THE EFFECTS OF AMBIENT POLLUTANTS ON THE PHYSIOLOGY AND MORPHOLOGY OF MATURE RED SPRUCE

A Thesis

by

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ABSTRACT

THE EFFECTS OF AMBIENT POLLUTANTS ON THE PHYSIOLOGY AND MORPHOLOGY OF MATURE RED SPRUCE.

(May 1993)

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Two stands of red spruce (Picea rubens Sarg.) trees growing in close proximity to each other on Whitetop Mountain in Virginia exhibit varying degrees of decline symptoms. The declining stand experiences higher precipitation and deposition amounts than the healthy stand. Average ozone levels are 50-60 ppb, with rain of pH 4.3 and cloudwater of pH 3.4. In both stands branch chambers were installed on mature trees to exclude ozone and acidic precipitation (filtered) or just acidic precipitation (non-filtered). Unchambered branches were used as controls. Morphological and physiological measurements were made on the branches over the growing season and comparisons were made among treatments for both the healthy and declining stands. Data from July to November 1990 show few significant differences between stands for any measurements on healthy needles of control

branches. Exclusion of ozone did not significantly alter any measured parameters, suggesting little effect of ambient ozone on red spruce physiology. There were occasional chamber effects, such as greater total chlorophyll, wax amounts, photosynthetic rates and contact angles in chambered branches as compared to unchambered branches, particularly in the healthy stand. There were numerous significant interaction effects, particularly those involving stand and needle age, and/or stand, needle age and time of year. In addition, many of the parameters measured exhibited decreases as needles aged, except for total chlorophyll, which peaked in one year old needles. In summary, healthy needles on declining trees showed little or no morphological and physiological differences from those on healthy trees. Consequently, the parameters chosen do not appear to be useful for diagnosing decline. Future studies should focus on reduced cold hardiness of red spruce as a result of acidic deposition.

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DEDICATION

This thesis is dedicated to my family without whom I could not have endured. My father and mother, Charles and Betty Hutcherson, have shown me true family love. I cannot begin to thank them for all of their care as well as their moral and financial support. My brother Ray has always been an inspiration to me. The magic he creates through his music is a true motivator that makes me realize that there are innumerable frontiers still left to discover. My precious wife Emily has freely taken the brunt of my frustrations as well as my celebrations throughout this time, and for this I am grateful. She has been my legs when I have felt I had none on which to stand. Melvin, Donna and Amy Smith, my second family, have opened their hands and hearts to me and I am truly thankful to call them family.

Lastly, this thesis is dedicated to the memory of my brother Charles Dean Hutcherson, Jr. whose loss has made me realize that we are only temporary guests in this world and that we should embrace each and every day as a new beginning. Our time should be dedicated to making life as full and rich as possible, for the fruits of life have been presented as a privilege, not a deserved right.

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INTRODUCTION

It has been known for more than a century that atmospheric pollutants have the potential to adversely affect the health of trees (Donaubauer, 1980). However, renewed concern about forest health has arisen because of the great decline known as Waldsterben occurring in European forests (Ashmore et al., 1985; Prinz et al., 1985; Krause et al., 1986). In the Forest Damage Survey, undertaken by the German Federal Ministry of Food, Agriculture and Forestry in 1984, an estimated 66% of the forests in the Baden-Wurtemburg region exhibited pathological symptoms including altered branching patterns, thinning crowns, altered morphology of foliage, needle drop, loss of fine-root biomass, radial growth reductions and mortality. In the same study it was estimated that 50% of the trees in the Federal Republic of Germany (FRG) were similarly damaged. Other countries in Europe experiencing forest decline include the former German Democratic Republic, France, Switzerland, Austria, Italy, Poland, Czechoslovakia and Sweden (Schutt & Cowling, 1985).

This phenomenon of decline is not limited to Europe. Radial growth reductions in loblolly and shortleaf pines have been shown in the Piedmont regions of Georgia, North

Carolina and South Carolina (Barnard, 1985). Declining trees have stunted needles, chlorotic needle mottling and reduced total foliar mass. But the cause is largely unexplained since it is not associated with any known pathogens. Visible injury, such as foliar damage and leaf fall, has been documented for Ponderosa and Jeffrey pines in the San Bernardino National Forest, California (Miller et al., 1977), and leaf injury on Eastern white pine in the Eastern United States has also been reported (McLaughlin et al., 1982).

A synchronous decline of spruce-fir forests typified by diameter growth reductions, loss of foliage, and tree mortality in both the northern and southern Appalachians has also recently been documented (Siccama et al., 1982; Johnson & Siccama, 1983; Bruck, 1984; McLaughlin, 1985). On Mount Mitchell in North Carolina, defoliation of the oldest leaves of red spruce and Fraser fir has been observed (Bruck, 1984). The spruce and fir that still have foliage exhibit a chlorotic tuft of needles at the branch ends. Degradation of roots in these two species has also been observed.

While regional declines involving only single species have been reported in the past, declines involving multiple species (such as Waldsterben) are a recent phenomenon. This decline has been called a "new-type" of forest decline since the temporal and spatial development

of needle loss, crown thinning and dieback has occurred rapidly in the forested areas of West Germany and more recently in the United States (McLaughlin, 1985). Unlike reversible, localized declines in the past that involved only single species, this "new-type" decline is thought to be progressive and irreversible. Air pollution has been suggested as the cause, although the particular pollutant and mechanism of damage is unknown (McLaughlin, 1985). However, hypotheses concerning the causes of "new-type" forest decline can be grouped into four general, causal categories: 1) aluminum-heavy metal toxicity; 2) nutrient leaching; 3) foliar fertilization; and 4) direct gaseous pollutant effects (Barnard, 1985).

At present, most of the research concerning pollution effects on trees has been conducted on seedlings in controlled greenhouses or open-top chambers because the small size of seedlings facilitates experimentation. One reason for the lack of information on the differential susceptibility of trees of varying sizes or ages is the difficulty in exposing large trees to controlled amounts of air pollutants. Also, experiments with whole tree chambers are costly and difficult to replicate. The present tree declines are in mature forests and this has led to a recent, concerted effort to work with mature trees in the field (Teskey et al., 1991).

Since mature trees and seedlings may react differently to pollution, we cannot assume that results from seedling experiments can be applied to mature trees. There are potential physiological differences between seedlings and mature trees for such things as carbohydrate reserves, nutrient acquisition, rooting depths, exposure to wind, pollutants and/or rime ice and hoar frost, light interception and competition (Cregg et al., 1989).

A modern approach to the problem is the use of branch chambers on large trees (Teskey et al., 1991). This approach is based on the "branch autonomy theory" (van der Wal, 1985 cited in Sprugel & Hinckley, 1988) which postulates that (1) a branch, after its first year, imports no carbohydrates from the parent tree and (2) before exporting any carbohydrates to the rest of the tree, each branch satisfies its own material and energy requirements. If these premises are true then it can be concluded that the branch is semi-independent of the tree to which it is attached. A branch's response to pollution would then be somewhat independent of stresses to the whole tree (Sprugel & Hinckley, 1988).

At the summit of Whitetop Mountain in Jefferson National Forest, southwestern Virginia, one stand of red spruce (*Picea rubens* Sarg.) is in a state of decline while another stand, only 100 m away and downslope, appears to be healthy. Symptoms observed in the declining

stand include needle chlorosis, crown thinning and widespread mortality. Red spruce from high elevation sites such as Whitetop are exposed to a number of stresses such as precipitation, fog, ice, high winds and long exposures to high levels of ozone (Friedland et al., 1984a; Harrington, 1986; Krause et al., 1986; Klein & Perkins, 1987; Pinkerton & LeFohn, 1987; Mueller & Weatherford, 1988). The question is if the decline in the upper stand on Whitetop is related to its increased pollutant load and greater climatic stresses relative to the lower, healthier stand.

The project described herein used branch chambers to determine causes of decline in mature red spruce trees. Physiological and morphological characteristics that have shown some changes with varying environmental conditions in previous studies were chosen for this investigation. Parameters chosen were chlorophyll concentration, gas exchange, total epicuticular wax, contact angles, wax tubule morphology and twig growth. Comparisons were made between needles of damaged and healthy red spruce, and among filtered (no ozone, no acidic precipitation), non-filtered (no acidic precipitation) and control branches. Some important questions addressed by this project are if physiology and morphology differ between healthy and declining red spruce, if filtering ambient pollutants (ozone and acidic precipitation) affects the

chosen parameter measurements, if the parameters studied are good indicators of tree decline and finally, if ozone affects mature trees in the same way that seedlings are influenced.

REVIEW OF LITERATURE

Forest declines may be caused by a variety of factors; biological, physical and chemical. Biotic factors such as fungi and insects alone are not believed to be responsible for the majority of declines since some declines have not been associated with any known pests or diseases. Nor can the majority of declines be explained solely by physical factors such as drought or harsh winters (McLaughlin, 1985). This is why recent studies have concentrated heavily on chemical factors such as gaseous pollutants, heavy metals and acid rain. Studying these pollutants and their effects on plants requires consideration of other stresses since pollutants may interact with natural stresses (Miller, 1983).

Manion (1981) has categorized stresses into three classes: predisposing, inciting and contributing. Predisposing factors, such as air pollution and climate, involve long-term stresses that make the trees more susceptible to the inciting factors. Inciting factors are short-term stresses that cause sudden physiological shocks to the tree. These include insect stresses and meteorological factors such as frost and drought. Air pollutants, while acting as predisposing factors, may also

be considered inciting factors. Contributing factors are those stresses which exert their effects once a tree is already in a state of decline. These factors usually "take the blame" as the primary cause of tree death because they are easiest to observe. Any of the stresses named previously could fall under this heading. Thus, the problem of proving which factor is the chief culprit in forest decline is difficult.

ALUMINUM-HEAVY METAL TOXICITY

Soil acidity determines the solubility of aluminum in soil. In most high pH soils aluminum is insoluble and thus non-toxic to plants. But, as the soil solution becomes more acidic, aluminum becomes more soluble, leading to a higher concentration in the soil solution (Barnard, 1985). Pulses of extremely acidic pH changes in soil occur in forest floors because seasonal changes in soil moisture, temperature, biological activity and climate may release sulfate and nitrate anions from the litter or soil (Hutterman & Ulrich, 1984; Turner et al., 1985). Ulrich (1980) proposes that during periods of warm weather with little rainfall, natural acidification rates increase. Therefore, in hot, dry years more nitric acid is produced from proteins in humus because of higher decomposition rates. However, Rehfuess et al. (1982),

observed that in Bavaria and Hessen, FRG, there was no abnormal rise in nitrate following a very warm, dry year.

The soluble aluminum released in soil as a result of acidic pulses may become toxic to roots (Foy, 1974), especially fine roots. This toxicity may increase moisture and/or nutrient stress, eventually leading to death if a drought is already occurring (Ulrich, 1980). Ulrich et al. (1980) found that aluminum concentrations of 1 to 2 mg/l could damage roots. In Solling, FRG, Matzner & Ulrich (1981) measured nearly 6 mg Al^{3+}/l in leachate from a beech forest and 15 mg Al^{3+}/l under a spruce forest.

McColl & Firestone (1984) performed experiments that supported Ulrich's hypothesis. They found that by increasing the acidity of the precipitation, the concentration of Al^{3+} increased and Ca^{2+} decreased. Ulrich (1983) discovered when the molecular ratio of Ca^{2+} to Al^{3+} in the soil solution or in roots was less than one, aluminum injury was more likely. Abrahamsen (1983) found that where soils had low Ca^{2+} and high Al^{3+}, the Al^{3+} would compete with Ca^{2+} for cation exchange sites. Magnesium uptake by spruce has also been found to be inhibited by Al^{3+} (Joslin et al., 1988a). More recent research in the field also strengthens the aluminum toxicity hypothesis of Ulrich (Joslin, personal communication).

Johnson & Siccama (1984) compared the Al^{3+} content of both roots and foliage from healthy and declining red spruce and found no consistent differences. Bauch (1983) found no substantial differences in Al^{3+} concentration in fine roots between healthy and declining firs.

Even though Ulrich's hypothesis of aluminum toxicity has been supported by some research (Thornton et al., 1987; Joslin et al., 1988a), its applicability is limited because the amount of organic matter in soil may moderate Al^{3+} effects. Organic matter acts as a chelator and can interfere with the toxic effects of Al^{3+} on roots. Since the forests exhibiting decline in North America are, for the most part, on organic soils, aluminum toxicity was not originally thought to be a significant factor (Barnard, 1985).

For the past 20 years, high elevation spruce-fir forests in the eastern United States have been accumulating heavy metals. Lead, copper and zinc have increased in concentration in forest soils (Friedland et al., 1984b). Throughout the Appalachians there is an increase in soil lead concentration with increased elevation (Bruck, 1984; Friedland et al., 1984b). While higher concentrations of soil Pb^{2+} are observed at sites where declines occur in the U.S., it has not been proven that metals affect plant function at these sites (Johnson & Siccama, 1983; Wargo et al., 1987). In a few laboratory

studies, heavy metals have been shown to cause reduced photosynthesis (Rolfe & Bazzaz, 1975), decreased growth (Davis & Barnes, 1973) and reduced root elongation (Wong, 1982). Geballe et al. (1990) found lead effects on the health of red spruce seedlings only when experimental concentrations were ten times the ambient level of forest floors at high-elevations. Toxic levels of lead have not, however, been found in leaves of declining spruce and fir trees (Prinz et al., 1982).

An alternative hypothesis is that heavy metals are toxic to soil microorganisms. Activities of soil organisms have been found to decrease with an increase in Pb^{2+} content in soil (Clark & Clark, 1981). This decrease in activity slows the litter decomposition rate and thus fewer nutrients are available to the trees growing in such soil.

NUTRIENT LEACHING

During rains, cations are normally leached from leaves (Joslin et al., 1988b). Nutrient leaching does not normally affect the plant, but increased H^+ loading in atmospheric deposition may accelerate leaching rates by altering the internal nutrient balance (Barnard, 1985). Acid precipitation may cause nutrient removal from the leaf by three processes: 1) erosion of the cuticle which

exposes the epidermal surface and increases the area available for nutrient loss; 2) release of nutrients through cation exchange because of increased concentrations of ions; and 3) increased nutrients in throughfall as a result of direct removal of atmospherically deposited substances (Kelly & Strickland, 1986). It is proposed that the major process, however, is cation exchange (Pfirmann, 1990).

Foliar leaching of K^+ and Ca^{2+} has been found to increase with decreasing pH under controlled studies (Wood & Bormann, 1975; Scherbatskoy & Klein, 1983). A number of studies have found leachate to have a higher pH than acid mist in comparison studies (Hoffman et al., 1980; Johnson et al., 1982; Scherbatskoy & Klein, 1983). In field studies, increased nutrients in throughfall have been observed in declining red spruce trees (Joslin et al., 1988c). Ozone in conjunction with acid fog has been shown to increase magnesium leaching from leaves (Krause et al., 1983). This increased nutrient loss may result from cuticle erosion. Exposure of conifers to acid mist (Mengel et al., 1987; Barnes et al., 1988) and ozone (Barnes et al., 1988; Grill et al., 1989) leads to degradation of cuticular waxes.

Joslin et al. (1988b) found that losses of Ca^{2+} and Mg^{2+} increased with the acidity of cloud water. Friedland et al. (1988) reported that lower foliar

concentrations of calcium, magnesium and zinc were found in apparently healthy red spruce at high-elevation relative to low-elevation sites in the eastern U.S. Foliar magnesium concentrations of Norway spruce at seven high-elevation sites in New York and Vermont were also found to be much lower than six matching sites at lower elevations (Bosch et al., 1986). Joslin et al. (1988b) hypothesize that these lower concentrations are a result of nutrient poor soils in combination with acidic deposition which amplify foliar cation losses.

Nonetheless, foliar leaching by acid mist may not harm the plant. Some nutrients are taken up in plants in excess of requirements and most research supports the contention that acid mist, in spite of enhanced leaching processes, does not result in foliar nutrient deficiencies (Skeffington & Roberts, 1985; Mengel et al., 1989; Pfirmann, 1990).

Acidic deposition increases the supply of H^+ ions, causing a higher exchange rate with base cations (Barnard, 1985). This has led to the leaching of base cations from surface soil horizons. Haman (1977), Stuanes (1980) and Lee & Weber (1982) demonstrated a reduction in base saturation, especially Ca^{2+} and Mg^{2+}, by irrigating with artificial rain of varying acidity. However, long-term changes in soil nutrients and pH in the field have not been shown to be consistent among sites (Barnard,

1985). This is understandable since weathering of minerals is continuous and resupplies nutrients to the soil. Also, trees continuously recycle nutrients.

While there has been little research on how increased nutrient leaching affects forest productivity, some research has correlated tree declines with nutrient deficiencies. Bauch (1983) found that in declining firs in Norway, the root cortex did not contain Ca^{2+} and Mg^{2+}. The cortex of declining spruce likewise had no Ca^{2+}. Hutterman & Ulrich (1984) showed that on fertilized plots there was less tree damage. They also found that when Mg^{2+} and Ca^{2+} were added to slightly damaged trees on a nutrient-poor site, the trees responded positively.

FOLIAR FERTILIZATION

The atmosphere can be a major source of nutrients for plants (Cowling, 1984), particularly nitrogen. This deposition certainly has the potential to be beneficial, however nitrogen, in great quantities, can have detrimental impacts in high elevation forests. In the fall, trees undergo a cold-hardening process. Growth decreases and sugar levels increase in the cells (Kramer & Kozlowski, 1979). Normally, the biochemistry of a plant changes to promote intracellular ice crystal formation by

the first frost. This is accomplished by increasing the solute concentration of the cell. Increased nitrogen fertilization, however, can prolong the growing period and the hardening process may not occur by the first frost. High-elevation red spruce forests have been found to experience high annual nitrogen deposition rates (around 40 kg $ha^{-1}yr^{-1}$) (Barnard, 1985). Tisdale & Nelson (1975) have shown that winter injury occurs when nitrogen has been added to forests. The red spruce decline in Vermont demonstrates support for the foliar fertilization hypothesis (Friedland et al., 1984a). Injury of mesophyll cells has been found in which the tonoplasts are barely discernible and vacuolar contents are granulated. Advanced injury in which the cell has completely disintegrated has also been observed (Friedland et al., 1984a). These same types of red spruce injuries have been seen in cases of excessive nitrogen fertilization (Soikkeli & Karenlampi, 1984). The visible injury, which includes red-brown discoloration and eventual needle loss of the proceeding years foliage, has been documented in high elevation spruce forests of Vermont (Friedland et al., 1984a). But since red spruce are especially sensitive to winter injury (Curry & Church, 1952) it is difficult to link their decline solely with excess nitrogen effects because the trees may simply be responding normally to climatic fluctuations.

Increased nitrogen may have deleterious effects in other ways. Kramer & Kozlowski (1979) found that increased nitrogen causes a plant to increase allocation of photosynthate to above-ground tissues. This offsets root growth causing a decreased root:shoot ratio. The decrease in root size may increase plant susceptibility to drought. Experimental results of nitrification on leaf palatability appear contradictory. An increase in foliar nitrogen may either increase herbivory because leaves become more nutritious or decrease it because they increase production of nitrogenous secondary compounds that deter herbivores (Foster, 1968). Similarly, increased nitrogen deposition has contrasting effects on fungal pathogens. It has been found that the addition of nitrogen to sugar maple and American elm causes an increased susceptibility to *Verticillium dahliae* (Zak, 1964). In contrast, *Phytopthora cinnamomi* root injury in southern pines is controlled by nitrogen fertilization (Copeland, 1962 cited in Kramer & Kozlowski, 1979).

Ectomycorrhizae may be adversely affected by excess nitrogen (Bjorkman, 1949 cited in Barnard, 1985). However, the evidence to support this hypothesis under field conditions is only circumstantial. For example, hormonal imbalances may occur and cause ectomycorrhizal roots to be decorticated. In addition, fungal auxin inactivation occurs, destroying the host plant's roots.

GASEOUS POLLUTANTS

Over half of the anthropogenic sulfur emissions, in the form of SO_2, come from the burning of coal. However, sulfur emissions can also come from volcanoes, anaerobic decomposition of plant material and evaporating ocean spray. Symptoms of SO_2 exposure include tip necrosis in conifers and marginal necrosis in broadleaf trees (Prinz et al., 1985).

The mechanism of SO_2 injury starts with the entrance of SO_2 through the stomates. The SO_2 reacts with internal water, resulting in sulfuric and sulfurous acids which form a film on cell walls (Smith, 1981). The mesophyll cells are attacked by these acids and collapse. Subsequently, photosynthesis and assimilation are reduced, and the plant might now be susceptible to other stresses.

Evidence of SO_2 damage to trees comes from studies in areas near smelters. In Canada, three types of conifers surrounding a smelter were all found to have decreased growth (Lathe & McCallum, 1939 cited in Barnard, 1985). Other studies have also found unusual numbers of dead and damaged plant life surrounding smelters (Gordan & Gorhm, 1963). High levels of SO_2 have been found in areas of damaged forests in the FRG (Tomlinson, 1983). In contrast, there are cases in the U.S where there is little correlation between high SO_2 levels and forest decline,

including the declining forests in Hubbard Brook, New Hampshire (Eaton et al., 1978).

In a controlled experiment in the FRG, Krause (1988) continuously fumigated *Picea abies* and *Fagus sylvatica* for a year with either 100 ppb SO_2 or with 60 ppb SO_2 accompanied by SO_2 peaks every 14 days at 500 ppb. The plants in the constant, high SO_2 treatment had slight growth reductions and some needle discoloration. The plants receiving SO_2 peaks showed no visible injury. Therefore Krause concluded that chronic, moderate doses of SO_2 caused injury rather than high peak concentrations. Since SO_2 exposure is usually in chronic, low doses, visible symptoms may not occur in some trees. This makes tissue assays necessary.

Ozone is another gaseous pollutant being studied as a possible cause of forest decline. Ozone is thought to have the highest degree of phytotoxicity of all gaseous pollutants and therefore may be a major contributing factor in the decline of forests today (Krause, 1988). It is formed when nitrous oxides (NO_x) and reactive hydrocarbons mix under the influence of UV-light (Krause, 1988). Ozone has been found in highest concentrations at moderate altitudes (500-1500 m above sea level) in remote areas away from urban and industrial centers. Background ozone levels in Europe and the U.S. are thought to have ranged from 20-40 ppb at the turn of the century

(Chameides & Lodge, 1992). In the southern Black Forest of the FRG, the monthly mean O_3 concentration in 1980 was 113 ppb, which is similar to that measured in some U.S. forests. Concentrations of nearly 250 ppb in higher elevations have been documented. These concentrations are high enough to injure sensitive plant species (Ashmore et al., 1985).

It is suggested that ozone injury occurs at the cellular level. The gas liquifies after diffusing into the leaf through the stomata, yielding a variety of free radicals which react with cellular components (Krause, 1988). In gymnosperms, injury occurs by destruction of mesophyll cells resulting in chlorotic bleaching of needle tips or mottling of the youngest leaves.

Ozone may act on cell membranes, leading to changes in membrane permeability of the plasmalemma and chloroplasts (Krause et al., 1983). Changes in membrane permeability may lead to stomatal closure and thus a decrease in CO_2 uptake. This eventually leads to a decreased photosynthetic rate. Damage to chloroplasts may also contribute to reduced photosynthesis. Nutrient leaching as well as weathering of the cuticle may result from ozone exposure (Krause, 1988). Many other effects have also been documented, such as reduction in assimilate translocation to roots and reduction in chlorophyll content (Krause, 1988; Krzak et al., 1988).

One of the most complete studies of ozone effects on forest ecosystems was conducted in the San Bernardino National Forest near Los Angeles, California (Miller et al., 1982). Ozone levels of 580 ppb were recorded during the growing season. This level is extremely high compared to maximum ozone peaks in the eastern U.S. of approximately 160 ppb. Many coniferous trees showed foliar injury and premature leaf fall. This coincided with decreased rates of photosynthesis and reductions in tree height, radial growth and seed production. These symptoms are characteristic of many tree declines at high-elevation forests in the U.S. (McLaughlin et al., 1982; Siccama et al., 1982).

MATERIALS AND METHODS

SITE

This study was conducted on Whitetop Mountain in southwestern Virginia in the Jefferson National Forest (Fig. 1). Whitetop Mountain is the second highest peak (1,689 m) in Virginia, with neighboring Mount Rogers (1,746 m) being slightly higher. The primary influences on air quality in this region are from the Tennessee Valley region which comprises the area from southern and eastern Tennessee to northwestern Alabama (TVA, 1987). Within the Tennessee Valley region are the urban industrial areas of Knoxville and Chattanooga in addition to four TVA coal-burning power plants. Also, numerous emissions in the Ohio Valley region contribute to high levels of pollution in the spring and summer. In the winter most airflow is from the Great Plains and Canada, which are generally cleaner regions (TVA, 1987).

The summit of Whitetop mountain contains a hectare of nearly pure red spruce, which are estimated to be 150 years old or less (Joslin et al., 1988c). A TVA atmospheric monitoring station is situated in the center of this stand in a fenced-in compound. Climatic data discussed herein come from this source.

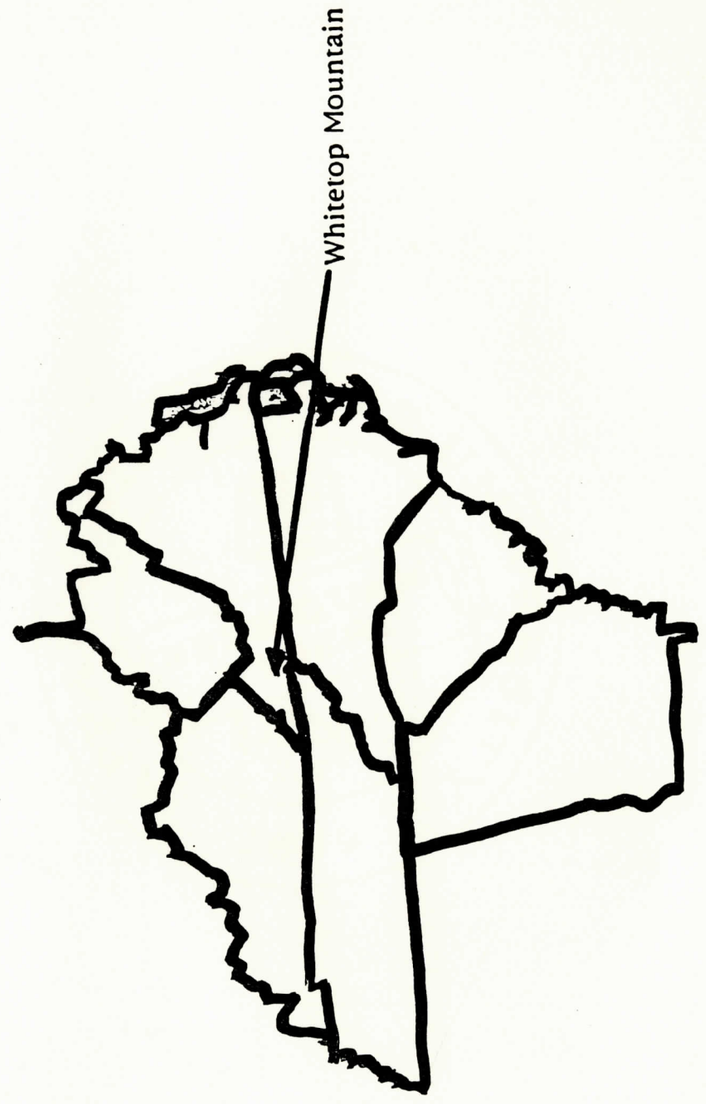


Figure 1. Location of Whitetop mountain.

The annual cloud frequency (percentage of time Whitetop is covered in clouds) at the summit is 31.3%. Thirty percent of the total hydrologic budget is from cloud moisture alone, delivering approximately 80% of the SO_4^{-2} and NO_3^- to the forest floor. The rest comes from rain water. Cloudwater SO_4^{-2} fluxes are between 5.3 and 9.1 kg ha^{-1} mo^{-1} and NO_3^- fluxes are between 2.8 and 5.4 kg ha^{-1} mo^{-1}. The average pH of rainfall is 4.3 with the lowest recorded pH at 2.6, while the average pH of cloudwater is 3.4. Mean 24-hour ozone levels at the site are 55 ppb, with little diurnal variation. However, mean hourly ozone concentrations have reached as high as 163 ppb. In the summertime, ozone concentrations can reach or exceed 85 ppb 13% of the time during the daytime hours (Mueller and Weatherford, 1988).

Weather data show Whitetop to have a typical montane environment with a cold and wet climate. Average windspeed is typically 3-5 m/s, but can be as much as 9-13 m/s. Wind blows from the west-northwest 45% of the time, and from the south-southeast 22% of the time (Mueller and Weatherford, 1988).

STAND CHARACTERISTICS

This study was conducted in two stands of spruce at the Whitetop site. One stand, nearest the summit and at the edge of the TVA compound, is undergoing a decline

while the other stand, 100 m away and slightly down slope, appears quite healthy. The upper stand has been shown to receive more precipitation in the form of rain and cloudwater (Joslin et al., 1988c). The forest floor of the upper stand also receives 15, 29 and 45% more sulfate, nitrate and ammonium, respectively. Morphological studies show the lower site to have greater foliar growth rates and needle retention, although bud mortality rates are higher. The litter-fall rate at the upper stand is also slightly higher. Consistently lower concentrations of Mg, Zn and Ca in foliage are found at the upper stand while K and B levels are higher. Soil solution chemistry reveals higher nitrate and aluminum levels at the upper stand. The higher nitrate levels are thought to be due to the higher rates of N deposition and N mineralization/nitrification (Joslin et al., 1988a).

The average height and diameter at breast height of the mature trees on and surrounding the summit are 11.7 m and 20.2 cm respectively. The density of mature trees at the declining site is 1475 live stems/ha. The area corresponding to the healthy stand has a mature tree density of 2100 live stems/ha. Average age of mature trees on and surrounding the summit is 96.5 years (Nicholas & Zedaker, 1992).

EXPERIMENTAL DESIGN

The purpose of this project was to study the effects of ambient air-pollutants on mature red spruce trees *in situ*. Many pollutant-effects projects have been performed in the past on seedlings in open top chambers. However, it is debatable whether a seedling will react to air pollutants in the same manner that a mature tree will, or whether a plant will react differently to pollutants in a highly-controlled situation relative to its natural environment.

Branch chambers were used to study air pollution effects on the mature trees. This necessitated the construction of platforms at both sites to reach the upper crown of the trees. Each platform consisted of 2 towers of scaffolding interconnected with walkways to provide access to the upper canopy level (15-20 m) of at least four trees. Both walkways were oriented so as to minimize shading effects on the sample trees. The towers were anchored to the ground with concrete footings and supported by guy wires.

Four trees that were in reach of the platforms were chosen for study. Three branches were selected from each tree and two of these were inserted into the branch chambers. These branches were chosen because they were accessible, in good health and exposed to the sun a majority of the daylight hours.

BRANCH CHAMBERS

Branch chambers were constructed of aluminum fashioned into cylinders 52 cm X 124.8 cm (20" by 48") with three struts connecting a hoop at either end (Figure 2). Branches were inserted two thirds of the way into a chamber. The chamber end in which the branch was inserted contained three aluminum strips connected to the branch over styrofoam sleeves and attached with two hose clamps. The distal end of the chamber was supported with a spring attached to a fixture on the platform. This relieved the branch of any undue weight stress by the chamber and prevented water from entering the chamber. The chamber frame was covered with 6 mil transparent, polyvinylchloride (PVC) plastic (Livingstone Coating Corp., Charlotte, NC). Air entered the chamber through a plenum on the distal end, which distributed and mixed the air. Air was supplied from a blower (Rexon Assoc. Inc., Roswell, GA) attached to the platform. Each tree contained one charcoal filtered chamber, one non-filtered chamber and an unchambered branch as a control. A polypropylene filter was connected to the blower input to exclude fog and large particulates. Filtered chambers contained both the particulate filter and a 74 cm^3 charcoal filter for exclusion of ozone.

The turnover rate of air in the chambers was 6.5 volumes per minute. A Kurz anemometer (Model 1440, Kurz

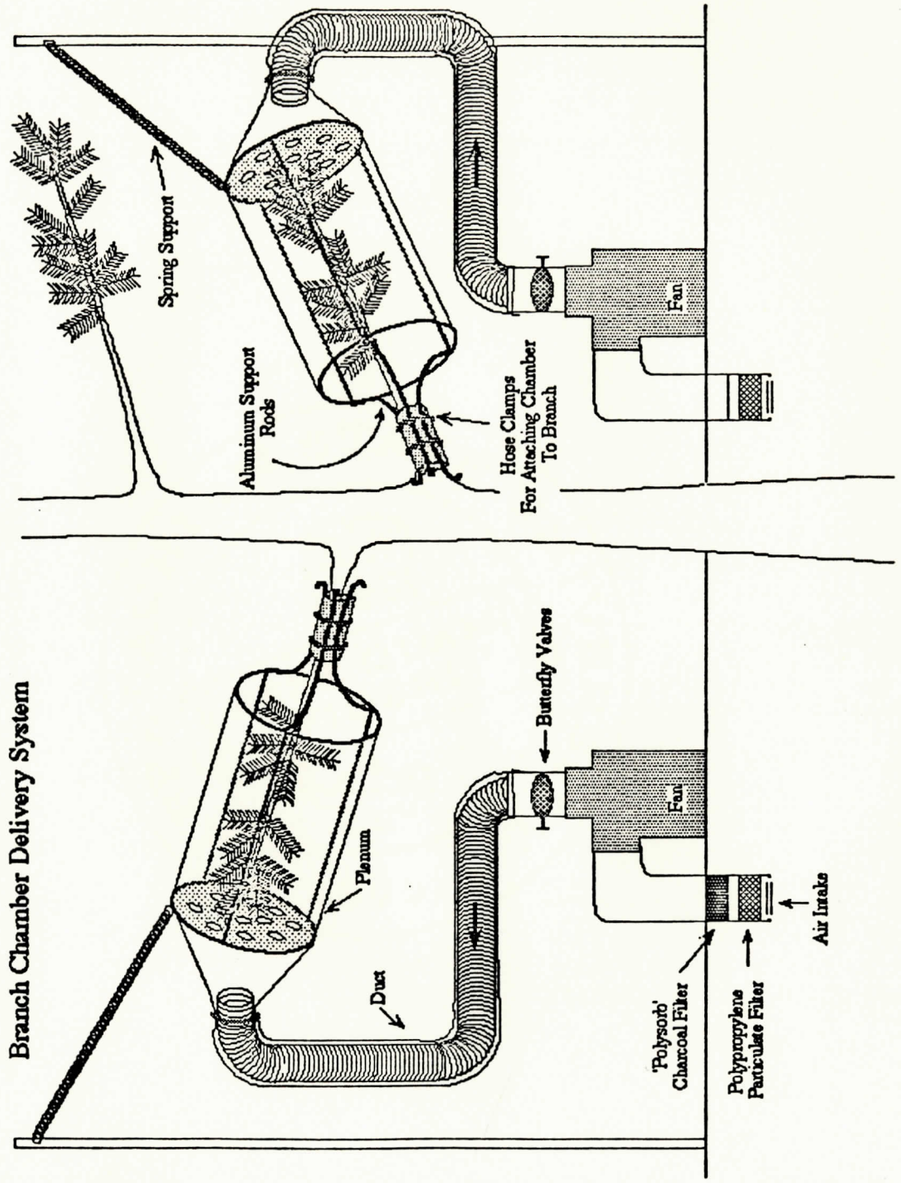


Figure 2. Branch chamber delivery system.

Instruments Inc., Monterey, CA) was used to check airflows in each chamber once a month.

Air temperature was measured by thermocouples (copper-constantan, 24 gauge) placed in every chamber. They were located inside of perforated 4 cm diameter PVC tubes to avoid solar heating effects. One control branch was used at each site to measure ambient air temperatures. Photosynthetically active radiation (PAR) sensors were placed in every chamber and on one control branch to monitor PAR. Sensors were GaAsP sensors (G1118, Hamamatsu Corporation, Japan) and were calibrated against a quantum sensor (Li-Cor 190S, Li-Cor Inc., Lincoln, NE). Ozone levels were sampled using FEP (6.44 mm, 1/4" O.D.) teflon tubing equipped with Zylon PTFE membrane particulate filters with a 5 um pore size (Gelman Sciences Inc., Ann Arbor, MI). The teflon tubes and sensor outputs were run down the side of the tower to a temperature controlled instrument shelter at the base. Solenoids were used to switch air streams for sampling. A Monitor Labs Ozone Analyzer (Model 8810, Lear Siegler Measurement Controls Corp., Inglewood, CO) at the upper site or a TECO Analyzer (Model 49, Thermo Electron Corporation, Franklin, MA) at the lower site were used to monitor ozone concentrations. Exhaust air from the ozone monitor was diverted to a thin-film capacitance relative-humidity sensor (Vaisala OY, Boston, MA) to determine chamber

and ambient relative humidity. The ozone analyzers were calibrated monthly (zero and span checks) with a Monitor Labs 8910 calibrator. The calibrator was certified as a secondary standard by EPA personnel in Athens, Georgia.

All monitoring data were collected using a Campbell 21x data logger (Campbell Scientific Inc., Logan, UT) interfaced with an AM32 multiplexer. Switching signals were sent to a control port multiplexer (SDM-CD16), which turned on solenoid valves to direct air flow from particular chambers or branch locations to the ozone monitor and relative humidity sensor.

Hourly maximum, minimum and average values were stored each hour for temperature, PAR, relative humidity and ozone values. An alarm warning was set off if chamber temperature rose more than 4°C above ambient and these data were marked by the data logger.

Data were retrieved once a week from the data logger using the PC208 software supplied by Campbell coupled with a Toshiba T1200 laptop computer. Data files were processed by a PASCAL program on a 286 personal computer back in the lab.

PARAMETER MEASUREMENTS

Measurements were made according to the following methods. Measurement dates are outlined in Table 1.

Table 1

Sampling Dates For All Analyses

Tests \ Dates	Oct. 89	Nov. 89	Dec. 89	Jan. 90	March 90	July 90	Aug. 90	Sept. 90	Oct. 90	Nov. 90
Contact Angles	X			X	X	X***	X*		X*	X*
Total Wax				X	X	X***	X*		X*	X*
SEM								X*		X*
Growth							X*	X*	X*	X*
Chlorophyll	X			X	X	X***	X*		X*	X*
Gas Exchange	X**	X	X**			X***	X*	X*		X*

X = Non-Chambered Branches Sampled For Both Stands
X* = Treatments Sampled For Both Stands
X** = Non-Chambered Branches Sampled in the Declining Stand Only
X*** = Treatments Sampled Only For Healthy Stand

Contact Angles

A contact angle is the angle that a drop of water makes with a leaf surface and is a measure of the wettability of a leaf. The smaller the angle the more hydrophyllic the leaf surface is and the more susceptible it may be to leaching by precipitation. A larger angle represents a more hydrophobic surface, with possibly a greater resistance to leaching.

Healthy, unshaded needles from each branch were excised and placed into labeled zip-lock bags and then stored on ice. Current (0) and 1 year old needles were taken from chambered branches and 0, 1 and 3 year old needles from unchambered controls.

Contact angles were measured on three needles per chamber per age class in the lab. A single excised needle was put in a clip on a micromanipulator and oriented horizontally with the abaxial side up. A slide projector with the lens removed was placed on one side of the needle and the projector lens placed on the opposite side of the needle so that the shadow of the needle projected onto a sheet of paper on the wall. To maintain consistency and precision, the projector and paper were always 2.8 meters apart and the lens and paper 0.44 meters apart.

Using a micropipette, 1 µl of deionized water was placed on the midsection of the abaxial side of the needle. The outline of needle and water drop was then

traced on the sheet of paper (Fig. 3). The contact angle was found using the following equation:

$$\theta = 2 \tan^{-1} (h/r) \qquad (1)$$

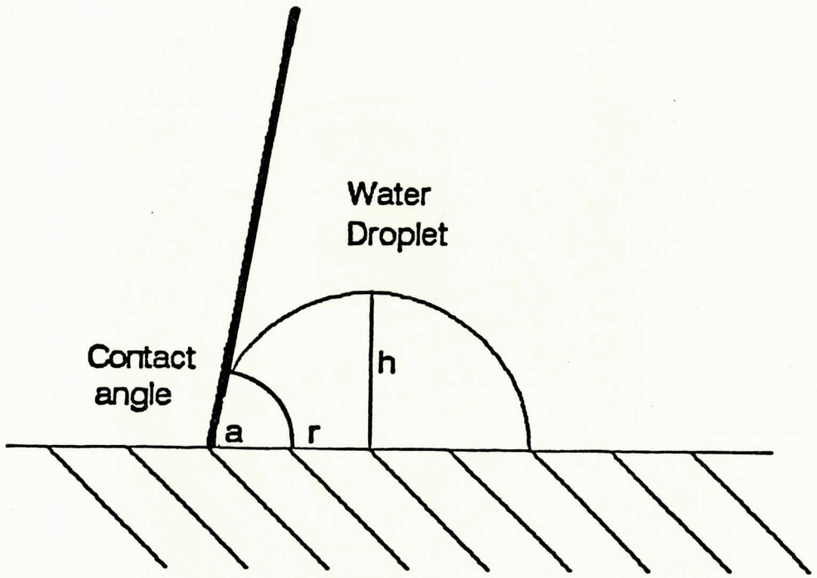
where h = height of the droplet and r = radius of the base (Cape, 1983).

Total Epicuticular Wax

Reductions in the amount of total epicuticular waxes on a leaf surface may be due to erosion from precipitation or natural ageing effects. If waxes are indeed being eroded with time, this may subsequently lead to foliar leaching of nutrients (Turunen & Huttunen, 1990).

The sampling protocol used for the contact angle measurements was also used for wax content. Total epicuticular waxes were determined within 48 hours of collection using a gravimetric method (Wilkins, USFS, personal communication). Two sets of 50 needles each were used per treatment per age class. The needles were placed into a 125 ml Erlenmeyer flask and washed three times (20 seconds each wash) with 5 ml of HPLC grade chloroform. After each washing the needles and chloroform were poured through #101 Whatman filter paper into a 50 ml beaker. After the final wash the flask was washed with 5 ml more of chloroform, which was then poured through the filter paper into the beaker. The needles were then put into labeled envelopes and dried in an oven at 70°C. The

Figure 3. Measurement of a contact angle. The contact angle (a) is the angle the water droplet makes with the leaf surface and is calculated as: a = 2/Tan(h/r), where h = droplet height and r = droplet radius.



extract in the beaker was poured into a pre-weighed aluminum pan, allowed to evaporate fully (usually about 5 hours) and then the pan was weighed on a microbalance (Sartorius, Model R2000, Goettingen, West Germany). Total waxes were expressed as mg wax per gram dry weight of needle.

Wax Ultrastructure

Wax tubules surround and encompass the stomatal chambers on red spruce needles. Any perturbations in wax structure can be easily seen and quantified by measuring wax tubule diameters and lengths from electron micrographs.

Samples for SEM analysis were collected on November 10, 1990 from each treatment branch. Current and 1 year old needles were collected from chambered branches and 0, 1 and 3 year old needles from unchambered branches. Only filtered (low ozone) chamber needles and controls were used for the analysis, since previous observations (Hutcherson, unpublished data) had shown little difference in filtered and non-filtered (ambient ozone) chambers in appearance of waxes.

Three needles from each age class per branch were randomly attached to metal stubs with silver glue. The stubs were then inserted into a Polaron SEM coating system (Biorad Laboratories, Cambridge, MA) and sputter coated

with gold-palladium twice at 45 seconds at each of two different angles. A Hitachi scanning electron microscope (Model S-570, Nissei Sangyo America, Ltd., Rockville, MD) was used to view the needles. The middle needle of each sample was chosen to make pictures. It was found that the average midpoint of the needles was 4.5 mm, thus the field of view was taken at this point. Magnification was then increased to 500X and the stomate that appeared to have the least-eroded waxes chosen. Magnification was increased to 10,000X and an area with the least erosion centered on the screen. After adjustment of focusing and contrast a picture was taken.

Wax tubule lengths and diameters were measured directly from the negatives by projecting the negative onto a printer table and increasing the negative's size 5 times (total magnification = 50,000X). Wax tubules were measured by projecting the negative onto a sheet of paper previously marked at 5 random points. The diameter and length of the tubule that touched or was closest to each point was then measured.

Growth

Growth measurements were made on 5 age classes of twigs (0, 1, 2, 3 and 4 years old) on each chambered and unchambered (control) branch. Five twigs per age class were used. Twig diameters were identified for all age

classes. In addition, length measurements were made on current year twigs. Length and diameter measurements were made on 4 seedlings (3-6 years old) and 4 saplings (10-13 years old) as a comparison to mature tree growth. Digital calipers (Brown and Sharpe, North Kingstown, RI) were used for all measurements.

Chlorophyll Test

Healthy, non-shaded needles were excised with scissors on each sampling date from each of the four trees in each stand. Three age classes, 0, 1 and 3 year old needles were chosen from non-chambered branches. Only 0 and 1 year needles were selected from chambered branches. These were immediately sealed in plastic zip-lock bags, labeled and put in a styrofoam ice box. Samples were taken back to Appalachian State University and kept refrigerated at 4°C. Extractions usually began within 24 hours.

A fresh weight to dry weight relationship was calculated for needles of each age class, using 5 samples of two needles each per tree. Fresh weights were obtained with a microbalance (Model R2000, Sartorius). Needles were then placed in a drying oven at 65°C for two days to obtain dry weights. A regression equation relating fresh weight to dry weight was then calculated for each age class per stand, grouping trees together in each stand.

Two samples of two needles each were taken from each tree per age class per treatment for chlorophyll extraction. Needles were cut into 2 mm sections and placed in a 20 ml polypropylene scintillation vial containing 5 ml of N,N-dimethylformamide. These were then covered and put into a refrigerator at 4°C for 48 hours to extract the chlorophyll (Moran and Porath, 1980; Moran, 1982; Inskeep and Bloom, 1985). Chlorophyll absorption was measured at 664 and 647 nm using a UV-visible spectrophotometer (Perkins-Elmer, model 3840) in high performance mode at a resolution of 0.25 nm and an average of 16 cycles per sample. Chlorophyll *a*, *b* and total chlorophyll concentrations (mg/ml) were then determined using the following formulae:

$$C_a = 12.64\ A_{664} - 2.99\ A_{647} \qquad (2)$$

$$C_b = -5.6\ A_{664} + 23.26\ A_{647} \qquad (3)$$

$$C_T = 7.04\ A_{664} + 20.27\ A_{647} \qquad (4)$$

where C_a = chlorophyll *a* concentration, C_b = chlorophyll *b* concentration, C_T = total chlorophyll concentration, A_{664} = absorbance at 664 nm and A_{647} = absorbance at 647 nm.

Gas Exchange

All gas exchange measurements were made between the hours of 10 AM and 2 PM, because preliminary experiments by Frank Thornton of TVA have shown rates to be the

highest during these times (unpublished data). Stands were measured on different days since time did not allow both stands to be sampled in a single day. Dates, however, were kept as close as possible (average = 2 days) to allow for stand comparisons.

Photosynthesis, total conductance to water vapor (includes boundary layer conductance) and internal CO_2 concentration were determined using a portable photosynthesis system (Model 6200, Li-Cor Inc., Lincoln, NE) with the 0.25 liter chamber. Light levels were kept constant at 600 moles m^{-2} s^{-1} by shading the Li-Cor chamber and using a 300 W quartz halogen lamp as the light source.

Gas exchange was measured on detached twigs of about 2.0 cm in length from the upper middle section of each branch. Two samples were taken from each tree per treatment per age class. Ages 0 and 1 were sampled from chambered branches and ages 0, 1 and 3 from unchambered branches.

Once a sample was measured it was put in a labeled envelope, taken back to the ASU lab and dried for two days at 70°C in a drying oven. Photosynthesis and total conductance were expressed on a unit needle dry weight basis.

STATISTICAL DESIGN

Two separate experimental designs were used. The first was analyzed using an analysis of variance with repeated measures. Parameters analyzed included contact angles, total epicuticular wax and gas exchange. Initial measurements were first made on control branches only, and included measurements made on the trees both prior to and after installation of the chambers. Samples from the fall and winter of 1989 and spring of 1990 were analyzed separately from the summer samples starting in July 1990 and going to the fall of that year, because these latter samples represented a new flush of needles which did not correspond to the same age classes as needles from sampling dates prior to July. This latter analysis included stand, age and time comparisons. Comparisons of 1989 needle cohorts were performed also and included stand and time effects. The repeated measures design was then used to analyze branch chamber effects. Here, chamber treatments were not compared between stands since chamber installation in the declining stand lagged one month behind those in the healthy stand. Therefore stands were analyzed separately. This analysis included treatment, age and time comparisons.

The second design was a nested factorial (trees nested within stands) and was analyzed using an analysis of variance, but did not include any time effects. It was

used for wax ultrastructure and growth measurements since these were one time measurements. Stand and age comparisons were first made on unchambered branches. Then stands were analyzed separately for treatment effects using treatment and age comparisons.

Comparisons among treatment means were made if there were significant treatment effects and these comparisons among means were made using Duncan's multiple range test. All statistical tests were considered significant at $p < 0.05$.

Measurements made in the lower stand after July 9, 1990 and in the upper stand after August 16, 1990 include branch treatment effects. All experiments prior to these dates were on control branches only, since the chambers had not yet been installed.

RESULTS

There are two major comparisons in this study. The first compares healthy and declining red spruce stands using various morphological and physiological parameters for vigorous unchambered branches. The second compares chambered and unchambered branches within each of the stands. Because technical difficulties delayed chamber operations by one month in the declining stand, I do not feel justified in making direct comparisons between stands for chamber treatment effects.

ENVIRONMENTAL PARAMETERS

A total of 2328 hours were logged with 1312 hours of usable data and 1016 hours of down time (43.6% down time). The non-chambered branches were exposed to 7.5% more ozone than the non-filtered chambers from July 31 to November 5, 1990 (Table 2). The control and non-filtered chambers were exposed to 2.98 times more ozone than the filtered chambers.

Ozone averages were similar (55 ppb) in the non-filtered and control branches from July 31 to September 19, 1990. These averages were almost three times as great as the filtered chambers (Figure 4).

Table 2

Total season ozone exposure by treatment for July - November 1990.

Treatment	Ozone Exposure (ppm * hrs)
Control	65.96
Non-Filtered	56.74
Filtered	20.54

Table 3

Total seasonal radiation load by chambered and unchambered treatments for July - November, 1990.

Treatment	Seasonal Radiation Load (umol/m^2)
Chambered	1031.4
Unchambered	934.5

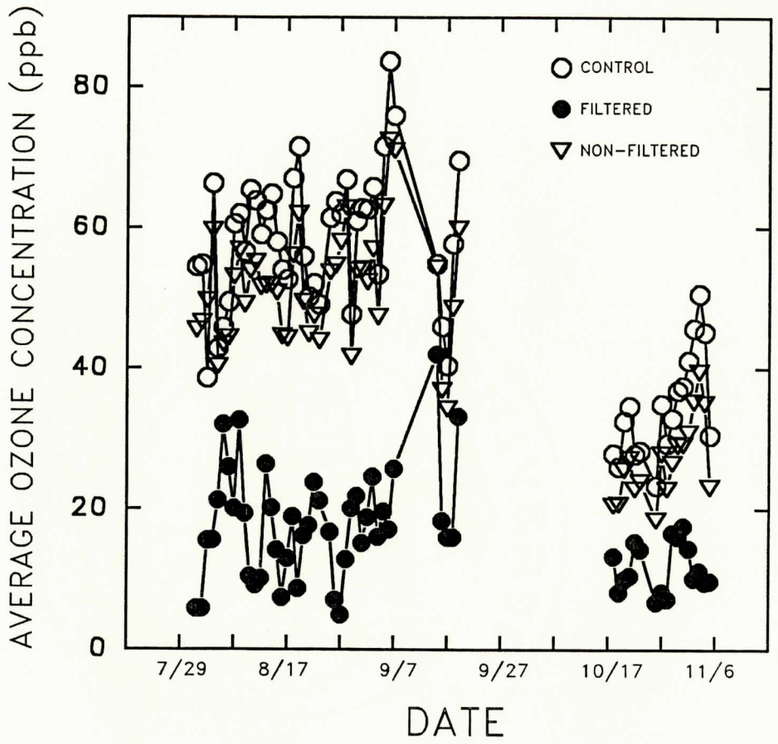


Figure 4. Daily ozone averages from July 29, 1990 to Nov. 6, 1990.

Starting October 17, the non-filtered and control branches exhibited only 2 times the ozone concentration as the filtered chamber. No data are available for September 20 to October 16 because the ozone analyzer malfunctioned.

The chambers did not decrease the overall amount of light striking the branches (Table 3). The quality of light may have been different but this was not tested.

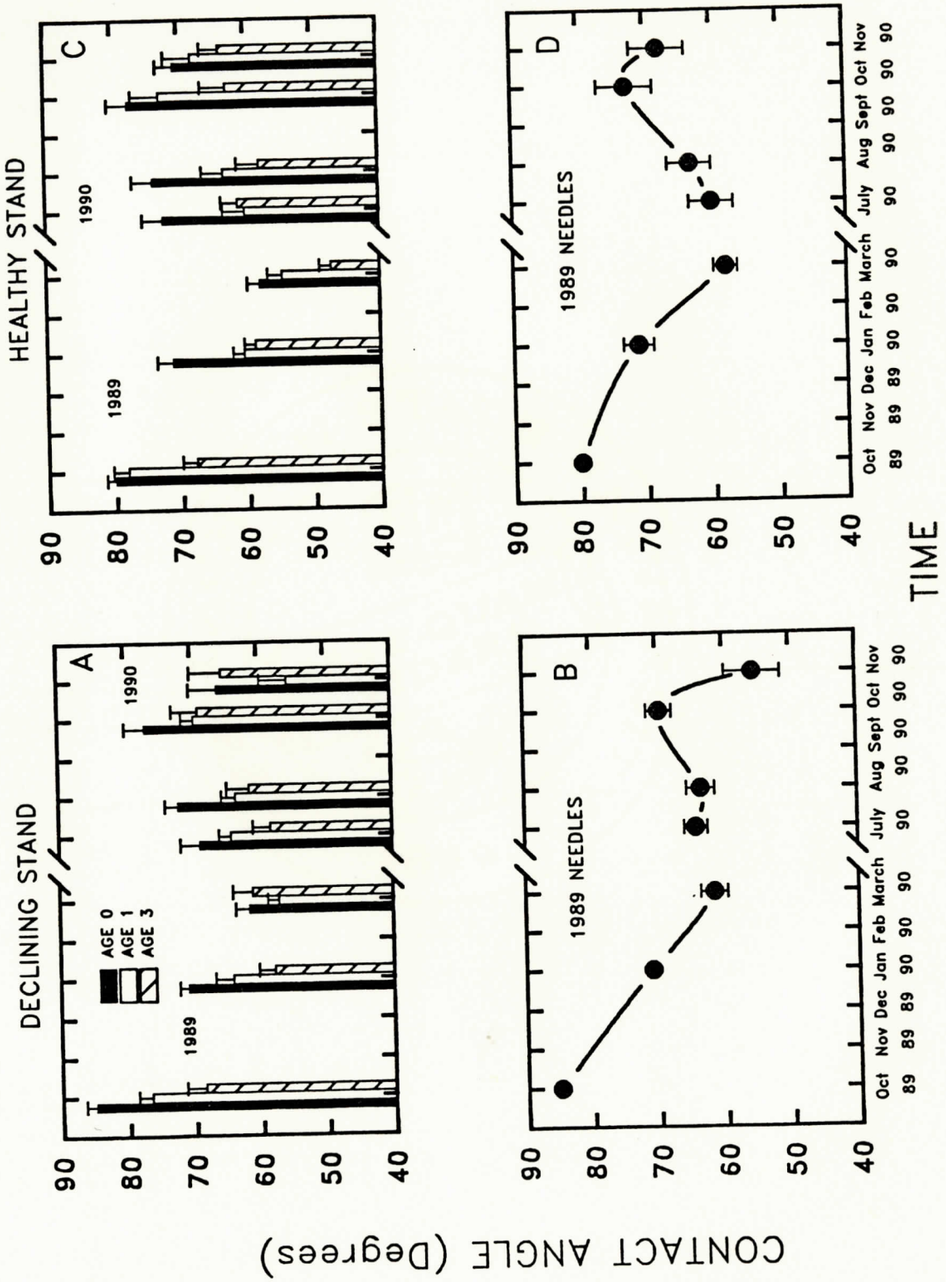
There were problems with the chambered branches overheating on sunny days. On a typical warm day of about 20°C, chambered branches exhibited average temperatures 5.6°C warmer than unchambered branches. Even on cooler days of about 14°C, chambered branches could be up to 4.8°C warmer than unchambered ones.

CONTACT ANGLES

Stand Comparisons Using Unchambered Branches

Contact angles in both stands decreased with needle age (Fig. 5a and c) from October 1989 to March 1990 and from July to November 1990. This effect was more pronounced early in the season, where 0 and 1 year needles had higher angles than 3 year old ones (82.53, 77.36 and 68.15 degrees for October 1989). By late in the season many of the age effects disappeared. Angles decreased from the fall of 1989 through winter up to March 1990. In 1990, angles increased through the summer, but began to

Figure 5. Contact angles of needles on unchambered branches. (A) Contact angles for 0, 1 and 3 year old needles in the declining stand as a function of time. (B) Contact angles for the 1989 needle cohort in the declining stand as a function of time. (C) Contact angles for 0, 1 and 3 year old needles in the healthy stand as a function of time. (D) Contact angles for the 1989 needle cohort in the healthy stand as a function of time. Lines are smooth fits to data. Points are sample means ± 95% CI.



fall again in November 1990. A stand difference was observed for October 1989 to March 1990 where the declining stand had higher angles (Table 4).

If we follow a single cohort of needles (0 year in 1989, 1 year old in 1990, Fig. 5b and d), a large drop in contact angles occurred at the end of 1989 and beginning of 1990 (82 degrees in October, 1989, 60 degrees in March, 1990), followed by an increase to October, with another drop in November, 1990 (62 degrees). There were no differences between the stands for these same needles.

Treatment Effects

Contact angle comparisons were made between needles in filtered, nonfiltered and control chambers to see what effects ambient pollutants had on the properties of the cuticle. There were significant treatment by time and time by treatment by age interactions in the declining stand (Fig. 6a and b, Table 5).

In the healthy stand (Fig. 6c and d) contact angles decreased as needles aged from 0 to 1 year old. A time effect was also observed, where angles for 0 year needles decreased from October 1990 to November 1990. The treatment effects were not significant. Filtered and non-filtered treatments were not consistently different (Table 5).

Table 4

Summary of contact angle analysis of variance using repeated measures on unchambered branches.

Oct. 89 through March 90

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
STAND	1	150.12	5.52	0.0304*
AGE	2	720.85	26.52	0.0001*
TIME	2	2300.62	70.72	0.0001*
ST*AG	2	13.90	0.51	0.6080
TM*ST	2	61.41	1.89	0.1661
TM*AG	4	56.14	1.73	0.1657
TM*ST*AG	4	49.81	1.53	0.2139

July 90 through Nov. 90

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
STAND	1	38.63	0.37	0.5525
AGE	2	673.77	6.44	0.0096*
TIME	3	128.26	3.86	0.0154*
ST*AG	2	53.69	0.51	0.6088
TM*ST	3	47.30	1.42	0.2486
TM*AG	6	45.11	1.36	0.2527
TM*ST*AG	6	34.83	1.05	0.4079

Values of $p < 0.05$ are considered significant and are highlighted by a *.

Figure 6. Contact angles for chambered and control branches in 1990. (A) Contact angles for 0 year old needles in the declining stand as a function of time. (B) Contact angles for 1 year old needles in the declining stand as a function of time. (C) Contact angles for 0 year old needles in the healthy stand as a function of time. (D) Contact angles for 1 year old needles in the healthy stand as a function of time. Lines are smooth fits to data. Points are sample means ± 95% CI.

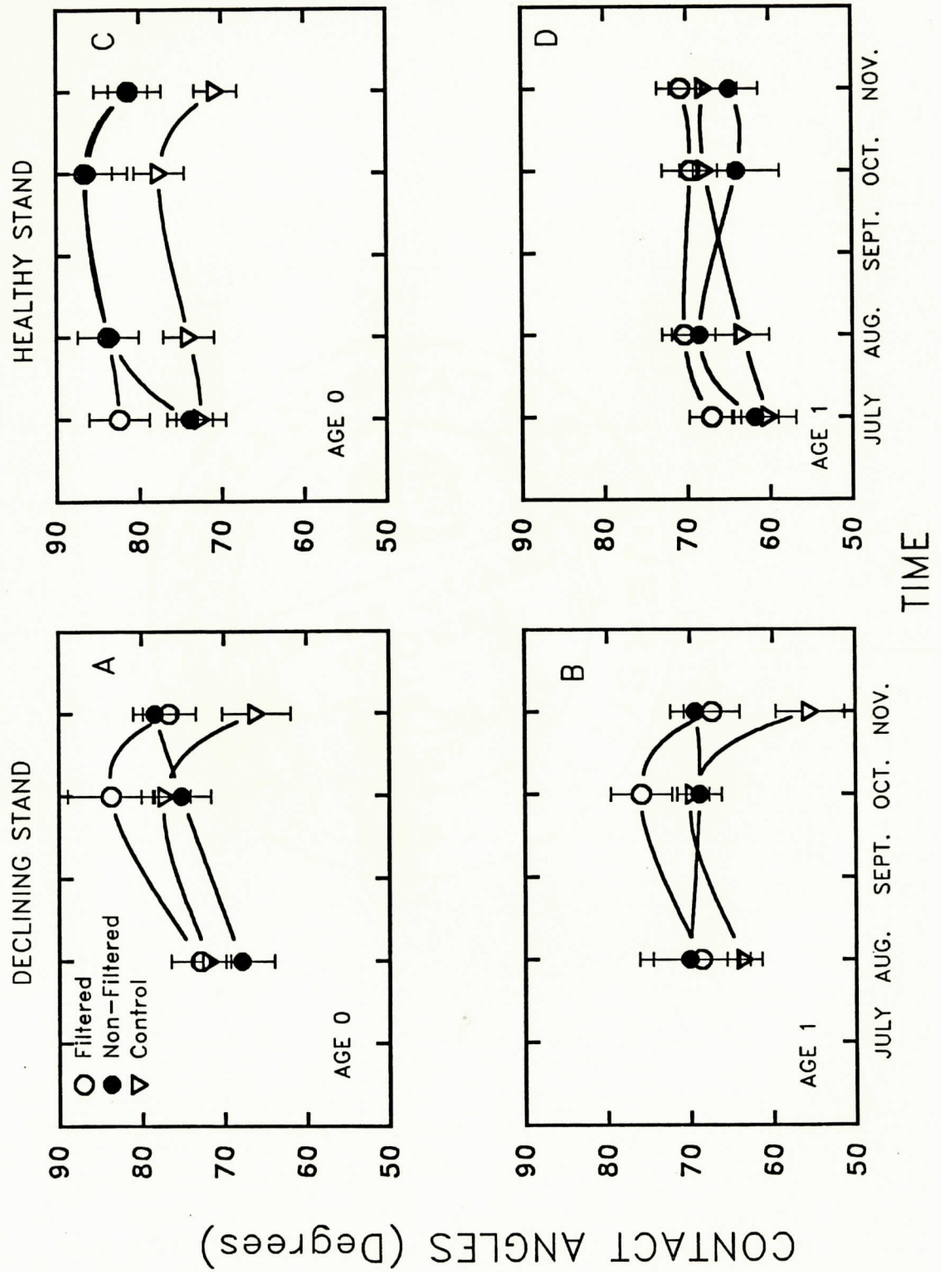


Table 5

Summary of analysis of variance using repeated measures for treatment effects on contact angles in chambered branches.

Declining Stand

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
TREATMENT	2	143.35	2.03	0.1734
AGE	1	573.11	8.13	0.0146*
TIME	2	254.93	4.44	0.0229*
TR*AGE	2	6.01	0.09	0.9188
TR*TIME	4	166.86	2.91	0.0430*
AGE*TIME	2	36.35	0.63	0.5396
TM*TR*AG	4	10.40	0.18	0.0460*

Healthy Stand

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
TREATMENT	2	356.67	3.15	0.0670
AGE	1	3937.75	34.80	0.0001*
TIME	3	177.17	3.39	0.0244*
TR*AGE	2	172.04	1.52	0.2454
TR*TIME	6	32.01	0.61	0.7192
AGE*TIME	3	24.30	0.46	0.7080
TM*TR*AG	6	34.49	0.66	0.6821

Values of $p < 0.05$ are considered significant and are highlighted by a *.

TOTAL EPICUTICULAR WAX AMOUNTS

Stand Comparisons using Unchambered Branches

In both stands 1 year old needles had more wax than either 0 or 3 year old needles from January 1989 to March 1990 (Fig. 7a and c). A time effect was noted in both stands, where wax amounts decreased from January 1990 to March 1990 for all age classes (Table 6). A significant time by stand by age interaction was observed for the summer of 1990. Wax amounts for 0 year 1989 needles dropped significantly in both stands from January 1990 to March 1990 (14 to 10 mg gdw^{-1}) (Fig. 7b and d). No significant changes occurred in the summer.

A regression of total epicuticular wax amounts (obtained from pooled samples of 50 needles per age class per tree) against contact angles (obtained on individual needles of a specific age class per tree) shows a strong, positive relationship (Fig. 8) ($r^2 = 0.62$, $p < 0.0001$), suggesting that one of the factors that determines the wettability of these leaves is the amount of epicuticular wax.

Treatment Effects

Wax amounts were significantly greater in the chambered needles than the unchambered ones in the declining stand (19 vs 16 mg gdw^{-1} in July, 1990) (Fig.

Figure 7. Total epicuticular wax of needles on unchambered branches. (A) Total epicuticular wax for 0, 1 and 3 year old needles in the declining stand as a function of time. (B) Total epicuticular wax for the 1989 needle cohort in the declining stand as a function of time. (C) Total epicuticular wax for 0, 1 and 3 year old needles in the healthy stand as a function of time. (D) Total epicuticular wax for the 1989 needle cohort in the healthy stand as a function of time. Lines are smooth fits to data. Points are sample means ± 95% CI.

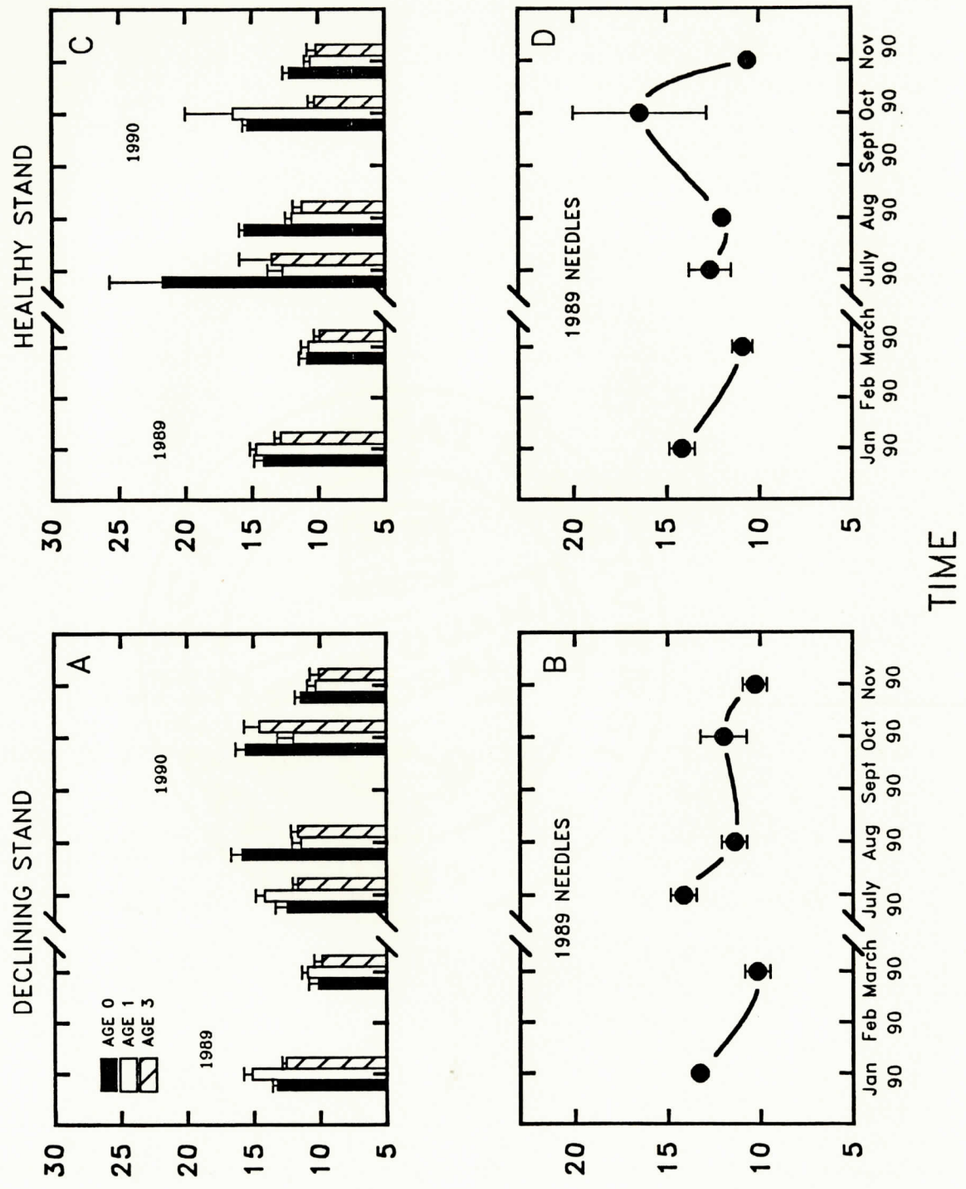


Table 6

Summary of total wax analysis of variance using repeated measures on unchambered branches.

Jan. 90 through March 90

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
STAND	1	1.80	0.82	0.3770
AGE	2	8.43	3.83	0.0410*
TIME	1	137.11	114.03	0.0001*
ST*AG	2	1.17	0.53	0.5955
TM*ST	1	0.00	0.00	0.9738
TM*AG	2	1.40	1.17	0.3343
TM*ST*AG	2	0.05	0.04	0.9591

July 90 through Nov. 90

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
STAND	1	1.56	0.44	0.5188
AGE	2	50.49	14.21	0.0004*
TIME	3	36.68	9.15	0.0001*
ST*AG	2	6.66	1.88	0.1898
TM*ST	3	6.32	1.58	0.2089
TM*AG	6	5.53	1.38	0.2453
TM*ST*AG	6	10.55	2.58	0.0321

Values of $p < 0.05$ are considered significant and are highlighted by a *

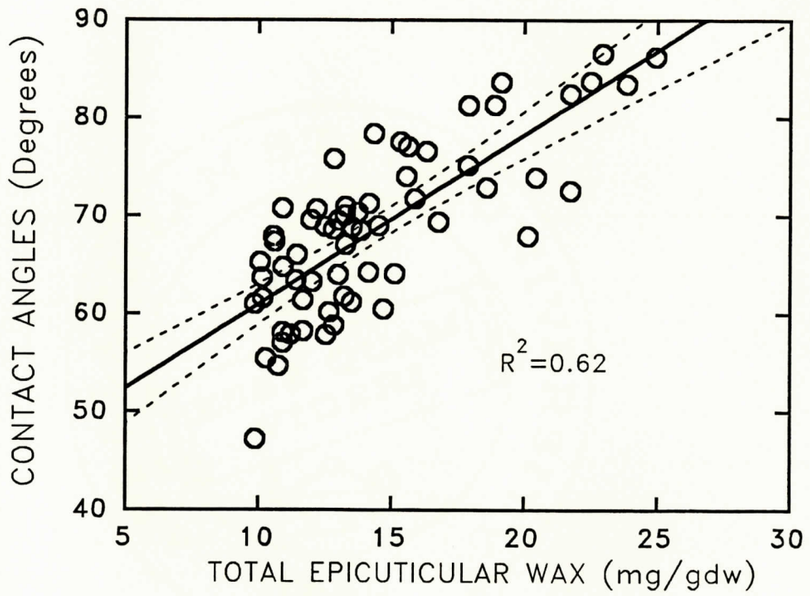


Figure 8. Linear regression of contact angle as a function of total epicuticular wax. Dotted lines indicate 95% confidence interval about the slope.

9a and b). There were no differences between the filtered and non-filtered treatments (Table 7).

Time by age interactions were significant for both stands, mainly because wax amounts dropped during the season in 0 year needles, but not the 1 year old ones. There was also a significant treatment by age interaction in the healthy stand since 0 year needles exhibited greater wax amounts in chambered branches than unchambered ones (Table 7).

WAX ULTRASTRUCTURE

Stand Comparisons Using Unchambered Branches

No significant differences existed among age classes or between stands for wax tubule length or diameter (Fig. 10a - d). A significant stand by age interaction was observed for tubule diameter due to age differences in the healthy stand but not in the declining stand.

Treatment Effects

An interaction effect of treatment by age was noted for tubule lengths in the declining stand because wax tubule lengths on needles in chambers decreased with age, but no change was observed for unchambered branches (Figure 10a, Table 8). Unchambered branches in the declining stand did have significantly greater tubule

Figure 9. Total epicuticular wax for chambered and control branches in 1990. (A) Total epicuticular wax for 0 year old needles in the declining stand as a function of time. (B) Total epicuticular wax for 1 year old needles in the declining stand as a function of time. (C) Total epicuticular wax for 0 year old needles in the healthy stand as a function of time. (D) Total epicuticular wax for 1 year old needles in the healthy stand as a function of time. Lines are smooth fits to data. Points are sample means ± 95% CI.

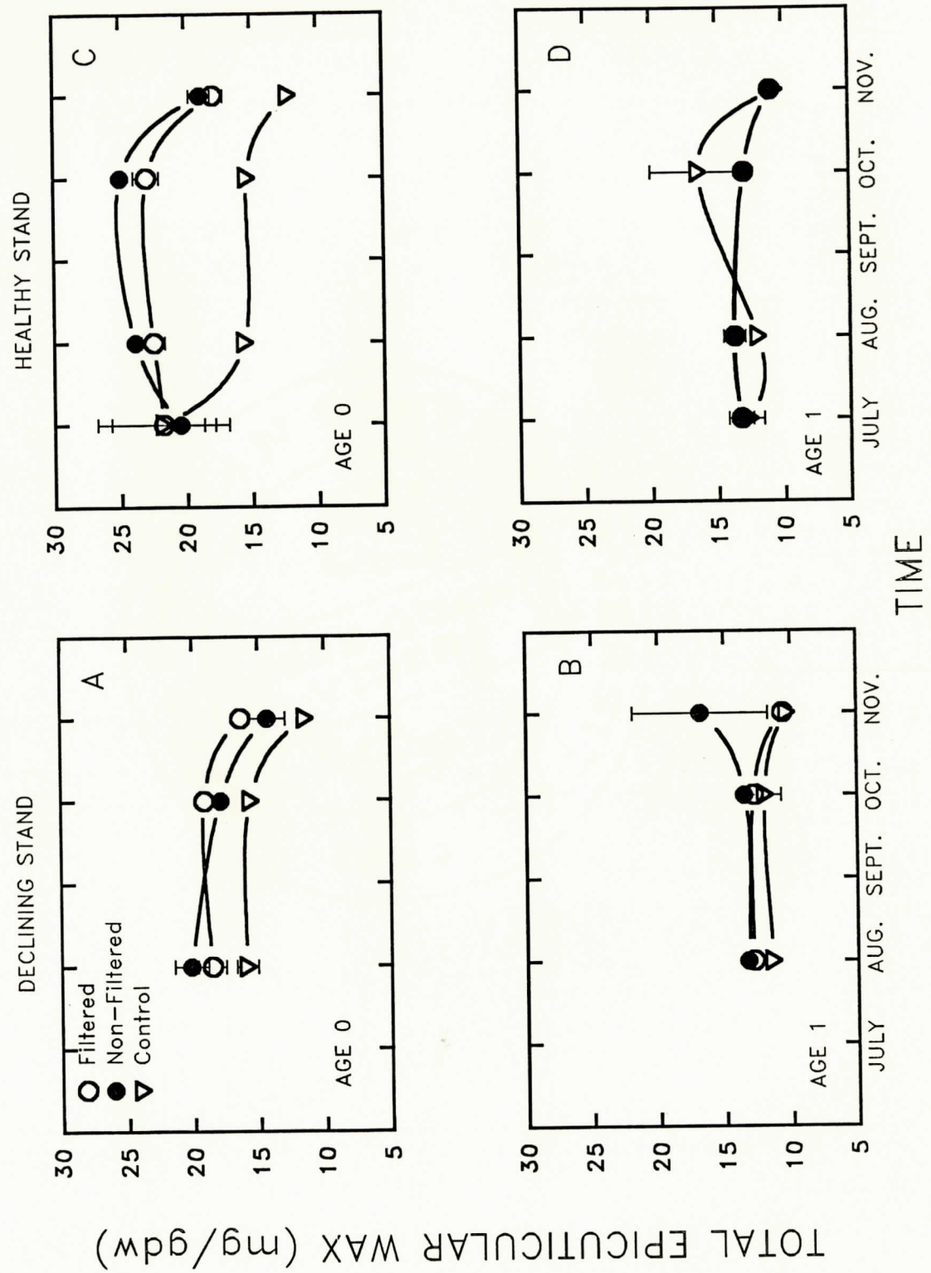


Table 7

Summary of analysis of variance using repeated measures for treatment effects on total wax amounts in chambered branches.

Declining Stand

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
TREATMENT	2	33.30	7.84	0.0077*
AGE	1	276.32	65.05	0.0001*
TIME	2	59.25	24.02	0.0001*
TR*AGE	2	7.19	1.69	0.2285
TR*TIME	4	1.93	0.78	0.5486
AGE*TIME	2	10.49	4.25	0.0275*
TM*TR*AG	4	2.28	0.92	0.4675

Healthy Stand

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
TREATMENT	2	115.83	14.48	0.0002*
AGE	1	913.51	114.17	0.0001*
TIME	3	62.32	25.90	0.0001*
TR*AGE	2	81.37	10.17	0.0012*
TR*TIME	6	4.09	1.70	0.1404
AGE*TIME	3	16.53	6.87	0.0006*
TM*TR*AG	6	5.41	2.25	0.0530

Values of $p < 0.05$ are considered significant and are highlighted by a *.

Figure 10. Wax tubule lengths and diameters for chambered and control branches in November 1990. (A) Wax tubule lengths for 0, 1 and 3 year old needles in the declining stand. (B) Wax tubule diameters for 0, 1 and 3 year old needles in the declining stand. (C) Wax tubule lengths for 0, 1 and 3 year old needles in the healthy stand. (D) Wax tubule diameters for 0, 1 and 3 year old needles in the healthy stand. Points are sample means + 95% CI.

Table 8

Summary of analysis of variance for treatment effects on wax tubule length and diameter.

Declining Stand (Length)

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
TREATMENT	1	0.001	0.00	0.9964
AGE	1	992.250	18.55	0.0001*
TRT*AGE	1	409.388	7.65	0.0075*

Healthy Stand (Length)

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
TREATMENT	1	495.01	6.29	0.0142*
AGE	1	17.11	0.22	0.6422
TRT*AGE	1	159.61	2.03	0.1583

Declining Stand (Diameter)

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
TREATMENT	1	11.33	4.46	0.0387*
AGE	1	0.81	0.32	0.5743
TRT*AGE	1	0.01	0.00	0.9502

Healthy Stand (Diameter)

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
TREATMENT	1	7.20	2.80	0.0983
AGE	1	12.80	4.98	0.0286*
TRT*AGE	1	5.51	2.14	0.1472

Values of $p < 0.05$ are considered significant and are highlighted by a *.

diameters than chambered ones (0.188 vs. 0.171 μm) (Fig. 10b).

Chambered branches in the healthy stand had needles with longer wax tubules than unchambered needles (0.594 vs. 0.494 um) (Fig. 10c), but no significant age effect was found. One year old needles had greater wax tubule diameters than 0 year needles (Fig. 10d). No significant difference in chambered and unchambered branches was found.

GROWTH

Stand and Size Comparisons Using Unchambered Branches

0 and 1 year old twigs on mature trees had similar diameters for both the declining and healthy stands (Fig. 11a and c). Twig diameters increased with age from 0 and 1 year old twigs to 5 year old twigs (2.14, 2.11, 2.55, 2.86 and 3.39 mm). No stand differences were observed. Length of 0 year twigs did not differ significantly between stands (Fig. 11b and d).

Twig diameters were significantly smaller in mature trees than either seedlings or saplings (2.14 vs. 2.68 and 2.96 mm) (Fig. 12a). Twig lengths were also reduced in mature trees compared to seedlings and saplings (26.93 vs. 102.29 and 99.80mm) (Fig. 12b). No significant

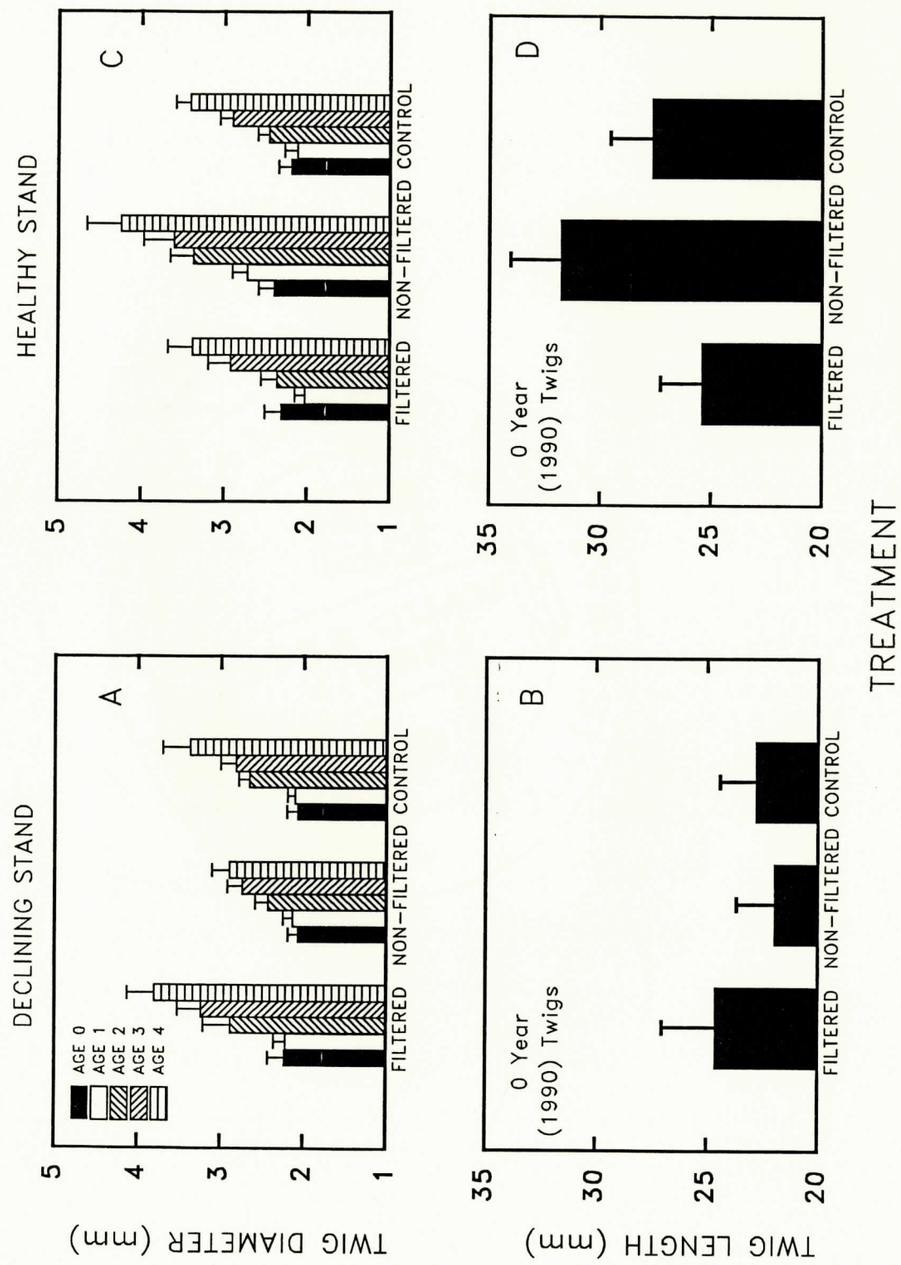


Figure 11. Twig diameter and length of chambered and control branches of mature trees in November 1990. (A) Diameter of 0, 1, 2, 3 and 4 year old twigs in the declining stand. (B) Length of 0 year old twigs in the declining stand. (C) Diameter of 0, 1, 2, 3 and 4 year old twigs in the healthy stand. (D) Length of 0 year old twigs in the healthy stand. Points are sample means ± 95% CI.

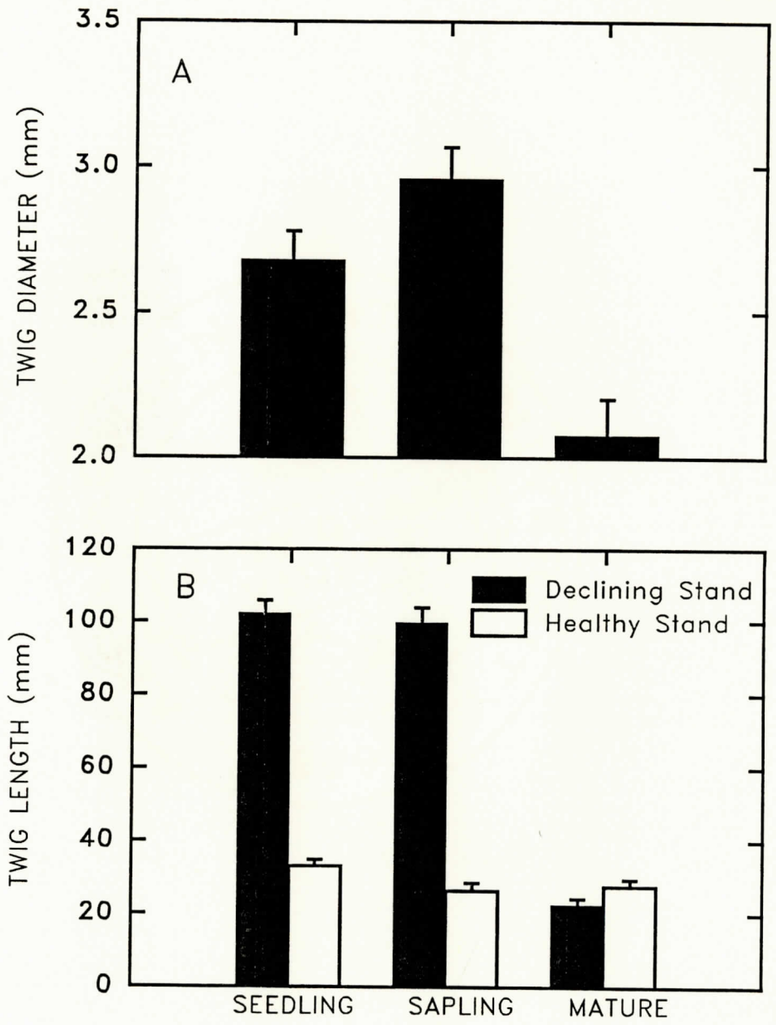


Figure 12. Diameters and lengths for current year twigs on unchambered branches of seedlings, saplings and mature trees in November 1990. (A) Twig diameters in the declining stand. (B) Twig lengths in the declining and healthy stands. Points are sample means + 95% CI.

differences were observed between saplings and seedlings for twig diameters or lengths.

Seedlings and saplings had greater 0 year twig lengths in the declining stand than in the healthy stand. A significant stand by size interaction was found because no differences were observed in twig lengths among seedlings, saplings and mature trees in the healthy stand.

Treatment Effects

Two, three and four year old twigs in the filtered treatment had significantly greater diameters than those in the non-filtered and unchambered treatments in the declining stand (Fig. 11a). There was also an increase in diameter with age for all treatments except for 0 and 1 year old twigs.

In the healthy stand, 1 through 4 year old twigs had significantly greater diameters in the non-filtered treatment than those in the filtered and unchambered treatments (Fig. 11c). Again the trend of increasing diameters with age was observed. The difference between 0 and 1 year old twigs was once again small. No significant differences were found in length or diameter growth for the declining or healthy stand when treatment effects on 0 year twigs were analyzed separately (Fig. 11b and d).

CHLOROPHYLL

Stand Comparisons Using Unchambered Branches

Chlorophyll *a* increased from 0 year needles to 3 year old needles from October 1989 to March 1990 and July to November 1990. No other significant differences were found except for a time by stand interaction from October 1989 to March 1990. This was due to a slight increase in chlorophyll *a* content of the healthy stand from October to March while no change was observed in the declining stand. Following the 1989 cohort through time, a significant increase was found for chlorophyll *a* (Table 9a) from March to August 1990 (1.15, 1.94 and 2.48 mg gdw^{-1}).

Chlorophyll *b* amounts were significantly greater for the declining stand than the healthy stand for the period October 1989 to March 1990. Amounts were also greater with age of the needle during this period. Amounts decreased with time, mostly in the declining stand. Only an age effect was found for the period July through November 1990 where concentrations increased with needle age (0.575, 0.602 and 0.804 mg gdw^{-1} for 0, 1 and 3 year old needles). A significant time effect was found for chlorophyll *b* amounts when following the 1989 cohort (Table 9b). Levels increased between March and August 1990.

Table 9

Summary of chlorophyll analysis of variance using repeated measures for a 1989 needle cohort.

Chlorophyll a

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
STAND	1	0.40	0.80	0.4128
TIME	6	2.76	29.17	0.0001*
ST*TM	6	0.15	1.61	0.1780

Chlorophyll b

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
STAND	1	0.13	1.48	0.2785
TIME	6	0.16	5.65	0.0005*
ST*TM	6	0.04	1.58	0.1870

Total Chlorophyll

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
STAND	1	1.04	1.01	0.3610
TIME	6	4.17	20.97	0.0001*
ST*TM	6	0.34	1.70	0.1552

Chlorophyll a to b ratio

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
STAND	1	0.34	0.12	0.7475
TIME	6	2.91	1.44	0.2318
ST*TM	6	2.02	1.00	0.4435

Values of $p < 0.05$ are considered significant and are highlighted by a *.

Total chlorophyll concentration increased with needle age (Fig. 13a and c). A time by stand interaction occurred probably due to the slight decrease with time in 0 and 1 year old needles for the declining stand while the healthy stand only exhibited a slight increase in 3 year old needles (Table 10). An age effect was found only from July to November 1990 where chlorophyll amounts increased from 2.54, 2.92 and 3.97 for the declining stand and 2.36, 2.88 and 3.23 for the healthy stand in 0, 1 and 3 year old needles, respectively.

A 1989 needle cohort exhibited an increase from March to August (Fig. 13b and d). No other significant differences were observed after this date (Table 9c).

The chlorophyll *a* to *b* ratio increased from October 1989 to January and March 1990 in all age classes (2.53, 3.71 and 3.21 for 0, 1 and 3 year old needles respectively). The *a* to *b* ratio increased from July to October 1990, while it decreased into November for all age classes. Although these trends appeared when the 1989 cohort was followed through time, no significant differences in the *a* to *b* ratio between stands or with time were found.

Treatment Effects

There were no significant differences in chlorophyll *a* amounts for treatment, age or time in the declining

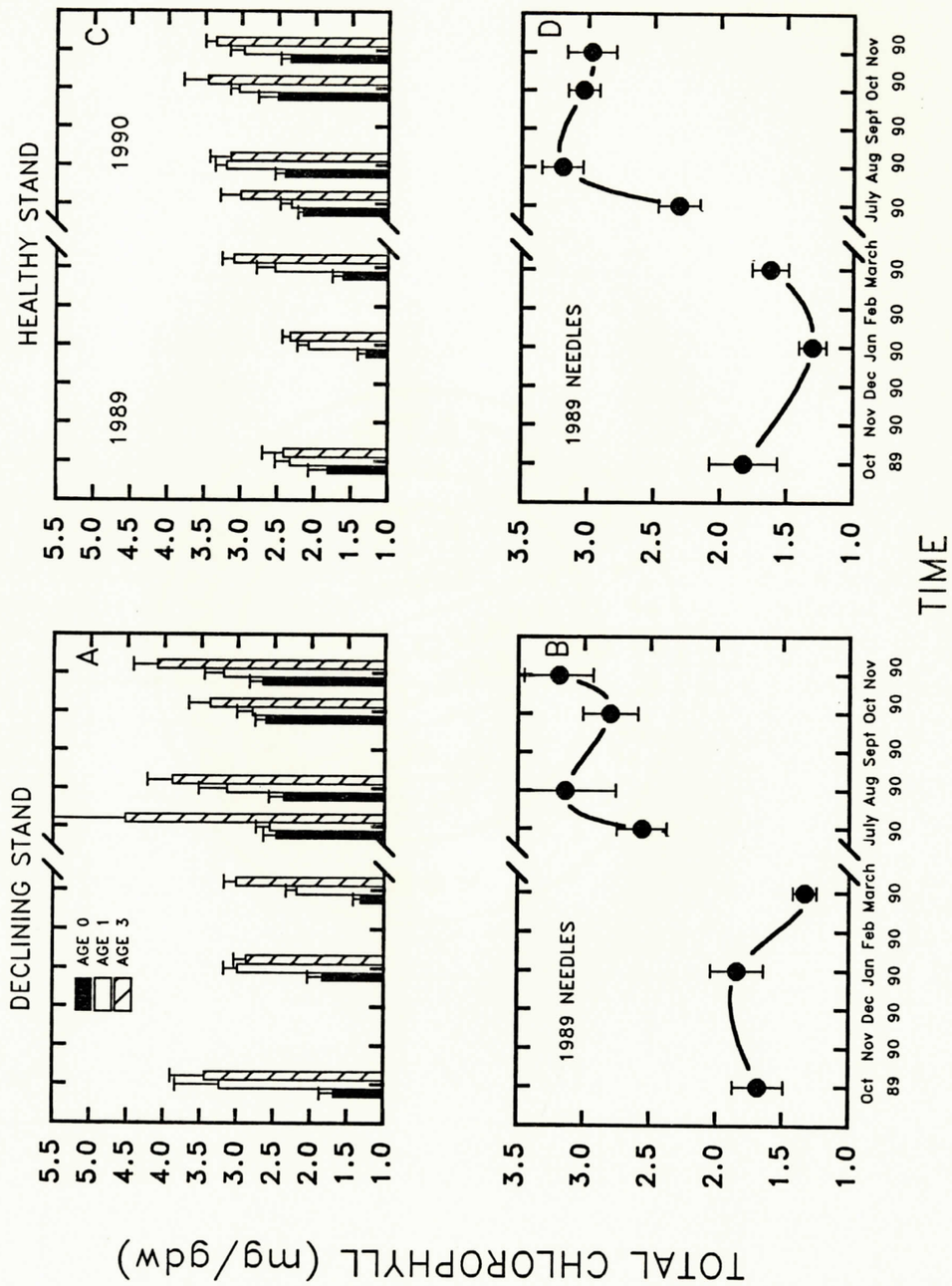


Figure 13. Total chlorophyll of needles on unchambered branches. (A) Total chlorophyll for 0, 1 and 3 year old needles in the declining stand as a function of time. (B) Total chlorophyll for the 1989 needle cohort in the declining stand as a function of time. (C) Total chlorophyll for 0, 1 and 3 year old needles in the healthy stand as a function of time. (D) Total chlorophyll for the 1989 needle cohort in the healthy stand as a function of time. Lines are smooth fits to data. Points are sample means ± 95% CI.

Table 10

Summary of total chlorophyll analysis of variance using repeated measures on unchambered branches.

Oct. 89 through March 90

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
STAND	1	2.07	5.06	0.0373*
AGE	2	10.43	25.44	0.0001*
TIME	2	0.47	1.77	0.1953
ST*AG	2	0.46	1.13	0.3444
TM*ST	2	1.50	5.69	0.0159*
TM*AG	4	0.26	1.00	0.4051
TM*ST*AG	4	0.27	1.02	0.3986

July 90 through Nov. 90

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
STAND	1	4.56	2.43	0.1397
AGE	2	10.90	5.81	0.0136*
TIME	3	0.05	0.09	0.8184
ST*AG	2	1.30	0.69	0.5156
TM*ST	3	0.90	1.77	0.2014
TM*AG	6	0.76	1.48	0.2530
TM*ST*AG	6	0.37	0.72	0.5255

Values of $p < 0.05$ are considered significant and are highlighted by a *.

stand. Significant differences did occur in the healthy stand for treatment, time and age effects. Chambered branches had higher chlorophyll *a* concentrations than unchambered branches for July through August 1990, especially in 0 year needles (2.2 vs. 1.74 mg gdw^{-1} in July). One year old needles also had higher concentrations than 0 year needles. Following these needles through time, amounts increased from July to October and then decreased into November. This change with time was especially apparent for 1 year old needles. There were no significant treatment, age or time effects for chlorophyll *b* amounts in the declining or healthy stands.

Total chlorophyll amounts were not affected by treatment, age or time in the declining stand (Fig. 14a and b). Chambered branches had higher total chlorophyll amounts than unchambered branches in the healthy stand (2.71 vs. 2.16 mg gdw^{-1} in July,respectively). There was also a significant time effect attributed to the dramatic increase from July to August, especially in 1 year old needles (Table 11). There were no significant treatment, age or time effects for chlorophyll *a* to *b* ratios.

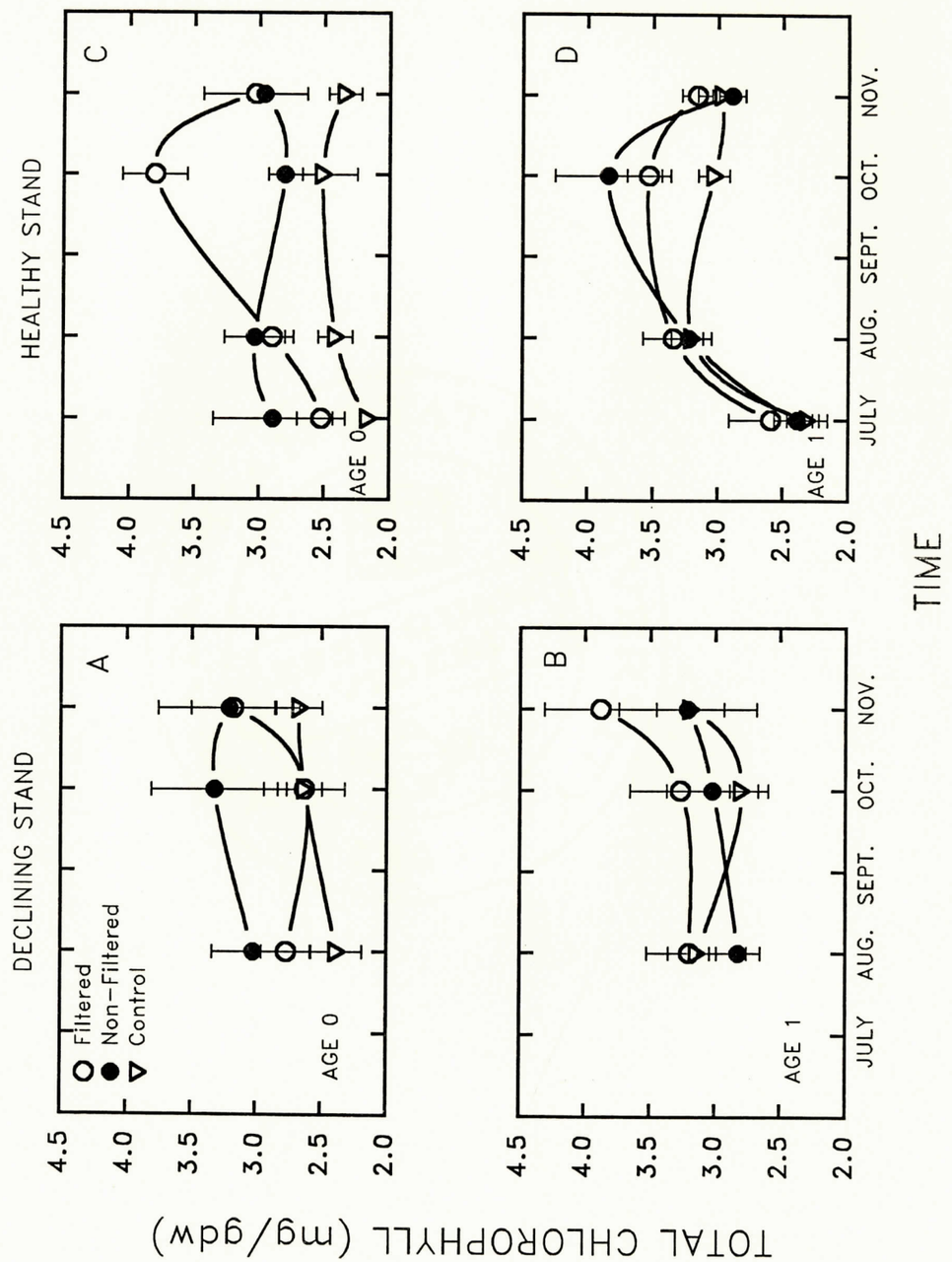


Figure 14. Total chlorophyll for chambered and control branches in 1990. (A) Total chlorophyll for 0 year old needles in the declining stand as a function of time. (B) Total chlorophyll for 1 year old needles in the declining stand as a function of time. (C) Total chlorophyll for 0 year old needles in the healthy stand as a function of time. (D) Total chlorophyll for 1 year old needles in the healthy stand as a function of time. Lines are smooth fits to data. Points are sample means ± 95% CI.

Table 11

Summary of analysis of variance using repeated measures, for treatment effects on total chlorophyll concentration in chambered branches.

Declining Stand

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
TREATMENT	2	0.41	0.43	0.6589
AGE	1	2.05	2.19	0.1651
TIME	2	0.39	1.08	0.3336
TR*AGE	2	0.87	0.93	0.4209
TR*TIME	4	0.16	0.43	0.7050
AGE*TIME	2	0.02	0.04	0.8853
TM*TR*AG	4	0.21	0.57	0.6174

Healthy Stand

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
TREATMENT	2	2.16	4.35	0.0287*
AGE	1	1.63	3.30	0.0861
TIME	3	2.54	11.03	0.0001*
TR*AGE	2	0.42	0.86	0.4416
TR*TIME	6	0.17	0.74	0.6235
AGE*TIME	3	0.39	1.71	0.1754
TM*TR*AG	6	0.37	1.63	0.1577

Values of $p < 0.05$ are considered significant and are highlighted by a *.

GAS EXCHANGE

Stand Comparisons Using Unchambered Branches

Gas exchange measurements were made between the months of October 1989 and November 1990. Since October and December 1989 dates are missing for the healthy stand, only November needles could be compared between stands in 1989. A significant stand by age interaction was observed since 0 year needles exhibited higher photosynthetic rates than 1 or 3 year old needles in the healthy stand but the age difference was not observed in the declining stand (Fig 15 a and c, Table 12).

Total conductance to water vapor was greater in the healthy stand than the declining stand for all age classes during November 1989 (0.70 vs. 0.22 mmol $gdw^{-1}s^{-1}$) (Fig. 16a and c). There were no significant differences among age classes (Table 12).

Internal CO_2 concentrations were higher for needles in the healthy stand than the declining stand for November 1989 (Fig. 17a and c). There was also a significant increase in internal CO_2 concentration with age from 0 to 3 year old needles (Table 12).

Age and time effects on all gas exchange parameters were analyzed in the declining stand for October through December 1989 separately from the healthy stand, since October and December dates were missing for the healthy

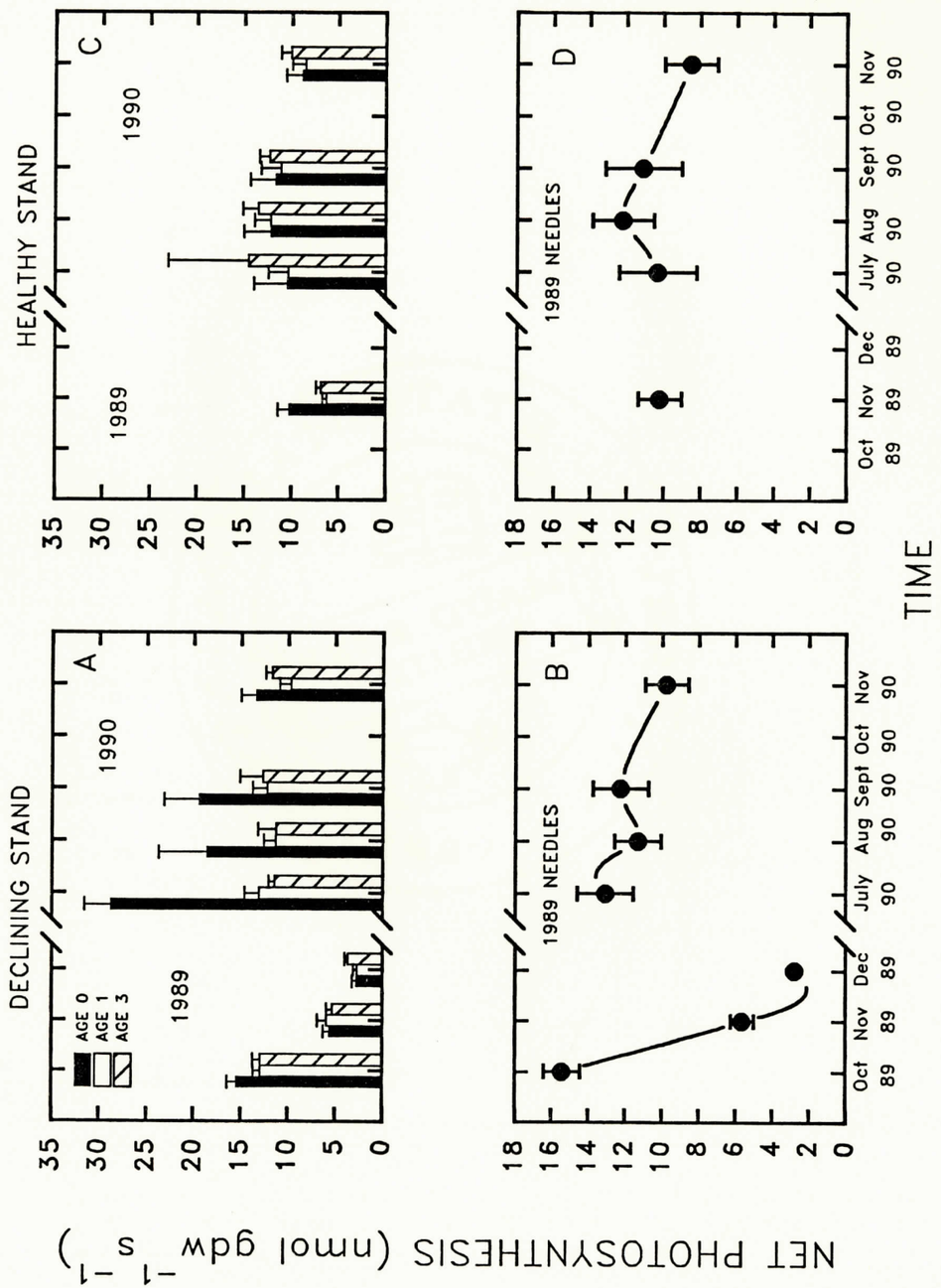


Figure 15. Net photosynthesis of needles on unchambered branches. (A) Net photosynthesis for 0, 1 and 3 year old needles in the declining stand as a function of time. (B) Net photosynthesis for the 1989 needle cohort in the declining stand as a function of time. (C) Net photosynthesis for 0, 1 and 3 year old needles in the healthy stand as a function of time. (D) Net photosynthesis for the 1989 needle cohort in the healthy stand as a function of time. Lines are smooth fits to data. Points are sample means ± 95% CI.

Table 12

Summary of gas exchange analysis of variance for Nov. 1989 on unchambered branches.

Net Photosynthesis

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
STAND	1	131.09	11.47	0.0010*
AGE	2	45.52	3.98	0.0214*
ST*AG	2	47.50	4.15	0.0182*

Total Conductance to Water Vapor

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
STAND	1	6.58	232.90	0.0001*
AGE	2	0.04	1.51	0.2257
ST*AG	2	0.08	2.84	0.0627

Internal CO_2 Concentration

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
STAND	1	3111.08	8.02	0.0055*
AGE	2	3307.97	8.53	0.0004*
ST*AG	2	806.61	2.08	0.1300

Values of $p < 0.05$ are considered significant and are highlighted by a *.

stand (Fig. 15a). In the declining stand, a significant decrease in net photosynthetic rate was found with time for all age classes (13.75, 5.61 and 3.04 nmol $gdw^{-1}s^{-1}$ for 0, 1 and 3 year old needles respectively). Total conductance to water vapor did not change between October and November but decreased from November to December (Fig. 16a). Internal CO_2 concentration increased from October to November but did not change through December (Fig. 17a). No significant age differences were found in net photosynthesis, total conductance to water vapor or internal CO_2 concentration in the declining stand for the period October to December, 1989.

Analysis of July through November 1990 data showed no stand, age or time effects for net photosynthesis (Fig. 15a and c). Total conductance to water vapor exhibited a significant time by stand interaction because 1 and 3 year needles reacted with an increase from July to August in the healthy stand and a decrease in the declining stand (Fig. 16a and c). A significant stand by age interaction was also observed since no age differences were found in the declining stand, but 1 and 3 year old needles in the healthy stand exhibited higher total conductance to water vapor than 0 year needles in August. Internal needle CO_2 concentrations did not change from July to August, but were followed by a decrease in September and again in

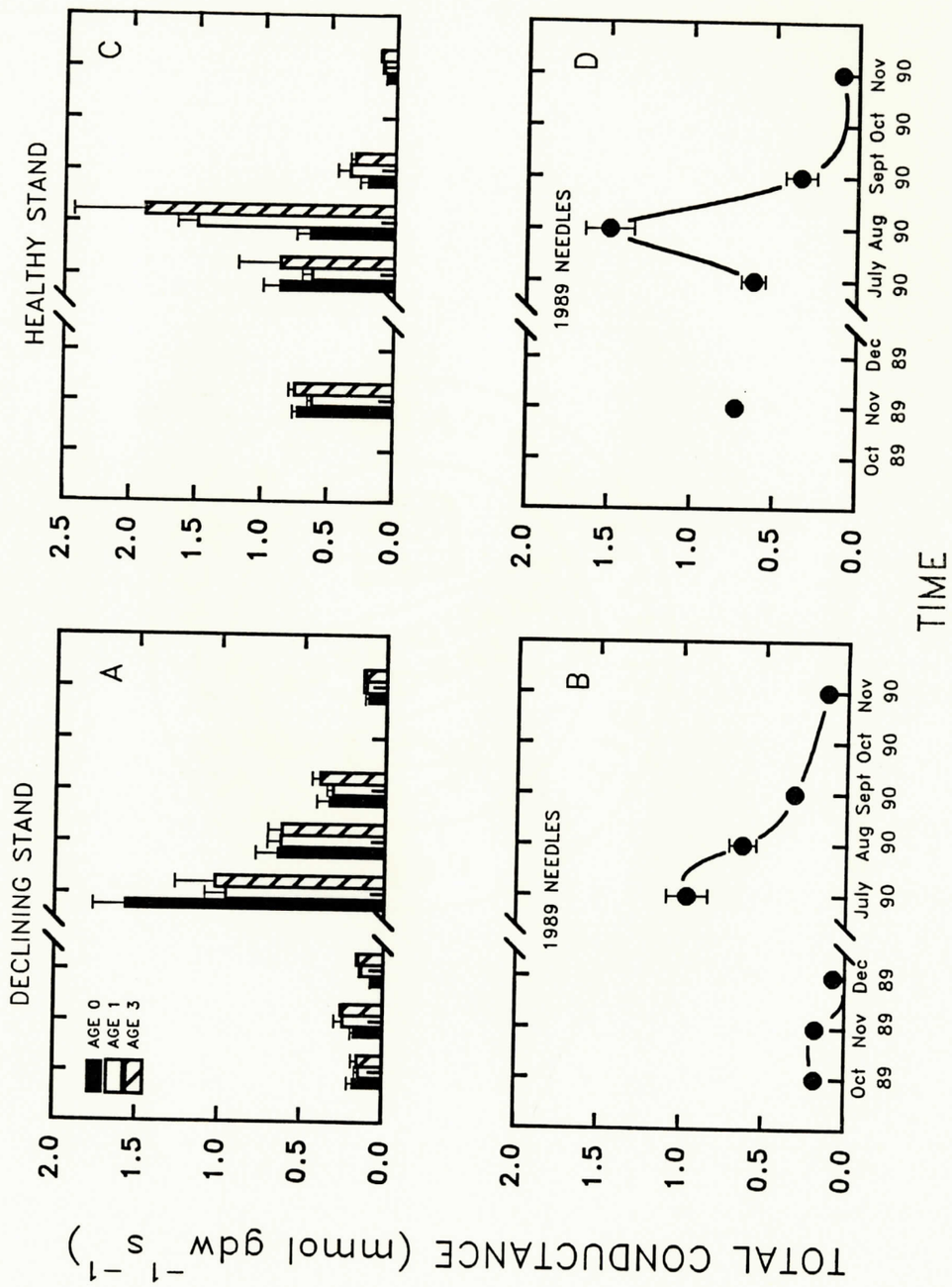


Figure 16. Total conductance to water vapor of needles on unchambered branches. (A) Total conductance to water vapor for 0, 1 and 3 year old needles in the declining stand as a function of time. (B) Total conductance to water vapor for the 1989 needle cohort in the declining stand as a function of time. (C) Total conductance to water vapor for 0, 1 and 3 year old needles in the healthy stand as a function of time. (D) Total conductance to water vapor for the 1989 needle cohort in the healthy stand as a function of time. Lines are smooths fits to data. Points are sample means ± 95% CI.

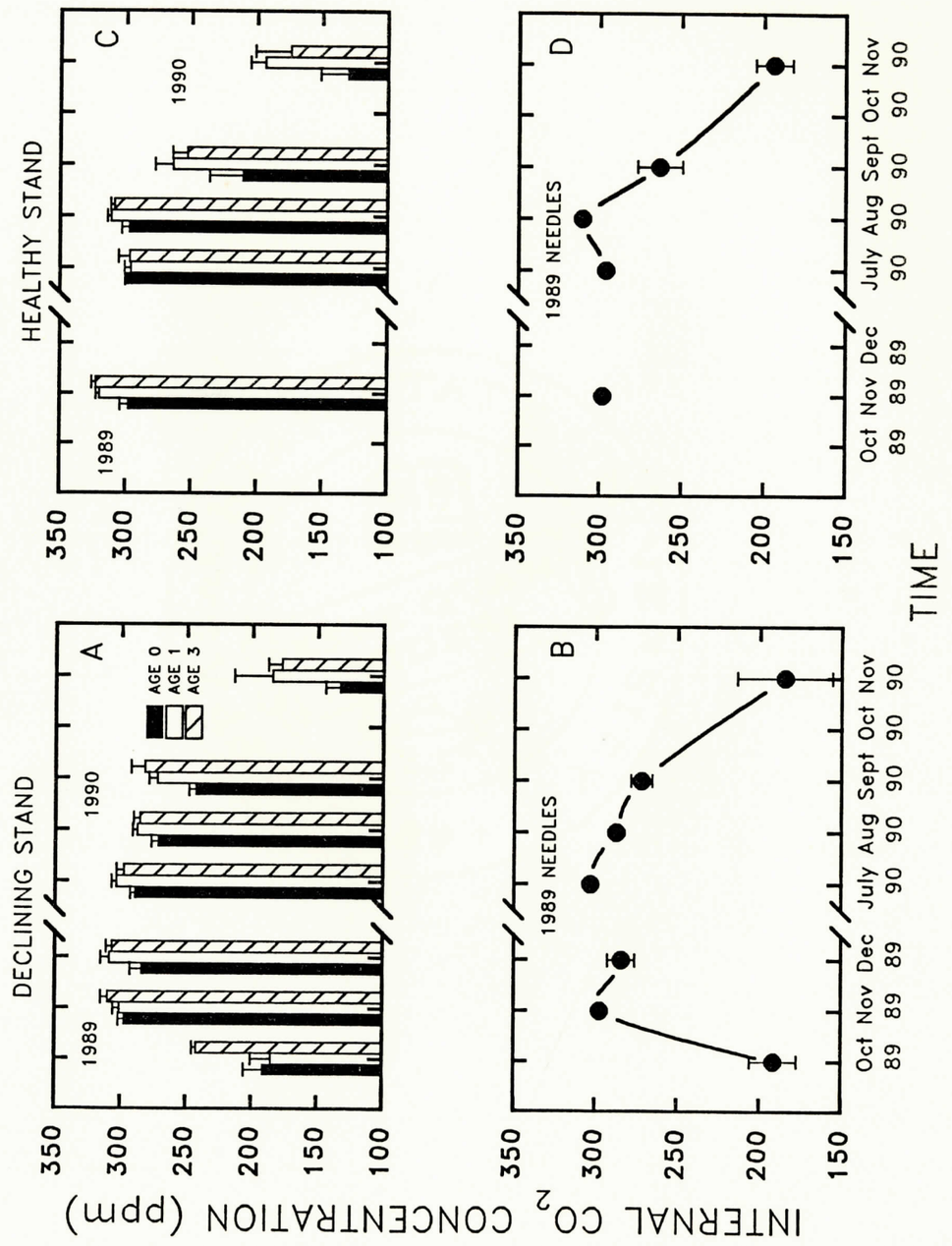


Figure 17. Internal carbon dioxide concentration of needles on unchambered branches. (A) Internal carbon dioxide concentration for 0, 1 and 3 year old needles in the declining stand as a function of time. (B) Internal carbon dioxide concentration for the 1989 needles cohort in the declining stand as a function of time. (C) Internal carbon dioxide concentration for 0, 1 and 3 year old needles in the healthy stand as a function of time. (D) Internal carbon dioxide concentration for the 1989 needle cohort in the healthy stand as a function of time. Lines are smooth fits to data. Points are sample means ± 95% CI.

November for all age classes in both stands (Fig. 17a and c).

Needle cohorts for 1989 were compared between stands using only the November 1989 sampling date and then July through November 1990 (Fig. 15b and d). No significant stand or time effects were found for net photosynthesis (Table 13). Differences in total conductance to water vapor between stands in time led to a significant interaction effect (Fig. 16b and d, Table 13). Internal CO_2 concentration changes were similar between stands (Table 13). Concentrations were similar in November 1989 and July 1990. A decrease was observed between August and November (Fig. 17b and d).

An analysis of the 1989 cohort in the declining stand showed a significant time effect for net photosynthesis, total conductance to water vapor and internal CO_2. Net photosynthesis decreased from October to December 1989 (Fig. 15b), however, it was greater in July 1990 than in December 1989. No changes were seen in net photosynthesis for July through November 1990. Total conductance to water vapor did not change between October and December 1989 (Fig. 16b). Conductance was much higher in July 1990 followed by a slow decline into November. Internal CO_2 increased dramatically form October to December 1989 (Fig. 17b), while no difference was found for December 1989 and

Table 13

Summary of gas exchange analysis of variance using repeated measures on unchambered branches for a 1989 needle cohort.

Net Photosynthesis

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
STAND	1	0.54	0.02	0.9030
TIME	4	21.77	2.64	0.0642
ST*TM	4	11.69	1.42	0.2643

Total Conductance To Water Vapor

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
STAND	1	0.41	8.34	0.0343*
TIME	4	0.95	19.45	0.0001*
ST*TM	4	0.45	9.25	0.0002*

Internal CO_2 Concentration

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
STAND	1	128.43	0.23	0.6540
TIME	4	15371.42	16.64	0.0001*
ST*TM	6	307.94	0.33	0.8522

Values of $p < 0.05$ are considered significant and are highlighted by a *.

July 1990. A decline in internal CO_2 was then observed into September followed by a dramatic decline in November.

Treatment Effects

Current year needles had higher net photosynthetic rates than 1 year old needles in the declining stand for August through November 1989 in all treatments (Fig. 18a and b). No significant treatment or time effects were found (Table 14).

Chambered needles in the healthy stand had higher net photosynthetic rates than unchambered ones for both age classes and at most times (Fig. 18c and d), except for 1 year old needles in September and November. There were no differences between filtered and non-filtered treatments (Table 14). Current year needles generally had greater photosynthetic rates than 1 year old needles, except for unchambered needles where the age difference was not as obvious (Fig. 18c and d). A time effect was also found. A slight increase was observed from July to August followed by a decline in November.

There were significant age by time and time by treatment by age interactions in total conductance to water vapor data. In addition the internal CO_2 concentration analysis revealed significant age by time, treatment by time and age by time interactions.

Figure 18. Net photosynthesis for chambered and unchambered branches in 1990. (A) Net photosynthesis for 0 year old needles in the declining stand as a function of time. (B) Net photosynthesis for 1 year old needles in the declining stand as a function of time. (C) Net photosynthesis for 0 year old needles in the healthy stand as a function of time. (D) Net photosynthesis for 1 year old needles in the healthy stand as a function of time. Lines are smooth fits to data. Points are sample means ± 95% CI.

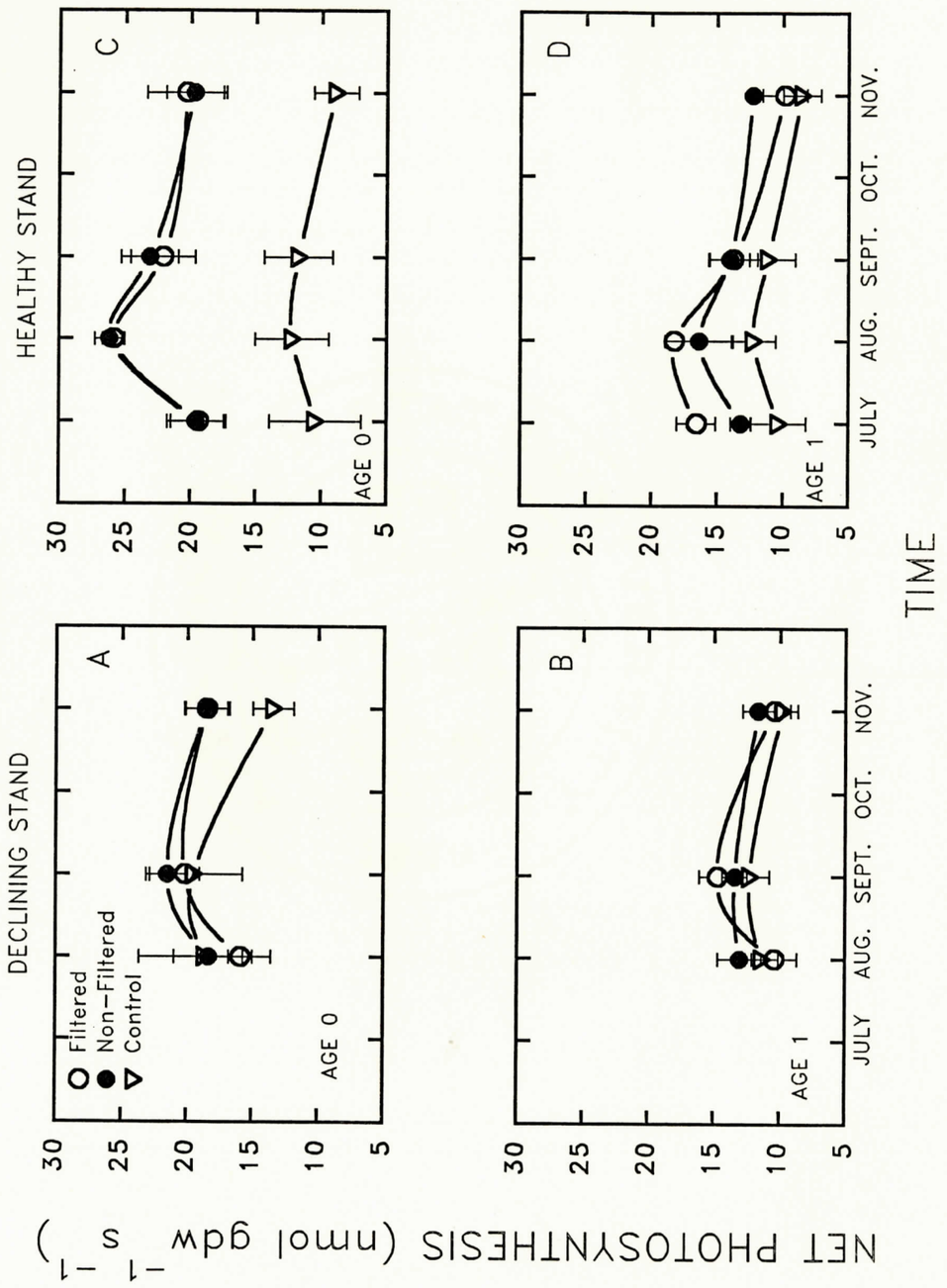


Table 14

Summary of analysis of variance using repeated measures for treatment effects on total conductance to water vapor in chambered branches.

Declining Stand

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
TREATMENT	2	0.010	0.87	0.4425
AGE	1	0.004	0.37	0.5554
TIME	2	1.150	85.65	0.0001*
TR*AGE	2	0.040	3.73	0.0550
TR*TIME	4	0.010	0.94	0.4568
AGE*TIME	2	0.002	0.15	0.8597
TM*TR*AG	4	0.010	0.95	0.4525

Healthy Stand

SOURCE	df	MEAN SQUARE	F VALUE	Prob.>F
TREATMENT	2	0.003	0.05	0.9531
AGE	1	0.010	0.21	0.6532
TIME	3	4.040	116.86	0.0001*
TR*AGE	2	0.140	2.13	0.1483
TR*TIME	6	0.020	0.52	0.7187
AGE*TIME	3	0.260	7.61	0.0002*
TM*TR*AG	6	0.130	3.79	0.0032*

Values of $p < 0.05$ are considered significant and are higlighted by a *.

DISCUSSION

CONTACT ANGLES AND WAX

The unchambered branch comparisons show no major differences in contact angles between stands during the summer months, and it appears that while contact angles change due to needle age and time, there are no distinct patterns that can be used to diagnose a tree in a state of decline. Similarly, Cape et al. (1989) found no significant differences in contact angles on needles from declining and healthy Scots pine and Norway spruce growing at the same sites. However, Cape et al. (1989) did observe consistently lower angles with needle age similar to the results of this study. These authors believe that leaf surface properties reflect environmental exposure rather than plant response to pollutants.

As a leaf becomes fully expanded, the rate of wax production slows down, but it is not clear if wax production ceases completely. Cape (1983) suggests that it may not completely cease while Turunen & Huttunen (1990) believe there is new evidence to support the idea of complete cessation of wax synthesis. However, the contact angle value observed depends not only on wax amounts and composition but also on the roughness of the underlying surface (Holloway, 1971; Cape, 1983). A

rough surface may lead to small airpockets being trapped between the wax surface and the water. This would reduce the effective area of contact. Exposure of the leaf cuticle, which is more hydrophobic than the surface of the wax, would also increase the contact angle (Holloway, 1969 cited in Cape, 1983). In contrast it has been demonstrated that an accumulation of dust on the needle surface can also cause a more hydrophyllic surface (Crossley & Fowler, 1986; Cape et al., 1989), a phenomenon that was not assessed in this study.

Total epicuticular wax amounts did not follow the exact pattern of the contact angle data in our study, especially for age effects. Since most or all wax production occurs in the first growing season, we might expect 0 year needles to have greater amounts of wax than 1 or 3 year old needles. This was not observed however. In January and March of 1990, for example, 1 year old needles had greater amounts of wax than 0 or 3 year old needles in the declining stand. No explanation can be offered for this anomalous pattern.

This study revealed no stand differences in total epicuticular wax amounts, supporting the suggestion that this parameter is not useful in diagnosing tree decline (Cape et al., 1989). Cape et al. (1989) did, however, find a decrease in wax with age of needles as well as a decrease with time for all age classes. Results from this

study show a similar decrease with time in 0 year needles but 1 and 3 year old needles exhibited no change except from January to March 1990. Cape (1988) suggested that any decrease with age could be explained by either a net loss of wax or an increase in needle dry weight per unit area. Perhaps waxes are most easily eroded in young needles as tubules are taller and more vulnerable to abrasion.

In this study there was an increase in contact angles for the 1989 needle cohort into the second summer for both stands. There was also a slight increase in wax amounts from March to July 1990, but the relationship between increasing wax mounts and contact angles with time did not correlate to a high degree. The regression of total wax amounts on contact angles in this study indicated a significant positive correlation, but only 62% of the variance in contact angles was explained by changes in wax amounts.

While there were no ozone effects on contact angles, wax amounts were greater on needles in chambers than on unchambered branches in the declining stand, with no differences between filtered and non-filtered chambers. This effect may relate to the higher temperatures maintained in chambered branches, which are known to increase wax synthesis (Baker, 1982). However, Bytnerowicz et al. (1989) found that increased

temperatures in chambers might actually cause structural damage to waxes on pine trees. Chambers may also have exerted effects by reducing weathering, which has been found to naturally erode waxes (Crossley & Fowler, 1986), or by reducing acidic precipitation, which may cause physical and chemical changes in waxes to a greater extent than normal precipitation (Barnes et al., 1988; Cape et al., 1988).

Percy et al. (1990) found no differences in wax yields of red spruce needles exposed to 70 and 250 ppb O_3. This suggested that the O_3 did not reduce the amount of carbon available for wax synthesis or inhibit steps in fatty acid biosynthesis. Other researchers, however, have reported greater erosion and losses of waxes on conifers with O_3 fumigated in chambers (Gunthardt-Goerg and Keller, 1987; Ashmore et al., 1988; Barnes et al., 1988; Grill et al., 1989).

One of the symptoms associated with declining trees at high elevations is premature degradation of the epicuticular wax (Bosch et al., 1983; Karhu & Huttunen, 1986). This was not observed in the present study when comparisons were made for contact angles, wax amounts or wax ultrastructure. No age effects were observed for wax tubule diameters or lengths in unchambered branch comparisons, which would have suggested erosion with time (Cape, 1983). Ambient levels of ozone appear not to have

any effect on wax ultrastructure at Whitetop mountain, since no differences were found between needles from filtered and non-filtered chambers. This lack of ozone effects on wax morphology has been reported by others (Trimble et al., 1982; Schmitt et al., 1987). However, erosion of waxes and changes in wax morphology due to ambient levels of O_3 has been reported as well (Gunthardt-Goerg & Keller, 1987; Grill et al., 1989). Percy et al. (1990), in particular, showed that for red spruce, exposure to 70 ppb O_3 did cause a coalescence of wax tubule ends within the epistomatal chambers.

Chambering effects, however, were observed in this study. If the chambers were protecting the wax on the red spruce needles, we might expect smaller tubule diameters and greater lengths in the chambered needles. Smaller diameters for chambered branches in the declining stand and greater tubule lengths in chambered branches in the healthy stand were observed. This may suggest either reduced weathering effects or reduced acidic deposition as discussed earlier.

Thornton et al. (1990) compared total epicuticular wax amounts among four treatments of red spruce seedlings on Whitetop mountain; clouds and ozone excluded, exposure to ambient ozone with clouds excluded, chambered but exposed to both ozone and clouds, and unchambered. No significant differences were found on 0 year needles

exposed to ambient levels of ozone and cloudwater and seedlings exposed to reduced pollution levels. They believe this supports other findings that artificial mist and ozone do not cause wax erosion during the first growing season (Percy et al., 1990). They did find, however, that 0 and one year old needles on native seedlings in unchambered plots had lower wax concentrations than needles on chambered seedlings. They suggested that since the unchambered plots were shaded, and sunlight stimulates wax synthesis (Baker, 1974 cited in Thornton et al., 1990), that the production of waxes was retarded because of low light. Results from this study fail to support Thornton's suggestion; i.e., unchambered branches on the mature trees exhibited lower needle wax amounts although they received less sunlight.

GROWTH

No stand differences were observed in twig lengths for mature trees in this study. Joslin et al. (1988c), however, found significantly greater lengths in twigs from the healthy site the year previous to this study. No differences were found between stands in diameter of 0 through 4 year old twigs. Seedlings and saplings had significantly greater 0 year twig diameters and lengths than mature trees in the declining stand. This might be explained by higher net photosynthetic rates in seedlings

and saplings (Tyszko, 1991). However, the differences in length of 0 year twigs was not observed among size classes in the healthy stand, although the same photosynthesis differences as in the declining stand were found. This led to a significant difference between stands in 0 year twig lengths of seedlings and saplings. Greater radiation levels might have explained this difference since seedlings and saplings in the healthy stand were much more shaded. No differences in net photosynthesis existed between the healthy and declining stand for seedlings and saplings (Tyszko, 1991) but since photosynthesis measurements were taken only at saturating light levels this is not in itself unusual. Greater soil nitrogen levels in the declining stand could also contribute to the difference. No foliar nutrient experiments were performed on seedlings and saplings, however. Except for Joslin et al. (1988c), twig lengths and diameters have not been used as comparative measures in the study of air pollution effects on conifers.

Treatment effects were observed in twig diameter growth in both stands. Two through four year old twigs in the filtered chambers were larger than in non-filtered chambers or control branches. However, just the opposite pattern was observed in the healthy stand. No length differences were found among treatments for 0 year twigs in either stand. Ultimate shoot length is controlled by

the environmental conditions of the previous year when bud formation occurred and not conditions during expansion (Kozlowski, 1979; Adams et al., 1990). Therefore, the lack of length differences among treatments in this study is not surprising.

Elevated levels of ozone have been found to decrease height in loblolly pine seedlings (Kress et al., 1982; Chevone et al., 1984; Shafer & Heagle, 1989; Horton et al., 1990), but have not been found to have an effect on above ground growth in Sitka spruce (Lucas et al., 1988) or Norway spruce (Payer et al., 1990). Effects of ozone on red spruce above-ground growth appear varied. Cumming et al. (1988) found decreased growth but Kohut et al. (1990) found no effects. Percy (1986) found decreased growth with decreasing pH of mist in red spruce but others found no effects (Deans et al., 1990; Kohut et al., 1990; Patton et al., 1991). Taylor et al. (1986) and Jensen et al. (1988) have found no interaction of ozone and acid mist on the growth of red spruce. The red spruce seedling study on Whitetop mountain found no effect of ambient ozone or acidic fog on growth. However, seedlings in the plot that allowed both ambient ozone and cloudwater exposure together exhibited increased height (Thornton et al., 1990), probably due to cloudwater fertilization.

It is believed that height by itself is not a sensitive measure of environmental influence (Ruehle et

al., 1984) and that even if other measurements are taken into account, genetics and soil-type ultimately determine the growth of a tree and are more important than ambient pollutant levels (Payer et al., 1990).

CHLOROPHYLL

Chlorophylls *a* and *b* increased proportionately with needle age as indicated by the lack of change in the ratio of *a* to *b*. This same phenomenon has been demonstrated in studies of other conifers as well (van Dijk & Roelofs, 1988; Wallin et al., 1990). Significant increases were also observed in chlorophylls *a* and *b* from winter to summer in our study with no change in *a* to *b* ratio, as has also been observed in other studies (Lewandowska & Jarvis, 1977; Duball & Wild, 1988). Chlorophyll content is thought to rise in the summer because of the vegetative cycle in chloroplasts (Senser et al., 1975 cited in Duball & Wild, 1988).

Pigment levels in green needles of declining conifers are often lower than those in healthy trees, and therefore chlorophyll amounts should be a good indicator of overall damage (Benner & Wild, 1987; Duball & Wild, 1988; Krzak et al., 1988; van Dijk & Roelofs, 1988). This decrease in chlorophyll in damaged trees has been attributed to nitrogen overload caused by high ammonium deposition (van

Djik & Roelofs, 1988), increased soil acidity and loss of nutrients in soil (Krzak et al., 1988; Bytnerowicz et al., 1990), as well as damage to chloroplastic membranes due to ozone exposure (Duball & Wild, 1988; Jung & Wild, 1988; Hopker et al., 1989). However, a decrease in acidity related to nitrate deposition has been found to increase chlorophyll concentration (Horsman & Wellburn, 1976 cited in Hendry et al., 1987; Barnes et al., 1990; Eamus & Fowler, 1990). In the present study the trees in the declining stand contained less foliage and therefore received more light. The healthy stand would tend to have more shading. Shade leaves have been found to have higher chlorophyll amounts since this provides a more efficient system of light capture. Even though our healthy stand was more shaded, chlorophyll amounts were still not significantly different between stands possibly because of the higher nitrogen concentration in the soil of the declining stand as well as an increased translocation of nutrients to the foliage remaining in the declining stand. However, no difference was observed in foliar nitrogen between stands at Whitetop mountain. Foliage from the declining stand did have lower magnesium and calcium levels (Joslin et al., 1988a), but not low enough to cause a decrease in chlorophyll content.

The only treatment effect was for significantly greater amounts of total chlorophyll in the chambered

branches as opposed to unchambered branches in the healthy stand. Apparently, ambient ozone levels are not high enough to cause any significant decreases in chlorophyll amounts at Whitetop mountain, at least for the brief period in which sampling was conducted. Similarly, others have found no effect of ambient levels of ozone on chlorophyll amounts (Bytnerowicz et al., 1990; Senser et al., 1990; Wallin et al., 1990). However, at increased levels using fumigation chambers, decreased chlorophyll amounts have been observed (Cumming et al., 1989; Hopker et al., 1989; Sasek & Richardson, 1989; Wallin et al., 1990). These decreases are mainly observed in older needles, which points to the possibility of less sensitivity to ozone in younger needles (Hopker et al., 1989; Sasek & Richardson, 1989).

Much research has focused on decreased synthesis of chlorophyll as opposed to oxidation of pigments (Jung & Wild, 1988; Sasek & Richardson, 1989). Chlorophyll amounts may decrease on a per dry weight basis because of an increased needle dry weight per unit surface area caused by ozone (Sasek & Richardson, 1989). Ozone is known to change starch accumulation characteristics. Therefore, expressing changes in chlorophyll content per dry weight might not be physiologically meaningful without characterizing the nature of carbon allocation patterns. Sasek & Richardson (1989) believe that ozone probably does

not penetrate past the cell plasmalemma and that it is the secondary products and consequences of ozone oxidation that affect chlorophyll concentration.

In the seedling experiment on Whitetop Mountain, Pier et al. (1992) found that red spruce in chambers that filtered clouds and ozone had higher concentrations of chlorophyll, suggesting that ambient ozone and cloud water lead to chlorophyll depletion.

GAS EXCHANGE

Current year needles in chambered branches had higher photosynthetic rates than 1 or 3 year old needles during the summer months for both stands. A decrease in photosynthetic rate with needle age has been observed in other studies (Coyne & Bingham, 1982; Vogels et al., 1986; McLaughlin et al., 1990). The age difference in non-chambered branches was not observed however and this point is unexplainable. A decrease in the net photosynthetic rate with time was also observed from July to November in chambered branches in the healthy stand. This may indicate the change in apparent quantum yield of CO_2 uptake from a summer maximum to the start of a winter minimum, as found by Leverenz & Oquist (1987). Generally, in November a reduction in day length and falling minimum temperatures induces a dormancy. However, decreases in total conductance to water vapor, internal

CO_2 and chlorophyll were not observed, therefore the changes in photosynthetic rate cannot be explained by changes in these parameters.

No significant differences in photosynthesis were observed between stands. Krzak et al., (1988), however, found higher photosynthetic rates in declining Norway spruce, and this was related to a higher stomatal conductance to water vapor. In the present study, with the exception of November 1989, no differences were observed between stands in stomatal conductance to water vapor. A stimulation of photosynthesis in response to defoliation and decreased self shading has been reported in other investigations (Heichel & Turner, 1983; Eamus & Fowler, 1990). Smith & Carter (1988) found greater needle packing in shoots grown in locations with higher exposure to sunlight, which led to increased photosynthetic rates per unit stem length. These environmental differences did not have a significant impact in the present study.

Other studies have found decreased net photosynthesis with increased tree damage (Coyne & Bingham, 1982; Vogels et al., 1986; Benner & Wild, 1987). However, these decreases were not always related to decreased stomatal conductance or chlorophyll amounts.

No treatment effects were observed in the declining stand, but in the healthy stand chambered branches had higher net photosynthetic rates than unchambered ones and

the difference was greater in 0 year needles. However, no differences were found between filtered and non-filtered chambers. Therefore, it is concluded that ambient levels of ozone do not affect photosynthetic rates of the mature red spruce on Whitetop mountain. The differences that were seen cannot be related to stomatal conductance to water vapor or to chlorophyll amounts.

The chambered branches were warmer than the unchambered ones and it has been observed that photosynthesis increases in plants with increasing temperatures (Bannister, 1976). Beyond an optimum temperature however, metabolic processes are inhibited and respiration is stimulated, causing a decrease in the net photosynthetic rate. Apparently, in this case, stimulation of photosynthesis may be occurring.

A few studies on Norway spruce (Barnes et al., 1990) and silver fir (Kuppers & Klumpp, 1988) have shown decreases in net photosynthesis with increasing ozone which have been related to an increase in respiration. Some investigations have even shown increases in net photosynthesis in conifers in responding to ozone (McLaughlin, 1988; Eamus et al., 1990). Stomatal closure (Freer-Smith & Dobson, 1989) and increases in stomatal conductance (Skarby et al., 1987) have both been reported in response to O_3 fumigation. But the majority of studies has shown red spruce (Taylor et al., 1986; Seiler

& Paganelli, 1987; Alscher et al., 1989; Cumming et al., 1989; Kohut et al., 1990), Norway spruce (Keller & Hasler, 1987; Barnes & Davison, 1988) and Sitka spruce (Lucas et al., 1988) to be relatively tolerant of high concentrations of ozone. Wallin et al. (1990), however, believe that when realistic concentrations of O_3 are used, short term experiments are not sufficient to evaluate impacts of O_3 on spruce gas exchange.

When an adverse effect of O_3 has been observed it has usually been in older needles beyond the first year (Kuppers & Klumpp, 1988; Hopker et al., 1989). It is believed that the response of young needles to O_3 is different from older needles because in young needles the predominant effect is on stomata and in older needles the effect is on chloroplasts (Wallin et al., 1990).

There is also little support for adverse effects of acid deposition on photosynthesis (Taylor et al., 1986; Seiler & Paganelli, 1987; McLaughlin et al., 1990). Rather, photosynthetic rates have been found to increase under acidic conditions as a result of N deposition (Eamus & Fowler, 1990; Kohut et al., 1990). This has led to the theory that increased N deposition causes trees to photosynthesize into the winter months thereby reducing their cold hardiness (Cape et al., 1988; Fowler et al., 1989; Thornton et al., 1990). Ozone has also been found to delay cold hardiness in red spruce (Cumming et al.,

1989), Norway spruce (Brown et al., 1987; Barnes & Davison, 1988), Sitka spruce (Lucas et al., 1988) and loblolly pine (Edwards et al., 1990). However, Klein et al. (1989) did not observe decreased hardiness in red spruce with increased ozone levels.

In this study, the higher levels of photosynthesis in 0 year needles of the declining stand in September and November, and the fact that damage to 1989 needles in April of 1990 resembled frost damage, might represent reduced winter hardiness, but the effects of increased N deposition and increased PAR cannot be separated.

In the seedling study on Whitetop mountain, cloudwater deposition enhanced photosynthesis, while trees growing in chambers excluding clouds and/or ozone had lower respiration rates (Pier et al., 1992). But ambient ozone levels did not appear to affect photosynthesis. Thornton et al. (1990) found that ambient cloud deposition impaired the development of cold tolerance of red spruce seedlings on Whitetop mountain, resulting in a 3° to 5°C reduction in cold hardiness from September through November, 1989.

Tyszko (1991) observed no significant differences in net photosynthesis among age classes of the mature trees at either site on Whitetop mountain, similar to results of this study. He also observed no significant differences between sites for mature tree or sapling photosynthesis.

He did observe that photosynthesis exhibited a significant decrease with tree size (seedling > sapling > mature tree). Stomatal conductance followed the same trend. There were no water potential differences among tree sizes at the upper site but mature trees had higher water potentials than seedlings or saplings at the lower site. Mature trees at the lower site had greater water potentials than those at the upper site during the 1990 growing season but there were no differences found for seedlings and saplings. Tyszko's data do not point to a difference in the health and vigor of the two stands of red spruce, with regards to the parameters that were studied, which is in agreement with my study. However, one might have expected higher net photosynthetic rates in saplings at the upper site since my growth data show 1990 twig lengths to be greater at the upper site.

CONCLUSION

It appears that none of the parameters measured in this study were consistently correlated with the condition of the trees. Declining trees had either the same or greater values than the healthy trees for the parameters measured. In part this may be due to the selection of visibly healthy needles from trees in both stands, and

possibly also because the parameters measured are not sensitive predictors of decline.

There were few if any treatment effects. For some parameters, such as wax amounts and photosynthesis, values were higher in the chambers compared to unchambered branches. With very few exceptions, there were no differences between filtered and non-filtered chambers, suggesting little or no effect of ozone on the parameters measured. This was consistent with the results of the seedling study at Whitetop (Pier et al., 1992), and with greenhouse fumigation studies showing red spruce to be insensitive to ozone at or near ambient levels (Taylor et al., 1986; Kohut et al., 1990; Senser et al., 1990).

The question of differential succeptibilities between young and mature trees remains unanswered. Not only is there a difference in the fraction of tissue that is photosynthetically active (Kramer & Kozlowski, 1979), but the foliage of a young tree and an old one are subjected to different microclimates. Net radiation, wind speed and air temperatures decrease going from above to below crown and humidity and CO_2 increase (Lee, 1978). It is possible that even ozone concentration could also vary with crown depth (Pye, 1988). Comparative studies between differently sized trees is currently an active area of research.

The results of this study suggest that other parameters should be studied for indicators of decline in red spruce, and that attributing changes in parameters investigated to pollution should be viewed with caution. Further studies should concentrate on 1) separating out confounding factors (i.e., acidic deposition, ozone and cold hardiness), 2) on longer treatment exposures to assess cumulative effects, and 3) on possibly setting up systems that add ozone and acid mist to the chambered branches. By controlling both ozone and acid mist levels, the researcher might separate out the individual contribution of each of these pollutants to the tree's response.

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