will cause death of microbes normally converting ammonium to nitrate in the soil.

Addition of heavy metals, such as copper, nickel, lead, and manganese, through atmospheric soil deposition compounds the effect. Soil deposition of manganese does not injure the roots directly but affects shoot development. Lead can result in a wide range of plant toxicity symptoms: root elongation is inhibited, roots form adventitiously, and stem elongation and leaf expansion are inhibited. Copper is associated with enzymes involved in oxidation-reduction in plants. Toxicity results in chlorosis and stunting with iron deficiency corresponding. Root system reduction appears first and implies faulty nutrient absorption and inhibition of growth. Nickel is readily absorbed. As a divalent cation, nickel competes with other cations, including calcium, magnesium, iron, and zinc, in root uptake mechanisms, leading to characteristic symptoms of chlorosis. Generally, inhibition of root elongation is the most sensitive parameter of heavy metal toxicity (Marschner 1986).

Compounding the acidifying soil effects is a leaching of nutrients from damaged needles. Nutrient deficiency normally stunts growth in conifers. However, with the current atmospheric deposition rates of nitrogen (as reflected in the current research project's nitrogen-to-sulfate ratio of 2.5:1) on foliage and soil, a fertilization effect takes place, spurring nutrient deficient conifers to attempt increased growth. A highly stressed state results.

Gaps exist in the current knowledge of foliar reactions to pollution levels, particularly heavy metals. The possibility of foliar absorption of heavy metals in *Ulmus* (elm), *Crataegus* (hawthorn), *Salix* (willow), and *Quercus* (oak) with heavy contamination using zinc, lead, and cadmium has been demonstrated (Little 1973). Cadmium reduces water uptake, photosynthesis, and transpiration, and increases dark respiration in maple (Lamoreaux and Chaney 1977). Sugarcane's leaf callus absorbs zinc and copper (Kannan 1986). Leaves accumulating heavy metals become inefficient due to waste accumulation and must therefore be replaced (Chapin *et al.* 1990).

Spruce in high-elevation areas exposed to acidic cloud deposition demonstrate susceptibility to pollution levels. However, absorbtion of heavy metals via spruce needle uptake is unknown, as are the physiological consequences of such absorption.

Objectives

The objectives of the study in relation to heavy metal uptake were twofold:

1. To determine if foliar absorption of heavy metals, in particular lead, copper, nickel, and manganese, was possible in Engelmann spruce, and
2. To determine the effects of heavy metal uptake on Engelmann spruce root and shoot growth alone, and in conjunction with varying acidity levels.

Hypotheses

H_0: Foliar absorption of heavy metals lead, copper, nickel, and manganese via an acidic fog treatment will not occur, and root and shoot growth will not be directly affected by heavy metal uptake either alone or in conjunction with varying acidity levels.

H_1: Foliar absorption of heavy metals lead, copper, nickel, and manganese via an acidic fog treatment will occur and affect root and shoot growth, both directly and in conjunction with varying acidity levels.

Methods and Materials

Methodology Selection

When selecting methodology for trace metal analysis the following factors were considered: (1) instrument detection limits, (2) concentration ranges of samples and compatibility with instrument, (3) spike recovery data (see Precision and Accuracy section), (4) precision and bias, (5) machine interferences from chemicals used in sample preparation, (6) instrument availability, (7) single and multi-element analytical techniques, and (8) sample preparation procedures. Graphite furnace atomic absorption spectrophotometry (GFAA) and inductively coupled plasma emission spectroscopy (ICP) are the two most frequently used techniques for precipitation chemistry and trace metal analysis. Since data is available showing comparable recovery for trace metal analysis between the GFAA and ICP, the ICP instrument was chosen because of its capability to simultaneously determine all metal levels (Keller *et al.* 1988).

Quality Assurance/Quality Control

General Laboratory Practice

Prior to biological preparation of needle material, all chemical glassware was soaked for 24 hours in a 50% reagent grade nitric acid soak followed by a 24-hour 50% reagent grade hydrochloric acid wash. Glassware was then soaked 48 hours in sterile water obtained from a water filtration system under the trade name Milli Q (manufactured by MilliPore Corporation, Bedford, Massachusetts [1-800-645-5476]). All water used in the following biological preparation was from a Milli Q system. Analytical grade hydrochloric acid was used in background solutions.

Plant Digestion Technique

Needle material was obtained from conifer foliage treated with an acidic heavy metal solution (Table 3.1). Plant stems, including foliage, were rinsed for 10 seconds in three separate washes of 10% HCL, were air dried, and then were placed in drying ovens at 112°C for 72 hours. The rinsing was done to remove any surface contamination from the treatment applications. Conifer needles were ground using a Wiley Mill equipped with a stainless steel blade. Care was taken to clean all parts of the machine coming in contact with needle material between each grinding of each tree needle treatment group. Approximately 0.5g of dry needle material was placed into 15ml porcelain crucibles and ashed at 500°C for 2.5 hours. A drop of water was used to settle the warm ash, and 10ml of 6 N HCL was added to each crucible and vaporized in a sand bath for 30 minutes, allowing 50% of the solution to remain. Contents of the crucible were then poured into a 50ml volumetric flask and diluted to full volume with 10% reagent grade HCL. Blanks, duplicates, spiked samples, and standard reference materials were included in analyses using the digestion technique for the ICP analyses.

Standard Reference Materials (SRM)

National Bureau of Standards (NBS) SRM 1575 Pine Needles were included, plus blanks, with each digestion process. Two standards and two blanks were included with every ash process. The original ashing furnace held 16 samples, of which two were standards and two were blanks. The blank was an empty crucible that was ashed as though it contained needle material; the 10ml of 6N HCL were added after removal from the furnace and then treated as a sample in all respects. The blank served as an indicator of contamination during preparation or analyses and as a calibration check during ICP processing. As more quality controls were added (see Precision and Accuracy) a larger ash furnace was used, and standards were 10% of the total samples for each furnace capacity.

Precision

Two sample duplicates were included for each ash process to obtain an estimate of precision for element analysis. During the ICP analysis, after every 10 samples analyzed, four were randomly picked and rerun to obtain an estimate of precision for that particular series of sample analyses and also to check the precision of the machine over time.

Accuracy

Accuracy for element analysis was determined using spiked samples. This is a standard accuracy check. The difference in concentration between the spiked and the unspiked sample is used to calculate a method percent recovery. An additional 10% of standards were prepared with a 50% spike of each heavy metal analyzed. Half were ashed and half were not. The spike was conducted with instrument standards for manganese, lead, copper, and nickel prepared 50% in excess of the NBS 1575 metal levels. An Eppendorf automatic pipet was used for delivery. Such a standard group gives an industry-accepted indication of any material lost due to ashing procedure and errors in preparation.

Instrumentation

Heavy metal uptake analyses were performed on a Jarrell-Ash Atom Comp 800 simultaneous ICP (Inductively Coupled Argon Plasma Emission Spectrometer) equipped with *Thermospec* hardware and software. Instrument calibrations included internal element corrections and interference and background corrections for each element analyzed.

Agreement with Standards

Initial runs of samples with standards during the fall of 1989 indicated a lack of agreement with our standard analysis data and NBS published standard data for #1575 pine needles. Thus results were unacceptable. After consulting with Dr. Jerry Bromenshenk, Department of Biology at the University of Montana and author of EPA methodology for heavy metal uptake by bees, a sample preparation problem seemed to be at fault.

Use of reagent grade acids, inadequate glass preparation, use of distilled water, inadequate number of standards, and standard methodology were addressed. Chemical glassware and nalgene containers used in sample preparation were soaked for 24 hours in a 50% reagent grade nitric acid distilled water bath followed by a 24-hour 50% reagent grade hydrochloric acid distilled water bath. The glassware was then rinsed for 48 hours in Milli Q water. Analytical grade hydrochloric acid and Milli Q water were used in chemical preparation of samples. New porcelain crucibles were employed in the ash portion of the sample preparation. In addition, standard samples were increased to include metal standards spiked with reference solutions for copper, nickel, lead, and manganese prior to ashing and a set of nonspiked standards; both the spiked and nonspiked group numbers were equal to 10% of the samples to be analyzed.

Further standard analyses indicated agreement with NBS #1575 except for magnesium. Samples were sent to the State Water Quality Laboratory at Helena and were run with a comparable preparation system and ICP. Their results for magnesium agreed with our results; therefore, I judged the laboratory chemical preparation was under control.

Results and Discussion

The levels of heavy metals manganese, lead, copper, and nickel uptake according to treatment level are given in Table 4.1. This was the final ICP analyses needed to double-check SRM precision and accuracy prior to a full analysis with complete treatment groups; not all quality control values are given, nor the complete breakdown by treatment groups. Rather, the table is to be used as an indication of the possibility of cation uptake.

In April 1992, during the analyses presented in Table 4.1, a fire broke out in the duct work above the ICP, terminating the analyses. In June 1992, I was in an auto accident. Due to these events and the fact that prepared sample viability is only six months and no further needle material was available, the final analyses were not completed.

From Table 4.1, an uptake of cations for needles receiving the fog treatment is noticeable in the ambient and twice-ambient metal levels at pH 3.5 and 5.4. The exception is manganese, which may not be part of the heavy metal uptake process, or part of a translocation process. At pH 2 and twice-ambient metal levels, a clear loss of heavy metals is apparent for needles receiving the fog treatment. This may be part of the foliar damage symptomology; a general leaching not only of nutrients, but also any metals present. Another possibility is translocation to the stem or root tissue. The roots were not analyzed for metal uptake. There would appear to be a correlation between the amount of heavy metal uptake by the needles receiving the fog treatment at the 5.4 and 3.5 pH levels and the high stem mass levels noted in Chapter 3, particularly at the pH 3.5.

Table 4.1 Foliar cation uptake by Engelmann spruce needles exposed to various treatment combinations of deposition type, acidity, and heavy metal concentrations. Results are preliminary and are based on 8 full ICP runs between 10/89 and 12/91.

Cation	Concentration (ug/g)	Mn	Pb	Cu	Ni
NBS standard 1575 published value		675 (±15)	10.8 (± .5)	3.0 (± .3)	3.5
NBS standard 1575 ICP values		660	11.7	2.6	3.4
Normal Plant Ion Content				3-30	20-30
pH	Metal concentration				
Immersion					
3.5	ambient	337	12.1	4.0	2.0
2.0	twice ambient	101	7.0	6.7	2.4
Fog					
7.0	no metals	631	12.1	4.1	5.8
5.4	twice ambient	437	20.3	15.5	2.7
3.5	twice ambient	502	26.4	8.5	2.5
2.0	twice ambient	141	5.7	1.9	1.3
5.4	ambient	426	19.6	9.3	5.6
3.5	ambient	104	13.0	21.0	26.2
2.0	ambient	337	12.1	4.0	2.0

Recommendations

The study needed to be more focused. As the proposal was originally written, only one age-class of newly germinating seedlings was to be treated. The 2/0 seedlings were added as a complete repetition of the treatment combinations to be applied to the germinating seedlings. The doubling of the workload with another age-class addition was an important factor during the processing of the 2/0 seedlings after the treatments were terminated in the fall of 1989. The three phases-processing 2/0 seedlings, germinating and transplanting seeds for the Conviron, and the initial ICP analyses-were conducted simultaneously. The author, because of demands on her time during this period due to growth problems in the Conviron and methodology problems with the ICP analyses, was not able to adequately monitor data sampling of the 2/0 seedlings, resulting in some data loss. This took place despite checklists being provided for workstudy help showing work completed.

Finishing all three phases in fall 1989 after the summer of 2/0 applications would have been complicated enough. However, problems were encountered with growing germinated seedlings in the Conviron. Test trials were conducted in growth chambers without the strong air circulation present in the Conviron. Five repeated transplants were carried out to overcome numerous problems with germination and transplanting in the large growth chamber. Further transplant trials could not be conducted because of competition for use of the Conviron.

In addition, supervision of the work-study students was not complete due to the compounding of problems in the fall demanding more of my time. This happened even though I had prepared checksheets for task completion to be filled out by the work-study students. Seedling sampling that appeared to be complete, was not. This was the major factor responsible for the unequal subclass numbers in subsequent ANOVAS presented in Chapter III. Thus either an experiment using 2/0 seedlings should have been attempted or one using newly germinated seedlings, but not both.

While the toxicology approach and the determination of useful treatment combinations were necessary, employing the immersion technique was not necessary. The aerosol particle as a unique entity and pollutant phenomena has been addressed by researchers interested in cloud and fog pollutant impact. It is my opinion that the aerosol particle research was adequate as a study. Verification of results did not require a comparison study. Thus the immersion portion of the study was not necessary. By eliminating the immersion portion of the study, the sample size could have been redistributed so that more sample material would have been available for the ICP analyses.

Needle dry biomass would have been the only posttreatment measurement. It would have been used in conjunction with heavy metal analyses. More emphasis should have been placed on foliar uptake of the heavy metals and other cations. ICP analyses could have been performed on the roots as well. Thus the study would have addressed (1) cation uptake by foliage of heavy metals, (2) foliar uptake of nitrogen, sulfate, and ammonium, (3) or emphasis placed on calcium and magnesium content (current literature contains aluminum, calcium, and magnesium ratios). However, a more accessible and reliable ICP would have been required, as well as access to better laboratory preparation facilities.

The ICP portion of the study should be regarded with a positive attitude. If the analyses had been sent to an outside laboratory, or if the analyses had been without problems, I would have been shortchanged a learning experience. I have a working knowledge of the ICP machine and software. I am able to troubleshoot sample preparation methodology problems, critique and implement quality assurance and quality control procedures acccording to Environmental Protection Agency standards. I can interact on a professional basis with published professionals using a sophisticated instrument analyzing organic or inorganic material.

References

Chapin , F.S. III, Schulze, E.D. and Mooneu, H.A. 1990. The ecology and economics of storage in plants. Annu. Rev. Ecol. Syst. 21:423-47.

Joslin, J.D. and Wolfe, M.H. 1992. Red spruce soil solution chemistry and root distribution across a cloud water deposition gradient. Can J. For. Res. 22:893-904.

Kannan, S. 1986. Foliar absorption and transport of inorganic nutrients. CRC Critical Reviews in Plant Sciences. 4:4:341-375.

Keller, B.J., Peden, M.E. and Skowron, L.M. 1988. Development of standard methods for the collection and analysis of precipitation: trace metals. Illinois State Water Survey, Analytical Chemistry Unit, Champaign, Illinois. United States Environmental Protection Agency, Sponsor.

Lamoreaux, R.J. and Chaney, W.R. 1977. Sulfur dioxide and plant response: an overview (Pollution injury). Biologist-Denver. 59:2:61-69.

Little, P. 1973. A study of heavy metal contamination of leaf surfaces. Environ. Pollut. 5:159-172.

Marschner, H. 1986. Mineral Nutrition of Higher Plants. Academic Press. London.

Raynal, D.J., Joslin, J.D., Thornton, F.C.1990. Sensitivity of tree species to Al: III. Red spruce and loblolly pine. J. Environ. Qual. 19: 180-187.

Schlegel, H., Amundson, R.G. and Huttermann, A. 1992. Element distribution in red spruce (*Picea rubens*) fine roots: evidence for aluminium toxicity at White Face Mountain. Can J. For. Res. 22:1132-1138.

Chapter V

A Perspective

In the late 1960s and 1970s, Scandinavian scientists recognized acid rain's regional influence and began a disciplined study of its effects. Concurrently, German scientists were studying acidic pollution effects in the Bavarian Fichtelgebirge Forest situated about 25km from the Czech border.

Ecologists Gene Likens and colleagues at Cornell University, after visits to Sweden (Likens *et al.* 1972; Cogbill and Likens, 1974; Likens and Bormann, 1974), identified regional acidic precipitation changes between 1950 and the 1970s in the United States and discussed terrestrial and aquatic ecosystem impacts. The reports were the basis for development of a new area of study in science (Zehr 1994).

Further opportunities for research were aided with the First International Symposium on Acid Precipitation and the Forest Ecosystem in 1975. At this symposium a precipitation monitoring network in the United States was proposed with coordinated programs of research on the effects of airborne chemicals (1978). The Acid Rain Network planned project was called the National Atmospheric Deposition Program (NADP) (Galloway and Cowling 1978).

In implementing the network, a standardized protocol for collection of precipitation was developed. Separate protocols for both wet and dry deposition were researched. However, agreements on procedures for collection of dry deposition proved controversial and research was discontinued.

The analytical laboratory at the Illinois Water Survey at Champaign was the central laboratory for sample analyses. This laboratory was selected because of the quality of the facility; it was a research organization already examining the chemistry of rain; the scientific members of the NADP trusted the laboratory's methodology and science; and the Department of Energy funded research at the lab. Thus, a strong tie to an important research funding agency was made by members of the NADP, facilitating research money to projects of the NADP. The network started with 18 stations and grew to more than 200 by 1987 (Zehr 1994). The acid rain collection protocol was published first by the Department of Energy and then by the Environmental Protection Agency. The protocol manual was important for two reasons: (1) it was a strong model for conducting precipitation chemistry analyses and almost a procedural requirement, if doing research in this area (these procedures were used in the present study); and,

(2) the protocol outlined also served to discourage criticism of data analyses procedures.

The NADP was aided in their efforts by the U.S. Department of Agriculture, the U.S. Forest Service, the U.S. Geological Survey, and various State Agriculture Experiment Stations. Such affiliations strengthened ties to possible research grants from such agencies but also helped to support a monitoring network linking researchers from a variety of disciplines.

To join the network, scientists needed to raise $4,000-$5,000 per monitoring site to purchase equipment and pay for laboratory analyses. Once a member, the scientists had access to data collected by fellow members. There was an interdependence and yet an independence because each researcher pursued individual research goals.

Moreover, the NADP increased the political and public impact of acid rain as a research problem. An interesting crossover took place in the scientific research community as NADP members sought support from bureaucrats, legislators, and the public. The scientists raised public awareness of acid rain and posed the solution as scientific research (Zehr 1994).

So successful was the group that the Council of Environmental Quality under President Carter in 1977 asked NADP administrators to develop a national acid rain research program. The National Acid Precipitation Assessment Program (NAPAP) was formally created in 1981 as a direct result of the work of the NADP. Funding for a 10-year research program on acid rain was in place. Furthermore, the Secretary of Agriculture, the Administrator of the Environmental Protection Agency, and the Administrator of the National Oceanic and Atmospheric Administration served as joint chairmen (NAPAP 1982). The outstanding feature of the program was the success of the NADP scientists in designing the federal program so that diverse and long-term research goals could be conducted and funded without allowing legislators and administrators to supervise the research or influence its outcome. The benefit of the NAPAP to the federal legislators and administrators was the appearance that the government was taking action on the problem (Zehr 1994).

During the 1980s, acid rain research was a predominant and publicized problem both in lay and scientific literature. NAPAP funding began at $11 million in 1980, increased to more than $80 million in the late 1980s, and to $560 million in the 10-year period. EPA administered about 60% of the money, while the remaining 40% funded university scientists' research. These scientists had published 1,000 papers by 1987 covering various aspects of acid rain's impact (NAPAP, 1987).

By the mid-1980s, NAPAP was expanded to include research on other pollutants, such as ozone. A great deal of atmospheric research would not have been conducted without the NAPAP.

My research problem, developed in 1986, was a direct result of interest shown by foresters and atmospheric research scientists in the impact of fog (or aerosol particles) on the acid rain problem. The research targeted areas with unanswered questions: (1) the relevance of the aerosol (fog) particle as an acidic deposition pollutant consideration; (2) the ability of the needle to sustain damage, thus addressing the question of foliar leaching and adsorption of heavy metals.

My research might better have been served with more emphasis on how the heavy metals might possibly interfere with key nutrient uptake processes. Rather than strictly focusing on the heavy metals, a broader perspective addressing foliar leaching of key nutrients might have been advisable.

Such a tact was taken by Turner and Tingey (1990). Douglas fir *(Pseudotsuga menziesii)* seedlings were exposed to twice weekly fogs over a 2-week period. Rates of foliar leaching and root uptake of calcium, magnesium, and potassium were determined. At the end of the treatment, seedlings were harvested for comparisons of dry weights and tissue nutrient concentrations using ICP methodology.

Throughfall enrichment of calcium, magnesium, and potassium was considerably higher in a pH 3.1 fog than in a pH 5.6 fog under similar nutrient regimes where nitrogen-to-sulfate ratios were 2:1. Increasing foliar biomass was noted at this pH. The greatest nutrient enrichment was for potassium; the ratio of potassium to calcium removed was greater at the 3.1 pH.

Root uptake rates were greatest for potassium, followed by calcium and magnesium. No effect of visible foliar injury was noted at pH 3.1. SEM analyses indicated no damage to epicuticular wax crystals.

Fog pH treatments at 3.1 and 5.6 proved not to be statistically significant; however, over the experimental time frame, growth and foliar nutrient concentrations responded to nutrient availability and not to pH. The seedlings were grown in a nutrient solution, and any leaching losses were compensated by nutrient uptake. (Note the similarity in biomass increase in both the Turner and Tingey [1990] work [pH 3.1] and present study at pH 3.5).

Conclusions reached in prior studies are not in agreement with the results of Turner and Tingey (1990) and my dissertation work. Mean shoot dry biomass production of soil-grown Douglas-fir seedlings in exposures to acid fog (Turner *et al.* 1989) or acid mist (McColl and Johnson 1983; McColl and Firestone 1987) was not detrimental in effect at pH ranges from 3.0 to 5.6.

Such research results further amplify this study's focus. Using a true fog particle, under approximately the same nitrogen-to-sulfate ratio of 2.5:1, but with the variance in heavy metal concentrations, mean shoot dry biomass increase was significant at pH 3.5. At pH 3.5 and ambient metal level in the fog treatment, mean shoot dry biomass increased 233% (2.1g to 4.9g) from the pH 5.4 and ambient metal level using the full data set means and 316% (3.1g to 9.8g) using the truncated data set means (Table 3.6). For the immersion treatment, mean shoot dry biomass increased at the pH 3.5 at all metal concentrations. When comparing Turner and Tingey's (1990) study to the present study, the question of heavy metals' role becomes more focused. Both studies show an increase in mean shoot biomass production at the pH 3.1 or 3.5. For the fog treatment, the ambient metal level showed the greatest increase. For the immersion treatment, biomass increase was non-metal concentration specific.

Further emphasis on the importance of nutrients and acid deposition effects are noted in two key papers highlighting soil effects since 1990. Schulze (1989) has argued that acid deposition containing nitrogen stimulates trees to shunt abnormal amounts of energy into growth. Studying in the Fichtelgebirge Forest of Germany, he believes these trees are suffering from pollution-caused chemical imbalances, weakening the trees to a state whereby insects, fungal attacks, and weather extremes become threats that a healthy tree could accommodate. Schulze *et al.* (1994) and Durka *et al.* (1994) noted a nitrogen saturation effect in these forests in the early 1990s. Atmospheric nitrate is passing through the forests without further adsorption by trees or microbes. In polluted stands of Norway spruce, nitrogen has affected forest vitality nutritionally, microbally, and hydrologically.

Schulze (1989) used ratios of heavy nitrate coming from air pollution to light nitrate coming from soil microbes. Nitrate ratios were measured in spring runoff. In the slightly declining stands, 23 to 30% of the nitrate in runoff came from air pollution. In two dying stands, 60 to 100% of the nitrate came from air pollution.

The effect on soil nutrition is evident. Being negatively charged, nitrate moves through the soil attracting the cations calcium and magnesium. Over a period of time, a forest can be transformed from a system readily absorbing nitrogen to a system satiated by nitrogen but starved for alkaline (base) cations, thus becoming more acidic. Acidity impairs plant roots, affects microbes using and storing nitrogen, and aides the movement of aluminum, toxic to plants and aquatic life if leached to streams. If a forest is not utilizing nitrogen efficiently, productivity drops. This has ramifications to carbon models and carbon storage.

In a 1996 paper, Likens *et al.*, using 30 years of data from the Hubbard Brooks Experimental Forest, demonstrated that acid rain has been leaching the soil of base minerals essential to neutralize acid. Given the rate at which calcium and magnesium are being depleted, recovery of the forests could take decades.

Vegetation growth has dramatically slowed in the U.S. Forest Service's Hubbard Brooks Experimental Forest, New Hampshire, since 1987. Calcium and magnesium levels have been dropping since 1970. The calcium pool, in itself, has dropped more than 50% in the past 45 years. Calcium to aluminum ratios for soil water from high-elevation stands are in a range affecting forest productivity.

Several implications are present in these papers. The depletion of calcium in the soils will not be readily corrected, since present levels were reached through centuries of soil weathering. Likens *et al.* (1996) argue that even with major reductions in pollutants as a result of 1990 amendments to the Clean Air Act, forest ecosystem recovery will be slow due to the calcium depletion.

Forest ecosystems seem to be more susceptible to strong acids than was apparent in the 1970s and 1980s. The limited availability of relatively long-term data sets such as those of Hubbard Brooks Experimental Forest poses a problem in ascertaining forest ecosystems susceptibility. Additionally, a great deal of previous acid deposition research was based on short-term studies lasting months.

Also, the acidity problem was believed to be reversible. As a corrective measure in the 1980s, liming of soil seemed a means of raising soil pH. It has proved to be relatively expensive and with negative ramifications for wildlife.

The situation is further complicated by recent research in atmospheric aerosol particles. Aerosols, composed primarily of sulfates, would seem to increase atmospheric reflection of sunlight back into space before it reaches the earth's surface and adds to the global warming effect (Charlson and Wigley, 1994). Because of a large amount of data collected by climatologists in studies of acid rain, sulfates are the best-understood aerosol particle. This knowledge is particularly important to researchers quantifying sulfate aerosol effects at Stockholm University in Sweden and the University of Washington, Seattle.

Scientists in climatology and those studying soil effects of acid deposition agree on the importance of reducing sulfate and nitrogen loads. In 1995, the Environmental Protection Agency had not backed new regulatory approaches. This is due to the EPA's uncertainty regarding scientific research related to nitrogen deposition. The bulk of the acid deposition research in the 1980s focused on the effects of sulfur. The EPA has concluded that scientific uncertainty of nitrogen's role in the acidification of forested watersheds is too great to warrant regulatory action (Renner 1995). The EPA has requested more funding for watershed research.

References

Charlson, R.J. and Wigley, T.M.L. 1994. Sulfate aerosol and climatic change. Scientific American. 270:2:48.

Cogbill, C.V. and Likens, G.E. 1974. Acid precipitation in northeastern United States. Water Resources Research 10:6:1133-1137.

Durka, W., Schulze, E-D., Gebauer, G., and Voerkelius, S. 1994. Effects of forest decline on uptake and leaching of deposited nitrate determined from 15N and 180 measurements. Nature. 372:765-769.

Galloway, J.N. and Cowling, E.B. 1978. The effects of precipitation on aquatic and terrestrial ecosystems: A proposed precipitation chemistry network. Journal of the Air Pollution Control Association. 28:229-235.

Likens, G.E., Bormann, F.H. and Johnson, N.M. 1972. Acid rain. Environment. 14:2:33-40.

Likens, G.E., Bormann, F.H. and Johnson, N.M. 1974. Acid rain: A serious regional environmental problem. Science. 184:1176-79.

Likens, G.E., Driscoll, C.T. and Buso, D.C. 1996. Long-term effects of acid rain: response and recovery of a forest ecosystem. Science. 272:5259:244-247.

McColl, J.G. and Johnson, R. 1983. Effects of simulated acid rain on germination and early growth of Douglas fir and Ponderosa pine. Plant and Soil. 74:125.

McColl, J.G. and Firestone, M.K. 1987. Cumulative effects of simulated acid rain on soil, chemical and microbial characteristics and conifer seedling growth. Soil Sci. Soc. Am. J. 51:794.

National Acid Precipitation Assessment Program. 1982. Annual report. Washington, D.C: U.S. Government Printing Office.

National Acid Precipitation Assessment Program. 1987. Interim Assessment: The causes and effects of acidic deposition. Washington, D.C: U.S. Government Printing Office.

Renner, R. 1995. "Scientific uncertainty" scuttles new acid rain standard: EPA study recommends no new measures to protect " sensitive areas" from continued acid deposition. Environ Sci and Technol. 29:10:464A-467A.

1978. Acid rain network planned. Science News. 113:407.

Schulze, E-D., Kelliher, F.M., Koerner, C., Lloyd, J. and Leuning, R. 1994. Relationships among maximum stomatal conductance, ecosystem surface assimilation rate, and plant nitrogen nutrition: A global ecology scaling exercise. Fautin, D.G. (Ed.). Annual review of ecology and systematics. 25:629-660.

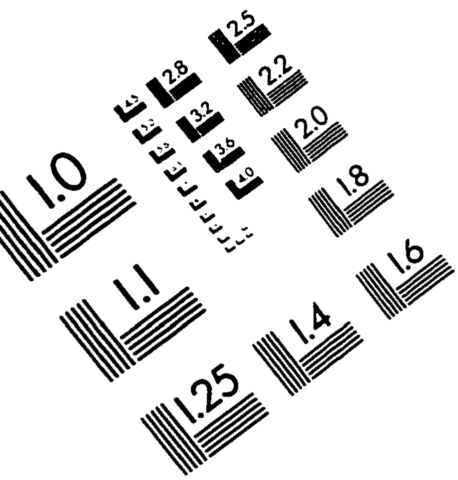
Schulze, E-D. 1989. Air pollution and forest decline in a spruce (*Picea abies*) forest. Science. 244:776-783.

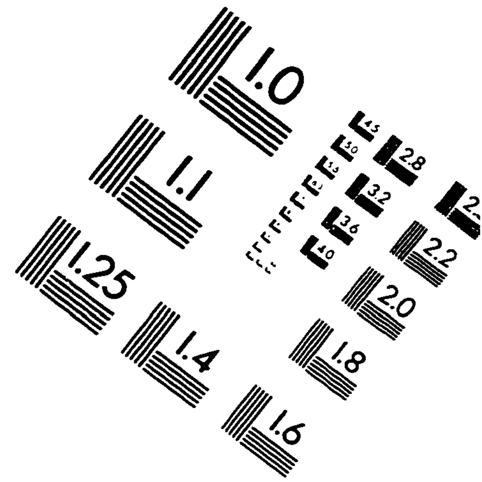
Turner, D.P., Tingey, D.T. and Hogsett, W.E. 1989. Acid fog effects on conifer seedlings. pp. 125-129. In: (J.B. Bucher and I. Bucher-Wallin, eds.) Proc. 14th int. meeting for specialists in air pollution effects on forest ecosystems, IUFRO. P2.05, Interlaken, Switzerland, Oct. 2-8, 1988. Birmensdorf.

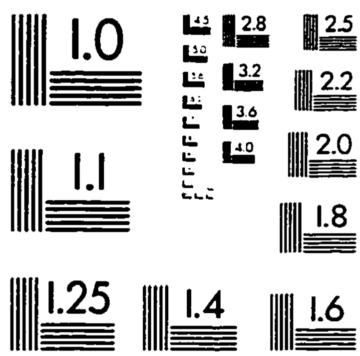
Turner, D.P., Tingey, D.T. 1990. Foliar leaching and root uptake of Ca, Mg and K in relation to acid fog effects on Douglas fir. Water, Air and Soil Pollution. 49:205-214.

Zehr, S.C. 1994. The centrality of scientists and the translation of interests in the U.S. acid rain controversy. The Canadian Review of Sociology and Anthropology. 31:3:325-354.

IMAGE EVALUATION TEST TARGET (QA-3)







150mm

6″