The Influences of Atmospheric Nitrates and Annual Climatic Variables in Predisposition to Winter Desiccation Injury in Fraser Fir and Red Spruce

by

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(ABSTRACT)

The occurrence of winter injury in red spruce (Picea rubens) L. sarg. and Fraser fir (Abies fraseri) pursh. poir. in relation to the level of atmospheric nitrates and climatic parameters of precipitation and temperatures was investigated. Data and foliage samples were collected from established field plots at 5500, 6000, and 6500 feet in the Black Mountains of North Carolina and from seedlings under 4 treatments of artificial rainfall, varying by NO_3 concentration. Samples were collected 4 times over the 1987 growing season.

Responses were similar in shadehouse and field samples. Wax content differed between collections but not between treatment levels, except for shadehouse spruce, and wax content decreased after collection 2. Between treatment levels, differences were found in the amount of water lost over 14 hours, but not in the average initial fresh weight dry weight ratio (RWT). Differences were found in both RWT and transpiration rate over the growing

season with field trees decreasing or remaining stable with each collection, and shadehouse seedlings increasing. No relationship between climatic parameters and annual leader growth was modeled because understory field trees were immature and exhibiting height growth, masking the effects of climate to understory trees. Winter injury ratings decreased from summer of 1987 to spring of 1988 and no significant differences in ratings were found between elevations. Classic winter injury symptoms were observed on one plot at 6500 feet, but most ratings greater than 0 were given because of the effects of shading from the overstory.

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INTRODUCTION

The ability of trees to survive and be productive at high elevation sites, such as the spruce-fir ecosystem, depends on adequate physiological and morphological protection from harsh environmental conditions. In undisturbed environments, cuticular development is adequate to protect needle tissue from excessive transpiration in winter. When that environment is disrupted with increased inputs of potentially harmful pollutants or an extended period of adverse climatic conditions, growth can be reduced.

Full development of the cuticle has been proven directly related to development and maturity of conifer needles. As elevation increases and timberline is approached, needle development is retarded, reducing deposition of the cuticle and ultimately weakening the ability of the cuticle to resist transpiration (Wardle,1971).

In unpolluted areas, cuticle development proceeds normally. Up to the point of timberline, shoots are able to ripen fully and therefore endure extremes in temperature, moisture stress, and wind. Light intensity, temperature, relative humidity, and climatic conditions will affect the density and distribution of epicuticular wax structures during formation.

In contrast, cuticle development in polluted areas proceeds relatively normally, but the mature state is altered. SO_2 causes accelerated fusion of wax structures, often called erosion, and accelerated aging of the cuticle, leading to increased transpiration during warm, sunny, winter days when the vapor pressure deficit between the leaf surface and the surrounding air increases. NO_3 is thought to prolong the growth period into the fall, delaying the normal hardening-off process and resulting in shoots which are unable to withstand the harsh environmental conditions of high elevation.

The Black Mountains of North Carolina, location of the highest peak in the eastern United States, experience climatic conditions which may induce winter injury and foliar tissue damage. Temperatures are mild to cool in summer and below freezing in the winter, favoring production of tubular wax structures rather than plate-like or dendritic. Relative humidity is high as ridgetops are frequently submerged in clouds, promoting less wax formation and consequently less cuticular resistance. Siccama (1974) observed that the boreal spruce forest of the Green Mountains is at or above cloud level for 900 to 2300 hours per year; a figure which may be projected well to the Black Mountains. Although high in elevation, radiant energy rates are low because of frequent cloud immersion. This would tend to favor sparse growth of wax structures. Taken together, these influences create a scenario of cuticular structure which is poorly developed and vulnerable to degradation and erosion. When one adds in the influences of air pollution, wind and the impact of raindrops, the cuticle is even more vulnerable and subject to winter desiccation injuries.

A second predisposing factor of winter injury may be prolonged drought stress over time. Initially, water stress during the growing season can stunt needle development and consequently cuticle development may not reach completion by the end of the growing season. Repeated summer water deficiencies could result in weakened trees and predisposition to other damages, especially winter desiccation if cuticle structure is inadequate. Johnson and Siccama (1983) used the Palmer Drought Index, based on annual precipitation values less than the amount required for an average growing season May to August, to indicate that approximately 70% of the years between 1900 and 1980 have experienced less than normal annual precipitation.

The effects of SO_2 pollutants have been well documented, but much less is known about atmospheric and rainwater NO_3 and little conclusive evidence on its benefits or damages has been presented. Currently, high concentrations of NO_3 have been found in rain and cloud water and its impact deserves recognition. Atmospheric nitrates appear to act as a fertilizing agent, possibly delaying the onset of dormancy initiation in the fall. This interaction has not previously been studied and it is not entirely clear why winter injury may increase as a result of it.

The intention of this research project was to investigate the role of NO_3 in wax development and cuticular transpiration of Fraser fir and red spruce seedlings at high elevations. Three aspects were studied: two direct measures of cuticular ability to resist transpiration along an elevational gradient (measurement of cuticular wax content and rate of cuticular transpiration), and an analysis of a single predisposing factor of winter injury (historical climate in relation to historical leader growth). The specific objectives of this study were:

1. Quantify wax content and cuticular transpiration rate of red spruce and Fraser fir to various treatment levels of atmospheric NO_3.

2. Evaluate the relationship of climatic variables to annual leader growth of red spruce and Fraser fir.

3. Campare the physical condition of red spruce and Fraser fir foliage before and after the winter of 1988 in relation to the presence of winter desiccation injury symptoms.

LITERATURE REVIEW

INTRODUCTION

Study Site Description

The spruce-fir ecosystem occupies a series of high elevation, island-like sites as far south as North Carolina and Tennessee, along the highest elevations of the Appalachian Mountains in the eastern United States. Spruce-fir forests also occupy a vast area as far west as Alaska in the Canadian provinces. *Picea rubens* (Sarg.) (red spruce), *Abies fraseri* ((pursh) poir.) (Fraser fir), and *Abies balsamea* (Britt.) (balsam fir), the dominant tree species, are found in forests at elevations greater than 700 meters in the north and 1400 meters in the south; balsam fir is located only in the northern Appalachians, Fraser fir only in the Southern Appalachians. Red spruce may be found at slightly lower elevations in transition zone forests in equal dominance with *Betula alleghaniensis* (Britt.) (yellow birch), *Acer saccharum* (Marsh.) (sugar maple), and *Fagus grandifolia* (Ehrh.) (American beech).

Two relatively distinct spruce-fir forests, northern and southern, can be found along the Appalachians; they are roughly separated at the area around the Alleghany Mountains in Pennsylvania and Northern Virginia. Westvald (1953) reported that three broad climax types are represented in the Southern Appalachian spruce-fir ecosystem: spruce-fir, spruce-fir-hardwoods, and hardwoods-spruce-fir.

Surrounded by a very temperate climate at lower elevations, the

Southern Appalachian spruce-fir forests approach a "boreal" climate, but are too far south in latitude to be considered anything other than "Appalachian montane". Average temperatures are 15.2°C in the summer and -5.0°C in the winter. Approximately 200 cm of rain and 400 cm of snow fall each year and the frost-free season is 120-160 days (White,1984).

Soils are typically Ochrepts, Umbrepts, or Udults and relatively sandy, with a pH between 3.2 and 5.0 (Johnson and Siccama,1983). Underlying rock is typically metamorphic or sedimentary granite (Rheinhardt and Ware,1984) . It has been hypothesized that after the last major continental glacial retreat, the climate was warmer than at present, creating unfavorable growing conditions for red spruce and Fraser fir. Growth of spruce and fir was only possible at the highest elevations, such as the Black Mountains of North Carolina or the Great Smokey Mountains of Tennessee. At this time a gap was produced in the continuous tree line where critical high elevation cooler temperatures were missing; presently where no spruce or fir exists in the Appalachian chain. Spruce and fir have never been able to regain their geographic spread, possibly due to a lack of seed source within this gap (Pielke,1981).

Observations of Winter Injury and Reductions in Growth

Since the early 1950's, observations have been made about an apparent reduction in growth in high elevation spruce-fir ecosystems of the eastern United States. Reductions in growth have been attributed to anthropogenic sources of pollutants, particularly acid rain, but there has been little conclusive evidence linking acid rain to a decline in growth rates. In fact, aside from possible increases in aluminum toxicity due to a decrease in soil pH, recent work has shown that acid precipitation has little detrimental effect on spruce-fir forests which are acid by nature. It has also been suggested that decline symptoms, manifested as decreased radial ring widths in red spruce, may be a natural result of advanced stand age in undisturbed forests (Zedaker et al.,1987).

To determine if a change in the forest environment has occurred in the spruce-fir ecosystems of the eastern United States and if those changes can be attributed to anthropogenic sources of pollution, one must first examine the natural environmental conditions specific to this ecosystem. Many authors have suggested that the most important physiological and morphological characteristics of trees that influence survival at high elevations near timberline are cuticular resistance to transpiration, stomatal control, and the amount of water reserves in needles during critical winter months (Baig and Tranquellini,1976; Wardle,1971; Tranquellini,1979).

Observed decline symptoms of red spruce and Fraser fir, thought to be caused by atmospheric pollutants, are very similar in nature to winter injury. Winter injury seems to result when late winter air temperatures warm temporarily causing accelerated transpiration, but soil remains frozen providing no source of replacement for transpired water. Winter injury has been observed in higher elevation spruce-fir forests of eastern North America. Symptoms of this type of stress include desiccated, excessively browned needles moving from the tips of branches inward, with loss of browned needles and occasionally death of whole trees (Friedland et al.,1984). Symptoms of red spruce decline have been described by a recent reduction in tree ring widths, dieback, lack of an obvious cause, and vulnerability to invasion by secondary organisms not normally occurring in healthy trees (Hornbeck et al.,1986; Johnson and Siccama,1983). These descriptions are very similar to descriptions given by many authors as early as the 1950's for frost injury.

Information from different studies has led to the hypothesis that three factors in combination, both natural and anthropogenic, may be responsible for the suggested "decline" of red spruce and Fraser fir at high elevations in the Southern Appalachians. These factors are:

1. Prevailing natural environmental conditions, such as relative humidity and temperature, which affect the formation of foliar cuticles.

2. The action of atmospheric pollutants in erosion of epicuticular waxes and disruption of stomatal control.

3. Harsh winter conditions amplified by a possible historic trend of decreased annual precipitation.

PHYSICAL AND CHEMICAL STRUCTURE OF THE CUTICLE

The ability of the cuticle to resist erosion and transpiration under harsh winter conditions depends partly on its physical and chemical structure (Baig and Tranquellini,1976). Development of cuticle structure can be greatly influenced by environmental conditions at the time of needle elongation. Differences exist in the wax structure and chemical composition of every tree species and the processes described are generally applicable to most species. However, the processes may not exactly describe the cuticle for red spruce or Fraser fir.

Morphology of the Plant Cuticle

DeBary (1884, from Holloway,1980a) was one of the first to describe what is presently called the "cuticular membrane". With the help of the light microscope, he described the entire membrane as being composed of four main structural forms: needles, rods, granular layers, and films. The discovery of scanning and transmission electron microscopes aided in more closely examining the cuticle, but even today DeBary's identifications hold true in the most rudimentary sense.

The cuticular membrane, made up of cuticular and epicuticular waxes, is found on the surface of all higher plants. Deposition of the cuticular membrane is initiated as soon as the leaf or needle unfolds from the shoot and ceases when needle or leaf elongation is complete (Chafe and Wardrop,1973; Hallum,1980; Juniper and Jeffree,1983).

The cuticular membrane is composed of four layers with the outermost layer being epicuticular wax. It is not known precisely where epicuticular wax precursors are synthesized, but it is most generally assumed to be in the epidermal cells. Wax precursors must traverse the plasmalemma, cell wall, pectin, cutin, and wax layers to reach the surface (Chabot and Chabot,1977). Epicuticular wax is composed of amorphous, crystalline, or semi-crystalline waxes and the particular structures formed are genetically determined and environmentally controlled (Jeffree et al.,1975; Holloway,1980b; Juniper and Jeffree,1983). Six wax structures have been described by Baker (1980): tubes, plates, ribbons, rodlets, filaments, and dendrites. During formation of the cuticular membrane, the density and distribution of these structures is environmentally controlled by light intensity, relative humidity, and temperature (Martin and Juniper,1970).

Below the epicuticular wax is the primary cuticle, composed of alternating layers of electron-lucent material and electron-dense material, called lamellae, which follow along the folds of the epidermal cells (Chafe and Wardrop,1973; Holloway,1980a). The lamellae increase in number as the plant tissue ages, until completion of needle elongation (Juniper,1960).

The secondary cuticle underlies the primary cuticle and is composed of cellulosic and lipoidal material in an amorphous reticulum. Cellulose microchannels, considered fibrils by some authors, are interlaced throughout the lipoidal material. These microchannels are generated at the subcuticular lamella below and extend to, but do not contact the primary cuticle (Jarvis and Wardrop,1974; Jeffree et al.,1976).

The thinnest structure, farthest from the surface, is the subcuticular lamella, composed of pectic substances. Opinions differ on whether or not this layer is part of the actual secondary cuticular layer or the primary wall of the epidermal cells. This layer is the site of origin of the fibrillae which permeate through the secondary cuticle (Holloway,1980b).

Structural and Chemical Components of the Cuticle

The chemistry of the cuticle is quite complex and still not fully understood; therefore, only the most common constituents are named as described by Martin and Juniper (1970).

Epicuticular waxes commonly contain long-chain hydrocarbons, alcohols, ketones, fatty and hydroxy-fatty acids and esters. Other compounds which appear, but to a lesser degree, are cyclic compounds such as terpenoids, flavones, and sterols.

Cutin is the most important structural component of the primary and secondary cuticles and it is composed of insoluble lipid polyesters (Holloway,1980b). Small amounts of phenolic compounds can also occur in the membranes which may be partly responsible for the ability of the cuticle to deter herbivores (Juniper and

Jeffree,1983). In addition, cellulose is embedded within the membranes of the zone adjacent to the epidermal cells.

Recent evidence has shown that the form and spatial distribution of crystalline wax deposits are determined principally by the chemical and structural composition of the wax exudates (Baker,1980). The specific form of wax structure is partially responsible for determining the ability of the plant to resist erosion and transpiration in dry weather.

The degree of resistance to erosion is well demonstrated in the "wettability" of a surface, measured by the contact angle of a drop of water 1mm in diameter and determined by the structural and chemical components of a specific cuticle . Tubular waxes, which are rich in B-diketone alkenes, are the least wettable, and structurally are the most erodable. Plate-type and dendritic waxes are rich in primary alcohols, most wettable, and structurally most resistant to erosion (Holloway,1971; Hallum and Juniper,1971). Jeffree et al. (1971) found short tubes on the surface of _Picea sitchensis_ (Bong.) carr. needles. These may be the typical wax structures found on _Picea rubens_, a closely related species, suggesting that red spruce needles may have low wettability, but are easily eroded.

NATURAL INFLUENCES ON EPICUTICULAR WAX DEVELOPMENT

During the period of needle elongation, environmental influences such as light intensity, temperature, relative humidity and ambient climatic conditions determine the density and distribution of epicuticular wax structures. To a degree, the specific wax structures can also be determined by environmental conditions at the time of development.

Light Intensity

Light is one of the most important influences in formation of wax structures. Development of wax structures requires the synthesis of palmitic acid from acetate (C_2); a process dependant of the supply of ultra-violet light (Juniper and Jeffree,1983). Juniper (1960), in his study on wax development in Pisum sativum L., suggested that wax development would not proceed unless leaves were exposed to at least 20% of full sunlight. He grew plants in the dark for 8 days and found that no wax was produced on the leaves until they were removed from the dark and exposed to light; significant results when one realizes that wax formation can normally be observed as soon as the leaves unfold in the shoot.

Hallum (1970) found that wax was produced on Eucalyptus L. leaves under light intensities as low as 1.2% of full sunlight, but development was abnormal and greatly retarded. Wax structures had little or no branching, were sparsely distributed, and sometimes appeared as little more than a thin film on the leaf surface. He determined, through testing a graduated set of light intensities, that normal wax production took place only above light intensities 20% of full sunlight. High radiant energy loads favor dense growth of wax structures while low radiant energy loads favor much sparser growth and little branching of surface wax structures (Juniper,1960; Baker,1974; Baker,1980).

Temperature

The effects of temperature on wax production are relatively clear. Moderately high temperatures (21°C) produce more wax on plant surfaces than very high (35°C) or very low (15°C) temperatures. Working with *Brassica oleracea* species, Baker (1974) found low temperatures (15°C) to produce only tubes oriented at a 90° angle from the surface of the cuticle while high temperatures (35°C) produced structures lying parallel to the surface, such as plates or dendrites. In the same study, the greatest quantities of wax, manifested as increased size of rods or tubes rather than increased overall density of structures, were produced at 21°C as opposed to 15°C or 35°C. However, Baker did not speculate as to where the actual peak of wax production would lie.

Relative Humidity

Opinions differ on the effect of relative humidity on epicuticular wax formation. Baker (1974) admits that research is lacking on the effects of humidity under controlled conditions on wax structure. He found that a decrease in relative humidity promoted the production of tubes rather than dendrites. In addition, an increase in relative humidity decreased the density of wax projections, suggesting that production of wax is inversely proportional to relative humidity.

In a later study Baker (1980) determined that decreases of relative humidity stimulated wax production. He found that changes in relative humidity most generally affected the distribution of predominant wax structure types and that increases in humidity reduced the density of tubular formations when temperature was low, but restricted the development of dendrites when the temperature was high. Martin and Juniper (1970) agree and also suggest that production of wax is inversely proportional to relative humidity.

Length of Growing Season

The amount of time available for cuticle formation may be limited by elevation. As elevation increases, the onset of the initial growth flush is progressively delayed and the number of frost-free days available for shoot formation and wax deposition decreases (Wardle,1971). Temperatures decrease, also limiting the amount of cuticular wax produced. First year seedlings are especially prone to winter desiccation damage because the start of the growing season is delayed, and shoots and leaves cannot begin growth until cotyledons have expanded, shortening the growing season even more.

Annual Precipitation

The amount of annual precipitation is very influential in cuticle development of conifers. As cuticle development follows needle growth and development, deficits of water during active growth will stunt growth and may retard cuticle development. Despite this, studies have shown that water stress in developing plants results in increased wax production, although the increase may not occur until after stress conditions have been relieved (Skoss,1955; Weete, et al.,1980; Baker and Procopiou,1978; Seiler,1985).

Larcher (1957, from Baig and Tranquellini,1976) stated that survival of trees at upper timberline depends in part on the amount of water reserves in the needles during critical winter months, which is determined by the amount of precipitation received the previous growing season. In many studies, mild to severe winter desiccation injury followed unusually hot, dry summers and/or autumns (Curry and Church, 1952; Horntvedt and Venn,1980; Johnson and Siccama,1984).

NATURAL INFLUENCES ON EPICUTICULAR WAX DEGRADATION

Epicuticular wax is synthesized and deposited only as long as needles are elongating. Some species are able to regenerate wax after injury, but only if injury occurs during elongation. The replaced wax is not synthesized de novo, but is simply that wax which remains to be synthesized and deposited (Martin and Juniper,1970). After needle maturation, epicuticular wax is subject to environmental stresses which can erode the cuticle, destroying its ability to resist transpiration and ultimately predisposing it to winter desiccation damage. Natural sources of stress are wind and rain. In addition, atmospheric pollutants, particularly SO_2, can erode cuticular waxes. Wax is not actually lost, but wax structures fuse, increasing wettability and losing effectiveness. Baig and Tranquellini (1976) state that the controlling mechanism of cuticular transpiration depends on the development and thickness of the cuticle on leaves and shoots. Cuticle thickness is often retarded at higher elevations, not reaching full morphological and physical development because of exceptionally short growing seasons (Wardle,1971).

Wind

Even gentle breezes can damage plant surfaces as needles brush against one other. Brushing can flatten and smooth waxes, reducing their ability to repel water and greatly increasing cuticular transpiration. Cape and Fowler (1981) found no weight loss in eroded needles, indicating cuticular wax remains in place during "erosion" in high winds. Hall and Jones (1961) observed increased wettability and cuticular transpiration when leaves were artificially weathered by brushing. According to Baig and Tranquellini (1980), an increased rate of cuticular transpiration may be attributed to the removal of the moist boundary layer of the needle, bringing drier air in contact with the epidermis, greatly increasing the vapor pressure gradient and consequently the transpiration rate.

Rain

The impact of raindrops is responsible for rapid rates of cuticular erosion and wax loss. Baker and Hunt (1986) suggested that because wax is not regenerated after injury, rain damage would be most extreme at the end of the growing season. Schonherr (1976) determined that because wax is a non-ionic substance, permeability of the cuticular membrane to water is not generally affected by changes in pH of applied water. This suggests that the pH of acid rainfall is not responsible for erosion of the cuticle, but it is more likely that other chemical components of polluted rain or simply the physical impact of raindrops may be responsible for reports of erosion in epicuticular wax.

NATURAL PROCESS OF COLD AND DESICCATION RESISTANCE IN TREES

Elevational Limits to Normal Plant Growth

Because of its southern latitude, the highest elevation in the eastern United States (6684 feet, 2037 meters, Mt. Mitchell, Black Mountains, North Carolina) cannot be considered alpine timberline. Yet, frequent winds and low temperatures create a situation in which growth on ridgetops is difficult at best and survival of vegetation is dependant on the ability to withstand periods of low moisture, high winds, and cold temperatures during the winter months.

Daubenmire (1954) discussed "modern" theories of timberline formation suggesting responsible factors such as excessive light, carbon dioxide deficiency, depth of snow, wind, desiccation during winter temperature inversions, heat and light deficiencies. Wardle (1971) has suggested that alpine timberline is the highest point at which shoots of woody upright plants can grow and ripen fully under the air temperatures which prevail at the height of the canopy. A ripened shoot has completed its seasonal elongation and production of leaves, and has lost the succulent appearance imparted by high water content and incompletely lignified cell walls. Above the point of timberline, where forest growth yields to herbaceous and shrubby vegetation less than 2 meters tall, growth forms are prostrate in order to take advantage of the sun-warmed layer of air just above the soil.

Marchand and Chabot (1978) consider the primary limiting factor in the growth, development, and distribution of high elevation trees to be the ability to withstand exposure to constant high winds during the winter when water supply is restricted; a theory well supported by other authors (Sowell et al.,1982; Hadley and Smith,1983; Baig and Tranquellini,1980).

Intrinsic in this ability to withstand exposure to wind is the possession of an adequate cuticular membrane. Although stomata do close rapidly in response to windspeeds greater than 2-4 miles per hour, a vapor pressure gradient is created between the leaf and its surrounding air which can cause cuticular transpiration to increase dramatically, especially with a high degree of insolation (Wardle,1971). Hadley and Smith (1983) found a significant relationship between wind velocity and direction and occurrence of needle mortality on individual shoots of spruce and fir with needles on the windward side of trees or krummholz mats receiving the most damage. The thickness of the cuticular membrane will determine the ability of a leaf or needle to resist cuticular transpiration once the stomata have closed. Cuticular resistance was markedly higher in Picea abies (L.) Karst trees at the valley bottoms in the Austrian Alps than in Picea cembra L. trees at the wind-exposed treeline (Baig and Tranquellini,1980).

Temperature increases or decreases can also be very detrimental to needle water balance, causing extreme desiccation at times. As temperatures drop, extracellular freezing and intracellular dehydration can occur. As temperatures increase, transpiration can accelerate even as relative humidity increases (Baig and Tranquellini,1980; Hadley and Smith,1983).

Physiological Processes of Cold Resistance

Trees are thought to use the mechanisms of supercooling and/or intracellular dehydration to increase cold resistance during winter dormancy (Larcher,1975). These mechanisms reduce the risk of intracellular ice formation with rapid drops in temperature, and the risk of death to trees. These mechanisms are initiated by specific environmental and physiological signals. For example, short days trigger supercooling or intracellular dehydration responses in trees (Larcher,1975).

Supercooling allows trees to chill intracellular water to the homogeneous nucleation point, where pure water will freeze spontaneously; about -40°C. This mechanism is most employed by tree species in climates whose average minimum temperature is no lower than a few degrees above the homogeneous nucleation point and so tends to be restricted to more southern latitudes (Quamme,1985).

Intracellular dehydration confers protection by first forming extracellular ice which creates a high vapor pressure deficit between the inside and outside cell. This draws water out of the interior cell, physically reducing the possibility of ice crystal formation. One of the dangers of this mechanism is extreme water stress or death from dehydration rather than freezing (Williams,1984).

Most often, angiosperm species exhibit supercooling while gynmosperms exhibit intracellular dehydration with latitude and elevation playing important roles in species distribution. It has recently been determined that many species of the genus Picea as well as other conifers exhibit supercooling as a means of cold resistance, particularly species commonly found in northern latitudes (Pukacki, 1985). Although this would appear to be detrimental to cold resistance in boreal spruce forests, many species live up to 300 years and it is unclear what other physiological factors play a part in this mechanism.

OTHER FACTORS INFLUENCING THE ABILITY OF HIGH ELEVATION CONIFERS TO RESIST FREEZING TEMPERATURES

Genetics

The role of genetic variation in cold resistance has become increasingly well documented in the past 20 years (Williams,1984; Berrang,1984). More northern than southern tree species have been found to be frost tolerant earlier in fall and to take longer in spring to come out of dormancy. This trend appears to be relatively consistent with elevation and latitude, but local unexplained sources of variation have been found (Sakai,1982).

Rime Ice

Rime ice has been considered as a factor in winter injury, although it is unlikely that it plays a great role. Causing secondary injuries, rime ice "smothers" a tree by forming a thin layer of ice over much of the tree, preventing any gas exchange. Damage is caused not from freezing, but from oxygen deficiency stress and the toxic build-up of CO_2 and other respiration products (Levitt,1980).

Cloud Immersion

According to Siccama (1974), the spruce-fir forests of the northern Green Mountains may be above the cloud base for 900-2300 hours per year, exposing them to atmospheric pollutants on a much larger scale than rainfall. The concentration of pollutants, including NO_3, is often much greater in clouds than rainfall. Lovett et al. (1982) estimated average NO_3 levels of 101.5 kg ha^{-1} yr^{-1} in bulk cloud water deposited on a balsam fir stand on Mount Moosilauke, New Hampshire. Mean depositions of NO_3 in bulk precipitation recorded from the same orographic events were 23.4 kg ha^{-1} yr^{-1}. The pH of clouds also tends to be very low, averaging pH 3.6 during the growing season (Johnson and Siccama,1984). Cloud immersion is undoubtedly one of the greatest sources of nitrates at high elevations on the Black Mountains also.

SO_2

Conifers growing in polluted areas have been seen to suffer from winter water stress and this has been attributed to disturbance of stomatal function, biochemical stress, and erosion of the wax covering (Huttunen and Laine,1983). Both atmospheric sulfate and nitrate can have a great influence on the ultimate ability of needles to resist cuticular transpiration and winter desiccation damage. SO_2 has been considered responsible for degrading the cuticle and disrupting stomatal control (Keller,1984; Soikkeli,1981). The effects of sulphur pollution are not being investigated in this project, but they must at least be recognized as an important influence on cuticular function.

Second to ozone, atmospheric SO_2 has been blamed for the greatest damage to vegetation in urban and rural areas (Morrison,1984; McLaughlin,1985). Depositions of 64.8 kg ha^{-1} yr^{-1} in bulk precipitation have been observed in a 10.3m tall balsam fir stand on Mt. Moosilauke, New Hampshire at an elevation of 1200m. At the same time and place, 275.8 kg ha^{-1} yr^{-1} were estimated in bulk cloud deposition (Lovett et al.,1982). The presence of SO_2 in the air has been shown to stimulate stomatal opening and reduce the rate of stomatal closure (Majernik and Mansfield,1970). Stomata were induced to open at night and duration of opening increased during the day when Vicea faba L. and Zea mays L. plants were exposed to concentrations between 28.6 and 143 ug m^{-3} (Unsworth et al.,1972).

Many authors have blamed SO_2 pollution for accelerated erosion of surface wax structures. What has been termed erosion throughout the literature seems not to be a loss of substance, but a fusion of wax structures which appears eroded and acts to reduce cuticular resistance. Cape and Fowler (1981) found no actual weight loss in eroded epicuticular wax in the presence of SO_2, but transpiration increased greatly. Huttunen and Laine (1983) observed that _Pinus sylvestris_ L. needles on trees in urban areas eroded 2 to 5 times faster than needles of trees from rural forested land. The most eroded needles also had the highest sulphur content. Cape and Fowler (1981) found the mean life time of needles from a polluted area (with depositions of about 100 ug SO_2 m^{-3}) to be about 25 months, while those from an unpolluted area (10 ug SO_2 m^{-3}) had a mean life time of 33 months. As stated previously, the erosion or fusion of epicuticular wax increases cuticular transpiration which can be very dangerous during winter.

NO_3

Although atmospheric nitrogen presumably has little direct effect on the cuticular membrane, it is thought that higher than normal nitrogen concentrations in growing plants may result in prolonged growth into autumn (Havas,1971). NO_3 is in a form readily usable by conifers and is not considered to be toxic in moderate concentrations, but the effects of higher concentrations are not quite as clear. Presently the effects of current levels of atmospheric nitrates to plant life are unknown.

Friedland et al. (1984) suggest predisposition to winter injury in the Green Mountains of Vermont may be due to the chronic inability of foliage to harden off in time for winter. They cite deposition estimates (of Lovett et al.,[1982]) of 37.5 to 44.1 kg N ha^{-1} yr^{-1} in bulk precipitation and cloud water at two high elevation sites. Scherbatskoy and Bliss (1983) reported NO_3 deposition estimates as high as 99.1 kg ha^{-1} yr^{-1} in cloud water and 66.8 kg ha^{-1} yr^{-1} in rainwater from the same area. Friedland et al. (1984) hypothesized that nitrogen supplied to foliage by cloud water and precipitation may delay dormancy initiation in late summer and cuticularization of the epidermis, but many authors have shown that that delay is unlikely because the cuticle would have begun formation at the time shoots unfolded (Chafe and Wardrop,1973; Baker,1980; Hallum,1980). Havas (1971) also suggests that excessive nitrogen in the growing base may result in prolonged physiological activity into autumn which may indirectly result in a shortage of water due to excessive transpiration.

Past research on cold hardiness of range plants has revealed that nitrogen fertilization has an inverse effect on winter survival of forage grasses. Howell and Jung (1965) found that orchardgrass (Dactylis glomerata L.) treated with high levels of ammonium nitrate and exposed to freezing temperatures of -8°C had significantly higher levels of electrical conductivity than orchardgrass treated with low levels of ammonium nitrate. Plants treated with the lowest level of nitrogen (125 pounds/acre) had the best survival and lowest electrical conductivity on 60% of 20 sample dates (over two years) while plants receiving the highest level of nitrogen (475 pounds/acre) were among the hardiest on only 10% of the sampling dates. The base level of nitrogen appears also to be important in the effects of excess nitrogen on plants. Pyiklik (1963) found that fall applications of nitrogen increased free sugars and cold resistance in winter cereals growing in soil deficient in nitrogen but decreased free sugars and net biomass of winter cereals growing in soils with ample nitrogen.

Applications of phosphorus and potassium, in addition to increasing carbohydrate levels, were observed to increase winter survival of pangolagrass (Digitaria decumbens Stout.), but the opposite effect was seen on the whole with applications of nitrogen (McCloud and Creely, 1957). At all treatment levels of potassium, winter survival decreased with increasing levels of nitrogen application.

It has been suggested that late applications of nitrogen fertilizer may delay initiation of dormancy as plants maintain growth without responding to normal signals of dormancy initiation (Smith, 1964; Friedland et al., 1984). Cold acclimation normally progresses in stages due to environmental and physiological signals such as photoperiod, temperature, and internal hormonal growth regulator balance. The greatest cold resistance occurs between November and February. Cold resistance was equal in all treatments until near November in orchardgrass, but after that, winter survival was lowest in those plants which received the highest nitrogen treatments (Howell and Jung,1965).

HYPOTHESIZED CAUSES OF WINTER INJURY

Winter injury is thought to be caused when periods of cold weather are followed by sudden increases in temperature, often accompanied by drying winds; it most often occurs in the spring. If soil is frozen, water transpired from the needles cannot be replaced (Curry and Church,1952; Sakai,1970; Havas,1971; Friedland et al.,1984). Two hypotheses have been postulated for the events in which a large number of trees in a single location have died, or in which numerous needles and shoots on parts of trees have turned brown and fallen off. Many authors agree that degradation or incomplete development of the cuticle may be responsible for predisposing conifers to winter injury (Marchand and Chabot,1978; Hadley and Smith,1983).

The effects of air pollutants on degradation and erosion of the cuticle have been studied by various investigators (Sharma,1975; Huttenen et al.,1984; Keller,1984). The following hypotheses most likely work in combination as it is unlikely that either one can be considered solely responsible for winter injury. Although all of the following factors have been discussed previously, they will be summarized here to describe these hypotheses.

The first hypothesis suggests that winter injury results from predisposition due to degradation of the cuticle. This suggests that atmospheric pollutants, SO_2 in particular, erode the cuticular membrane by fusion of wax structures and accelerate its natural aging up to 5 times faster than normal (Huttunen and Laine,1983). Erosion of the cuticle seems to result in an increased rate of transpiration which is harmful to needle tissues. At higher elevations, the greatest damage to needles occurs because of frequent winds, freezing temperatures, and high insolation during the winter months.

A second hypothesis suggests that predisposition to winter injury is due to prolonged annual drought stress. The presence of brown, desiccated needles in the spring after a drier than normal summer and/or fall has been observed (Johnson and Siccama,1984). It has been suggested that perpetuated drought stress slows development of the leaf and predisposes the tree to other damages. Predisposition to other injuries is the key to this hypothesis. Possibly, as growth rates slow from continued water deficits, cuticular development will be retarded and trees will be less able to resist both direct and indirect damages of pollutants and pathogens.

It has recently been suggested that climate may play a role in the decline of red spruce at high elevations. Temperature and precipitation have been found to be major variables in modeling red spruce decline in various eastern U.S. forests. Hepting (1963) discussed the validity of "climax" forests, suggesting that climate has never remained stable long enough for a forest type to completely self-perpetuate in response to any particular climate. He also discussed the sensitivity of forests to even slight changes in seasonal temperature or precipitation stating that some pathogens need only a 2°C increase or decrease in temperature and a minimum period of available moisture to cause a sustained outbreak which could weaken or debilitate a particular tree species in a local area.

Changes in temperature or precipitation may be responsible for declining tree growth rates. Johnson et al. (1986) were able to successfully model tree ring widths using temperature and precipitation as predictors in two observed (and confirmed) periods of red spruce mortality in 1871-1885 and in 1968-1976. They created an index which transformed temperature extremes in August and December to standard normal deviates and plotted those years which exceeded the 90% probability level. They found that ring widths were reduced during a reported (and confirmed) period of heavy red spruce mortality from 1871-1885. Cook et al. (1987) found that periods of temperature stress coincided with the two periods of observed red spruce mortality. "Tree ring calenders," which express radial wood growth over a period of time (commonly centuries) also show a depression in radial growth in the 1870's and 1970's (Hepting, 1963).

A third hypothesis, which this research project addresses, may also be postulated. It states that high elevation winter injury may be the result of the two factors in combination. Primarily, the increase in atmospheric nitrates may be unnaturally extending the length of time in which physiological activity is occurring in high elevation conifer species. Although this would in turn increase the amount of time for cuticle development, it may be delaying the onset of physiological mechanisms which increase desiccation resistance. Trees may not be fully prepared for excessive transpiration in late winter allowing membrane damage and desiccation of needle tissues. Secondarily, perpetuated annual drought may be weakening trees, predisposing them to winter injuries as described in the preceding paragraph.

METHODS AND MATERIALS

OVERALL APPROACH

Measurements were taken and samples collected from red spruce and Fraser fir regeneration from field plots at the highest elevations of the Black Mountains of North Carolina. In order to isolate specific effects of nitrogen, development over the growing season, or their interactions, red spruce and Fraser fir seedlings were also raised in a shadehouse in Blacksburg, Virginia. Because environmental conditions between Blacksburg, Virginia and the Black Mountains of North Carolina differed, responses of seedlings grown under controlled conditions did not always mimick responses of trees from field plots in absolute numbers. Information was for comparitive purposes only.

Foliage samples taken from both field plots and shadehouse seedlings were analyzed for epicuticular wax content and cuticular transpiration rate (on intact twigs). Internodal distance of regeneration was measured for 10 to 20 previous years on and around field plots. Finally, field plot trees were rated twice for physical condition and visual damage to foliage; in June of 1987 and April of 1988. All field plot and shadehouse seedlings were sampled for epicuticular wax content four times in 1987: June 22-25 (collection 1), July 27-30 (collection 2), August 31-September 3 (collection 3), and October 24-26 (collection 4). All field plots and shadehouse seedlings were sampled the latter 3 times for cuticular transpiration rate starting with collection 2.

PLOT DESCRIPTION AND LOCATIONS

Permanent field plots were randomly established by the Southern Appalachian Research and Resource Management Cooperative (SARRMC) (Figure 1). Data is currently being collected to determine the relationships between site and stand characteristics as they relate to degree of decline and regeneration success of the spruce-fir type over its geographic range in the Southern Appalachians (Zedaker et al.,1986)

The plots are 400m^2 (20m x 20m) each with a 600m^2 (20m x 30m) extension for additional data collection, and are established along elevational (5000', 5500', 6000', 6500'), exposure (S-SE: exposed, N-NE: protected), and topographical (ridge, slope, draw) gradients. Data collection for this project was from both exposed and protected ridges, and from four plots at 5500 feet, three plots at 6000 feet and three plots at 6500 feet. Appendix A lists plots, their elevations, and the number and species of samples removed from each plot.

Up to ten trees of each species per plot were chosen to sample in the field. Only the most vigorous trees with greater than 75% of the foliage healthy and intact were chosen, limiting the available number of trees to sample on some plots. At least six trees of each species were always chosen. All trees were between 1 and 2.5 meters tall in order to be at least 10 years old, but with the tip of the leader within reach of the sampler.

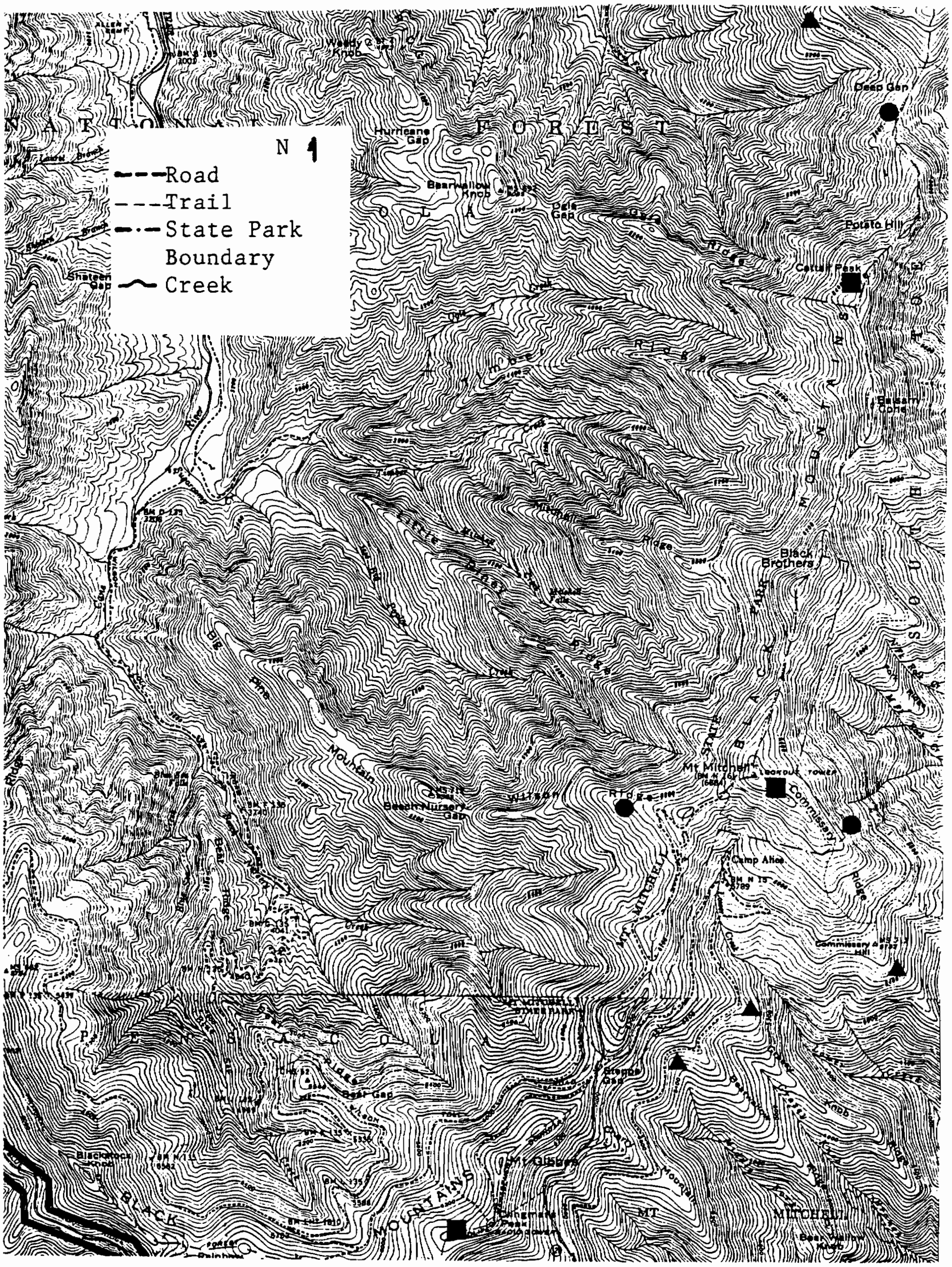

Figure 1. General study site and plot locations in the Black Mountains, North Carolina. Plots in each elevation band are marked by : 5500 feet ▲ , 6000 feet ● , 6500 feet ■ .

EXPERIMENTAL TREATMENTS

Eighty Fraser fir and 160 red spruce three year old bare-root seedlings were purchased from Blevins Land Development Company in Whitetop, Virginia and from the Nova Scotia Department of Lands and Forests in Truro, Nova Scotia, respectively. Fraser fir seedlings were planted in 1 gallon plastic pots with soil collected from beneath a spruce-fir forest canopy near Mt. Rogers National Recreation Area, Virginia. Red spruce seedlings were planted in 4-inch plastic pots with soil collected under a spruce-fir forest canopy in the Black Mountains of North Carolina. All seedlings were placed in a shadehouse adjacent to the laboratory used for nitrate rainfall treatments. Treatment levels corresponded to 5 ppm (control/low exposure), 50 ppm (low intermediate exposure), 500 ppm (high intermediate exposure), and 1000 ppm (high exposure) nitrate. Rainfall was applied to seedlings from April, 1987 until October, 1987 as diagrammed in Appendix B.

Rainfall treatments were applied using the rainfall simulator housed at the Laboratory for Air Pollution Impact to Agriculture and Forestry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia. The simulator applies artificial rainfall based on the principle of droplet formation from needle tips (Chevone et al.,1984). Four artificial rainfall solutions were used, varying by the nitrate concentrations described above, which were similar to measured wet deposition rates between 5500 and 6500 feet in the Black Mountains (Bruck, 1987 personal communication). The pH of the solution was adjusted to 3.6, the ambient pH of cloud cover on the top of the Black Mountains. Rainfall treatments were applied 58 times between May and October of 1987. Total NO_3 deposition varied from approximately 11 gh ha^1 to 2900 kg ha^1 depending on species and treatment level (Table 1).

METHODS ASSOCIATED WITH SPECIFIC OBJECTIVES

Analysis of Epicuticular Wax Content

In the field, two twig samples were clipped from each tree which included at least two years of growth. In most cases samples were taken from the upper 50% of the crown and from twigs on the perimeter of the tree. Foliage samples were identified by tree number, plot, and species, sealed in a plastic bag, and kept on ice until they reached Blacksburg where they were stored in a cold room at a constant 2°C temperature until analysis.

Because of the small size and limited new growth, 1/4 of the total shadehouse seedlings of each species and treatment level were harvested at each collection date and the foliage divided between the wax content and transpiration rate analyses. Nine spruce and six fir seedlings from each treatment level were harvested each collection date. Each sample was identified by species, treatment level and pot number and stored in a plastic bag in a cold room at 2°C until analysis. Shadehouse samples used in the transpiration

TABLE 1. Total NO_3 deposition on shadehouse grown seedlings over the 1987 growing season[1].

	NO_3 Concentration			
Species	5	50	500	1000
	kg/ha			
Fraser fir	14.5	145.0	1450.0	2900.0
Red spruce	11.2	112.3	1123.7	2247.5

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[1] Treatments were applied 58 times between May and October of 1987.

rate analysis were subjected to testing immediately upon harvesting.

Projected surface area of Fraser fir and red spruce needles was measured with a Li-Cor Li-3000 leaf area meter (Li-Cor, Lincoln, NE) prior to chloroform wax extraction. Total weight of epicuticular wax was divided by total projected surface area to obtain average epicuticular wax per projected cm^2 of foliage (one side only).

Epicuticular wax content of field and shadehouse samples was measured by gravimetric analysis. Fifty current year needles, randomly chosen from the center of the clipped twig, were agitated in 5 cm^3 of chloroform for 20 seconds. The chloroform-wax mixture was then poured through a Whatman no. 1 filter paper (medium-fast filter speed) into a pre-weighed glass test tube. After the chloroform evaporated, the test tube was reweighed. The filter paper and needles were saved in individual envelopes and oven-dried to determine the dry weight of the needles. Wax content was then calculated on a dry weight and surface area basis.

Analysis of Cuticular Transpiration Rate

A branch tip and first branch whorl were clipped from each of 10 spruce and 10 fir trees sampled in the field and from 9 spruce and 6 fir seedlings from each treatment level in the shadehouse. Testing of transpiration rate over time followed methods similar to those used by Baig and Tranquellini (1980) and DeLucia and Berlyn (1984).

In the laboratory, gravimetric analysis was used to determine if the average rate of water loss varied due to elevation or nitrate treatment level. Each sample was tagged and clipped under water leaving the current year and previous year's growth and left upright in water to rehydrate for 24-72 hours. At the end of rehydration, the sample was weighed on a Mettler balance to two decimal places and set upright in a styrofoam tray. The trays were then placed in a darkened growth chamber at a constant 25°C and <=10% relative humidity. A household fan was set in the growth chamber which blew directly on the samples with an approximate wind speed of 1.8 m/sec.

After the initial weighing, the samples were weighed after 1 hour, once hourly for the next three hours, and once every 2 hours after that for 14 hours with 10 actual weight measurements. All samples were oven-dried to determine transpiration rate per gram of foliage and stem.

Analysis of Climate/Leader Growth Relationship

In the summer of 1987 internodal distance for the previous 20 years was measured on a maximum of 15 trees per species per plot for all three elevations. Temperature and rainfall data were collected from regional climatic data (NOAA, Asheville, North Carolina) records for the same 20 years to determine if a relationship exists between temperature, rainfall, and annual leader growth.

Mean annual temperature and precipitation should change little (<1.5°C) over 300 meters (1000 feet) in elevation, so no adjustments were made for elevation in regional temperature records. Temperature parameters used in analysis of winter injury included the number of days in individual winter months which were unseasonably warm (TOTJAN, TOTFEB). Data on these parameters originating from Mt. Mitchell were available only for the years 1980-1984, so instead the more complete records of Grandfather Mountain, North Carolina were used. Grandfather Mountain is within 50 miles of Mt. Mitchell and at 5300 feet, experiences a climate similar but slightly milder than that of Mt. Mitchell. A statistical comparison of the two sites yielded simple R^2' values of .85 and .90 for TOTJAN and TOTFEB data respectively. Because of the high R^2 values, it was decided to use the values for Grandfather Mountain in order to increase the midwinter temperature data base.

Winter Injury Ratings

While foliage samples were being collected in June of 1987 from sample trees on each plot, each tree was subjectively rated on its physical condition according to the following scale:

0: Healthy tree, no sign of any desiccation damage and all needles green,
1: 1-25% of the needles brown or damaged,
2: 26-50% of the needles brown or damaged,
3: 51-75% of the needles brown or damaged,
4: 76-99% of the needles brown or damaged (but some green needles still present on the tree),
5: Tree completely dead.

A return trip to all plots was made in April of 1988 and each tree was again rated according to this scale. Trees were rated in April because red needles were still present winter injury symptoms were most obvious at that time In analysis, the two separate scores were compared to determine if damage had occurred over the growing season.

EXPERIMENTAL DESIGN AND STATISTICAL ANALYSIS

Both field plots and shadehouse treatments had a completely randomized design. Whole field plots acted as units of observation in the field while individual seedlings acted as units of observation at each nitrate concentration level in the shadehouse. Generalized analysis of variance tables for both species in the field and in the shadehouse are shown in Appendices C and D.

Differences in epicuticular wax content between elevation/nitrate treatment levels were determined by a two factor analysis of variance. To insure validity of statistical tests, a Shapiro-Wilk test for population normality and independence was performed initially and transformed data used where necessary. significant differences in treatment level or significant interactions were present at the p = .1 level, Tukey's Range Test was used to separate means. Duncan's Multiple Range Test was not used because it was considered inappropriate for unbalanced designs when using SAS (Statistical Analysis System, Cary, North Carolina).

The cuticular transpiration rate experiments were analyzed to find differences between elevation/nitrate treatment level and between sample collection time at the p = .1 level. Equations describing mean relative weight loss over 14 hours at each treatment level and sample collection time were determined by both multiple and simple linear regression analyses. A two-sample t-test was used to find differences in individual slopes of transpiration rate while differences in mean relative weight over 14 hours and in the change in the fresh weight/dry weight ratio over 14 hours were examined by one-way analysis of variance.

Multiple and simple linear regression analyses were used to model a relationship between selected climatic variables and annual leader growth of spruce and fir in the field. The RSQUARE procedure, including C_p and MSE diagnostics in SAS were employed to determine significant parameters for model building with a $p = .1$ significance level.

Contingency table analysis was used to find differences in injury ratings between elevations and between years. All six rating categories were tested and a significance level of .1 was used.

RESULTS AND DISCUSSION

WAX CONTENT OF FRASER FIR AND RED SPRUCE

Elevation/Nitrate Concentration Level Effects

Wax weight per square centimeter surface area (WWTSA) or gram dry weight (WWTDWT) did not differ significantly at the p = .1 level between elevation or nitrate concentration treatments for field spruce or field and shadehouse fir. Shadehouse grown spruce differed wax content in response to nitrate treatments at all sample collection times except collection 2.

A lack of effect of elevation was also found by DeLucia and Berlyn (1984) with *Abies balsamea* at or near alpine timberline. Although the tested elevations in that study were lower (732m - 1402m) than those in the Black Mountains (1675m - 2000m), samples were collected from either red spruce-balsam fir or solitary balsam fir forests. In addition, the relative amounts of wax per gram dry weight in theri study, 25.1 - 27.8 mg/g dry wt., were in general agreement with those measured on Fraser fir in this study.

Field and shadehouse fir seedlings responded only to temporal treatmnets, while shadehouse spruce responded to both temporal and nitrate concentration treatments and their interaction. Highest WWTSA and WWTDWT values were seen in the 500 ppm treatment at all sample collections except for WWTSA at collection 2 (Table 2). Within each nitrate treatment level, highest WWTSA values were not consistent, ranging from collection 1 to collection 4, but highest WWTDWT values within each nitrate treatment level were always at

TABLE 2. Wax weight (per unit foliage surface area (WWTSA) and dry weight (WWTDWT)) values for shadehouse grown red spruce over 4 sample collection times[1]

Sample Collection Time	EXPERIMENTAL TREATMENTS (ppm NO_3) 0005	0050	0500	1000
	WWTSA (mg/cm^2)			
1	0.75bc[2]	0.33c	1.21abc	1.14abc
2	1.24abc	1.67abc	1.14abc	1.00abc
3	0.65c	0.46c	0.87b	0.54c
4	0.68c	2.34ab	2.37a	0.51c
	WWTDWT (mg/g)			
1	16.66bc	18.07bc	31.37a	25.22ab
2	10.38cd	10.59cd	10.98cd	9.77cd
3	9.09cd	5.07d	9.35cd	6.25d
4	5.79d	9.83dc	13.81cd	4.57d

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[1] Sample collection times are 1: June 22-25, 1987; 2: July 27-30, 1987; 3: August 31-September 3, 1987; 4: October 24-26, 1987. Shadehouse seedlings were harvested and collected within 5 days of field sample collections.

[2] Values followed by different letters are different (p= 0.10) based on Tukey's range test, which is based on the interaction of sample collection time and NO_3 treatment level. WWTSA and WWTDWT were tested independently.

collection 1, suggesting that dry weight continued to increase over the growing season while wax content remained constant or decreased after collection 1.

Temporal Treatment Effects

Since no main elevation/nitrate treatments effects or interactions of collection time and treatment level could be found for field fir, field spruce, or shadehouse fir, data from all elevations or nitrate treatments were combined to test for significant differences in WWTSA and WWTDWT over the growing season.

Significant differences between sample collection times were found for all groups for both parameters WWTSA and WWTDWT. Table 3 lists WWTSA and WWTDWT values for field spruce and fir and shadehouse fir.

WWTSA values for field spruce and fir were highest at mid-growing season. Although WWTSA increased slightly in field spruce from collection 2 to collection 3, the increase was not significant. This increase may have resulted from below normal amounts of rainfall in the Black Mountains of North Carolina in July and August of 1987, and field spruce may have been more sensitive to this stress than fir. Without needed moisture, growth could have decreased or stopped altogether. Table 4 shows that dry weight did in fact decrease slightly in late August (collection 3) while it increased progressively in mid-July and October from previous collections.

If respiration continued in trees while photosynthesis was

TABLE 3. Wax weight (per unit foliage surface area (WWTSA) and dry weight (WWTDWT)) values for field and shadehouse grown Fraser fir and field red spruce over 4 sample collection times[1]

Species	Group	Sample Collection Time	WAX CONTENT WWTSA (mg/cm^2)	WWTDWT (mg/g)
Fraser fir	field	1	0.341bc[2]	24.380a
		2	0.481a	24.266ab
		3	0.402b	20.717b
		4	0.323c	13.408c
Fraser fir	shadehouse	1	0.349b	27.009a
		2	0.431a	20.446b
		3	0.278b	16.843cb
		4	0.271b	13.714c
Red spruce	field	1	0.705b	10.396b
		2	1.347a	14.900a
		3	1.459a	14.967a
		4	0.927b	10.226b

[1] Sample collection times are 1: June 22-25, 1987; 2: July 27-30, 1987; 3: August 31-September 3, 1987; 4: October 24-26, 1987. Shadehouse seedlings were harvested within 5 days of field sample collections.

[2] Values for each group within columns (of four values) followed by different letters are different ($p = 0.10$) based on Tukey's range test. Each species and treatment was tested independently.

TABLE 4. Oven dry weight of field foliage samples used in wax content analyses[1].

Species	Elevation (Feet)	Sample Collection 1	2	3	4
		mg/g			
Fraser fir	5500	1.63	2.86	2.60	3.65
	6000	1.80	3.13	2.76	4.05
	6500	1.29	2.80	2.30	3.81
Red spruce	5500	0.83	1.70	1.35	1.70
	6000	0.70	1.68	1.60	2.38
	6500	0.47	1.35	1.42	1.88

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[1] Values per needle, based on 50 needles per foliage sample.

reduced by drought stress, carbohydrate reserves would have been consumed and not replaced, resulting in a loss of dry weight. The decrease at collection 3 is not large relatively, and may be a result of the shortage of rainfall in July and August. Wind was present to degrade epicuticular waxes, but rainfall was negligible, probably resulting in slightly less wax degradation than usual.

Despite low rainfall amounts, temperatures were not unseasonably high and soil moisture reserves may have been adequate to allow for growth throughout the summer. It is unlikely that growth stopped in July and August of 1987 and as expected, WWTSA decreased after mid July, indicating that physiological activity continued in needles throughout the summer.

WWTSA in shadehouse fir decreased in value after collection 2 as did field fir. The highest WWTSA value was found at collection 2 and decreased significantly in the next sample collection, but little decrease was seen from collection 3 to collection 4.

It is not unexpected that highest WWTSA values were observed early in the growing season for shadehouse fir. Growth most likely continued in shadehouse seedlings over the entire experimental period due to inputs of NO_3 2-5 times per week throughout the study. This presumably resulted in increases in biomass, and possibly foliar surface area, with progressively lower WWTSA values over time. In addition, the impact of raindrops on seedlings in the shadehouse may have contributed to loss of wax throughout the study period. Baker and Hunt (1986) observed erosion of wax structures on

Brassica leaves in response to raindrops applied at low and medium velocity (.25 to 5 m s^{-1}) and suggested that rain damage would be greatest at the end of a growing season, possibly allowing increased permeability of pollutants.

Many authors suggest that wax deposition is complete by the time needle elongation is finished (Martin and Juniper, 1970; Hallum, 1980), but that characteristic was not so obvious in this study. Observations made in the field revealed that fir and spruce did generally complete needle elongation after collection 1, which can be seen in the peak WWTSA values at collection 2. But while it was documented that shadehouse spruce and fir completed elongation previous to collection 1 (by June 15), peak WWTSA values were highest at collections 2 or 3. Needles may not have hardened off completely, but buds had begun to set for the following year by collection 1 on all shadehouse seedlings. No factors were intentionally isolated or controlled to test date of hardening and it is difficult to determine the reason for this difference in pattern in shadehouse seedlings.

As with WWTSA values, WWTDWT values for all groups were significantly different between collections over the growing season, but not over treatment levels, with the exception of shadehouse spruce. In all groups but shadehouse spruce, WWTDWT values followed a general pattern of decrease over time after collection 2. With the removal of some outliers from the data set, shadehouse spruce would have followed a similar pattern, but the number of outliers was large enough that removal may have caused bias in the results, so they were not excluded. The relatively consistent pattern seen in the other 3 groups over time suggests that biomass increased progressively over the growing season, but that wax content stayed constant or decreased from the initial deposition amount.

An interesting response to nitrate concentration treatments in shadehouse spruce is seen in Table 2. WWTSA for all treatments were somewhat similar within each measurement variable at collections 1, 2, and 3, but at collection 4 differed much more. The two intermediate nitrate concentration treatments (50 and 500 ppm) resulted in significantly higher WWTSA and values for the most part at collection 4 than the high and low concentration treatments (5 and 1000 ppm).

Spruce seedlings responded with constant or decreasing biomass to added increments of NO_3 above 5 ppm and up to the level of 500 ppm. The decrease in WWTDWT with the 1000 ppm treatment suggests that increments of NO_3 above 500 ppm were enough to cause seedlings to continue biomass production after August, a response which was not seen even at the highest elevations in the field. The slight increase in WWTDWT values for intermediate treatments was expected if seedlings from those treatments did in fact initiate dormancy with a consequent reduction of growth and consumption of carbohydrate reserves.

In normal environments, trees respond to environmental signals of decreasing daylength and temperature with a reduction of photosynthesis, respiration, and growth rate, and initiation of cold resistance mechanisms such as supercooling or intracellular dehydration. These actions protect trees from harsh winter conditions and ultimately death from freezing (Kramer and Kozlowski, 1979). The consequences of a progressive increase in biomass in the fall in relation to dormancy initiation and winter cold resistance are important.

It is not clear why a similar response occurred with the 5 ppm treatment. Even with the decrease in WWTDWT values seen with the 5 and 1000 ppm treatments in shadehouse spruce, further measurements, such as differential thermal analysis or tests of spring survival are needed to determine if seedlings at any particular treatment level in the shadehouse responded to normal signals of dormancy initiation.

WWTSA values for both field and shadehouse spruce were up to 4 times greater than both fir groups. Although spruce may in fact produce more wax per unit of surface area, this is at least partly a result of the measurement process. The Li-Cor leaf area meter yielded a "projected" surface area, measuring only one side of needles. Because fir needles are fairly flat while spruce have a thinner, trapezoidal shape, it is a natural outcome that WWTSA will be relatively higher for spruce than for fir. WWTDWT values were greater for both fir groups than both spruce groups, indicating absolute meaurements throughout the study are at least partially a result of their measurement process. Spruce and fir species in this study were not intended to be tested against each other, only general comparisons made.

TRANSPIRATION RATES OF FIELD SPRUCE AND FIR OVER THE 1987 GROWING SEASON

Actual transpiration was not measured in this experiment; it was assumed that loss in weight due to ongoing respiration was minimal so that change in the fresh weight/dry weight ratio (FWDW) during the experiments reflected transpiration. Each FWDW ratio reflects a single hourly or bi-hourly measurement only. Initial relative weight (RWT) was the average initial FWDW ratio measured at time 0 in an experiment. All samples at a particular elevation or nitrate concentration level were averaged to report a single RWT measurement for each treatment level at a single collection.

Slope of Transpiration Over Time

For all groups at all sample collections, the pattern of RWT over 14 hours was generally parallel with no RWT x hourly measurement interaction. Individual curves and their regression equations are shown in Figures 2 - 5. DeLucia and Berlyn (1984) reported significant differences between slopes of transpiration over time between samples collected at 3 elevations, but the pattern of the rate of water loss over time was similar between elevations.

Transpiration rate experiments were performed in the dark and water loss was cuticular throughout. Nevertheless, hour 1 weight measurements probably reflect water lost before complete stomatal closure, in the first 15-20 minutes. All weight measurements after

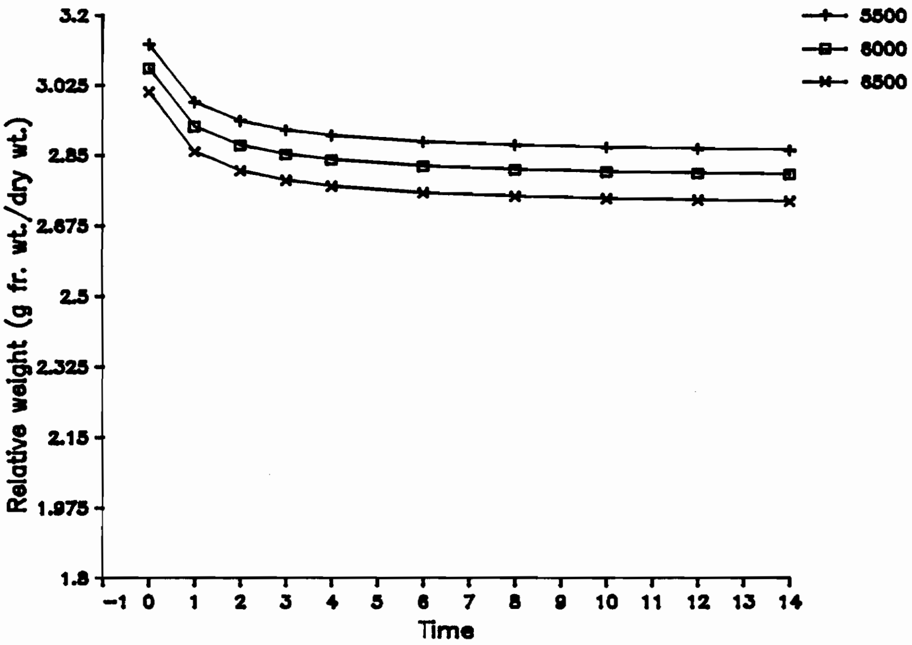

Figure 2a. Curves of mean relative weight loss over 14 hours for 3 elevations (in feet) for collection 2, field fir. Individual curve equations are: 5500 feet, logRWT = 1.122 + 0.094(1/Time) - 0.038(Dry wt.); 6000 feet, logRWT = 1.024 + 0.097(1/Time); 6500 feet, logRWT = 1.190 + 0.102(1/Time) - 0.066(Dry wt.).

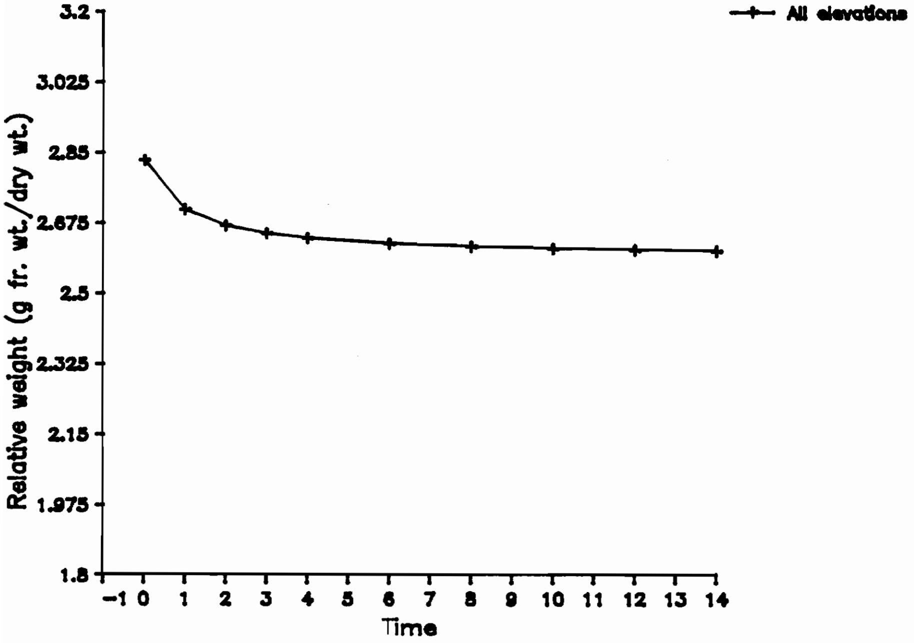

Figure 2b. Curve of mean relative weight loss over 14 hours for 3 elevations (in feet) combined (no significant difference between elevations) for collection 3, field fir. Curve equation is: logRWT = 1.046 + 0.089(1/Time) - 0.062(Dry wt.).

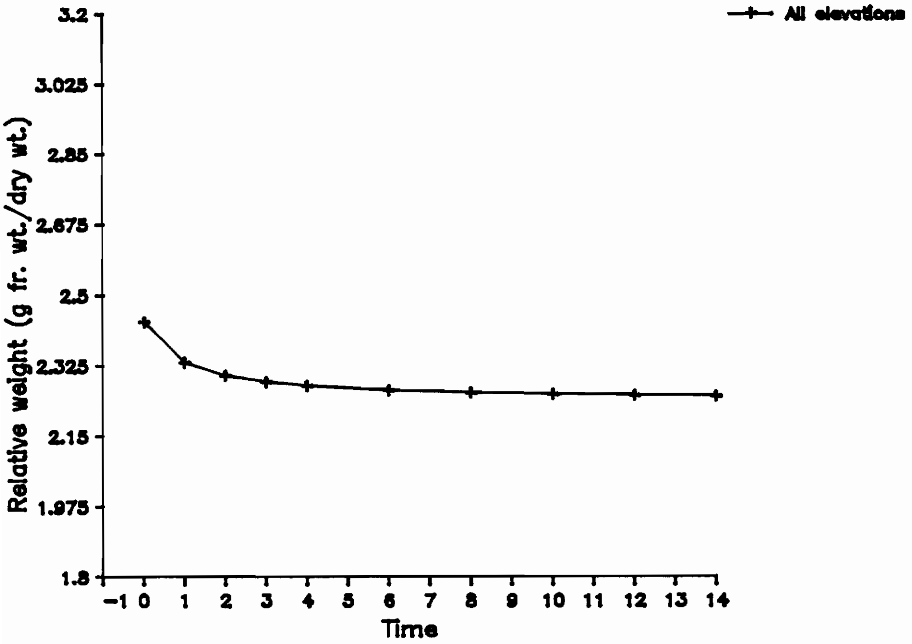

Figure 2c. Curve of mean relative weight loss over 14 hours for 3 elevations (in feet) combined (no significant difference between elevations) for collection 4, field fir. Curve equation is: LogRWT = 0.969 + 0.085(1/Time) - 0.099(Dry wt.).

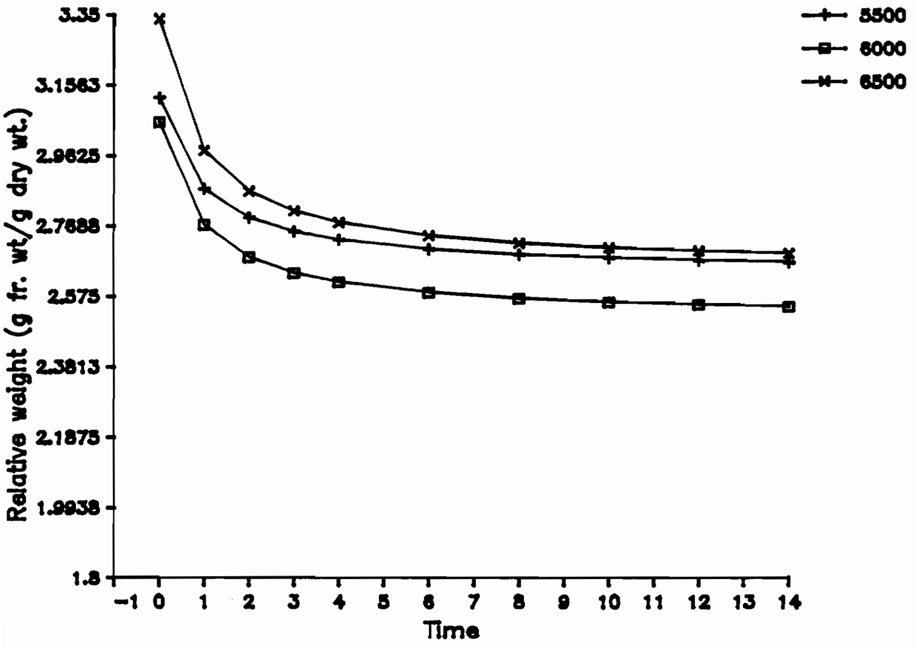

Figure 3a. Curves of mean relative weight loss over 14 hours for 3 elevations (in feet) for collection 2, field spruce. Individual curve equations are: 5500 feet, logRWT = 0.970 + 0.166(1/Time); 600 feet, logRWT = 0.923 + 0.195(1/Time); 6500 feet, logRWT = 1.020 + 0.230(1/Time) - 0.036(Dry wt.).

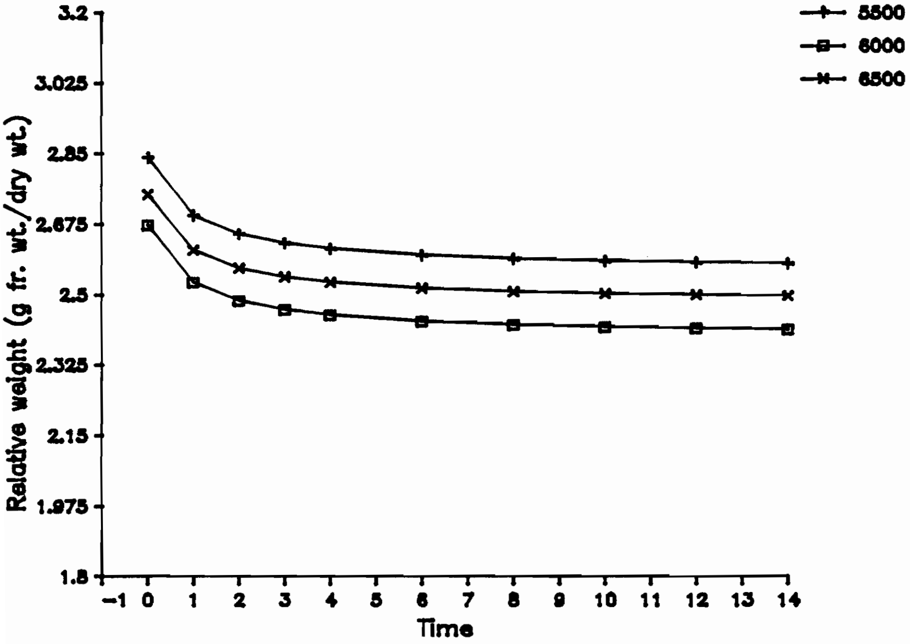

Figure 3b. Curves of mean relative weight loss over 14 hours for 3 elevations (in feet) for collection 3, field spruce. Individual curve equations are: 5500 feet, logRWT = 0.982 + 0.104(1/Time - 0.030(Dry wt.); 6000 feet, logRWT = 0.874 + 0.109(1/Time); 6500 feet, logRWT = 0.972 + 0.103(1/Time) - 0.053(Dry wt.).

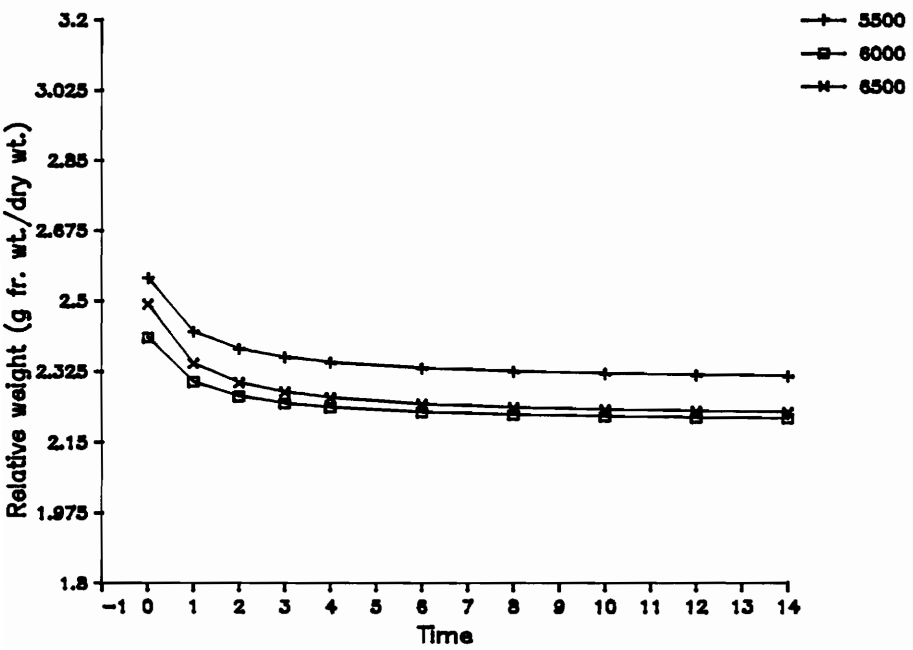

Figure 3c. Curves of mean relative weight loss over 14 hours for 3 elevations (in feet) for collection 4, field spruce. Individual curve equations are: 5500 feet, logRWT = 0.944 + 0.107(1/Time) - 0.100(Dry wt.); 6000 feet, logRWT = 0.876 + 0.093(1/Time) - 0.061(Dry wt.); 6500 feet, logRWT = 0.888 + 0.122(1/Time) - 0.088(Dry wt).

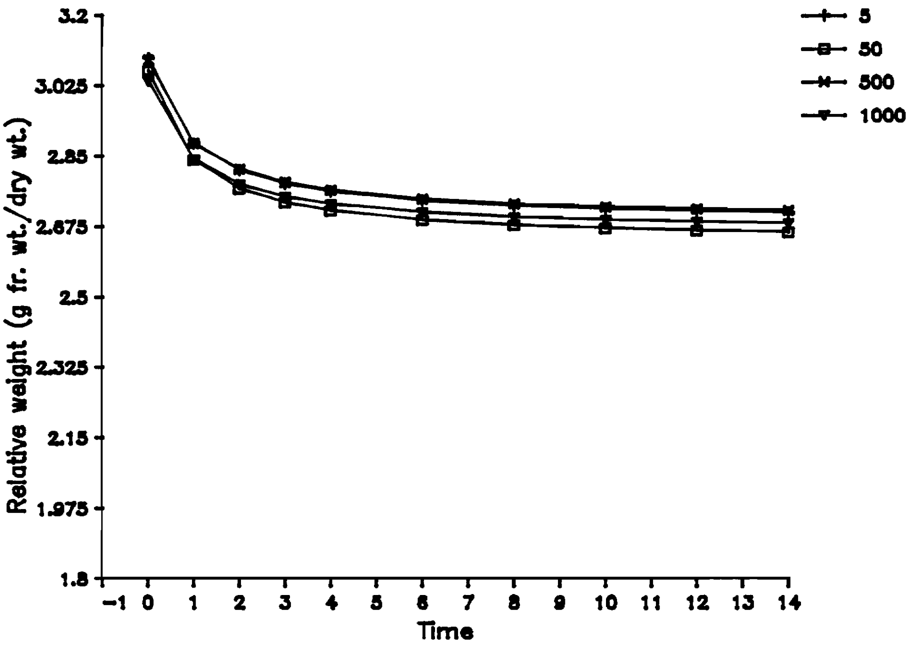

Figure 4a. Curves of mean relative weight loss over 14 hours for 4 nitrate concentration levels (in ppm) for collection 2, shadehouse fir. Individual curve equations are: 5ppm, logRWT = 0.987 + 0.142(1/Time); 50 ppm, logRWT = 0.902 + 0.150(1/Time) + 0.069(Dry wt.); 500 ppm, logRWT = 0.947 + 0.138(1/Time) + 0.034(Dry wt.); 1000 ppm, logRWT = 0.978 + 0.133(1/Time).

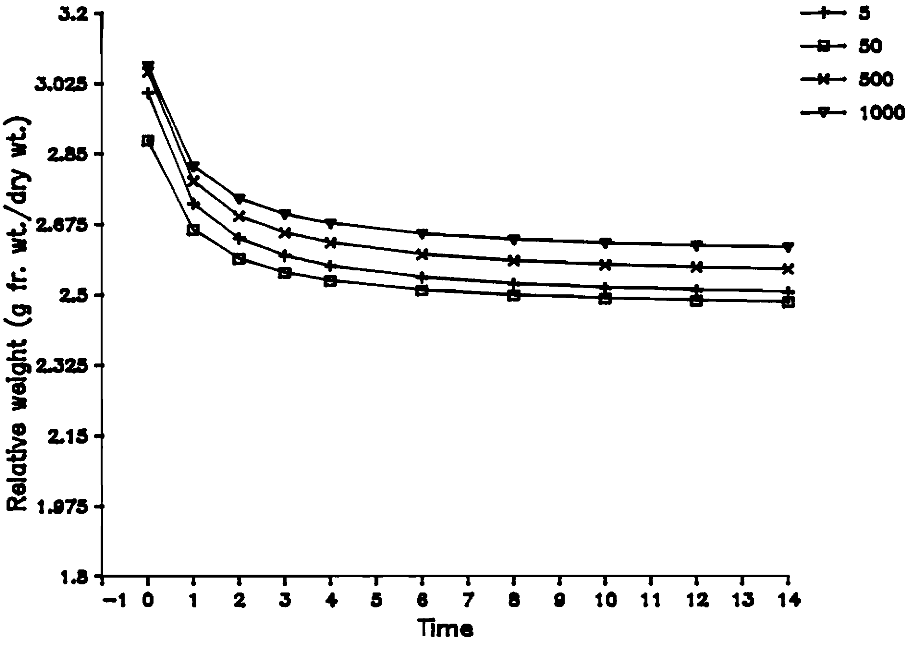

Figure 4b. Curves of mean relative weight loss over 14 hours for 4 nitrate concentration levels (in ppm) for collection 3, shadehouse fir. Individual curve equations are: 5 ppm, logRWT = 0.905 + 0.193(1/Time); 50 ppm, logRWT = 0.898 + 0.161(1/Time); 500 ppm, logRWT = 0.892 + 0.188(1/Time); 1000 ppm, logRWT = 0.950 + 0.171(1/Time).

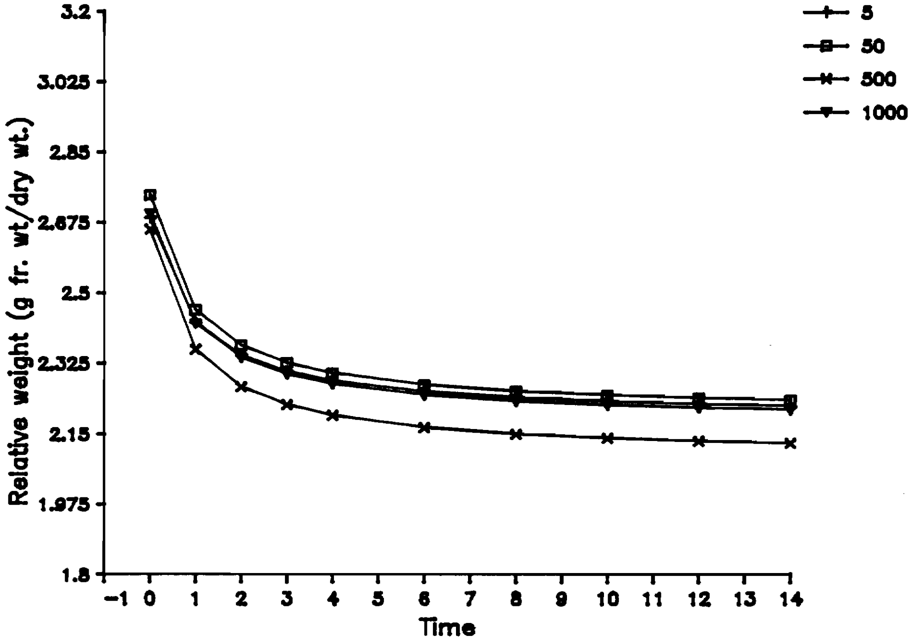

Figure 4c. Curves of mean relative weight loss over 14 hours for 4 nitrate concentration levels (in ppm) for collection 4, shadehouse fir. Individual curve equations are: 5 ppm, logRWT = 0.783 + 0.205(1/Time); 50 ppm, logRWT = 0.740 + 0.220(1/Time) + 0.048(Dry wt.); 500 ppm, logRWT = 0.699 + 0.239(1/Time) + 0.027(Dry wt.); 1000 ppm, logRWT = 0.725 + 0.213(1/Time) + 0.043(Dry wt.).

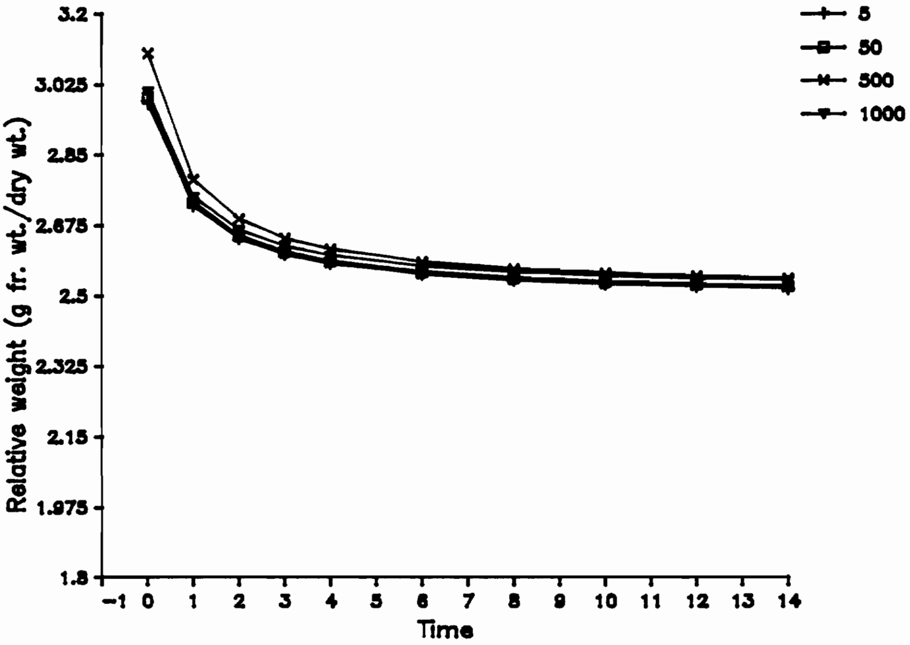

Figure 5a. Curves of mean relative weight loss over 14 hours for 4 nitrate concentration levels (in ppm) for collection 2, shadehouse spruce. Individual equations are: 5 ppm, logRWT = 0.911 + 0.180(1/Time); 50 ppm, logRWT = 0.860 + 0.184(1/Time) +0.130(Dry wt.); 500 ppm, logRWT = 0.917 + 0.214(1/Time); 1000 ppm, logRWT = 1.041 + 0.182(1/Time) - 0.275(Dry wt.).

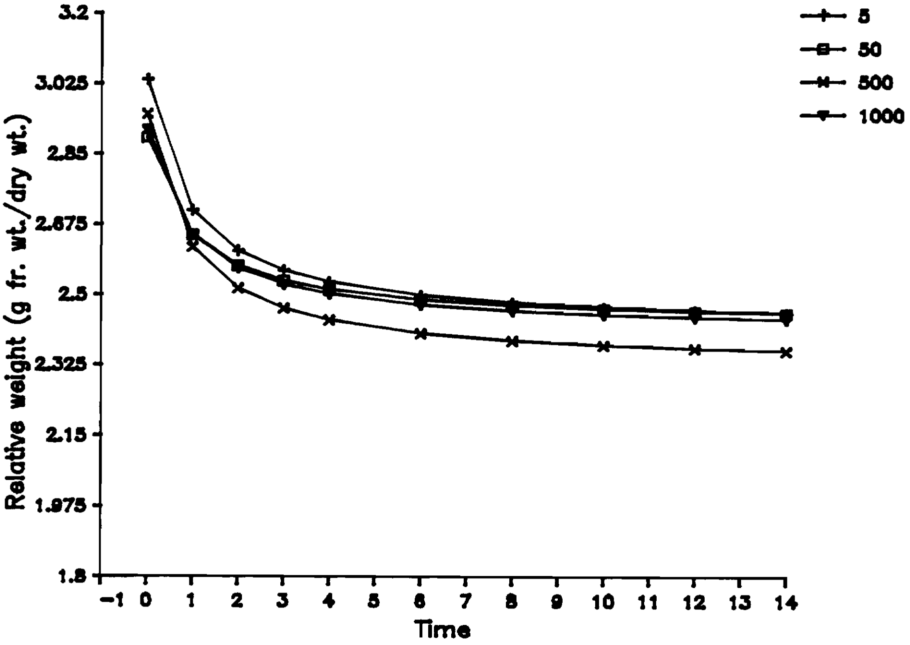

Figure 5b. Curves of mean relative weight loss over 14 hours for 4 nitrate concentration levels (in ppm) for collection 3, shadehouse spruce. Individual curve equations are: 5 ppm, logRWT = 0.992 + 0.227(1/Time) - 0.246(Dry wt.); 50 ppm, logRWT = 0.960 + 0.176(1/Time) - 0.155(Dry wt.); 500 ppm, logRWT = 0.752 + 0.239(1/Time) - 0.268(Dry wt.); 1000 ppm, logRWT = 0.979 + 0.190(1/Time) - 0.232).

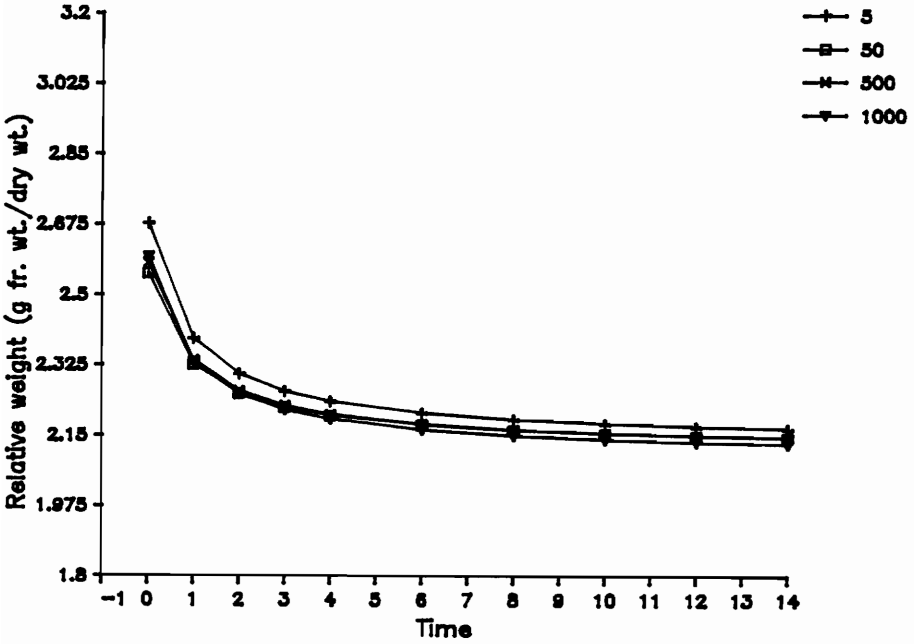

Figure 5c. Curves of mean relative weight loss over 14 hours for 4 nitrate concentration levels (in ppm) for collection 4, shadehouse spruce. Individual curve equations are: 5ppm, logRWT = 0.881 + 0.223(1/Time) - 0.219(Dry wt.); 50 ppm, logRWT = 0.809 + 0.186(1/Time) - 0.084(Dry wt.); 500 ppm, logRWT = 0.822 + 0.198(1/Time) - 0.124(Dry wt.); 1000 ppm, logRWT = 0.808 + 0.212(1/Time) - 0.097(Dry wt.).

hour 1 are much smaller relatively. With stomata closed, the cuticle provides a high degree of resistence to water loss as can be seen after hour 3 in Figures 2-5.

Elevation/Nitrate Concentration Level Effects

There were no differences in initial RWT between treatment levels either in the field or shadehouse (Table 5). In addition, no increasing or decreasing trends with treatment level were found within each group. DeLucia and Berlyn (1984) found significant differences in mean RWT between the lowest and highest elevations on Mt. Moosilake, but not between the middle elevation and either of the other two (732m, 1143m, and 1402m). In most studies a direct relationship has been found between increasing elevation and transpirational water loss which has been strongly associated with amount and thickness of epicuticular wax (Baig and Tranquellini,1976; Sowell et al.,1982; DeLucia and Berlyn,1984). It is not surprising that no treatment level differences were found as wax weight per unit measurement did not differ between treatment levels at any of 4 sample collections either.

Most studies of this type have tested samples from below, at, or above the natural treeline, with contrasting environments of wind, temperature, and degree of insolation between the sites. Timberline in sites in those studies was established partly as a result of the inability of foliage to resist excessive transpiration stimulated by increases in foliar temperatures from solar radiation.

TABLE 5. Average initial relative weight of field and shadehouse grown seedlings at each treatment level.

Treatment	Species	Treatment Level			
		Elevation (feet)			
		5500	6000	6500	
Elevation	Fraser fir	2.77[1]	2.74	2.73	
	Red Spruce	2.80	2.67	2.81	
		NO_3 Concentration (ppm)			
		0005	0050	0500	1000
Shadehouse	Fraser fir	2.88	2.84	2.83	2.87
	Red Spruce	2.83	2.74	2.82	2.81

[1] No significant differences were found between treatment levels in the field or in the shadehouse at the p = .1 level.

The lower intensity of the responses in this study compared to others of this type can be explained by the fact that actual treeline limits are approached only on a few of the most exposed ridgelines of the Black Mountains. The temperature differences between plots at the three elevations sampled is probably not greater than 1.5°C, the standard elevation lapse rate, at any one time. Wind can decrease temperatures somewhat, but all sampled plots were relatively protected from wind.

Temporal Treatment Effects

RWT is the average initial FWDW ratio which reflects differences in the relative amount of water held by groups over 14 hours. RWT decreased significantly with each collection except collection 3 in shadehouse spruce (Table 6). It is not unexpected for needle biomass to increase over the summer from its initial succulent state, keeping more water per gram dry weight over time.

Athough useful for measuring differences in FWDW ratio between a treatment level at any one time, RWT does not indicate actual rates of transpirational water loss over a single experiment. The more relavant information is that of the difference in relative weight of each group of samples at the beginning and end of each experiment, or transpirational water loss over time. Because significant differences in the change in FWDW ratio over time were not found between treatment levels for any particular species or group, treatment levels were grouped at each collection.

TABLE 6. Average initial relative weight of field and shadehouse grown seedlings at each sample collection.

Treatment	Species	Sample Collection		
		2	3	4
Elevation	Fraser fir	3.03a[1]	2.80b	2.40c
	Red Spruce	3.07a	2.74b	2.47c
Shadehouse	Fraser fir	3.02a	2.92b	2.62c
	Red Spruce	2.96a	2.90a	2.52b

[1] Values in rows followed by the same letter were not significantly different at the p = .1 level by Tukey's range test.

Significant differences were not always found, but the amount of water transpired, or the change in FWDW ratio, did change over the growing season (Table 7). FWDW ratios remained stable or decreased with each collection in field seedlings and increased with each collection in shadehouse seedlings.

Marchand and Chabot (1978) also found that transpiration rates of Abies balsamea, Picea mariana, and Betula papyrifera in the field declined over the growing season, with maximum water loss early in the summer and minimum loss during winter months. Similar results were found by Sowell et al. (1982).

Field spruce FWDW ratios showed much greater change over time in July (collection 2) than in October (collection 4) indicating that the ability to resist water loss was much more developed at the end of the growing season than at the beginning. Field fir values did not decrease in the same manner, but remained low throughout the growing season. At collection 2, most sample trees in the field appeared to have flushed, with needles fully elongated, but many may still have had succulent tissue more susceptible to excessive transpiration as July temperatures warmed foliage. By collection 3 (late August) the change in FWDW ratio over time had decreased to a value equivalent to that seen at collection 4.

Field fir showed the least amount of transpirational water loss at all collections over the growing season. Field spruce values at collection 2 were relatively high, but decreased to a rate similar to field fir at collection 3. Observations in the field indicated

TABLE 7. Percent of total water lost in transpiration over 14 hours in 4 groups and 3 collections.

Treatment	Species	Sample Collection		
		2	3	4
Elevation	Fraser fir	0.14[1]a[2]	0.13a	0.14a
	Red Spruce	0.32a	0.17b	0.17b
Shadehouse	Fraser fir	0.21a	0.25b	0.32c
	Red Spruce	0.27a	0.30ab	0.33b

[1] Percent water lost was computed as the difference in fresh sample weight between the beginning and end of a single experiment divided by the total water in an average foliage sample.

[2] Values in rows followed by the same letter were not significantly different at the $p = .1$ level by Tukey's range test.

that, on the whole, spruce trees broke bud after fir trees. Even at collection 2 (mid July), some spruce trees had only recently broke bud and many samples had obvious succullent tissue, whereas this characteristic was not observed on fir sample trees. This lag in shoot flush could account for the high rate of transpirational water loss seen at collection 2 in field spruce.

Despite the high transpiration rate at collection 2, the cuticle appeared to be fully developed, or close to complete, in field samples. This is in agreement with others who state that wax development proceeds with needle elongation and that the cuticle is fully developed when elongation ceases (Martin and Juniper,1970; Hallum,1980).

Shadehouse spruce and fir responded to nitrate treatments in a manner opposite that of field seedlings, and cuticular transpiration generally increased with each collection over the growing season (Table 7). Although mean RWT was of about the same order at each collection (Table 6), most likely cuticle development was complete before collection 2 and continual degradation by raindrops may have been responsible for the progressive increase in transpiration rates throughout the growing season. In wax content per unit measurement experiments, WWTSA and WWTDWT also decreased in the latter half of the growing season in shadehouse seedlings, with raindrop impact again the probable cause of continual decrease.

Environmental factors are most likely responsible for the contrasting trends of field and shadehouse seedlings; in particular, rainfall and growing season temperatures. Throughout the summer, air temperatures were 10-20°C higher in Blacksburg than in the Black Mountains and the date of last frost was much earlier in Blacksburg. The Black Mountains received below normal amounts of precipitation in July and August of 1987 while rainfall was obviously not a limiting factor for shadehouse seedlings.

If cuticles were fully developed on foliage of shadehouse seedlings well before the second sample collection (budset was in progress by June 21, 1987), progressive wax degradation and consequent increased cuticular transpiration could occur throughout the summer. If, on the other hand, the growing season, and therefore cuticle formation, began later in the summer, full cuticle development and/or needle hardening may have reached completion at a much later date in the field than in the shadehouse. This, coupled with the absence of degrading forces of rainfall in the field, would allow for a decrease in the relative amount of water transpired between collections 2 and 3. It is quite possible that foliage had not fully finished elongation and wax development in the field by collection 2, as had originally appeared.

Another possible explanation would be acclimation made by field spruce and fir to prolonged water deficits in July and August. At the onset of water stress in July, osmotic adjustments may have taken place, decreasing the vapor pressure deficit between leaves and surrounding air. This could have contributed to smaller transpirational losses at collections 2 and collection 3. Normal processes of dormancy probably played a larger role in the decrease in the change in FWDW ratio seen at collection 4 as normal rainfall amounts had resumed in the Black Mountains by the September.

Shadehouse seedlings did not mimic transpirational rate trends of field seedlings over the growing season because of the difference in growing season environments between the Black Mountains and Blacksburg. Therefore it is difficult to determine if shadehouse seedling responses accurately predicted the responses of field seedlings. Despite these trends, significant differences in the change in FWDW ratio over time between elevation/nitrate treatment levels were not found in any group. As in the wax content experiments, neither spruce nor fir responded to nitrate treatment levels.

RELATIONSHIP OF CLIMATIC VARIABLES AND ANNUAL LEADER GROWTH OF FRASER FIR AND RED SPRUCE

Internodal distances on red spruce and Fraser fir were measured in the field in the summer of 1987. Local climatic data was also collected from NOAA records of the Mt. Mitchell area. The climatic parameters used to predict annual leader growth (measured as internodal distance) and their descriptions are listed in Table 8 and can be most generally grouped as parameters of growing season precipitation, growing season temperature, and unseasonably warm mid-winter temperatures. The previous year's values of these parameters were also modelled.

The 10 parameters listed in Table 8 were chosen because they have been used successfully in past studies to predict tree ring widths, a growth characteristic which closely parallels that of annual leader growth (Cook et al.,1987; Johnson et al.,1986). In order to avoid spurious correlations or collinearity generated by excessive parameters, each variable was regressed independently against internodal distance initially, then in combination with other parameters considered "best" by means of C_p and Maximum R^2 diagnostic statistics in stepwise analyses. Both simple linear and multiple regression analyses were used to investigate potential models. Because of the problem of autocorrelation in time-series models, internodal distance was initially regressed against year (spruce) or year + $year^2$ (fir) and the other climatic parameters

TABLE 8. Climatic variables used to predict annual leader growth of Fraser fir and red spruce[1].

Parameter	Variable Name	Description
Precipitation	GSPPT	Sum of current year growing season precipitation including June, July, August, and September
	PRGSPPT	Sum of previous year growing season precipitation including June, July, August, and September
Temperature	JUNF	Average temperature over month of June of current year
	PREJUNF	Average temperature over month of June of previous year
	JULF	Average temperature over month of July of current year
	PREJULF	Average temperature over month of July of previous year
Unseasonably warm winter temperature	TOTJAN	Total number of days >=40°F in January of current year
	PRTOTJAN	Total number of days >=40°F in January of previous year
	TOTFEB	Total number of days >=40°F in February of current year
	PRTOTFEB	Total number of days >=40°F in February of previous year

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[1] No variables were found to be significant in the model at the p = .1 level after removal of the effects of height growth with time.

were then regressed against the residuals of the appropriate species model.

The regression of annual leader growth (ALG) of fir over time alone, $ALG = 26053.84 - 264.4(YEAR) + 0.671(YEAR^2)$, had an R^2 value of .92. The higher order of the equation suggests that height growth begins earlier in fir than in spruce. The regression of annual leader growth of spruce over time was $ALG = -1138.5 + 0.583(YEAR)$ with $R^2 = .86$. (Figure 6).

Although absolute age was not measured on these trees, between 15 and 20 whorl scars were counted on most trees, so it was assumed that average tree age was somewhere between 15 and 20 years. Because red spruce is a slower growing tree than Fraser fir (Harlow et al.,1979), it is not unexpected that Fraser fir shows exponential growth for the years 1967 through 1987, rather than more linear growth as is seen in red spruce for the same years.

After collection of the data, it became apparent that internodal distance could not simply be predicted from climatic parameters because all measured trees were currently experiencing typical rapid height growth following an establishment phase of about 10 years. Height growth in Fraser fir and red spruce is very slow initially while seedlings establish themselves (Harlow et al., 1979). Conifers in general exhibit a short establishment phase followed by a 20-30 year period of rapid height growth until maturity is approached (Avery and Burkhart, 1983). During this rapid growth phase understory trees may be relatively insensitive to

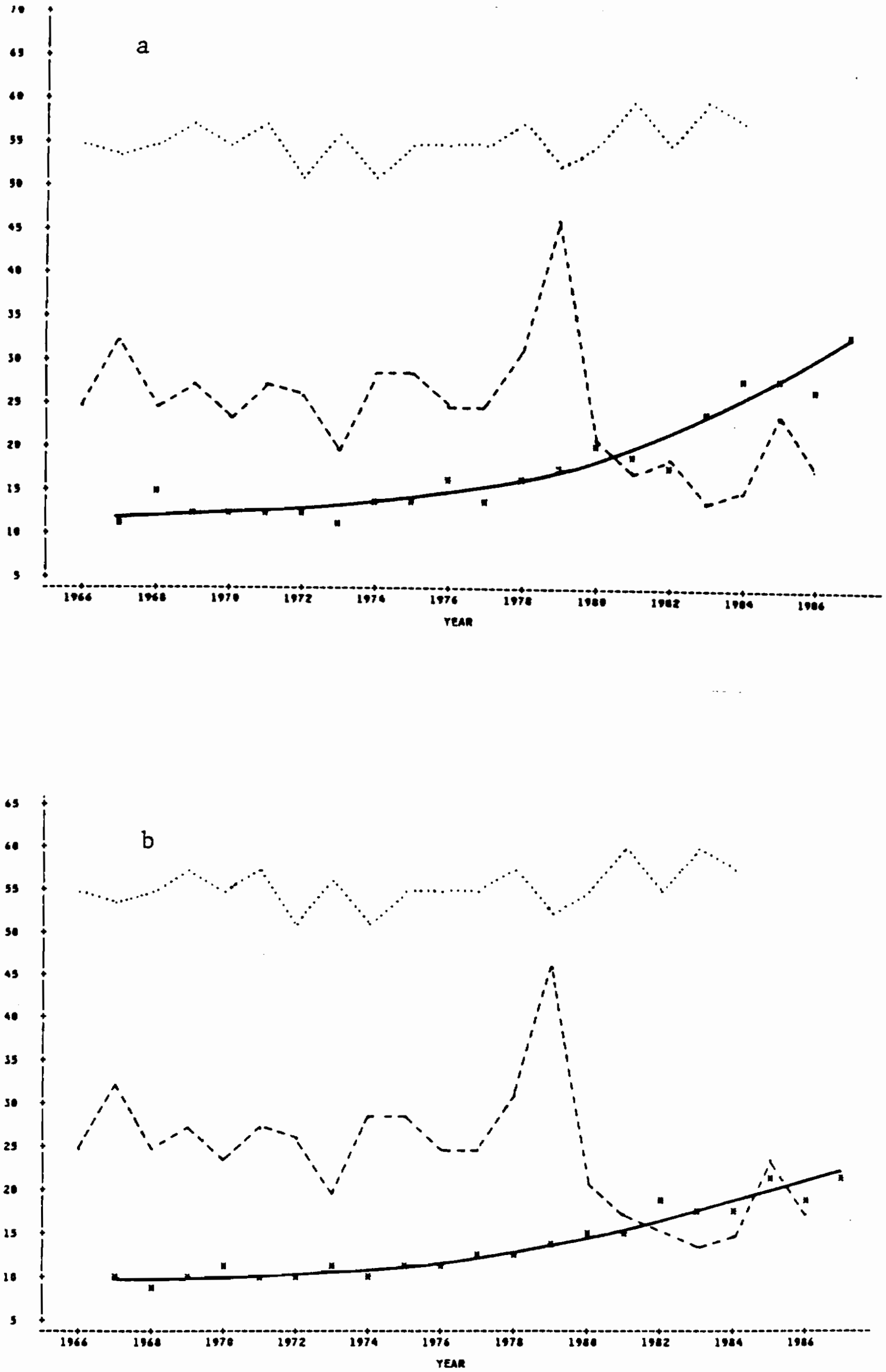

Figure 6. Relationship of the climatic variables, Growing Season precipitation (- - -), and Mean June Temperature (.....) to annual leader growth(_____) of Fraser fir (a) and red spruce (b) over the years 1967 to 1987. Y-axis units are in inches (growing season precipitation), °F (June temperature),or centimeters (annual leader growth).

deviations in normal climatic characteristics because of the buffer provided by the overstory. However, this is not necessarily true of understory trees in openings, which will show greater response to climatic changes because of the increased exposure to weather elements.

Jacoby (1984, unpublished study) found that June rainfall singularly explained 56.4% of tree ring width variation in Loblolly pines in Arkansas and Oklahoma. Johnson et al. (1986) determined that periods of warm late summer temperatures (August) in combination with cold early winter temperatures (December) coincided with periods of reported red spruce decline in the Northern Appalachians during at least two periods in the past 150 years. Both periods, 1871 to 1885 and 1962 to the present.

Cook et al. (1987) also suggest the importance of warm August/cold December temperatures and possible effects of warm, late autumns on carbon allocation in relation to dormancy initiation of red spruce. In addition, they suggested that previous year temperatures were more important than previous year periodic rainfall or current year temperatures or rainfall in tree ring response because of its effect on bud set and shoot formation in the following year. The latter two studies both suggested observed declines may be much too widespread over tree age classes, elevation, and geographical location to be attributed to atmospheric pollutants alone which tend to cause damage in more of a gradient-like manner. Nevertheless, atmospheric pollutants may further weaken trees under climatic stress.

The three different conclusions of these studies demonstrate the widely varying opinions as to most influential climatic factors on tree ring widths over time, but all seem to agree that natural environmental causes of reported declines may be more important than anthropogenic causes. The hypothesis generated in this study was that at least one climatic parameter, if not more, would influence annual leader growth in high elevation fir and spruce. The parameters chosen were felt appropriate as the spruce-fir ecosystem was not expected to deviate much from most other ecosystems in the general effects of climate on annual leader growth. Namely, annual leader growth should increase with increasing growing season precipitation and increasing growing season temperatures and decrease with increasing number of unseasonably warm winter days. The effects of growing season temperature and precipitation on height growth are well documented (Kramer and Kozlowski, 1979; Kozlowski,1979) and unseasonably warm winter days act to increase transpiration with adverse effects on bud survival (Hygen,1963; Cannell and Smith,1984; Friedland et al.,1984).

In Figure 6 an obvious change in average annual growing season precipitation is seen in 1979 and between 1980 and 1987. These two precipitation shifts, especially the one between 1980 and 1987, could be expected to affect a decrease in internodal distance in older trees, as both red spruce and Fraser fir are relatively drought intolerant (USDA, 1965). But in fact, the effects of this period of below normal precipitation may have been "washed out" by the current juvenile growth beginning around 1972 in both species. Had mature trees or immature trees in gaps been measured, it is quite possible that the response of annual leader growth to changes in climatic parameters would have been more evident. Unfortunately destructive sampling, the only way to measure internodal distance on mature trees, was not within the scope of this study.

DESICCATION INJURY OVER THE WINTER OF 1988

All field sample trees were given ratings before and after the winter of 1988 to determine if winter injury symptoms occurred over the winter of 1988. Contingency table analysis was used to determine if the degree of winter injury symptoms differed significantly between elevations.

Both field spruce and fir sample trees were given lower ratings in the spring of 1988 than in the summer of 1987 (Table 9). Red spruce trees with ratings of 1 averaged 10% of total sample trees in 1987, decreasing to 4.4% in 1988. Fir with ratings of 1 averaged 16.4% in 1987 and decreased to 8.7% in 1988. The majority of trees given a rating of 1 were seen at 6500 feet in 1987 and at 5500 feet in 1988. Only 1 tree was given a rating greater than 1 in either 1987 or 1988. Ratings assigned trees at each elevation are listed in Table 6.

Only one plot at 6500 feet contained trees with injured foliage similar to winter desiccation injury described by other authors (Curry and Church,1952; Horntvedt and Venn,1980; Friedland et al.,1984). Injury was seen in the most current foliage only and more injury was seen at the top of trees than farther down the crown. Injury was noted on both spruce and fir on this plot, but was more prevalent on spruce than on fir.

The observations in this study agree with those of Horntvedt and Venn (1980) on young <u>Picea abies</u> (L. Karst.), 1-8 meters in

TABLE 9. Ratings given and occurrence of winter injury symptoms on field spruce and fir before and after the winter of 1988 at 3 elevations[1].

		Injury Rating							
		1				2			
		Spruce		Fir		Spruce		Fir	
Elevation (feet)	n[2]	1987	1988	1987	1988	1987	1988	1987	1988
		number[3]							
5500	66(1987) 50(1988)	3	1	1	3	0	1	0	0
6000	56(1987) 40(1988)	3	1	4	2	0	0	0	0
6500	46	3	1	5	1	0	0	0	0

---

[1] Ratings: 0: healthy tree, no sign of any desiccation damage and all needles green and present, 1: 1-25% of the needles brown, damaged, or missing, 2: 26-50% of the needles brown, damaged, or missing, 3: 51-75% of the needles brown, damaged, or missing, 4: 76-99% of the needles brown, damaged, or missing (but tree still living), 5: tree dead.

[2] n= number of trees sampled in all plots at a specific elevation.

[3] All other trees had ratings of 0.

height, in southeastern Norway. They observed injury on the most recent foliage, injury at random throughout the stand, a large variation in the degree of injury to each tree, and no relationship of injury to elevation, aspect, or terrain. The observations of winter injury symptoms made on the one high elevation plot in this study were very similar but of lesser magnitude. The Southern Appalachians, although containing peaks of higher elevation than the study sites described in southeastern Norway (sites in Norway were not higher than 1640 feet), experience a warmer climate even in winter because of their more southerly latitude.

In 1988 the number of trees given ratings of 1 was 100% greater at 5500 feet than at 6500 feet, and 50% greater at 6000 feet than at 6500 feet. The percentage of total trees at each elevation was very small and the actual difference between elevations was not greater than 3 trees. If climate and/or input of atmospheric nitrates were to increase sensitivity of trees to winter desiccation damage, it would seem that the highest elevations would receive the greatest amount of injury, which was not observed in this study. Although classic winter injury symptoms were indeed seen only on a plot from the 6500 foot elevation band, the degree of injury was not severe enough on any single tree to warrant a rating of 1. Other trees not included in the sample but on and around the plot, showed a relatively small amount of damage.

Although trees with ratings of 1 were found at all elevations, little evidence of classic winter injury symptoms was seen except on the previously described plot. The factor more likely responsible for higher ratings was the amount of shading received by trees with ratings of 1 or 2. In 1987 47.4% and in 1988, 70% of the trees given 1's were located under moderate to heavy canopy cover, and absent or dead branches and needles were typically found in the lower half of the crown. For most trees the rating of 1 or 2 was not given for red needles, but for the effects of shading.

SUMMARY AND CONCLUSIONS

Summary

For all groups except shadehouse spruce, wax content per square centimeter or gram dry weight of foliage was not significantly different between treatments at the p = .1 level. Significant differences in wax content were found between sample collections, with wax weight per gram dry weight (WWTDWT) and wax weight per square centimeter foliage (WWTSA) values generally decreasing over the growing season after collection 2 (July 27). In shadehouse spruce, apart from highest WWTSA values being typically found in the 500 ppm treatment and WWTDWT values highest over all treatment levels at collection 1, no consistent trends in wax weight per unit measurement could be found.

Curves of cuticular transpiration over 14 hours were parallel in all groups overall, and no significant differences in slope could be found between treatment levels, collections, or groups. Initial relative weight did not differ between treatment levels at each collection and no consistent trends in RWT differences between elevations could be found. The change in FWDW ratio from the beginning to the end of each experiment increased or remained stable with each collection in field species and decreased with each collection in shadehouse species.

Climatic variables could not be used to model a relationship between climate and annual leader growth in this study because field trees were immature and experiencing rapid yearly height growth; any effects of climate were masked by accelerated leader growth. Fraser fir were found to have slightly more advanced growth than red spruce, although trees of both species were approximately the same age, between 15 and 20 years.

The number of sample trees given ratings of 1 decreased from the summer of 1987 to the spring of 1988. Overall, 9 spruce and 10 fir, out of 168 total trees were given ratings of 1 in 1987, and 4 spruce and 6 fir out of 136 total trees were given ratings of 1 or 2 in 1988. All other sample trees were rated 0. One plot at 6500 feet showed classic winter injury symptoms of red needles on most recent year branches only. This symptom was seen randomly throughout that plot, mostly on red spruce.

Conclusion

According to the results of this study, the presence or level of atmospheric NO_3 has no effect on susceptibility to winter desiccation injury in the Black Mountains of North Carolina. These results may not necessarily be found with duplicate experiments at other sites, especially more northern ones. Except for shadehouse spruce, wax weight per unit measurement showed no response to changes in predicted NO_3 levels at any sample collection either in the field or in the shadehouse. Similar responses were found in all groups with the transpiration rate experiments, in addition to a lack of differences in the curves of cuticular transpiration over time between elevation or NO_3 concentration level. Winter injury ratings before and after the winter of 1988 also showed no relationship between predicted treatment level and severity of rating, with symptom ratings actually decreasing after winter.

Decline symptoms have been described similarily to winter injury symptoms. Atmospheric nitrates have been cited as an aggravating influence on the degree of winter injury in high elevation spruce-fir forests. NO_3 may be a responsible injury factor in the Northern Appalachians, but this study indicates that neither the presence nor concentration of NO_3 has any injurious effect on cuticular transpiration or foliar wax content.

Other studies of this type have reported symptoms of winter injury to increase with proximity to sources of pollutants or with increasing elevation. But few, if any, have simulated field levels of nitrogen concentrations to determine responses of wax weight per unit of measurement or cuticular transpiration rate over time. These studies have been performed in the field, but not simulated experimentally in relation to NO_3 concentration.

Local climate may explain why results of tests in this study are not in agreement with results of similar tests from other studies. The Southern Appalachians have a milder winter climate than the Northern Appalachians or Northern European mountains where most other study sites are located. This milder climate results in a longer growing season and greater development of protective tissues, thus trees which are better able to resist winter desiccation injury. Results of this study indicate only that atmospheric nitrogen has no effect on susceptibility or occurrence to winter desiccation injury in the Southern Appalachians, where growing seasons are longer. Because of the variable local climates, these results should not be applied to other areas.

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Appendix A. Elevations, species sampled, and number of foliage samples collected (in parentheses) per collection trip, of plots sampled in the Black Mountains.

Plot Number	Elevation (Feet)	Species Sampled
B39	5500	Spruce (6), Fir (10)
B40	6000	Spruce (6), Fir (10)
B42	6000	Spruce (10), Fir (10)
B44	6500	Spruce (7), Fir (10)
B46	5500	Spruce (10)
B47	5500	Spruce (10), Fir (10)
B48	5500	Spruce (10), Fir (8)
B50	6000	Spruce (10), Fir (10)
B52	6500	Fir (10)
B54	6500	Spruce (9), Fir (10)

Appendix B. Rainfall simulator treatment schedule[1]

Month	Number of days per week rainfall treatments applied[2]
May	5
June	4
July	3
August	3
September	2
October	2

---

[1] Based on average days of precipitation per month over years 1981, 1982, 1983, 1984, 1985.

[2] Based on a rainfall rate of 0.75cm per treatment.

APPENDIX C. Two-factor ANOVA on epicuticular wax content per unit of measurement for field and shadehouse grown spruce and fir.

FIELD

Source of Variation	DF
Elevation	2
Time	3
Elevation x Time	6
Experimental Error	24
Total	35

SHADEHOUSE

	DF	
Source of Variation	Fir	Spruce
NO_3 Concentration	3	3
Time	3	3
NO_3 Conc. x Time	9	9
Experimental Error	80	128
Total	95	143

APPENDIX D. Two-factor ANOVA on cuticular transpiration rate over 14 hours for field and shadehouse grown spruce and fir.

FIELD

Source of Variation	DF
Elevation	2
Time	2
Elevation x Time	4
Experimental Error	19
Total	27

SHADEHOUSE

	DF	
Source of Variation	Fir	Spruce
NO_3 Concentration	3	3
Time	2	2
NO_3 Conc. x Time	6	6
Experimental Error	60	96
Total	71	107

Appendix E. Computer Documentation

The following pages contain computer file documentation which is representative of the data files used in various analyses in this thesis project. Files are grouped by the particular study to which they apply. Also included are some file manipulations which were used to transform raw data to a more usable condition.

Appendix E1. Computer documentation for wax content study.

```
*******FILE:  WAX DATA  *******************
   EXPERIMENT:  EPICUTICULAR WAX PER UNIT MEASUREMENT.  FILES WITH THIS T
                YPE OF FILENAME ARE THE RAW DATA FOR WAX CONTENT OF FEILD
                AND SHADEHOUSE SAMPLES OVER 4 COLLECTIONS (OVER 1987
                GROWING SEASON), 2 SPECIES IN THE BLACK MTN., NC.  FILES
                WHICH HAVE THIS SETUP AND SIMILAR VARIABLES ARE:
FWAX1 DATA (FIR, FIELD, COLLECTION 1)
FWAX2 DATA (FIR, FIELD, COLLECTION 2)
FWAX3 DATA (FIR, FIELD, COLLECTION 3)
FWAX4 DATA (FIR, FIELD, COLLECTION 4)
SWAX1 DATA (SPRUCE, FIELD, COLLECTION 1)
SWAX2 DATA (SPRUCE, FIELD, COLLECTION 2)
SWAX3 DATA (SPRUCE, FIELD, COLLECTION 3)
SWAX4 DATA (SPRUCE, FIELD, COLLECTION 4)
FGHWAX1 DATA (FIR, GREENHOUSE, COLLECTION 1)
FGHWAX2 DATA (FIR, GREENHOUSE, COLLECTION 2)
FGHWAX3 DATA (FIR, GREENHOUSE, COLLECTION 3)
FGHWAX4 DATA (FIR, GREENHOUSE, COLLECTION 4)
SGHWAX1 DATA (SPRUCE, GREENHOUSE, COLLECTION 1)
SGHWAX2 DATA (SPRUCE, GREENHOUSE, COLLECTION 2)
SGHWAX3 DATA (SPRUCE, GREENHOUSE, COLLECTION 3)
SGHWAX4 DATA (SPRUCE, GREENHOUSE, COLLECITON 4)
   COLLECTION DATE:  WEEK OF     7/27/87
   STAT DESIGN:  COMPLETE RANDOMIZED,TWO WAY FACTORIAL WITH
                 ELEV (NLEVEL) AND COLLECTION AS FACTORS
   VARIABLES:  ELEV REDUCED BY A FACTOR OF TEN (550=5500 FEET)
               NLEVEL IS NOT TRANSFORMED
   COLLECTOR:  SUSAN ERWIN;
*PLT IS PLOT NUMBER, REFER TO ERWIN THESIS FOR PLOT LOCATIONS AND
     DESCRIPTIONS IN THE BLACK MOUNTAINS;
*SAMPLE IS SAMPLE NUMBER;
*ELEV IS ELEVATION BAND (5500, 6000, 6500) IN FEET;
*SPP IS SPECIES--SPRUCE OR FIR=S OR F;
*IWT=INITIAL TUBE WEIGHT IN GRAMS;
*FWT=FINAL TUBE WEIGHT IN GRAMS;
*SA1=SQCM SURFACE AREA OF FOLIAGE MEASUREMENT NO. 1 (OF 3);
*SP=FOLIAGE SAMPLE DRY WEIGHT WITH PAPER;
*PAP=WEIGHING PAPER WEIGHT;
*SA=AVERAGE SURFACE AREA (OF 3 MEASUREMENTS) IN SQUARE CENTIMETERS;
*DWT=FOLIAGE SAMPLE DRY WEIGHT IN GRAMS;
*WWTSA=WAX WEIGHT PER SQCM.;
*WWTDWT=WAX WEIGHT PER GRAM DRY WEIGHT;
CARDS;
B54 S 01 650 7.07956 7.08109 1.74 1.69 1.62 0.3209 0.2123
B54 S 02 650 7.78679 7.78809 0.48 1.08 1.01 0.2829 0.2137
B54 S 03 650 6.33873 6.34025 1.08 1.30 1.48 0.3159 0.2122
```

:

Appendix E2. Computer documentation for cuticular transpiration study.

```
DATA RATES;
INPUT PLT$ ELEV$ SPP$ SAMPLE$ DRYWT T0 T1 T2 T3 T4 T6 T8 T10 T12 T14;
FORMAT PLT$ 3.0 ELEV$ 3.0 SPP$ 1.0 SAMPLE$ 2.0 DRYWT 6.4 T0 5.2 T1 5.2
  T2 5.2 T3 5.2 T4 5.2 T6 5.2    T8 5.2 T10 5.2
  T12 5.2 T14 5.2;
****************FILE: TRANRUN DATA***************************
'TRANRUN2 FLDFIR'=TRANSPIRATION EXPERIMENT,COLLECTION 2, FIELD FIR
THIS FILE HAS THE RAW DATA FOR THE CUTICULAR TRANSPIRATION EXPERIMENTS.
THE DATA ARE THE WEIGHTS OF CLIPPINGS DURING THE EXPERIMENT, ON AN
HOURLY OR BI-HOURLY BASIS.  REFER TO ERWIN THESIS FOR FULL EXPLANATION
OF THE EXPERIMENT.  MEASUREMENTS ARE IN GRAMS AND ARE UNTRANSFORMED.
VARIABLES:  PLT=PLOT NUMBER IN THE BLACK MTN. REFER TO THESIS FOR
                LOCATION AND DESCRIPTIONS
            ELEV=ELEVATION BAND (5500, 6000, OR 6500) (550=5500 FEET)
            SPP=SPECIES, SPRUCE (S) OR FIR(F)
            SAMPLE=INDIVIDUAL CLIPPING IDENTIFICATION FROM PLOT
            DRYWT=OVEN-DRY WEIGHT OF SAMPLES
            T0=WEIGHT OF CLIPPING AT INITIATION OF EXPERIMENT
               FRESH WEIGHT OF SAMPLE
            T1=WEIGHT OF CLIPPING AFTER ONE HOUR OF EXPERIMENT
            T12=WEIGHT OF CLIPPING AFTER 12 HOURS OF EXPERIMENT
DRYWT IS OVEN DRY WEIGHT OF TWIGS + NEEDLES
RELATED FILES: TRANRUN2 FLDFIR      TRANRUN2 GHFIR
               TRANRUN3 FLDFIR      TRANRUN3 GHFIR
               TRANRUN4 FLDFIR      TRANRUN4 GHFIR
               TRANRUN2 FLDSPR      TRANRUN2 GHSPR
               TRANRUN3 FLDSPR      TRANRUN3 GHSPR
               TRANRUN4 FLDSPR      TRANRUN4 GHSPR
****************************************************************;
CARDS;
B39 550 F 01 3.1567 10.33 10.20 10.14 10.07 10.02 9.87 9.78 9.73 9.58 9.48
B39 550 F 02 2.2467 7.09 7.00 6.92 6.85 6.79 6.65 6.56 6.46 6.40 6.29
B39 550 F 03 0.6812 2.42 2.27 2.25 2.24 2.20 2.20 2.14 2.13 2.07 2.04
```

```
DATA WEIGHT;
INPUT PLOT$ SPP$ IND$ TYPE$ BOTH ENV;
************FILE:  TRUN DATA*****************************************
EXPERIMENT: THIS FILE GIVE THE OVEN-DRY WEIGHTS OF SAMPLES USED IN THE
            TRANSPIRATION TESTS.  NEEDLE WEIGHT AND TWIG (ANY BIOMASS
            OTHER THAN FOLIAGE) IS GIVEN INDIVIDUALLY FOR EACH SAMPLE.
            WEIGHTS ARE IN GRAMS.
VARIABLES:  NOT TRANSFORMED.
            TRUN2 FLDFIR=BIOMASS WEIGHTS FOR COLLECTION 2,FIELD,FIR
           TRUN4 GHSPR=BIOMASS WEIGHTS FOR COLLECTION 4,SHADEHOUSE,SPRUCE
            PLOT=PLOT NUMBER IN THE FIELD, REFER TO ERWIN THESIS FOR
                 SPECIFIC LOCATIONS AND ELEVATIONS.
            SPP=SPECIES, SPRUCE(S) OR FIR(F)
            TYPE=TYPE OF BIOMASS,NEEDLES(N) OR TWIG(T)
            BOTH=WEIGHT OF ENVELOPE AND BIOMASS COMBINED
            ENV=WEIGHT OF ENVELOPE ALONE.
RELATED FILES:  TRUN2 FLDFIR        TRUN2 FLDSPR
                TRUN3 FLDFIR        TRUN3 FLDSPR
                TRUN4 FLDFIR        TRUN4 FLDSPR
                TRUN2 GHFIR         TRUN2 GHSPR
                TRUN3 GHFIR         TRUN3 GHSPR
                TRUN4 GHFIR         TRUN4 GHSPR
*********************************************************************;
CARDS;
B39 F 05 N 2.177 1.7753
B48 F 07 T 2.512 1.8247
B42 F 01 T 2.1414 1.8149
```

```
DATA TAGWT;
INPUT TRTMT$ SPP$ SAMPLE$ TAGWT;
***************FILE:  TAGS DATA*************************
  THIS FILE CONTAINS THE WEIGHT OF TAGS USED IN THE CUTICULAR
       TRANSPIRATION TEST OF  EACH COLLECTION .  WEIGHT
       IS IN GRAMS.  AIR DRY WT.  BOTH FIELD AND SHADEHOUSE
       SAMPLES IN THIS FILE.  COLLECTION NUMBER FOLLOWS "TAGS"
VARIABLES:  UNTRANSFORMED.
            TAGS3 DATA IS TAGWT'S FOR COLLECTION 3
            TRTMT=PLOT (B39-B54) OR NO3 TREATMENT LEVEL
                  (5PPM - 1000 PPM)
            SPP=SPECIES, SPRUCE (S) OR FIR (F)
            TAGWT=AIR DRY WEIGHT OF IDENT. TAG FOR TEST
            SAMPLE=INDIVIDUAL TREE NUMBER OR SEEDLING SAMPLED
RELATED FILES:  TAGS2 FLDFIR  TAGS2 FLDSPR
                TAGS3 FLDFIR  TAGS3 FLDSPR
                TAGS4 FLDFIR  TAGS4 FLDSPR
                TAGS2 GHFIR   TAGS2 GHSPR
                TAGS3 GHFIR   TAGS3 GHSPR
                TAGS4 GHFIR   TAGS4 GHSPR
*******************************************************************;
B39  S 02 0.0494
B39  S 05 0.0488
B39  S 01 0.1219
0005 F 03 0.1312
0050 S 29 0.0412
1000 F 32 0.0538
```

Appendix E3. Computer documentation for annual leader growth/climate variables study.

```
DATA MINTEMP;
INPUT YEAR$ MONTH$ MINTEMP;
************FILE:  XMINTEMP FILE************************************
EXPERIMENT:  THIS FILE CONTAINS THE AVERAGE MINIMUM MONTHLY
             TEMPERATURES RECORDED AT MOUNT MITCHELL, 1980-1984.  DATA
             WAS USED IN THE STUDY WHICH MODELLED A RELATIONSHIP
             BETWEEN CLIMATIC VARIABLES AND ANNUAL LEADER GROWTH
             OF FIELD SPRUCE AND FIR.
VARIABLES:  YEAR=YEAR FOR WHICH DATA POINT COLLECTED
            MONTH=MONTH FOR WHICH DATA POINT COLLECTED
            CURRTEMP=INDIVIDUAL MINIMUM TEMPERATURE DATA POINT
RELATED FILES: XMAXTEMP FILE (AVERAGE MAXIMUM TEMPS FOR THE SAME MONTHS)
               XMINTEMP DATA (SAME DATA, BUT UNMANIPULATED)
********************************************************************;
CARDS;
1980 JAN 21.8
1980 FEB 12.5
1980 MAR 23.5
1980 APR 33.5
```

```
DATA LENGTH;
INPUT PLOT$ SPP$ YR0 YR1 YR2 YR3 YR4 YR5 YR6 YR7 YR8 YR9 YR10 YR11 YR12
      YR13 YR14 YR15 UR16 YR17 YR18 YR19 YR20;
***********FILE:  NODE DATA  ******************************
   EXPERIMENT:  CLIMATE/ANNUAL LEADER GROWTHE RELATIONSHIP.  THE DATA IN
                THIS FILE ARE THE MEASUREMENTS
                OF INTERNODAL DISTANCES ON FIELD SPRUCE AND FIR IN THE
                GROWING SEASON OF 1987 IN BLACK MTNS., NC.  MEASUREMENTS
                WERE TAKEN ON TREES ON AND AROUND THE SAMPLE PLOTS USED
                FOR WAX CONTENT AND CUTICLE TRANSPIRATION TESTS.  SEE
                ERWIN THESIS FOR INFORMATION ABOUT THESE PLOTS.

   VARIABLES:  UNTRANSFORMED, YR# MEASUREMENTS ARE IN CENTIMETERS.
               PLOT=PLOT NUMBER IN FIELD, REFER TO THESIS FOR LOCATION
                    AND DESCRIPTION.
               SPP=SPECIES, FIR OR SPRUCE.
               YR1=INTERNODAL DISTANCE FOR YEAR 1 (1986).
                  YR0=1987      YR11=1976
                  YR1=1986      YR12=1975
                  YR2=1985      YR13=1974
                  YR3=1984      YR14=1973
                  YR4=1983      YR15=1972
                  YR5=1982      YR16=1971
                  YR6=1981      YR17=1970
                  YR7=1980      YR18=1969
                  YR8=1979      YR19=1968
                  YR9=1978      YR20=1967
                 YR10=1977
RELATED FILES:  FNODE DATA   FINT FILE(ALL CLIMATE VARIABLES)
                SNODE DATA   SINT FILE(ALL CLIMATE VARIABLES)
*************************************************************;
CARDS;
B42 F 36 28 19 18 28 14 17 21 15 23 08 10 15 12 11 05 07 04 05 02  .
B42 F 38 06 16 32 32 30 18 14 16 16 10 11 18 06 07 19 20 11 06 08 03
B42 F 27 25 11 31 16 16 16 28 24 15 18 27 17 17 15 05 06 04  .  .  .
```

```
DATA PPT;
INPUT YEAR$ YRTOT CURRPPT MONTH$;
************FILE:  PRECIP FILE***********************************
EXPERIMENT: THIS FILE CONTAINS MONTHLY PRECIPITATION TOTALS
            AT MOUNT MITCHELL OF NORTH CAROLINA FOR THE YEARS 1922-1987
            DATA WAS USED TO MODEL A RELATIONSHIP BETWEEN ANNUAL LEADER
            GROWTH OF FIELD SPRUCE AND FIR AND CLIMATIC
            VARIABLES.
VARIABLES:  NOT TRANSFORMED.
            YEAR=YEAR OF DATA POINT
            MONTH=MONTH OF YEAR OF DATA POINT
            CURRPPT=MONTHLY TOTAL OF RAINFALL
            YRTOT=SUM OF MONTHLY TOTAL OF RAINFALL, YEAR TOTAL
RELATED FILES:  FINT FILE   PRECIP DATA
                SINT FILE
******************************************************************;
CARDS;
1922  68.51 6.05  JAN
1922  68.51 6.01  FEB
1922  68.51 8.45  MAR
1922  68.51 5.10  APR
```

```
DATA TEMPS;
INPUT YEAR$ MONTH$ CURRTEMP;
******************FILE:  TEMPS FILE*********************************
EXPERIMENT:  THIS FILE CONTAINS DATA FOR THE STUDY MODELLING A
             RELATIONSHIP BETWEEN CLIMATIC VARIALES AND ANNUAL
             LEADER GROWTH OF FIELD SPRUCE AND FIR.  THIS FILE
             CONTAINS THE AVERAGE MONTHLY TEMPERATURES ON MT.
             MITCHELL FOR THE YEARS 1964-1984.
VARIABLES:   NOT TRANSFORMED, TEMPS IN DEGREES FAHRENHEIT
             YEAR=YEAR FOR WHICH DATA POINT COLLECTED
             MONTH=MONTH FOR WHICH DATA POINT COLLECTED
             CURRTEMP=AVERAGE TEMPERATURE FOR INDIVIDUAL MONTH
                      IN DEGREES FAHRENHEIT.
RELATED FILES:  TEMPS DATA (SAME DATA, BUT UNMANIPULATED)
*******************************************************************;
CARDS;
1964 JAN 25.8
1964 FEB 19.5
1964 MAR 31.6
1964 APR 42.7
```

```
DATA MAXTEMP;
INPUT YEAR$ MONTH$ CURRTEMP;
************FILE:  XMAXTEMP FILE***********************************
EXPERIMENT:  THIS FILE CONTAINS THE AVERAGE MAXIMUM MONTHLY
             TEMPERATURES RECORDED AT MOUNT MITCHELL, 1980-1984.  DATA
             WAS USED IN THE STUDY WHICH MODELLED A RELATIONSHIP
             BETWEEN CLIMATIC VARIABLES AND ANNUAL LEADER GROWTH
             OF FIELD SPRUCE AND FIR.
VARIABLES:  YEAR=YEAR FOR WHICH DATA POINT COLLECTED
            MONTH=MONTH FOR WHICH DATA POINT COLLECTED
            CURRTEMP=INDIVIDUAL MAXIMUM TEMPERATURE DATA POINT
RELATED FILES: XMINTEMP FILE (AVERAGE MINIMUM TEMPS FOR THE SAME MONTHS)
               XMAXTEMP DATA (SAME DATA, BUT UNMANIPULATED)
*******************************************************************;
CARDS;
1980 JAN 37.2
1980 FEB 33.8
1980 MAR 41.4
1980 APR 50.1
1980 MAY 57.3
```

Appendix E4. Computer documentation for winter injury symptoms study.

```
DATA RATING;
INPUT YEAR$  CODE ELEV;
************FILE: INJURY DATA***************************************
EXPERIMENT: THIS FILE CONTAINS THE INDIVIDUAL RATINGS GIVEN TO
            SAMPLE FIELD TREES IN THE WINTER INJURY SYMPTOM RATING
            TEST.  SEE ERWIN THESIS FOR COMPLETE DETAILS, WINTER
            INJURY SUBSECTION IN THE RESULTS AND DISCUSSION SECTION.
            1987 RATINGS ARE GIVEN IN THE SUMMER OF 1987(JUNE), 1988
            RATINGS ARE GIVEN TO SAME TREES AFTER THE WINTER OF
            1988(APRIL)
VARIABLES:  NOT TRANSFORMED.
            INJURY FIR= DATA FOR FIR SAMPLE TREES.
            YEAR=YEAR THAT OBSERVATION/RATING WAS GIVEN, 1987 OR 1988
            CODE=RATING GIVEN
            ELEV=ELEVATION AT WHICH THE INDIVIDUAL SAMPLE TREE WAS
                 LOCATED.
RELATED FILES:  INJURY FIR
                INJURY SPRUCE
*******************************************************************;
CARDS;
1987 1 5500
1987 0 5500
1987 0 5500
```

Appendix E5. Computer documentation for auxiliary shoot growth study.

```
DATA SHOOTS;
INPUT NLEVEL$ SPECIES$ SAMPLE$ M1 M2 M3;
*******************FILE:  SHOOTL DATA **********************
  EXPERIMENT:  AVERAGE SHOOT LENGTH OF SPRUCE AND FIR SEEDLINGS
               UNDER 4 NITRATE CONCENTRATION TREATMENTS IN THE
               SHADEHOUSE.  NOT PART OF THE THESIS PROJECT, JUST
               A SIDE PROJECT.
  VARIABLES:   NOT TRANSFORMED, MEASURED IN MILLIMETERS (21=21MM)
               NLEVEL=PPM NO3, TREATMENT LEVEL (5, 50, 500, 1000 PPM)
               SPECIES=SPRUCE(S) OR FIR(F)
               SAMPLE=INDIVIDUAL SEEDLING NUMBER
               M1=MEASUREMENT 1 OF 3 MEASUREMENTS ON AN INDIVIDUAL
               SEEDLING.  AVERAGE OF 3 MSMNTS. USED FOR STAT. TESTS.
  RELATED FILES:   FSHOOTL DATA
                   SSHOOTL DATA
********************************************************************;
LENGTH = (M1 + M2 + M3)/3;
CARDS;
0005 F 01 50 68 69
0005 F 02 35 43 41
0005 F 03 69 51 57
```

Appendix E6. Miscellaneous file manipulation procedures to transform raw data into a more usable state for statistical analyses.

```
********FILE:  GHRELWT FILE******************************************
*********THESE COMMANDS ARE USED TO GET RELATIVE WEIGHTS FROM ABSOLUTE
  MEASUREMENTS.  RELATIVE WEIGHT = (WATER LOST OVER 1 OR 2HOURS)
  DIVIDED BY OVEN-DRY WEIGHT OF SAMPLE.************************
PROC SORT; BY NLEVEL;
DATA ALL;
SET RATES;
RW1=(T0-T1)/DRYWT;
RW2=(T1-T2)/DRYWT;
RW3=(T2-T3)/DRYWT;
RW4=(T3-T4)/DRYWT;
RW5=((T4-T6)/2)/DRYWT;
RW6=((T6-T8)/2)/DRYWT;
RW7=((T8-T10)/2)/DRYWT;
RW8=((T10-T12)/2)/DRYWT;
RW9=((T12-T14)/2)/DRYWT;
PROC SORT;
BY NLEVEL;
DATA _NULL_;
SET ALL;
FILE PUNCH;
PUT NLEVEL$ 4.0 SPP$ 2.0 RW1 5.2 RW2 5.2 RW3 5.2 RW4 5.2 RW5 5.2
   RW6 5.2 RW7 5.2 RW8 5.2 RW9 5.2;
```

```
************FILE CONVERT FILE***************************************
THIS PROCEDURE IS USED TO CONVERT A FILE OF HORIZONTALLY ARRANGED
     DATA TO ONE OF VERTICALLY ARRANGED DATA, WITH ONE DATA
     POINT PER OBSERVATION.
DATA LENGTH;
INPUT PLOT$ SPP$ YR0 YR1 YR2 YR3 YR4 YR5 YR6 YR7 YR8 YR9 YR10 YR11 YR12
      YR13 YR14 YR15 YR16 YR17 YR18 YR19 YR20;
DROP YR0-YR20;
INTDIST=YR0;YEAR=1987;OUTPUT;
INTDIST=YR1;YEAR=1986;OUTPUT;
INTDIST=YR2;YEAR=1985;OUTPUT;
INTDIST=YR3;YEAR=1984;OUTPUT;
INTDIST=YR4;YEAR=1983;OUTPUT;
INTDIST=YR5;YEAR=1982;OUTPUT;
INTDIST=YR6;YEAR=1981;OUTPUT;
INTDIST=YR7;YEAR=1980;OUTPUT;
INTDIST=YR8;YEAR=1979;OUTPUT;
INTDIST=YR9;YEAR=1978;OUTPUT;
INTDIST=YR10;YEAR=1977;OUTPUT;
INTDIST=YR11;YEAR=1976;OUTPUT;
INTDIST=YR12;YEAR=1975;OUTPUT;
INTDIST=YR13;YEAR=1974;OUTPUT;
INTDIST=YR14;YEAR=1973;OUTPUT;
INTDIST=YR15;YEAR=1972;OUTPUT;
INTDIST=YR16;YEAR=1971;OUTPUT;
INTDIST=YR17;YEAR=1970;OUTPUT;
INTDIST=YR18;YEAR=1969;OUTPUT;
INTDIST=YR19;YEAR=1968;OUTPUT;
INTDIST=YR20;YEAR=1967;OUTPUT;
CARDS;
```

VITA

The author was born in Vancouver, Washington on April 23, 1959. She grew up in Beaverton, Oregon, where she attended St. Mary of the Valley Academy for Girls from 1973-1977. From 1977-1980 she studied liberal arts at University of Oregon in Eugene then transferred to Oregon State University in 1981. There she studied forestry for 3 years and transferred to Northern Arizona University in Flagstaff, Arizona in 1984. In May of 1986 she received her B.S.F. and began her master's program in forest biology at Virginia Tech in June of 1986. Eleven years after starting college, she is finally getting a real job and doesn't plan on being a student for a while.

Susan A. Erwin

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