

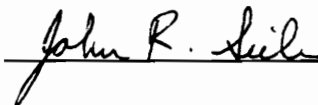
RED SPRUCE PHYSIOLOGY AND GROWTH IN RESPONSE TO
ELEVATED CO₂, WATER STRESS AND NUTRIENT LIMITATIONS

by


Lisa Jean Samuelson

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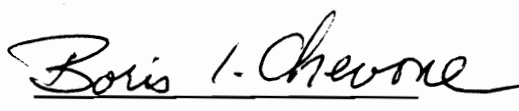
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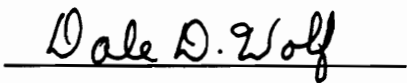
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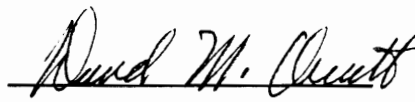
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(ABSTRACT)

Spruce-fir ecosystems of the eastern United States interest scientists because of reported changes in population growth. Whether red spruce (*Picea rubens* Sarg.) populations are declining because of disease, pollution or environmental stress or experiencing natural changes in stand development is unclear. This research examined the growth and physiological responses of red spruce seedlings to changes in atmospheric CO₂, water and nutrient availability to determine the response of this species to potential climatic changes. Red spruce seedlings were grown from seed for 1 year in ambient (374 ppm) or elevated (713 ppm) CO₂ in combination with low or high soil fertility treatment, and well-watered or water-stressed conditions. Red spruce seedlings grown with limited nutrient and water availability increased growth in elevated CO₂ as did seedlings grown with high soil fertility treatment and ample water. At 12 months of age, elevated CO₂-grown seedlings

had greater dry weight, height, diameter and specific leaf weight than ambient CO₂-grown seedlings. Seedlings that formed a bud in elevated CO₂ at 5 months of age produced more total fixed growth than seedlings grown in ambient CO₂. Mean relative growth rate was greater in elevated than ambient CO₂-grown seedlings only from 3 to 5 months of age. Growth was greater at 12 months despite a lower photosynthetic rate in elevated CO₂-grown seedlings compared to ambient CO₂-grown seedlings. Transplanting seedlings from 175-cm³ pots into 646-cm³ pots at 7 months did not change growth and physiological responses to elevated CO₂ at 12 months. Dry weight allocation patterns to leaf, stem and root were not influenced by growth in elevated CO₂ for 1 year. Drought-conditioning had a greater influence on the physiological responses of red spruce to decreasing water potential than did growth in elevated CO₂. Results from this research suggest that red spruce seedlings will grow bigger faster in a future elevated CO₂ atmosphere even if water and nutrients are limiting.

ACKNOWLEDGEMENTS

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CHAPTER I

INTRODUCTION

The predicted increase in atmospheric CO₂ by the year 2050 may enhance plant growth by increasing plant carbon uptake. However, plant growth in elevated CO₂ may be limited by higher mean global temperatures and lower precipitation regimes over much of the world (Manabe et al. 1981, Gates 1983, Mitchell 1983 and Gleick 1987). How plant species will respond is unknown. Large differences between species in the amount of growth induced by an elevated atmospheric CO₂ concentration have been reported (Kramer 1981). Since plant growth is often dependent on factors such as source sink relations, nitrogen metabolism, leaf duration and light interception and utilization (Nelson 1988), greater photosynthesis in elevated CO₂ may not result in greater growth.

Short-term experiments that have studied the effect of elevated CO₂ on plant growth and physiology generally predict positive results. But, most studies are conducted under an optimum, highly controlled environment where interactions between species, resource availability and plant developmental stage are eliminated. Studies that have examined CO₂ effects on species competition suggest that

competitive potentials of species may change in an elevated CO₂ environment. For example, Wray and Strain (1987) found that the C₃ species Aster pilosus, was the more aggressive competitor against the C₄ species Andropogon virginicus when grown together in elevated CO₂. The C₄ species was more competitive when mixtures were grown in ambient CO₂. Williams et al. (1988) determined that a low nutrient supply in combination with competition between six species of grassland herbs decreased the beneficial effects of CO₂ on growth. In addition, growth habit may effect how a species responds to climatic change. Bazzaz et al. (1990) predict that shade tolerant species will respond differently than shade intolerants to increasing CO₂ concentration.

Clearly, if tree species respond differently to predicted changes in global climate and atmospheric CO₂ concentration, forest community compositions may shift gradually or rapidly depending on species adaptability and the severity of global climate change. Changes in species hierarchy within a forest community will be a result of the combined influence of changing CO₂ concentration, water availability, temperature and nutrient status on photosynthesis, carbon allocation and growth. Some scientists predict drastic climate change and massive ecosystem alterations while others foresee small climatic

changes and predict minimal shifts in species range and forest community composition.

Red spruce (Picea rubens Sarg.) is a dominant component of the unique spruce-fir ecosystem of the eastern United States. Because reductions in growth have been reported for some red spruce populations, recent research has attempted to determine the cause, if any, of population changes. Insects, disease, water and nutrient stress, pollution and natural stand development have been suggested as the source of changes in stand growth (Hornbeck et al. 1986, Zedaker et al. 1987, McLaughlin et al. 1987 and 1990). The main objective of this research was to examine how changes in resource availability influence the physiology and growth of red spruce seedlings to determine how responsive this species is to environmental fluctuation. This research examined (1) the growth and gas exchange characteristics of red spruce seedlings in response to an elevated CO₂ growth environment in combination with nutrient limitations and water stress and (2) the gas exchange responses of drought-conditioned and well-watered red spruce seedlings to temperature, light, and CO₂ concentration.

HYPOTHESES

The general hypotheses tested by this research were:

- 1.Ho: Gas exchange characteristics and growth of red spruce will not change with elevated CO₂ exposure.
- 2.Ho: Red spruce response to elevated CO₂ will not be influenced by nutrient and water limitations.
- 3.Ho: The physiological responses of red spruce to varying temperature, light level, and CO₂ concentration will not change with drought conditioning.

Five specific studies were undertaken to test these general hypotheses. The first study examined the growth and gas exchange characteristics of red spruce seedlings grown in elevated CO₂ in combination with nutrient and water stress. Fixed growth in response to elevated CO₂ was examined in the second study and gas exchange responses to decreasing water potential and elevated CO₂ were investigated in the third study. The fourth study examined the gas exchange responses of red seedlings to temperature, light and CO₂ concentration in combination with drought stress. The final experiment consisted of a field study of the drought tolerance of red spruce seedlings growing on Whitetop Mountain, Virginia.

CHAPTER II

LITERATURE REVIEW

The physiological and growth characteristics of plants are influenced by the interaction of numerous factors including light, temperature, CO₂, O₂, humidity, water and nutrients. Plant growth is dependent on photosynthesis and photosynthesis is, in turn, subject to plant and environmental constraints. The influence of elevated CO₂ on plant growth has been suggested as being extremely dependent on nutrient and water availability (Tolbert and Zelitch 1983, Acock and Allen 1985). Future shifts in red spruce distribution will be a result of the combined influence of changing CO₂ concentration, water availability, temperature and nutrient status on photosynthesis, carbon allocation and growth. This review will examine existing information on plant responses to atmospheric CO₂, water stress and temperature.

PLANT RESPONSES TO ELEVATED CO₂

Influence of elevated CO₂ on plant physiology

When other resources that influence photosynthesis are optimal, photosynthesis is limited by ambient CO₂

concentration (Tolbert and Zelitch 1983). Even with moderate limitations in resource supply, higher CO₂ concentrations may enhance photosynthesis by lowering the stomatal limitation to photosynthesis and decreasing the ratio of photorespiration/photosynthesis in the mesophyll of C₃ species. The ratio of CO₂/O₂ influences the extent to which rubisco fixes CO₂, the primary substrate and activator of rubisco in the Calvin cycle, and O₂ in the glycolate pathway (Ogren and Bowes 1971).

How plants sense changes in atmospheric CO₂ concentration is unknown. Plants most likely respond to changes in atmospheric CO₂ concentration (Ca) by sensing changes in intercellular CO₂ concentration (Ci) (Mott 1990). Photosynthesis and stomatal conductance appear to respond to changes in the ratio of Ci/Ca to maintain a constant Ci/Ca ratio (Bell 1982, Ball and Berry 1982).

Often a lower rate of photosynthesis is observed in elevated CO₂-grown plants when photosynthetic rates are compared between ambient and elevated CO₂-grown plants (Stitt 1991). A lower photosynthetic rate in elevated CO₂-grown plants may be a result of both stomatal and mesophyll changes in the leaf. For example, decreased carboxylation efficiency at low CO₂ concentration has been reported for cotton and oleander grown in high CO₂ (Pearcy and Bjorkman 1983). In Oryza sativa grown for a season in 160, 250, 330,

500, 660 or 900 ppm CO₂, leaf starch to sucrose ratios were positively correlated with CO₂ growth concentration (Rowland-Bamford et al. 1990). Canopy photosynthesis measured at ambient CO₂ and leaf sucrose and starch concentration increased with CO₂ growth treatment only up to 500 ppm CO₂. Caemmerer and Farquhar (1984) observed a decrease in CO₂ assimilation and RuBP carboxylation activity with an increase in internal p(CO₂) in Phaseolus vulgaris plants grown in elevated CO₂. Porter and Grodzinski (1984) also examined the acclimation to high CO₂ in Phaseolus vulgaris. After seven days of growth in 1200 ppm CO₂, bean plants had greater total plant dry weight but less carbonic anhydrase and RuBP carboxylase activity than ambient grown plants. Peet et al. (1986) observed lower carbon exchange rates measured under CO₂ growth conditions in Cucumis sativus grown in 1000 ppm CO₂ than in plants grown in 350 ppm CO₂. Decreased carbon exchange rates in plants grown in 1000 ppm CO₂ were associated with decreasing stomatal conductance and less RuBP carboxylase and carbonic anhydrase activity compared to ambient CO₂-grown plants. In Gossypium hirsutum grown from seed in either 350, 675 or 1000 ppm CO₂ for four weeks, elevated CO₂-grown plants had lower photosynthetic rates than ambient grown plants when measured at 350, 675 and 1000 ppm CO₂ (Delucia et al. 1985). Decreased photosynthesis was accompanied by higher leaf

starch levels. Stitt (1991) summarized the photosynthetic response to CO₂ concentration in ten agronomic species grown in ambient or elevated CO₂. First, in the majority of species, the photosynthesis/Ci response decreased in elevated CO₂-grown plants. Secondly, elevated CO₂-grown plants had lower photosynthetic rates than ambient CO₂-grown plants when measured at ambient CO₂. Finally, the O₂-sensitivity of photosynthesis was maintained and sometimes greater in elevated CO₂-grown plants.

A lower photosynthetic rate in elevated than in ambient CO₂-grown trees has also been reported. Fetcher et al. (1988) found that after 14 months of growth in 500 ppm CO₂, the carbon exchange rate of Pinus taeda increased but the rate decreased in Liquidambar styraciflua at any Ci. Photosynthetic rates were lower in Citrus sinensis grown in 800 ppm CO₂ for 12 months than in ambient CO₂-grown trees when measured at the same CO₂ partial pressure (Downton et al. 1987). But, rates of photosynthesis were always higher in both CO₂ treatments when measured at 800 ppm CO₂ than at ambient CO₂. In 9-month-old Pinus radiata, Pseudotsuga menziesii and Nothofagus fusca seedlings exposed to 340 or 640 ppm CO₂ for 120 days, photosynthetic rates were lower in elevated CO₂-grown seedlings than in ambient CO₂-grown seedlings when measured at ambient CO₂ concentration (Hollinger 1987). In one-year-old Carya ovata, Liriodendron

tulipifera, Quercus rubra, Platanus occidentalis, Acer saccharinum and Fraxinus lanceolata seedlings exposed to 350 or 700 ppm CO₂ for 90 days, photosynthetic rate and RuBP carboxylase concentration tended to decline in elevated CO₂-grown seedlings (Williams et al. 1986). Stomatal conductance and net photosynthesis measured at 350 and 675 ppm CO₂ and chlorophyll content were reduced in Ochroma lagopus and Pentaclethra macroloba seedlings grown in 675 ppm CO₂ for at least 60 days (Oberbauer et al. 1985). Wullschleger et al. (1992) exposed Liriodendron tulipifera and Quercus alba seedlings to ambient CO₂, ambient plus 150 ppm CO₂ and ambient plus 300 ppm CO₂ for 24 weeks in open-top chambers in the field. The carbon exchange rate of Liriodendron tulipifera and Quercus alba was 60% and 30% greater, respectively, in elevated CO₂-exposed seedlings than in ambient-exposed seedlings when measured at their CO₂ growth concentration. No change in stomatal conductance between CO₂ treatments was observed, but lower chlorophyll contents and higher starch/sucrose ratios were found in elevated CO₂-grown leaves than in ambient CO₂-grown leaves.

In contrast, other studies have reported no change in photosynthetic rate in plants grown in elevated CO₂. Ziska et al. (1990) found no photosynthetic change in elevated CO₂ in the salt marsh species Scirpus olneyi grown in 680 ppm CO₂ for two growing seasons. The authors suggested that the

high organic content of the salt marsh supported increased productivity and carbon demand in elevated CO₂. After four growing seasons, the photosynthetic rate of Scirpus olneyi was increased by 32% as a result of exposure to 340 plus ambient CO₂ (Arp and Drake 1991). Elevated CO₂-grown Scirpus olneyi had generally greater rates of photosynthesis at any C_i above the compensation point. Grulke et al. (1990) measured the whole ecosystem CO₂ flux of Eriophorum tussock tundra exposed to 340 or 680 ppm CO₂ in open top chambers for one growing season. The authors found that the tussock tundra grown in elevated CO₂ had greater ecosystem carbon gain than ambient-grown tundra only at high light levels. Sage et al. (1989) found that the initial slope of the photosynthetic response to CO₂ concentration and the CO₂ saturated rate of photosynthesis decreased or increased in plants grown in 900-1000 ppm CO₂ for at least three weeks, depending on which C₃ species was examined.

Many hypotheses have been proposed to explain the changes in photosynthetic rate in elevated CO₂-grown plants. A lower photosynthetic rate in elevated CO₂-grown plants when compared to ambient CO₂-grown plants may be a result of assimilate accumulation in leaves limiting photosynthesis through feedback inhibition (Herold 1980). Inhibition of sucrose synthesis in elevated CO₂-grown leaves may inhibit recycling of phosphate to the chloroplast and subsequently

reduce photosynthesis (Stitt 1991). Starch build-up in leaves has been shown to lower photosynthesis by crushing the thylakoid system (Madsen 1975). A decrease in rubisco protein and activity and a reduction in other Calvin cycle enzymes in a high CO₂ environment may limit photosynthesis (Stitt 1991). A reduction in the number and strength of plant sinks and an increase in the ratio of source to sink may limit photosynthesis in elevated CO₂-grown plants (Bowes 1991). Stomatal acclimation to elevated CO₂ may also reduce photosynthesis (Arp 1991, Stitt 1991). Elevated CO₂-grown plants may decrease the size and number of stomates per unit leaf area (Eamus and Jarvis 1989). In addition, pot size may influence photosynthetic capacity in an elevated CO₂ environment. Pot-binding and restricted root growth in elevated CO₂-grown plants may further increase starch loading of leaves (Arp 1991). Finally, low internal plant nitrogen may limit photosynthesis in plants that initially grew rapidly in elevated CO₂ (Stitt 1991).

Elevated CO₂ may also influence plant dark respiration. Wullschleger et al. (1992) found lower leaf dark respiration in Liriodendron tulipifera and Quercus alba seedlings grown and measured in ambient + 150 and ambient + 300 ppm CO₂ for 24 weeks than in ambient CO₂-grown seedlings. Reduced maintenance respiration was reported for Medicago seedlings exposed to 950 ppm CO₂ at night (Reuveni and Gale 1985).

Spencer and Bowes (1986) observed lower leaf photosynthesis and dark respiration in Eichhornia crassipes grown in 600 ppm CO₂ for four weeks than in plants grown in 330 ppm CO₂.

The existing data on physiological responses of trees to growth in elevated CO₂ suggests that red spruce will respond to an elevated CO₂ environment by reducing the rate of photosynthesis. Exactly how red spruce will respond physiologically to elevated CO₂ has not been previously investigated.

Influence of elevated CO₂ on tree growth

Kimball (1983) compiled data from over 70 reports of elevated CO₂ effects on agricultural yields that included more than 37 species. He concluded that, in general, yields should increase by 33% with a doubling in ambient CO₂ concentration. Agronomic species response to elevated CO₂ may be different than tree species response because of differences in lifespan and growth habit. Acclimation to elevated CO₂ in tree species may occur over a timespan of hours to generations, depending on the physiological process studied (Eamus and Jarvis 1989). Although little information is available on the physiological responses of tree species to growth in elevated CO₂, much more work has been done on growth responses. In general, most studies have reported enhanced growth in response to short-term

elevated CO₂ exposure. These reports of a positive growth enhancement in elevated CO₂ suggest that red spruce growth will also increase in elevated CO₂, at least on a short-term basis.

Exposure to elevated CO₂ initially increases the height, leaf area, stem diameter and dry weight of most tree species but growth responses can be extremely variable (Eamus and Jarvis 1989). Increased leaf growth in elevated CO₂ has been reported for both agronomic and tree species (Overdieck and Reining 1986, Conroy et al. 1988). Thomas and Harvey (1983) found increased leaf thickness in the leaves of 1-year-old Pinus taeda and Liquidambar styraciflua grown in elevated CO₂ for one growing season. The authors found indications of the development of a third mesophyll layer in pine but no change in any specific cell type was found in sweetgum. Increased biomass of stems, roots and secondary leaves was observed in Pinus contorta seedlings grown for five months at 1000 ppm CO₂ (Higginbotham et al. 1985). Canham and McCavish (1981) found greater dry weight, height and diameter in Picea sitchensis and Pinus contorta grown in 1500 and 3000 ppm CO₂ during the day for 16 weeks but no significant effect of CO₂ on growth was observed in Pinus nigra var. Maritima. In Pinus taeda and Liquidambar styraciflua seedlings grown in 350, 500 and 650 ppm CO₂ for one growing season, stem length, basal diameter and stem,

root and leaf dry weights increased with increasing CO₂ concentration in both species (Sionit et al. 1985). The number of branches and leaves of Liquidambar styraciflua was greater in 650 ppm CO₂ than in ambient CO₂. O'Neill and others (1987) found increases in leaf dry weight, root dry weight and specific leaf weight in Liriodendron tulipifera seedlings grown in 692 ppm CO₂ for 24 weeks. A 79% and 30% increase in total plant weight was observed in Ochroma lagopus and Pentaclethra macroloba, respectively, grown from seed in 675 ppm CO₂ for 60 and 123 days, respectively (Oberbauer et al. 1985). A CO₂ environment of 1000-1300 ppm increased the height and diameter growth of Ilex aquifolium cuttings after eight months of exposure but had no effect on the height and diameter growth of Pseudotsuga menziesii, Picea glauca and Lagerstroemia indica grown from seed for 5, 10 and 4 months, respectively (Lin and Molnar 1982). Yeatman (1970) found a 32%, 40%, 61% and 78% increase in the total dry weight of 3-week-old Pinus sylvestris, Pinus banksiana, Picea glauca and Picea abies, respectively, sown and grown in 900 ppm CO₂. Dry matter production was increased by 30 to 40% by exposure to 660 ppm CO₂ for 22 weeks in Pinus radiata seedlings supplied with abundant phosphorus and water (Conroy et al. 1986a). Tinus (1972) found greater total plant weight, height and needle number in Pinus ponderosa grown in 1200 ppm CO₂ during the day for

12 months. One and two-year-old Pinus ponderosa seedlings exposed to a CO₂-enriched atmosphere for 2.5 years had greater stem diameter, height and volume than ambient CO₂-grown seedlings (Surano et al. 1986). In Poncirus trifoliata x Citrus sinensis and Poncirus trifoliata x Citrus paradisi seedlings exposed to 330 or 660 ppm CO₂ for five months, new shoot growth, rootstock dry weight, leaf weight and size, and leaf RuBP carboxylase activity was greater in elevated CO₂-grown seedlings (Koch et al. 1986). The authors suggest that positive long-term effects of increased CO₂ concentration may occur in species that have a large sink capacity. Downton et al. (1987) also examined elevated CO₂ effects on Citrus sinensis scions on C. sinensis x Poncirus trifoliata rootstock over a 12 month period. An 800 ppm CO₂ concentration caused a 70% increase in fruit retention. Fruit from the elevated CO₂ trees was of the same fresh weight, soluble solid content, dry weight and seed number as the ambient exposed trees. The authors suggest that fruit yields will increase in species that experience source limitation during fruit development when ambient CO₂ concentrations rise. After two years of growth in 300 ppm + ambient CO₂ concentration, Citrus aurantium trees had 79% more leaves, 56 more primary branches and greater trunk and branch volume than trees grown in ambient CO₂ (Idso et al. 1991). Bazzaz et al. (1990) found

increased plant biomass in Fagus grandifolia, Betula papyrifera, Prunus serotina, Acer saccharum and Tsuga canadensis grown in 700 ppm CO₂ for at least 60 days. Pinus strobus seedlings grown in 1000 ppm CO₂ for four months had greater height, stem diameter and crown diameter than seedlings grown in ambient CO₂ (Funsch et al. 1970). Populus clones grown in 700 ppm CO₂ for 92 days were taller, had more leaves per plant and had greater leaf area, leaf thickness and total biomass than clones grown in ambient CO₂ (Radoglou and Jarvis 1990). Norby et al. (1987) found that exposure to 694 ppm CO₂ for 34 weeks without fertilizer increased the total dry weight of Pinus echinata seedlings by increasing the amount of fine root mass. No difference in seedling mass was found between CO₂ treatments after 41 weeks due to a steep decline in the relative growth rate in the high CO₂ treatment. Campagna and Margolis (1989) suggest that the influence of elevated CO₂ on plant growth varies with the stage of development and the duration of exposure. They found that Picea mariana seedlings did not respond to 1000 ppm CO₂ during a 3 or 6 week exposure time when the seedlings had ceased shoot growth and were going into dormancy. However, actively growing seedlings showed increased total biomass in response to short-term elevated CO₂ exposure.

Interaction between elevated CO₂ and water availability

Water influences almost every cellular metabolic process and is a necessary constituent, solvent and reactant in plants (Kramer 1983). Plant cell enlargement and growth are dependent on the maintenance of turgidity by water. Boyer (1982) suggests that drought is the greatest limitation to plant productivity on a global scale. A higher ambient CO₂ concentration may alleviate the effects of increasing temperature and drought frequency by enhancing the water use efficiency of some species during water stress. Exposure to elevated CO₂ concentration during water stress may increase plant water use efficiency by maintaining photosynthesis at lower leaf conductances or by increasing photosynthesis in relation to transpiration (Pearcy and Bjorkman 1983). Elevated CO₂ increases leaf water use efficiency in most species on a short-term basis (Eamus 1991). Short-term responses are usually a result of a 30-40% decrease in leaf conductance and a large increase in carbon assimilation (Eamus 1991). For example, Aster pilosus plants grown in 500 and 650 ppm CO₂ for 49 days retained higher photosynthetic rates after two weeks of drought than plants grown in 380 ppm CO₂, although reductions in leaf conductance in elevated CO₂ plants were similar to ambient CO₂-grown plants (Wray and Strain 1986). Valle et al. (1985) found greater WUE in Glycine max leaves

grown in 660 ppm than in leaves grown in 330 ppm CO₂ mainly because leaves grown in high CO₂ had higher photosynthetic rates at any CO₂ level above 160 ppm. Whole canopy WUE may also increase in response to elevated CO₂. A 63% improvement in canopy water use efficiency was observed in well-watered Trifolium repens exposed to 600 ppm CO₂ (Nijs et al. 1989). CO₂ assimilation rates of water-stressed Glycine max plots were similar to rates of irrigated plots when measured in 1800 ppm CO₂ (Frederick et al. 1990). Long-term WUE responses may not be as positive because the large initial increase in assimilation in elevated CO₂ may not be maintained (Eamus 1991).

Relative water content, turgor maintenance and subsequent drought postponement during water stress may be improved in elevated CO₂. An increase in organic solutes from enhanced photosynthesis in high CO₂ may lower plant osmotic potentials (Percy and Bjorkman 1983). Lower plant osmotic potentials during drought may prolong turgor maintenance, stomatal opening and photosynthesis (Hinckley et al. 1980, Parker et. al. 1982). Sionit et al. (1981) observed more positive turgor pressures and higher leaf relative water contents at the end of a drought cycle in Triticum aestivum plants grown in 1000 ppm CO₂. Water potentials of Amaranthus retroflex, Ambrosia artemisiifolia and Setaria faberii increased in elevated CO₂ (Garbutt et

al. 1990). Three-month-old Ipomoea batatas plants grown in 666 ppm CO₂ for 90 days experienced water stress later than plants grown in 438 ppm CO₂ (Bhattacharya et al. 1990). Plants subjected to elevated CO₂ had a greater yield of fresh storage root and increased root starch even under water stress.

Elevated CO₂ may also compensate for water stress induced reductions in photosynthesis and growth in tree species. Tolley and Strain (1984) grew Liquidambar styraciflua and Pinus taeda from seed for eight weeks under 350, 675 or 1000 ppm CO₂. When subjected to a drought, elevated CO₂-grown sweetgum seedlings had higher water potentials, photosynthetic rates and WUE than ambient CO₂-grown seedlings. CO₂ enrichment had little effect on the water potentials or gas exchange of water-stressed pine. In Pinus radiata grown for 22 weeks in 330 or 660 ppm CO₂, whole plant WUE during water stress was increased 34% by CO₂ enrichment because of an increase in plant dry weight (Conroy et al. 1988). Conroy et al. (1986b) also looked at the chlorophyll a fluorescence of Pinus radiata grown in ambient or 660 ppm CO₂ for 22 weeks during exposure to drought. In elevated CO₂-grown seedlings, drought stress did not lower the electron flow to photosystem II as it did in ambient CO₂-grown seedlings. Possibly, water stress induced reductions in growth will not be as severe in

elevated CO₂-grown red spruce seedlings because of increased leaf and plant water use efficiency.

Interaction between elevated CO₂ and nutrient availability

Plants require nutrient elements as constituents of plant tissue, components and activators of enzymes, and regulators of protoplasm hydration (Larcher 1980). CO₂-induced growth enhancement may depend on the availability of nutrients, especially nitrogen and phosphorus (Tolbert and Zelitch 1983) and species specific responses to growth in a nutrient poor environment. McMurtrie (1991) used a carbon and nutrient balance model to simulate the influence of nutrient supply and increased photosynthesis in elevated CO₂ on forest growth. He suggests that plants in a nutrient poor environment will respond positively to elevated CO₂ only if nutrient uptake increases or internal nutrient recycling increases.

The nutrient, elevated CO₂ interaction has been examined in some plant species. However, conclusions vary as to how plants will respond to growth in elevated CO₂ when nutrients are limiting. Israel et al. (1990) grew nonnodulated Glycine max in 350 or 700 ppm CO₂ for 27 days with growth limiting concentrations of nitrogen and phosphorus. Elevated CO₂ increased growth, and nitrogen and phosphorus utilization efficiencies but did not affect

uptake efficiency. CO₂-enriched Triticum aestivum grown in 1500 ppm for 8 weeks with low or high nitrogen supply produced two times the amount of dry matter than ambient grown plants at all nitrogen levels (Hocking and Meyer 1991). The proportionally greater increase in dry matter growth than in nitrogen accumulation and the lower nitrate reductase activity in elevated CO₂-grown plants than in ambient CO₂-grown plants suggested an increase in nitrogen-use efficiency. O'Neill et al. (1987) observed that total plant nitrogen in Liriodendron tulipifera seedlings grown in unfertilized forest soil was not affected by enhanced growth in 692 ppm CO₂. The authors suggest that an increase in nitrogen-use efficiency occurred through internal nitrogen recycling to support the increase in growth in elevated CO₂. Norby et al. (1986) found similar total nitrogen content in Quercus alba seedlings grown in either 362 ppm or 690 ppm CO₂ for 40 weeks even though the high CO₂-grown seedlings were larger. Seedlings grown in elevated CO₂ had greater nitrogen use efficiency and a greater proportion of nitrogen in fine roots and leaves and less in storage. Luxmoore et al. (1986) found greater nutrient use efficiency (carbon gain per element uptake) for potassium and phosphorus but not for nitrogen in one-year-old Pinus virginiana grown in 600 ppm CO₂ for 16 weeks. Nitrogen uptake was proportional

to growth with greater nitrogen uptake with increased biomass.

Conversely, Mousseae and Enoch (1989) found premature leaf yellowing, decreased chlorophyll content, less leaf area and nutrient dilution in 2-year-old Castanea sativa seedlings grown in 700 ppm CO₂ for two growing seasons. Total biomass of the grass Bromus mollis was not affected by exposure to 652 ppm CO₂ for 129 days when nitrogen was limiting (Larigauderie et al. 1988). In Populus tremuloides, exposure to 750 ppm CO₂ for 100 days lowered plant nutrient status despite fertilization with nitrogen (Brown 1991). Elevated CO₂ accelerated whole plant declines in nitrogen and phosphorus. Brown and Higginbotham (1986) found increased total biomass and leaf weight in Picea glauca grown for 100 days in 750 ppm CO₂ only when nitrogen was supplied. When nitrogen was limiting, exposure to elevated CO₂ enhanced root mass. Only with adequate phosphorus supply, did two years of CO₂ enrichment increase dry matter production, wood density and tracheid wall thickness in Pinus radiata (Conroy et al. 1990a). Conroy et al. (1990b) also determined that the maintenance of higher photosynthetic and growth rates in Pinus radiata and Pinus caribaea in an elevated CO₂ environment was possible only if adequate sinks existed, such as stem growth, and large amounts of foliar phosphorus were available.

Red spruce may respond positively to an elevated CO₂ environment despite nutrient limitations by increasing internal nutrient use efficiencies. Alternatively, growth and physiological responses to elevated CO₂ may be diminished if nutrient uptake is limiting or internal plant recycling of nutrients does not increase.

PLANT RESPONSES TO WARM, DROUGHT-STRESSED ENVIRONMENTS

A stomatal response to water stress, osmotic adjustment and heat tolerance are adaptive strategies in plants exposed to warm, moisture-limited environments (Hall et al. 1976, Ludlow 1980). Decreases in osmotic potential with increased water stress may result from passive mechanisms, such as a change in the relative water content or symplastic water fraction, or active mechanisms such as osmotic adjustment (Kramer 1983). Seiler and Cazell (1990) found no osmotic adjustment in red spruce although photosynthesis continued to -3.0 MPa. Osmotic adjustment was measured using pressure-volume curves and oversaturation may have influenced osmotic potential estimates (Parker and Pallardy 1987). Drought-conditioned Pinus taeda seedlings lowered needle osmotic potentials and maintained leaf turgor and photosynthesis to lower needle water potentials during water stress than well-watered seedlings (Seiler and Johnson

1985). A reduction in osmotic potential during drought was observed in Quercus saplings and Juniperus virginiana, species characteristic of more xeric sites (Bahari et al. 1985). Drought-preconditioned Picea mariana maintained lower osmotic potentials and higher turgor pressures during severe water stress while unconditioned plants wilted (Zwiazek and Blake 1989). Osmotic adjustment and subsequent turgor maintenance were observed in Lolium perenne during water stress (Thomas and Evans 1989). High yields have been associated with osmotic adjustment in wheat, sorghum and millet (Blum et al. 1983, Morgan 1983, Blum and Sullivan 1986) while reduced yields have been associated with osmotic adjustment in barley and cotton (Quisenberry et al. 1984, Grumet et al. 1987).

Seasonal fluctuations in the temperature optimum for net photosynthesis have been reported for desert plants and woody plants in response to a warm environment (Kramer and Kozlowski 1979, Smith et al. 1984). The acclimation range for the photosynthetic temperature optimum in plants is approximately 10°C (Raison et al. 1980, Seeman et al. 1984). A broad summer temperature optimum for photosynthesis has been reported for Pinus taeda (Teskey et al. 1986) and a photosynthetic temperature range between -5°C and 45°C has been documented for Picea sitchensis (Ludlow and Jarvis 1971). The optimum temperature range for photosynthesis in

Pseudotsuga menziesii was found between 10 and 15°C (Doehlert and Walker 1981). Changes in the rate of photosynthesis with temperature are a result of changes in rubisco specificity and activity, CO₂ and O₂ solubility, and feedback inhibition of photosynthesis (Laing et al. 1974, Ku and Edwards 1977, Schnyder et al. 1984, Leegood and Furbank 1986). Downward shifts in the temperature optimum for photosynthesis with water stress have been reported (Boyer 1971, Lange et al. 1974, Lange et al. 1975, Nobel et al. 1978) and are a result of water stress effects at the stomatal and mesophyll level (Nobel et al. 1978).

CHAPTER III

RED SPRUCE SEEDLING GAS EXCHANGE AND GROWTH RESPONSES TO ELEVATED CO₂, WATER STRESS AND NUTRIENT LIMITATIONS

INTRODUCTION

Atmospheric CO₂ concentration is expected to double by the year 2050, and the rise in ambient CO₂ concentration may increase mean global temperatures and absolute humidity within the near-surface air layer (Manabe et al. 1981). Corresponding increases in temperature with elevated CO₂ may lower annual precipitation regimes, increase summer drought frequency and raise evapotranspirational demands over the central United States, eastern Europe and Russia (Gates 1990, Gleick 1987). Because plant photosynthesis uses CO₂ as a substrate and activator of rubisco, predicted increases in atmospheric CO₂ concentration may influence plant physiology and resulting growth. How the plant kingdom will respond as a whole is unknown. Large differences between species in the amount of photosynthesis and growth induced by an elevated CO₂ concentration have been reported (Kramer 1981). The potential influence of a changing CO₂ environment on plant physiology and growth will depend on the availability of resources such as water and nutrients, as well as plant developmental stage, source-sink relations

and species (Williams et al. 1986, Campagna and Margolis 1989). Studies of long-term CO₂ effects suggest that CO₂-enhanced growth may continue provided adequate sinks and unrestricted rooting volume are provided (Tinus 1972, Idso et al. 1991). However, species distribution and forest community composition may change if plant species respond differently to elevated CO₂. Since forest management is based on the production of select tree species for wood products, a change in species distribution in elevated CO₂ may directly affect forestry practices (Bazazz et al. 1990). For example, an increase in ambient CO₂ may shorten rotation lengths but increase the need for intensive weed control.

Red spruce, a component of the unique spruce-fir ecosystem, is an important timber species in the northeastern United States and Canada, and an esthetically valuable species in the southern Appalachians of the United States. Recent research efforts have focused on red spruce because of reported changes in population growth (McLaughlin et al. 1987, Zedaker et al. 1987). The future success or failure of this species may depend on how it will respond to an increase in atmospheric CO₂ and climatic changes. To understand how an increase in atmospheric CO₂ may influence red spruce, this study examined gas exchange and growth responses of red spruce seedlings grown from seed for 1 year in elevated CO₂ coupled with varying nutrient and water

availability. Because pot size may influence photosynthesis and subsequent growth in an elevated CO₂ environment by restricting the sink demand of root growth (Arp 1991), a subset of seedlings was transplanted into larger pots at 7 months. Gas exchange and growth responses to elevated CO₂ were compared between seedlings grown in small and large pots.

MATERIALS AND METHODS

CO₂ treatment

Two CO₂ treatments, an ambient treatment (a daily mean of 374 (±21 standard deviations) ppm CO₂ and a nightly mean of 409 (±27) ppm CO₂) and an elevated treatment (a daily mean of 713 (±32) ppm CO₂ and a nightly mean of 748 (±47) ppm CO₂), were administered to two exposure chambers. Exposure chambers were of dimension 1.5m x 0.91m x 0.91m and constructed of thin-walled electrical conduit and clear, heat-resistant, teflon film plastic in a greenhouse. Light transmittance through each chamber was over 90% of greenhouse light. Ambient air was pulled into the chambers from outside the greenhouse at 4.6m above the ground and distributed to each chamber at the same rate with a regenerative blower. Blower flow to a chamber was set with in-line pvc ball valves to yield approximately 6 exchanges

per hour per chamber. The elevated CO₂ treatment was accomplished by injecting pure (99.99%) liquid CO₂ (Scott Specialty, Plumsteadville, PA, USA) into blower air at a flow rate set by a variable area flowmeter with a high resolution valve. An infrared gas analyzer (ADC Mk III, Hoddeson, England) and strip chart recorder were used to monitor CO₂ concentration within each chamber. The analyzer was calibrated daily. Chamber sampling was controlled by a diverter valve which was energized by a relay clock (Tork, Mount Vernon, NY, USA) to switch between chambers every 10 min. An air-conditioner was mounted in each chamber to control temperature and facilitate air mixing. The CO₂ concentration did not vary with position within a chamber.

This study was arranged as a completely randomized design and any inherent chamber differences were averaged across treatment combinations by rotating CO₂ treatments and seedlings between chambers every week. Seedlings were rotated within a chamber every other day to average any within chamber variation. Each CO₂ treatment and accompanying seedling and treatment, therefore, spent equal time in each chamber and in various positions within a chamber.

Recording hygrothermographs were placed in each chamber to monitor temperature and relative humidity (RH). The hygrothermographs were calibrated monthly with a thermo-

hygro probe (Solomat, Stamford, CT, USA). Chamber temperature and RH averaged 22.8 (± 1.8 standard deviations) $^{\circ}$ C and 68 (± 11)%, respectively, during the day and 20.6 (± 1.4) $^{\circ}$ C and 79 (± 13)%, respectively, at night in the high CO₂ treatment. In the ambient CO₂ treatment, chamber temperature and RH averaged 22.5 (± 1.7) $^{\circ}$ C and 63 (± 13)%, respectively, during the day and 20.6 (± 1.3) $^{\circ}$ C and 76 (± 18)%, respectively, at night.

Soil fertility and water stress treatments

Red spruce seed (F.W Schumacker, Sandwich, MA, USA) of unknown parentage collected in the northeastern United States was stratified for 30 days at 3 $^{\circ}$ C and sown in a 2:1 mix (v:v) of soil from Whitetop mountain Virginia (a high elevation, loamy-skeletal, mixed, frigid Typic Haplumbrept soil of pH 4.4) and perlite into 175-cm³ Ray Leach Conetainers (Stuewe & Sons, Inc., Corvallis, OR, USA). The high soil fertility treatment was applied by hand mixing nonsoluble N-P-K at 150 kg ha⁻¹, 66 kg ha⁻¹ and 124 kg ha⁻¹, respectively, into the soil. Calcium and Mg were applied at 50 kg ha⁻¹ each using CaSO₄ and MgSO₄, respectively. Nitrogen, P, K, Ca and Mg were applied at 75 kg ha⁻¹, 33 kg ha⁻¹, 62 kg ha⁻¹, 25 kg ha⁻¹ and 25 kg ha⁻¹, respectively, in the low fertility treatment.

The containers were placed in the appropriate CO₂ treatment on 25 March 1991. Seedlings were maintained on a 16 hr photoperiod for 3 months using sodium vapor lamps (400 W) suspended over each chamber (approximately 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the seedling level after sunset). After 3 months of growth under 16 hr photoperiods, seedlings were subjected to short days by covering each chamber with white on black poly film (A.H. Hummert Seed Co., St. Louis, MO, USA) after 8 hr of daylight to induce bud set. Bud formation and subsequent bud break were induced to avoid loss of apical dominance which is typical of red spruce grown under greenhouse conditions without chilling application. After 2 months of an 8 hr photoperiod when the 5-month-old seedlings had set a bud and appeared visibly hardened (elongation had ceased and new growth had thickened and darkened), all seedlings were watered and placed in a cold room at 3°C for 7 weeks. Seedlings were not exposed to elevated CO₂ in the cold room. After 7 weeks, seedlings were returned to their appropriate CO₂ treatment and a 16 hr photoperiod to induce bud break and continue the study.

Because restricted root growth may limit plant growth responses to elevated CO₂ (Arp 1991), a subset of seedlings was transplanted into larger pots at 7 months of age to eliminate any potential influence of pot-binding on plant responses to elevated CO₂. A subset of 10 seedlings from

each treatment combination was transplanted after removal from the cold room into 646-cm³ Deepots (Stuewe & Sons, Inc., Corvallis, OR, USA) with the appropriate soil fertility treatment (described previously) reapplied only to transplanted seedlings. Fertility treatments were reapplied during transplanting because new soil was added to the large pots and to insure that seedlings had adequate nutrients for growth. Pot size treatment most likely included plant responses to nutrient readdition as well as rooting volume because, in all probability, soil fertility treatments were either depleted or leached out of the soil in seedlings grown in 175-cm³ pots by the end of the study. Leaf N concentration and the concentration of elements in the soil from each fertility and pot size treatment measured at 9 months of age (2 months after seedlings were transplanted) suggests that transplanted seedlings had more N, P and K available for growth (Table 3.1). At 9 months, leaf total Kjeldahl N was measured on 4 samples from each soil fertility, pot size treatment. A leaf sample consisted of needles removed from 2 seedlings. A soil sample consisted of soil subsampled from 4 pots from a treatment combination. Needles and soil were oven-dried at 60°C and ground. Total Kjeldahl nitrogen of leaf and soil samples was determined by block digestion and analysis using the ammonia salicylate colorimetric technique (Bremner and Mulvaney 1982) with a

Table 3.1 Red spruce seedling leaf nitrogen and the concentration of soil elements as influenced by soil fertility treatment and pot size treatment at 9 months. Soil fertility treatments were reapplied at 7 months to seedlings transplanted into 646-cm³ pots.

Pot size (cm ³)	Fertility treatment	N-leaf ($\mu\text{g g}^{-1}$)	N-soil -----	P ($\mu\text{g g}^{-1}$)	K ($\mu\text{g g}^{-1}$)	Ca -----	Mg -----
175	low	8500	3200	0.1	55	190	46
175	high	8700	3200	0.2	58	197	48
X ₁₇₅		8600§	3200	0.2§	56§	193§	47§
646	low	14300	3100	1.5	64	146	29*
646	high	14800	3300	3.7	92	163	41
X ₆₄₆		14500	3200	2.6	78	154	35

* Indicates a significant difference between soil fertility treatment within a pot size treatment at the $p \leq 0.05$ level (n=4 for leaf and soil N and n=8 for soil P, K, Ca and Mg).

§ Indicates a significant difference between pot size treatment means (X) in the concentration of leaf or soil elements at the $p \leq 0.05$ level.

Technicon Auto Analyzer II (Technicon Instruments Corp., Tarrytown, NY, USA). Exchangeable cations in the soil were determined by extraction with 0.05 N HCl and 0.025 N H₂SO₄ and subsequent colorimetric analysis using inductively coupled plasma (ICP) spectrometry.

Well-watered treatments were watered daily. Water stress treatment was initiated when the seedlings were 10 weeks old and defined as the number of days between waterings (Table 3.2). Needle relative water content (RWC) was measured at the end of cycles 4-6 when the seedlings were larger. Cycles were then adjusted accordingly to approximate equal stress levels between CO₂ treatments (Table 3.2). RWC was determined by subsampling needles from 5 seedlings in each treatment combination for a total of 4 repetitions for a drought cycle and CO₂ treatment. Needles were immediately weighed, placed in distilled water in the dark at 24°C overnight (24 hr), and then blotted dry and reweighed to determine saturated weight (SW). Samples were then oven-dried for 48 hr at 60°C and RWC was calculated as:

$$\text{RWC} = (\text{FW}-\text{OD}) \div (\text{SW}-\text{OD}) \times 100$$

where FW = fresh weight and OD = oven-dried weight. To avoid removing too much leaf area from the small seedlings, plant water status was not measured for cycles 6-9 when the seedlings were placed on short days and cycles 10-11 when

Table 3.2. Drought cycles and corresponding duration and needle relative water content (RWC) or water potential (WP) of red spruce seedlings in response to CO₂ and pot size treatment.¹

Drought cycle	Pot size (cm ³)	CO ₂ treatment (ppm CO ₂)					
		374			713		
		Days	RWC(%)	WP(MPa)	Days	RWC(%)	WP(MPa)
1	175 ²	7	- ³		7		
2	175	7			7		
3	175	7			7		
4	175	7	90		7	83	
5	175	11	86		9	86	
6	175	11	89		10	89	
7	175	11			9		
8	175	11			10		
9	175	12			10		
10	175	13			10		
10	646	13			10		
11	175	12			9		
11	646	12			9		
12	175	16	87		11	93	
12	646	16	90		11	94	
13	175	16		-1.50	14		-1.82
13	646	16		-1.25	14		-1.54
14	175	16	88		14	89	
14	646	16	88		14	90	
15	175	15		-1.68	13		-1.69
15	646	15		-1.50	13		-1.51
16	175	12		-1.53	13	87	
16	646	12		-1.25	13	88	
17	175	15		-1.50	13		-1.69
17	646	15		-1.45	13		-1.51
18	175	16		-1.56	12		-1.20
18	646	16		-1.48	12		-1.48
19	175	11			13		-1.35
19	646	11			13		-1.30
20	175				14		
20	646				14		
\bar{X}	175		86	-1.52		88	-1.55
X	646		89	-1.32*		90	-1.51

Table 3.2 continued.

¹ Control treatments were watered daily and RWC and WP averaged 94(±2)% and -0.65(±0.16) MPa, respectively, for seedlings in the elevated CO₂ treatment. RWC and WP averaged 93(±2)% and -0.73(±0.16) MPa, respectively, for control seedlings in the ambient CO₂ treatment. Mean standard deviations are noted in parentheses.

² Seedlings were transplanted into 646-cm³ pots after drought cycle 9.

³ Plant water status was not measured where RWC or WP values are omitted due to limited plant material.

* Indicates a significant difference in RWC or WP averaged across all drought cycles (\bar{X}) between CO₂ treatments within a pot size treatment at the p≤0.05 level. No significant differences were found between pot size means within a CO₂ treatment at the p≤0.05 level.

the seedlings were breaking bud. After drought cycle 12, whole plant water potential (WP) was measured using a pressure chamber on seedlings from the 175-cm³ pot treatment when extra seedlings became available. The water potential of a branch was measured on seedlings grown in the 646-cm³ pots. Water potential values were an average of 2 samples from a treatment combination. No measures of plant water status were made for the final drought cycles due to limited amount of available tissue. RWC and WP means were calculated across all drought cycles for each CO₂ treatment within a pot size treatment. No significant difference in RWC means between CO₂ treatments was observed within either pot size (Table 3.2). In seedlings grown in 646-cm³ pots, elevated CO₂-grown seedlings had a lower mean WP than ambient CO₂-grown seedlings (Table 3.2).

Gas exchange measurements

Gas exchange measurements were made 2, 3, 5, 9 and 12 months after germination on 5 randomly selected seedlings from each treatment combination. Different seedlings were measured at each measurement period. Previous to each measurement session, all treatment combinations were watered for 2 days to ensure complete hydration. Water potentials were measured after gas exchange measurement and averaged -0.72 (± 0.17) MPa for the well-watered treatment and -0.73

(± 0.21) MPa for the water-stressed treatment. Seedlings were removed from the exposure chambers immediately prior to gas exchange measurement. Gas exchange of each sample seedling was measured at a cuvette CO_2 concentration of 358 (± 7) ppm and 728 (± 4) ppm CO_2 in the greenhouse using a portable photosynthesis system (LI-6250, LI-COR Inc., Lincoln, NE, USA). Average photosynthetic photon flux density at the leaf surface was 939 (± 259) $\mu\text{mol m}^{-2} \text{s}^{-1}$ and cuvette temperature and RH averaged 26.0 (± 4.3) $^{\circ}\text{C}$ and 37 (± 13) %, respectively, for all gas exchange measurements. Measurements were made between 0800 and 1400 hr on attached leaf tissue. The entire upper portion of the crown (included both developing and fully expanded leaf tissue) of a seedling was placed in the cuvette. After measurement, projected area of the needles within the cuvette was determined using a digital scanner (Hewlett Packard ScanJet Plus, Hewlett-Packard Co., Boise, ID, USA). A known area was scanned and converted to number of pixels. This ratio of known area to number of pixels was used to translate pixel number to projected needle area.

At 12 months, gas exchange of new and old foliage of seedlings transplanted into 646- cm^3 pots was also measured at 363 and 730 ppm CO_2 to determine if the photosynthetic response to growth in elevated CO_2 differed by leaf age. Younger foliage was defined as leaf growth on the top 5 cm

of stem and older growth was defined as the leaf tissue on the next lower 5 cm of stem. New tissue was easily distinguished from old visually (younger needles were a lighter green and more succulent when compared to older needles which were darker and fully expanded). Leaf age differences in gas exchange were not measured in seedlings grown in 175-cm³ pots because of their small size.

Dark respiration (Rd) of new and old foliage of transplanted seedlings (646-cm³ potted seedlings) and of the entire upper crown of 175-cm³ potted seedlings was also measured at 12 months at a CO₂ concentration of 363 ppm. Respiration was measured immediately following photosynthesis by covering the leaf chamber to eliminate light.

Biomass Measurements

Biomass measurements coincided with gas exchange measurements at 2, 3, 5, 9 and 12 months after germination. At each of the harvests, 10 randomly selected seedlings (5 of which were used in gas exchange measurement) from each treatment combination were oven-dried at 60°C for at least 48 hr and seedling shoot weight, root weight, and leaf weight were measured and used to calculate mean relative growth rate (RGR) and organ dry weight allocation patterns. At the final harvest total height, diameter at the root

collar, and branch number were measured on 10 seedlings from each treatment combination. Because the number of branches formed on a stem are dependent on stem height, the tabulated number was divided by stem height to estimate branch density. Specific leaf weight (SLW) was also measured at 12 months of age on a sample of 5 seedlings from each treatment combination. Projected leaf area was measured using a digital scanner (Hewlett Packard ScanJet Plus, Hewlett-Packard Co., Boise, Idaho, USA).

The mean RGR between harvests was calculated as :

$$\text{RGR} = (\ln W_2 - \ln W_1) / (t_2 - t_1)$$

where W_2 is total plant weight at time 2 (t_2) and W_1 is total plant weight at time 1 (t_1).

Organ growth is dependent on total plant size, therefore, an increase in organ growth may be due to a treatment effect on total plant size rather than to an effect on organ allocation to root and shoot. Eamus and Jarvis (1989) concluded that the majority of CO_2 effects are a result of plants getting bigger faster in high CO_2 rather than a direct effect of CO_2 on organ allocation. To determine if differences in dry weight organ allocation patterns existed between CO_2 treatments, allometric growth analysis as described by Causton and Venus (1981) was used. Dry weight allocation patterns to root, stem and leaf organs in elevated and ambient CO_2 were examined by comparing the

regression parameters 'c' and 'd' in the transformed equation:

$$\ln(W_i) = \ln c' + d \ln(W_j)$$

derived from the allometric growth equation:

$$W_i = cW_j^d$$

where W_i and W_j are the dry weights of two plant parts, the intercept 'c' is the constant of proportionality (or initial dry matter investment) and the slope 'd' is the exponential factor (or relative organ growth rate) of the allometric growth equation. Data were combined across soil fertility and water stress treatments and the studentized range statistic was used to test for differences in the intercept and the slope estimates between CO₂ treatment and pot size treatment. Regression lines were separated by pot size treatment because slope and intercept parameters from the regression equations describing organ allocation were significantly different between seedlings grown in 175 and 646-cm³ pots (p≤0.05). Differences in dry weight allocation patterns between CO₂ treatments were determined by identifying different intercept values when slopes were similar or by identifying divergent slopes.

Statistical analysis

Gas exchange variables were analyzed using a 3 factor analysis of variance with CO₂ growth concentration, soil

fertility and water stress treatment as main effects at 3 and 5 months. Water stress was not included as a main factor in analysis of gas exchange variables at 2 months because water stress treatment had been initiated for only 2 weeks. Pot size was added as a fourth factor in the analysis of gas exchange responses at 9 and 12 months. Differences in gas exchange responses between young and old foliage were analyzed within the 646-cm³ pot size treatment using foliage age, soil fertility, water stress and CO₂-growth treatments as main effects. Dry weight responses at 12 months were compared between treatments using a 4 factor analysis of variance with CO₂, soil fertility, water stress and pot size as main effects. Mean RGR was compared between treatments within a pot size treatment using a 3 factor analysis of variance with CO₂, soil fertility and water stress treatments as main effects. When appropriate, the analysis of variance was performed on the natural log of a variable to satisfy heterogeneity of variance assumptions.

RESULTS

No significant ($p \leq 0.05$) two-way, three-way or four-way interactions between CO₂ growth treatment, soil fertility treatment, water stress treatment and pot size treatment on red spruce net photosynthesis (Pn), leaf conductance (gl),

water use efficiency (WUE) or internal CO₂ concentration (Ci) were observed at any of the measurement periods. At 2 months of age, red spruce gas exchange physiology was not significantly ($p \leq 0.05$) influenced by CO₂ growth environment or soil fertility (data not shown). After 3 months of growth, elevated CO₂-grown seedlings had greater g_l than ambient CO₂-grown seedlings at both CO₂ measurement levels, but no effect of CO₂ growth concentration on P_n, Ci or WUE was found (Table 3.3). Seedlings grown in the high fertility treatment had greater g_l when measured at 728 ppm CO₂ than seedlings grown in low fertility (Table 3.3).

No main effect of water stress on any gas exchange variable was observed in 3-month-old, rehydrated seedlings (water-stressed seedlings were well-watered prior to gas exchange measurement) (Table 3.3). However at 5 months, water stress reduced P_n and g_l at both CO₂ measurement levels despite similar water potentials between control and water-stressed seedlings (-0.72 MPa and -0.73 MPa, respectively) (Table 3.3). Seedlings grown in high soil fertility treatment for 5 months had greater P_n when measured at 728 ppm CO₂ (Table 3.3). No significant effect of an elevated CO₂ growth environment on gas exchange was observed at 5 months.

At 9 months of age, P_n was lower in elevated CO₂-grown seedlings when measured at a CO₂ concentration of 358 ppm,

Table 3.3. Net photosynthesis (Pn), leaf conductance (gl), water use efficiency (WUE) and internal CO₂ concentration (Ci) of red spruce seedlings measured at a cuvette CO₂ concentration of 358 ppm or 728 ppm in response to CO₂, soil fertility, water stress and pot size treatment over 12 months.

	Growth treatment (ppm)	CO ₂ (ppm)	Pn (μmol m ⁻² s ⁻¹)			gl (mmol m ⁻² s ⁻¹)			WUE (μmolCO ₂ mol ⁻¹ H ₂ O)			Ci (ppm)						
			Age (months)	3	5	9	12	3	5	9	12	3	5	9	12			
CO₂ (ppm) environment																		
	374	358	1.2	1.7	3.0*	2.9*	22*	52	29	50	50	30	110	60*	260	287	189	250*
	713	358	1.6	1.4	2.6	2.3	30	48	26	45	50	30	100	50	264	286	194	268
	374	728	3.6	5.6	6.3	6.6*	21*	41	26	47	180	150	270	160	416	460	339	460*
	713	728	4.2	5.5	5.9	5.4	27	44	24	42	160	130	270	150	442	491	313	495
Soil fertility																		
	low	358	1.2	1.3	2.8	2.6	24	45	28	48	50	30	110	60	263	290	193	258
	high	358	1.5	1.9	2.7	2.5	28	56	27	47	50	30	110	60	261	283	190	262
	low	728	3.8	4.8*	6.1	6.0	21*	40	25	45	180	130	260	160	414	486	341	487
	high	728	4.1	6.2	6.0	6.0	27	44	25	44	160	150	280	150	450	462	311	469
Water stress																		
	control	358	1.4	2.0*	3.1*	2.6	27	62*	34*	53*	50	30	90*	50*	265	285	204*	266*
	stressed	358	1.3	1.2	2.5	2.5	24	37	22	42	50	30	120	60	258	289	177	253

Table 3.3. continued.

Treatment	CO ₂ (ppm)	Pn (μmol m ⁻² s ⁻¹)			g ^l m ⁻² s ⁻¹			WUE (μmolCO ₂ mol ⁻¹ H ₂ O)			Ci (ppm)						
		Age (months)	3	5	9	12	3	5	9	12	3	5	9	12			
Water stress																	
control	728	4.1	6.3*	6.7	6.0	25	51*	31*	49*	170	130	230*	130	435	488	356*	498
stressed	728	3.8	4.5	5.5	6.1	23	31	19	39	170	150	310	180	426	456	287	457
Pot size (cm³)																	
175	358	- ²								26	42*			100*	50*	201*	272*
646	358									29	51			120	60	179	252
175	728									24	38*			240*	130*	351*	494
646	728									26	48			300	170	297	468

* Indicates a significant difference in a gas exchange variable between a CO₂, soil fertility, water stress or pot size treatment within a CO₂ measurement concentration at the p≤0.05 level (n=5).

2 A photosynthetic photon flux density of 939 (±259) μmol m⁻² s⁻¹, temperature of 26.0 (±4.3)°C, relative humidity of 37 (±13)% and CO₂ concentration of 358 (±7) ppm or 728 (±4) ppm CO₂ was provided in the leaf cuvette during gas exchange measurement. Standard deviations are noted in parentheses.

1 Seedlings were transplanted into 646-cm³ pots at 7 months of age.

but g_l , C_i and WUE were similar between elevated and ambient CO_2 -grown seedlings (Table 3.3). No effect of soil fertility on gas exchange was observed. Water stress lowered P_n and g_l when measured at 358 ppm CO_2 , and reduced g_l and C_i and increased WUE at both CO_2 measurement concentrations despite rehydration (Table 3.3). Seedlings transplanted into 646-cm³ pots had greater P_n and WUE, and lower C_i than seedlings grown in 175-cm³ pots at both CO_2 measurement levels (Table 3.3).

At 12 months, P_n was lower and C_i were higher in elevated CO_2 -grown seedlings than in ambient CO_2 -grown seedlings at both CO_2 measurement levels (Table 3.3). Water stress reduced g_l at both CO_2 measurement levels but had no influence on P_n (Table 3.3). Water stress increased WUE and decreased C_i when measured at 358 ppm CO_2 (Table 3.3). Gas exchange was not influenced by soil fertility treatment at 12 months (Table 3.3). P_n , g_l and WUE were greater in seedlings transplanted into 646-cm³ pots than in seedlings grown in 175-cm³ pots at both CO_2 measurement levels (Table 3.3). C_i was lower in transplanted seedlings only when measured at 358 ppm CO_2 (Table 3.3).

No significant interactions between age of foliage and CO_2 growth treatment, soil fertility, water stress treatment or pot size treatment for any gas exchange variable ($p \leq 0.05$) were found at 12 months. P_n and R_d were significantly

greater in older foliage than in new foliage ($p=0.05$). Mean P_n and R_d were $8.0 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $5.2 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively, in the older foliage and $5.9 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $1.5 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively in the younger foliage. No significant ($p \leq 0.05$) effect of CO_2 growth treatment, soil fertility treatment or pot size on R_d was found, but an interaction between water stress treatment and pot size treatment on R_d was observed. Water stress increased R_d in seedlings grown in 646-cm^3 pots CO_2 (Table 3.4).

Growth responses

After 12 months of growth, no significant ($p \leq 0.05$) two-way or three-way interactions between soil fertility, water stress and CO_2 treatments on any growth variables were observed in red spruce. In addition, no significant ($p \leq 0.05$) three-way or four-way interactions between CO_2 , soil fertility, water stress and pot size treatments for any growth variable were observed. The only statistical interaction detected was between pot size and water stress treatment for dry weight growth and height. A significant water stress induced reduction in organ and total dry weights and height was found only in seedlings grown in the 646-cm^3 pots (Table 3.5). Water stress reduced seedling diameter in both pot sizes but had no effect on branch density or SLW (Table 3.6).

Table 3.4. Dark respiration in response to pot size treatment in control and water-stressed red spruce seedlings.¹

Pot size treatment (cm ³)	Water stress treatment	
	control	stressed
	----- $\mu\text{mol m}^{-2} \text{s}^{-1}$ -----	
175	5.1	1.6
646	1.8*	5.0

* Indicates a significant difference between water stress treatment at the $p \leq 0.05$ level.

¹ A temperature of 24.1 (± 1.6) °C, relative humidity of 36 (± 12) % and CO₂ concentration of 363 (± 7) ppm was provided in the cuvette during gas exchange measurement. Standard deviations are noted in parentheses.

Table 3.5. Root, stem, leaf and total dry weights and height responses to pot size treatment in control and water stressed 12-month-old red spruce seedlings.

Growth parameter	Pot size (cm ³)	Water stress treatment	
		control	stressed
root (mg)	175	292	183
stem (mg)	175	63	46
leaf (mg)	175	187	159
total (mg)	175	542	388
height (mm)	175	61	51
root (mg)	646	757*	313
stem (mg)	646	293*	114
leaf (mg)	646	680*	327
total (mg)	646	1737*	768
height (mm)	646	146*	86

* Indicates a significant difference between water stress treatment at the $p \leq 0.05$ level.

Table 3.6. Red spruce seedling dry weights, diameter, height, branch density and specific leaf weight (SLW) responses to CO₂, water stress, soil fertility and pot size treatment after 1 year.

Treatment	leaf	stem	root	total	diameter (mm ²)	height (mm)	branch density (# cm ⁻¹)	SLW (mg cm ⁻²)
CO ₂ (ppm)	-----mg-----							
374	286*	100*	250*	641*	1.3*	81*	0.82	7.5*
713	483	156	419	1065	1.5	91	0.87	8.8
Soil fertility								
low	298*	112*	328*	738*	1.3	83*	0.77*	8.1
high	371	145	443	959	1.4	90	0.94	8.4
Water stress								
control	- ¹	-	-	-	1.6*	-	0.89	8.3
stressed	-	-	-	-	1.2	-	0.81	8.1
Pot size (cm ³)								
175	173*	55*	238*	465	1.1*	56*	0.81	8.6*
646	502	205	535	1252	1.7	116	0.89	8.0

* Indicates a significant difference between CO₂ or soil fertility treatment at the p≤0.05 level.

¹ Means are not presented when a significant interaction between main effects occurs.

Root weight, stem weight, leaf weight, total seedling weight, stem diameter, height and SLW were greater in elevated than in ambient CO₂-grown seedlings after 1 year, but elevated CO₂ did not influence branch density (Table 3.6). Red spruce seedlings grown in the high soil fertility treatment had greater dry weight, height and branch density than seedlings grown in the low soil fertility treatment, but no influence of soil fertility on diameter or SLW was observed (Table 3.6). Seedlings transplanted into 646-cm³ pots had greater dry weight, height and diameter and lower SLW than seedlings grown in 175-cm³ pots (Table 3.6).

The mean RGR was not affected by CO₂ growth concentration, soil fertility treatment or water stress up to 3 months of age (Table 3.7). Between 3 and 5 months, elevated CO₂ increased and water stress decreased the mean RGR (Table 3.7). From 9 to 12 months of age, no significant effect of CO₂ treatment or soil fertility treatment on the mean RGR was observed in seedlings grown in either 175-cm³ or 646-cm³ pots (Table 3.7). Water stress reduced the mean RGR from 9 to 12 months in seedlings transplanted into 646-cm³ pots (Table 3.7). Seedlings transplanted into 646-cm³ pots showed a significantly ($p \leq 0.05$) greater mean RGR from 9 to 12 months than seedlings grown in the 175-cm³ pots (0.012 versus 0.008 mg mg⁻¹ day⁻¹, respectively).

Table 3.7. Mean relative growth rates ($\text{mg mg}^{-1} \text{ day}^{-1}$) in response to CO_2 , soil fertility, water stress and pot size treatments of red spruce seedlings.

Treatment	Pot size (cm^3)	Time (months)		
		2 to 3	3 to 5	9 to 12
<u>CO_2 (ppm)</u>				
374	175	0.0220	0.0072§	0.0084
713	175	0.0176	0.0120	0.0075
374	646	¹	-	0.0117
713	646	-	-	0.0120
<u>Soil fertility</u>				
low	175	0.0184	0.0089	0.0079
high	175	0.0212	0.0103	0.0080
low	646	-	-	0.0129
high	646	-	-	0.0107
<u>Water stress</u>				
control	175	0.0218	0.0125*	0.0079
stressed	175	0.0178	0.0067	0.0080
control	646	-	-	0.0146*
stressed	646	-	-	0.0090

¹Seedlings were transplanted into 646- cm^3 pots at 7 months of age.

§ Indicates a significant difference between a treatment at the $p=0.07$ level.

* Indicates a significant difference between a treatment at the $p\leq 0.05$ level.

Use of the transformed allometric equation to describe dry weight allocation patterns between leaf, stem and root resulted in high R^2 values (Table 3.8). Parameters of the regression equations describing organ allocation were initially compared between pot size treatments within a CO_2 treatment. In all cases, slope estimates were significantly lower for seedlings transplanted into 646-cm³ pots than seedlings grown in the 175-cm³ pots, indicating greater dry weight allocation to leaf rather than root and stem (Table 3.8). CO_2 effects on organ allocation were therefore examined within a pot size treatment. No significant differences between CO_2 treatment in the intercept and slope estimates of the transformed allometric equations for root versus leaf allocation, and stem versus leaf allocation were observed from 2 to 12 months of age in seedlings grown in 175-cm³ pots and from 9 to 12 months in seedlings transplanted into 646-cm³ pots (Table 3.8).

Table 3.8. Regression models and coefficients of determination of the transformed allometric equations in response to CO₂ treatment from 2 to 12 months in red spruce seedlings grown in 175-cm³ pots and from 9 to 12 months in seedlings transplanted into 646-cm³ pots.

Dry weight relationship	CO ₂ treatment (ppm)	Pot size (cm ³)	Model		R ²
			α	β	
stem-leaf	374	175	ln(stem) = -3.44 ¹	1.43ln(leaf)	0.91
	713	175	ln(stem) = -3.28	1.36ln(leaf)	0.91
stem-leaf	374	646	ln(stem) = -2.42 ²	1.24ln(leaf)	0.89
	713	646	ln(stem) = -2.12	1.17ln(leaf)	0.94
root-leaf	374	175	ln(root) = -1.30	1.28ln(leaf)	0.86
	713	175	ln(root) = -1.10	1.23ln(leaf)	0.82
root-leaf	374	646	ln(root) = -0.59	1.08ln(leaf)	0.90
	713	646	ln(root) = -0.66	1.09ln(leaf)	0.92

¹ No significant differences in intercept and slope estimates between CO₂ treatments were found at the p≤0.05 level (n=200 for 175-cm³ treatment and n=80 for 646-cm³ treatment).

² Intercept and slope estimates of the regression equations describing stem and leaf, root and leaf allocation were significantly different between the 175-cm³ and 646-cm³ pot size treatment within a CO₂ treatment at the p≤0.05 level.

DISCUSSION

No consistent effect of soil fertility treatment on gas exchange was observed throughout the study. Water stress treatment reduced g_l at 5, 9 and 12 months and reduced P_n at 5 and 9 months despite the high WP of previously water-stressed seedlings on a measurement day. The reduction in P_n in rehydrated seedlings was possibly a result of decreased g_l and changes in the photosynthetic capacity of the mesophyll as a result of long-term exposure to water stress (Farquhar and Sharkey 1982, Fredeen et al. 1991, Mathews and Boyer 1984).

The reduction in P_n observed at 9 and 12 months in elevated CO_2 -grown red spruce seedlings when compared to ambient CO_2 -grown seedlings has been reported for Liquidambar styraciflua, Citrus sinensis, Pinus radiata, Pseudotsuga menziesii, Nothofagus fusca, Ochroma lagopus and Pentaclethra macroloba (Oberbauer et al. 1985, Hollinger 1987, Fetcher et al. 1988). Elevated CO_2 -grown seedlings had lower rates of P_n than ambient CO_2 -grown seedlings in both the small and large pot size treatments. Arp (1991) compiled data on the interaction between photosynthetic rate and pot size in elevated CO_2 -grown plants and found a strong correlation between photosynthetic rate and rooting volume. A lower photosynthetic rate was observed in plants grown in

elevated CO₂ with a limited rooting volume and reduced sink demand from growing roots. Red spruce seedlings were transplanted into larger pots at 7 months to avoid confounding elevated CO₂ effects on physiology and growth as the seedlings grew larger. Transplanting seedlings to a larger rooting volume did increase seedling growth and leaf gas exchange rates but did not affect gas exchange and growth responses of red spruce to elevated CO₂. Upon examination at 2, 3, 5, 9 and 12 months, ambient and elevated CO₂-grown seedlings in both pot size treatments had large amounts of soil volume without any roots present. Despite unexplored rooting volume in the elevated the CO₂ treatment, a lower leaf Pn rate was observed in elevated CO₂-grown seedlings. The greater Ci that accompanied the reduction in Pn in elevated CO₂-grown seedlings suggests that the reduction was due, in part, to mesophyll limitations. CO₂ research has suggested that a desensitization of photosynthesis in response to growth in elevated CO₂ may be a result of starch accumulation in leaves, feedback inhibition, decreased rubisco amount and activity or a reduction in the number and strength of plant sinks (Madsen 1975, Herold 1980, Bowes 1991, Stitt 1991). In this study, seedlings transplanted into 646-cm³ pots showed greater rates of Pn and gl, and lower Ci as well as greater plant dry weight, size and mean RGR than seedlings

grown in 175-cm³ pots. The increased growth and gas exchange of transplanted seedlings suggests an increase in the number or size of plant sinks. Yet, transplanted seedlings grown in elevated CO₂ also showed a reduction in Pn compared to ambient CO₂-grown plants. Greater SLW observed in elevated CO₂-grown needles suggests starch accumulation but whether starch accumulation is the cause of photosynthetic change is unclear.

Interestingly, an increase in gl in elevated CO₂-grown needles was observed at 3 months. Leaf conductance was possibly greater in elevated CO₂-grown leaves because of increased stomatal density or aperture. However, this CO₂ effect did not persist.

Older foliage had greater rates of Pn and Rd than younger foliage possibly because older foliage was more developed and hence enzymatically more active than younger foliage. But, gas exchange responses of both young and old foliage to growth in an elevated CO₂ environment were similar. That is, Pn was lower in elevated than in ambient CO₂-grown needles in both undeveloped and fully-expanded needles.

Greater stem diameter, height and dry weight growth in red spruce seedlings exposed to elevated CO₂ for 1 year was likely due to an increase in total plant carbon uptake. Pn more than doubled in both ambient and elevated CO₂-grown

seedlings when the CO₂ concentration was raised from 358 to 728 ppm. Other studies have reported similar growth enhancement in tree species in response to long-term (a year or longer) CO₂ enrichment (Tinus 1972, Surano et al. 1986, Downton et al. 1987, Idso et al. 1991).

Although total seedling dry weight was greater in elevated than in ambient CO₂-grown seedlings at 1 year, the mean RGR was greater in elevated than in ambient CO₂-grown seedlings only from 3 to 5 months of age. Greater dry matter accumulation at 1 year in elevated CO₂-grown seedlings may be explained by a greater mean RGR from 3 to 5 months and from germination to 2 months of age in elevated CO₂-grown seedlings. Total plant dry weight was greater ($p \leq 0.05$) in elevated CO₂-grown seedlings than in ambient CO₂-grown seedlings at the first harvest at 2 months of age (81 mg versus 63 mg, respectively). The mean RGR was therefore greater in elevated CO₂-grown seedlings sometime between germination and 2 months of age. A similar mean RGR response to elevated CO₂ was reported after the first 6 weeks of exposure in Picea mariana seedlings (Campagna and Margolis 1989). These initial gains in dry matter investment from germination to 2 months of age and from 3 to 5 months in elevated CO₂ yielded greater total plant weight at 12 months even though the mean RGR, or average efficiency of dry matter production by each existing unit of dry

matter, was not greater in elevated CO₂-grown seedlings from 9 to 12 months. We should also note that from 3 to 5 months seedlings were subjected to an 8 hr photoperiod to induce bud set. We visually observed that seedlings in the elevated CO₂ treatment continued above ground growth longer and set a bud later than the ambient CO₂ treatment. Growth responses between 3 and 5 months may be confounded by elevated CO₂ effects on plant developmental stage.

How plant physiology and growth will respond to an elevated atmospheric CO₂ level may depend on the supply of available water and nutrients (Tolbert and Zelitch 1983, Acock and Allen 1985). Soil fertility and water stress treatments were crossed with CO₂ exposure treatments to determine if growth and physiological responses of red spruce to elevated CO₂ were dependent on nutrient and water supply. A dependence of CO₂ growth enhancement on nutrient supply has been reported for Pinus radiata, Populus tremuloides, Pinus contorta and Picea glauca (Canham and McCavish 1981, Brown and Higginbotham 1986, Conroy et al. 1990, Brown 1991). In contrast, growth enhancement and gas exchange responses to elevated CO₂ were not dependent on nutrient and water supply in red spruce. No interactions between water stress treatment and CO₂ treatment were detected for any growth variable, although a difference in WP between CO₂ treatments was observed in seedlings grown in

646-cm³ pots. This difference in WP, although statistically significant, does not appear to be great enough to influence growth responses to elevated CO₂. Red spruce seedlings subjected to low soil fertility treatment and water stress responded to elevated CO₂ similarly to seedlings grown in the high soil fertility treatment with adequate water. Seedlings grown in the low soil fertility treatment possibly increased internal nutrient use efficiencies to support increased growth in elevated CO₂ as did Liriodendron tulipifera, Quercus alba and Pinus virginiana grown in elevated CO₂ (Luxmoore et al. 1986, Norby et al. 1986, O'Neill et al. 1987).

Seedlings grown in the larger pots were bigger most likely because the soil fertility treatments were reapplied (when additional soil volume was added) only to seedlings that were transplanted at 7 months of age. Leaf N and soil P and K were lower in the small pots than in the large pots at 9 months (Table 3.1). Whether greater growth in the large pots was due to rooting volume or nutrient readdition, physiological and growth responses to elevated CO₂ were still similar between seedlings grown in the low soil fertility treatment in small pots and seedlings grown in the high fertility treatment in large pots.

The interaction between pot size and water stress treatment on organ weights, total seedling weight, height

and R_d was possibly a result of a greater impact of water stress on the growth of seedlings with a high mean relative growth and more dry weight allocated to leaf rather than stem and root. But, measures of plant water status did not indicate greater water stress in seedlings transplanted into larger pots.

Little is known about the influence of elevated CO_2 on the allometric coefficient 'd' in plants (Farrar and Williams 1991). In response to elevated CO_2 , increased partitioning to roots has been reported for herbaceous species while no long-term changes in partitioning have been reported for cereals (Farrar and Williams 1991). Several studies have examined elevated CO_2 effects on root to shoot ratios in tree species (Tolley and Strain 1984, Hollinger 1987), but very little research has utilized allometric analysis to examine elevated CO_2 effects on organ allocation. In this study, CO_2 effects were examined across all soil fertility and water stress treatments for simplicity and because no statistical interactions between these parameters were observed. Transplanting seedlings to a larger pot size increased leaf allocation in relation to root and stem allocation but growth in elevated CO_2 did not change dry weight allocation patterns to root, stem and leaf in seedlings grown in either pot size. Red spruce seedlings

grown in elevated CO₂ simply got bigger sooner than seedlings grown in ambient CO₂.

In conclusion, after 12 months of growth in elevated CO₂, red spruce seedlings were bigger in both size and weight than seedlings grown in ambient CO₂. Gains in seedling dry weight in elevated CO₂ were initiated early in growth. Seedlings grown in elevated CO₂ grew bigger sooner but allocation patterns to root, stem and leaf did not change after one year of exposure. Gas exchange characteristics of red spruce seedlings were not influenced by growth in elevated CO₂ until 9 months of age. Lower Pn and greater leaf weight in elevated CO₂ seedlings when compared to ambient CO₂-grown seedlings suggests that greater growth in elevated CO₂ was due to an increase in total seedling photosynthesis. The observation of photosynthetic change in developed and undeveloped needles of actively growing seedlings provided with an adequate rooting volume and renewed soil fertility suggests that leaf age, pot-binding, nutrient limitation and a decrease in sink/source ratio were not the causes of photosynthetic change in red spruce seedlings. The lack of interaction between soil fertility treatment, water stress and CO₂ treatment suggests that red spruce seedlings growing in natural ecosystems with limited nutrients and water will still benefit from an increase in ambient CO₂ concentration.

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CHAPTER IV

FIXED GROWTH OF RED SPRUCE SEEDLINGS IN RESPONSE TO ELEVATED CO₂

INTRODUCTION

Ambient CO₂ concentrations are predicted to double by the year 2050 (Gates 1990) and subsequent effects on plant growth are complicated by varying resource availability and species specific responses. Little is known of the influence of elevated CO₂ in combination with varying resource availability on the fixed growth of woody plants. In tree species that express fixed growth, winter buds contain preformed primordia of shoots that will expand the following growing season (Kramer and Kozlowski 1979). In fixed growth species, the environment during bud formation affects resulting shoot growth the following season. Alternatively, free growth species simultaneously initiate and elongate new stem units during the current growing season. In free growth species, shoots may develop as a result of continued initiation and expansion of preformed stem and leaf primordia during the growing season. Fixed growth is viewed as a protective adaptation in species that are subjected to early frosts.

The amount of shoot growth during the seedling stage is important in seedling survival and potential volume growth (Teich and Khalil 1973, Pollard and Logan 1974). Elevated CO₂ may influence seedling survival and establishment in fixed and free growth species by affecting terminal shoot primordia formation and resulting height growth. However, an elevated CO₂ environment may be more beneficial to fixed growth species who have a limited time frame in which to develop shoot primordia.

Red spruce (*Picea rubens* Sarg.) is an economically valuable timber species in the northern United States and Canada as well as an ecologically unique component of the Appalachian spruce-fir ecosystem of the southeastern United States. This study examined the fixed growth responses of red spruce seedlings to elevated CO₂ in combination with limited nutrient and water availability to determine the potential influence of an elevated CO₂ atmosphere on lateral and terminal shoot growth in red spruce. *Picea* species supplement their predetermined growth with free growth in the seedling stage (Jablanczy 1971). Red spruce seedlings were exposed to an 8 hr photoperiod while flushing to isolate fixed growth. Exposure to an 8 hr photoperiod was shown to inhibit free growth while allowing fixed growth expression in *Picea mariana* (Pollard and Logan 1974), a

species that hybridizes with red spruce where range overlap occurs (Harlow et al. 1978).

MATERIALS AND METHODS

Red spruce seed (F.W. Schumacker, Sandwich, MA, USA) from the northeastern United States was stratified for 30 days at 4°C and sown in a 2:1 mix (v:v) of soil from Whitetop mountain, Virginia (a high elevation, loamy-skeletal, mixed, frigid Typic Haplumbrept soil of pH 4.4) and perlite into 175-cm³ containers (Ray Leach Nursery, Canby, OR, USA). The containers were placed in either an ambient CO₂ (362 (±14) ppm CO₂) treatment chamber or an elevated CO₂ (711 (±29) ppm CO₂) treatment chamber. The CO₂ chambers and dispensing and monitoring system were previously described in Chapter III. Seedlings were rotated within a chamber every other day and CO₂ treatment and seedlings were rotated between the two chambers weekly to average any within chamber and between chamber variation across all treatments. Each CO₂ treatment and accompanying seedling and CO₂ treatment spent equal time in each chamber. Daily temperature and relative humidity were measured by recording hygrothermographs placed in each chamber. Temperature and relative humidity averaged 22 (±3)°C and 68 (±10)%, respectively, during the day in the elevated CO₂ treatment.

In the ambient CO₂ treatment, daily temperature and relative humidity averaged 22 (±3)°C and 64 (±13)%, respectively.

Soil fertility treatments were administered by hand mixing nonsoluble N-P-K, CaSO₄ and MgSO₄ into the soil. Nitrogen, P, K, Ca and Mg were applied at 150 kg ha⁻¹, 66 kg ha⁻¹, 124 kg ha⁻¹, 50 kg ha⁻¹ and 50 kg ha⁻¹, respectively, in the high fertility treatment. In the low soil fertility treatment, elements were applied at half the high fertility rate.

Due to the small size of the seedlings, water stress treatments were not initiated until the seedlings were ten weeks old. Water stress treatments were defined as the number of days between watering (Table 4.1). Needle relative water content (RWC) was measured at the end of cycles 4-6 when the seedlings were larger. The length of drought cycles was adjusted so that CO₂ treatments were subjected to approximately the same degree of stress. RWC values for a drought cycle were based on a sample size of 4 from each CO₂ treatment. A sample included 3 needles from 5 seedlings. Needles were weighed immediately to determine fresh weight (FW) and then placed in distilled water overnight and reweighed to determine saturated weight (SW). Needles were then oven-dried for 48 hr at 60° to determine oven-dried weight (OD). RWC was calculated as:

$$\text{RWC} = (\text{FW}-\text{OD})/(\text{SW}-\text{OD}) \times 100.$$

Table 1. Duration of drought cycles for water-stressed, elevated and ambient CO₂ treatments and corresponding needle relative water contents (RWC) of red spruce seedlings.¹

Drought cycle	CO ₂ treatment (ppm)	Duration (days)	RWC (%)
1	362	7	- ²
	711	7	-
2	362	7	-
	711	7	-
3	362	7	-
	711	7	-
4	362	7	90
	711	7	83
5	362	11	86
	711	9	86
6	362	11	89
	711	10	89
7	362	11	-
	711	9	-
8	362	11	-
	711	10	-
9	362	12	-
	711	10	-

¹ RWC of well-watered seedlings averaged 92 (±1) %.

² The RWC of needles were not measured before cycle 4 because of the small size of the seedlings and after cycle 6 to avoid removing too much leaf area while the seedlings were subjected to short days.

RWC was not measured after cycle 6 to avoid removing too much leaf area after seedlings were placed on short days and above ground growth had ceased.

Seedlings were grown for 3 months under a 16 hr photoperiod provided by 400 W sodium vapor lamps suspended over the chambers (an approximate photosynthetic photon flux density of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$ at the leaf surface after sunset). After 3 months, seedlings were subjected to short days by covering each chamber with white on black polyfilm (A.H. Hummert Seed Co., St. Louis, MO, USA) after approximately 8 hr of daylight to induce bud set. After 2 months on short photoperiods when buds had formed and the seedlings appeared visibly hardened (elongation had ceased, new growth had darkened and thickened), 10 seedlings from each treatment combination were harvested, oven-dried at 60°C for 48 hr and root, stem and leaf dry weights were determined. The remaining 5-month-old seedlings were placed in a cooler at 3°C for 7 weeks. A subset of 5 seedlings from each treatment combination was placed in a growth chamber (Conviroon E15, Conviroon Products of America, Pembina, ND, USA) after the 7 weeks. The remaining seedlings were put back into their respective CO_2 treatments to continue the study.

Seedlings in the growth chamber were placed on an 8 hr photoperiod for 8 weeks so that only fixed growth was

expressed. Pollard and Logan (1974) determined that only fixed growth was expressed in flushing Picea mariana seedlings subjected to an 8 hr photoperiod. An average daily temperature and relative humidity of 25 (± 0.5) $^{\circ}\text{C}$ and 60 (± 2)%, respectively, and nightly temperature and relative humidity of 25 (± 0.5) $^{\circ}\text{C}$ and 60 (± 2)%, respectively, were maintained in the growth chamber. The growth chamber supplied ambient air and a photosynthetic photon flux density of 700 $\mu\text{mol m}^{-2} \text{s}^{-1}$ at the leaf surface. All seedlings were kept well-watered. Seedlings were rotated within the growth chamber every other day.

Measurements were made on each seedling when stem and needle elongation were complete and a new terminal bud had formed. Stem and leaf dry weights of old growth were measured on all seedlings. Dry weight of all new shoot growth (lateral plus terminal) and total leaf dry weight, total leaf area, stem dry weight and stem height of the terminal leader were also measured. Samples were oven-dried for 48 hr at 60 $^{\circ}$ and weighed. Projected leaf area was measured using a digital scanner (Hewlett-Packard ScanJet Plus, Hewlett-Packard Co., Boise, Idaho, USA). A ratio of known area to scanned number of pixels was used to translate pixel number to projected needle area.

The study was arranged as a completely randomized design. A 3 factor analysis of variance was used to test for CO₂, soil

fertility and water stress effects. Since plants grown in elevated CO₂ were already significantly larger by 5 months of age (Table 4.2), an analysis of covariance was performed to isolate the effect of elevated CO₂ on bud size independent of plant size. Total preformed above ground (stem plus leaf) dry weight was used as a covariate to remove the influence of seedling size on bud formation.

RESULTS AND DISCUSSION

In red spruce seedlings, fixed total (lateral plus terminal) and fixed total (stem plus leaf) terminal growth were greater from buds that developed in elevated CO₂ (Table 4.3). Soil fertility treatment had no significant ($p \leq 0.05$) influence on any fixed growth parameter (data not shown), and no significant ($p \leq 0.05$) two-way interaction between soil fertility and CO₂ treatment or three-way interaction between soil fertility, water stress and CO₂ treatments was observed. However, an interactive effect between CO₂ treatment and water stress on terminal stem height, total leaf weight and total leaf area originating from the terminal bud was observed.

Addition of the covariate in the analysis improved the significance level for total new growth, total terminal dry weight and total terminal leaf weight but had no effect on

Table 4.2. Red spruce seedling dry weight growth in response to CO₂ treatment at 5 months of age.

Dry weight growth (mg)	CO ₂ treatment (ppm CO ₂)	
	362	711
leaf	43 *	78
stem	8 *	14
root	52 *	85
total	102*	178

* Indicates a significant difference between CO₂ treatment at the $p \leq 0.05$ level.

Table 4.3. Fixed growth of red spruce seedlings in response to CO₂ treatment.

Fixed growth variable	CO ₂ treatment (ppm CO ₂)	
	362	711
Total new growth (mg)	44 *	56
<u>Terminal Growth</u>		
total terminal weight (mg)	24 **	28
total leaf weight (mg)		
well-watered	20	19
water-stressed	19 *	28
total leaf area (cm ²)		
well-watered	280	244
water-stressed	245 *	391
terminal height (mm)		
well-watered	10	8
water-stressed	9 *	14

* Indicates a significant difference between CO₂ treatment at the p≤0.05 level.

** Indicates a significant difference between CO₂ treatment at the p≤0.10 level.

the significance level for terminal height and total terminal leaf area. The effect of elevated CO₂ on total and new terminal dry weight growth, therefore, may be by way of an increase in total preexisting photosynthetic tissue, which provided the carbon during bud formation. At the time of bud set, seedlings grown in elevated CO₂ had greater leaf dry weight than seedlings grown in ambient CO₂ (Table 4.2). In addition, the observed increase in fixed growth may be due to enhanced photosynthesis per unit of preexisting leaf area in an elevated CO₂ environment. Increased photosynthesis at an elevated CO₂ level has been reported for Pinus taeda, Pinus radiata, Picea abies and Pseudotsuga menziesii (Mortensen and Sandvik 1983, Conroy et al. 1986, Hollinger 1987, Fetcher et al. 1988) and red spruce (Seiler and Cazell 1990, and Chapter III).

Currently, we know of no study that has examined the influence of elevated CO₂ on the fixed growth potential of woody plants. However, Norby et al. (1986) observed an increase in the average dry weight per bud in 1-year-old elevated Quercus alba seedlings exposed to 690 ppm CO₂ for 40 weeks that suggested a possible increase in subsequent shoot elongation in an elevated CO₂ treatment.

Exposure to drought during bud development in fixed growth species has been shown to reduce shoot growth the following season (Kozlowski et al. 1991). Clements (1970)

found that smaller buds that developed during drought elongated into shorter shoots in Pinus resinosa trees despite irrigation during shoot expansion. In this study, a significant influence of elevated CO₂ was found in seedlings that developed terminal shoot primordia during water stress. Elevated CO₂ increased the height, total leaf weight and total leaf area of the terminal leader only in water-stressed seedlings (Table 4.3). An increase in ambient CO₂ supply may be more beneficial to growth when plants are carbon limited (Arp 1991), and exposure to elevated CO₂ during stress may overcome stress induced growth reductions (Eamus and Jarvis 1989). For example, in Liquidambar styraciflua seedlings, growth reductions in response to drought were ameliorated by concurrent exposure to elevated CO₂ (Tolley and Strain 1984). Greater CO₂-induced growth enhancement was observed by Conroy et al. (1988) in water-stressed Pinus radiata seedlings than in well-watered seedlings grown in 660 $\mu\text{l/l}$ CO₂ for 22 weeks. In red spruce, exposure to elevated CO₂ overcame water stress induced reductions in the fixed growth potential of the terminal shoot.

In summary, red spruce seedlings that set bud in elevated CO₂ produced more total shoot dry weight and total terminal dry weight than seedlings grown in ambient CO₂. As seedlings grow older and the capacity for free growth

diminishes, the ability of trees to develop preformed shoot primordia becomes more critical (Pollard and Logan 1974). This study suggests that predetermined shoot growth will be greater when ambient CO₂ concentrations rise. The greater terminal height and terminal leaf dry weight observed in seedlings exposed to elevated CO₂ and water stress during bud formation suggests that seedling survival during water stress may be enhanced in fixed growth species in elevated CO₂.

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CHAPTER V

INFLUENCE OF DROUGHT-CONDITIONING AND CO₂ ENRICHMENT ON THE GAS EXCHANGE RESPONSES OF RED SPRUCE SEEDLINGS TO WATER STRESS

INTRODUCTION

Boyer (1982) suggests that drought is the greatest limitation to plant productivity on a global scale. Predicted changes in global climate may increase the drought frequency over large areas of the United States, eastern Europe and Russia and further limit plant productivity (Manabe et al. 1982, Gates 1983, Mitchell 1983, Gleick 1987). However, future increase in ambient CO₂ concentration may reduce the effects of increasing drought frequency by enhancing plant water use efficiency. Exposure to an elevated CO₂ concentration during water stress may increase leaf water use efficiency by maintaining photosynthesis at a lower leaf conductance or by increasing photosynthesis in relation to transpiration (Pearcy and Bjorkman 1983). Short-term increases in WUE in response to elevated CO₂ exposure are usually a result of a 30-40% decrease in leaf conductance and a large increase in carbon assimilation (Eamus 1991).

Research efforts have focused on red spruce because of reported changes in population growth in some stands in the eastern United States (Hornbeck et al. 1986, Zedaker et al. 1987, McLaughlin et al. 1987 and 1990). Whether these changes in population growth are due to insects, disease, water and nutrient stress, or natural stand development is still debated. This study examined the influence of growth in elevated CO₂ and drought conditioning on the physiological responses of red spruce seedlings to water stress to determine how potential changes in global atmospheric CO₂ concentration and drought frequency may influence this species. We tested the specific hypothesis that well-watered and drought-conditioned red spruce seedlings grown in ambient or elevated CO₂ will respond similarly to water stress.

MATERIALS AND METHODS

Red spruce seed (F.W. Schumacker, Sandwich, MA, USA) was stratified for 30 days at 3°C and then sown in a 2:1 mix (v:v) of soil from Whitetop Mountain, Virginia (a loamy-skeletal, mixed, frigid Typic Haplumbrept soil of pH 4.4) and perlite in 175-cm³ Containers (Stuewe & Sons, Inc., Corvallis, OR, USA). Nonsoluble N-P-K, CaSO₄ and MgSO₄ were hand-mixed into the soil at 150 kg ha⁻¹, 66 kg ha⁻¹, 124 kg

ha⁻¹, 50 kg ha⁻¹ and 50 kg ha⁻¹, respectively. The containers were placed in either an ambient CO₂ treatment (374 (±21) ppm) or elevated CO₂ treatment (713 (±32) ppm) chamber in a greenhouse on 25 March 1991 and seedlings were allowed to grow for 1 year. Please see Chapter III for chamber and CO₂ dispensing and monitoring descriptions. Average daily temperature and RH over the 12 months were 73.1 (±3.3)^oC and 68 (±11)%, respectively, for the elevated CO₂ treatment chamber and 72.5 (±3.1)^oC and 63 (±13)%, respectively, for the ambient CO₂ treatment chamber.

The study was arranged as a completely randomized design. CO₂ treatments and accompanying seedlings were therefore rotated between the 2 CO₂ treatment chambers on a weekly basis and seedlings were rotated within a chamber every other day. Each CO₂ treatment and respective seedlings spent equal time in each chamber and in various locations within a chamber.

After 3 months of growth on a 16 hr photoperiod (supplied by sodium vapor lamps suspended over each chamber) seedlings were placed on an 8 hr photoperiod for 2 months to induce bud set. Bud formation and subsequent flushing were induced to avoid loss of apical dominance typical of red spruce seedlings grown under greenhouse conditions without chilling. Chambers were covered with white on black polyfilm (A.H. Hummert Seed Co., St. Louis, MO, USA) after 8

hr of daylight. After the 2 months, seedlings were placed in a cooler for 7 weeks at 3°C to promote bud break. After the 7 weeks, seedlings were transplanted into 646-cm³ Deepots (Stuewe & Sons, Inc., Corvallis, OR, USA) with the soil mixture and soil fertility treatments described previously and placed back into the appropriate CO₂ treatment on a 16 hr photoperiod.

The well-watered treatment was watered daily. Drought-conditioning treatments were initiated when the seedlings were 10 weeks old. Drought cycles were defined by the number of days between watering (Table 5.1). After drought cycle 3 when the seedlings were larger, needle relative water contents (RWC) were measured at the end of a drought cycle to determine if CO₂ treatments were receiving the same degree of water stress. The drought cycles were then adjusted to deliver approximately the same degree of stress to each CO₂ treatment. Needle RWC values were based on a sample size of 4. Three needles were removed from each of 5 seedlings within a CO₂ treatment to make up 1 sample. After the needles were removed, they were weighed immediately to determine fresh weight (FW) and then placed in distilled water overnight to determine saturated weight (SW). Leaf samples were oven-dried for 48 hr to

Table 5.1. Drought cycles and corresponding plant water status (relative water content, RWC or water potential, WP) of drought conditioned red spruce seedlings in response to CO₂ treatment.¹

Drought cycle	CO ₂ treatment (ppm CO ₂)			
	374		713	
	Days	Water status	Days	Water status
	RWC(%)	WP(MPa)	RWC(%)	WP(MPa)
1	7	- ²	7	
2	7		7	
3	7		7	
4	7	90	7	83
5	11	86	9	86
6	11	89	10	89
7	11		9	
8	12		10	
9	13		10	
10	12		9	
11	12		9	
12	16	90	11	94
14	16	88	14	90
15	15		13	-1.51
16	12		13	88
17	15		13	-1.51
18	16		12	-1.48
19	11		13	-1.30
20			14	

¹ Control treatments were watered daily and measured RWC and WP averaged 94 (± 2)% and -0.65 (± 0.16) MPa, respectively, in the elevated CO₂ treatment. In the ambient CO₂ treatment, average RWC and WP of the control treatment were 93 (± 2)% and -0.73 (± 0.16) MPa, respectively. Standard deviations are noted in parentheses.

² No measures of plant water status were made for cycles where RWC or WP values are omitted.

determine oven-dried weight (OD). The RWC was then calculated as:

$$\text{RWC} = ((\text{FW}-\text{OD})/(\text{SW}-\text{OD})) \times 100.$$

No measures of plant water status were made during drought cycles 6-11 when seedlings were placed on short days and top growth had ceased and when seedlings were transplanted into larger pots and were breaking bud to avoid removing too much leaf area. After drought cycle 11, needle RWC or the water potential of a branch were measured at the end of a cycle. Plant water potentials were measured using a pressure chamber. No measures of plant water status were made at the end of the final drought cycles to avoid removing too much leaf tissue.

Both the well-watered and drought-conditioned treatments were subjected to water stress in their respective CO₂ treatments at 12 months. Seedlings were initially watered for 3 days before the water stress treatment began. Gas exchange was measured on 4 seedlings from a treatment combination after the 3 days and every 3 days thereafter using a portable photosynthesis system (LI-6200, LI-COR Inc., Lincoln, NE, USA) between 0800 and 1100 hr. Gas exchange of each seedling was measured at a CO₂ concentration of 366(±6) ppm and 731(±8) ppm. Leaf area on the top 3 cm of stem was marked and the same leaf tissue was measured during each session. The water potential of a

branch was measured immediately following gas exchange measurement using a pressure chamber. The study was concluded after each seedling had reached a water potential of approximately -2.0 MPa. The projected area of needles measured during gas exchange was determined using a digital scanner (Hewlett Packard Scanjet Plus, Hewlett-Packard Co., Boise, ID, USA).

Linear regression was used to examine the relationship between gas exchange variables measured at a CO₂ level of 366 ppm and 731 ppm CO₂, and water potential. Slopes and intercepts were compared between ambient and elevated CO₂ growth treatments and well-watered and drought-conditioned treatments within a CO₂ measurement level using a t statistic.

RESULTS AND DISCUSSION

The log of water potential and cuvette CO₂ concentration during gas exchange measurement in the regression equation describing net photosynthesis resulted in the best fit across all CO₂ growth and drought conditioning treatments (overall R²=.52). However, the log of water potential and CO₂ concentration were better at predicting photosynthesis in elevated CO₂-grown seedlings and in well-watered seedlings than in ambient CO₂-grown

seedlings and drought-conditioned seedlings at both CO₂ measurement concentrations (Table 5.2). The log of water conductance resulted in the best fit for all CO₂-growth and drought conditioning treatments at both CO₂ measurement levels (Table 5.3). Seiler and Cazell (1990) also found the log of water potential to be a good predictor of leaf conductance in water-stressed red spruce seedlings.

Drought-induced reductions in stomatal conductance and photosynthesis may be delayed and less severe in an elevated CO₂ environment (Eamus and Jarvis 1985). In red spruce seedlings, growth in elevated CO₂ did not affect the response of net photosynthesis and leaf conductance to decreasing water potential. During the dry down, water was withheld from seedlings while they remained in their respective CO₂ growth environments. Despite receiving an elevated CO₂ concentration during the dry down, no significant differences in intercept and slope estimates of the regression equations describing photosynthesis and conductance were found between ambient and elevated CO₂-grown seedlings at either CO₂ measurement level (Tables 5.2 and 5.3). Tolley and Strain (1985) also found no influence of CO₂ enrichment on the physiological response of Pinus taeda to water stress. In contrast, CO₂ enrichment improved drought acclimation in Liquidambar styraciflua and Pinus radiata seedlings (Tolley and Strain 1985, Conroy et al.

Table 5.2. Linear regression equations and R² values for the relationship between net photosynthesis (Pnet), the log of water potential (log WP) and cuvette CO₂ concentration (CO₂) measured at 366 ppm and 731 ppm CO₂ in response to CO₂ growth treatment and drought conditioning treatment in 1-year-old red spruce seedlings.¹

Treatment	CO ₂ (ppm)	Regression model	R ²
CO ₂ treatment (ppm)			
374	366	Pnet = 20.6 -1.28(log WP)	0.26
713	366	Pnet = 53.8 -0.83(log WP)	0.47
374	731	Pnet = 84.0 -0.09(log WP)	0.13
713	731	Pnet = 84.4 -0.83(log WP)	0.42
Drought conditioning treatment			
well-watered	366	Pnet = 37.9* -1.77(log WP)*	0.72
drought-conditioned	366	Pnet = 30.4 -0.62(log WP)	0.18
well-watered	731	Pnet = 101.2* -0.09(log WP)*	0.41
drought-conditioned	731	Pnet = 43.0 0.41(log WP)	0.07

¹ A photosynthetic photon flux density of 1233 (± 139) $\mu\text{mol m}^{-2} \text{s}^{-1}$, temperature of 23.5 (± 1.2) °C, relative humidity of 36 (± 10)% and CO₂ concentration of 366 (± 6) ppm or 731 (± 8) ppm were provided in the cuvette during gas exchange measurement.

* Indicates a significant difference in intercept or slope estimates between drought conditioning or CO₂ growth treatment at the p \leq 0.05 level (n=68).

Table 5.3. Linear regression equations and R² values for the relationship between leaf conductance (gl) and the log of water potential (log WP) measured at 366 ppm and 731 ppm CO₂ in response to CO₂ growth treatment and drought conditioning treatment in 1-year-old red spruce seedlings.

Treatment	CO ₂ (ppm)	Regression model	R ²
CO ₂ treatment (ppm)			
374	366	gl = 24.2 - 32.3(log WP)	0.61
713	366	gl = 21.2 - 28.8(log WP)	0.68
374	731	gl = 22.3 - 29.3(log WP)	0.59
713	731	gl = 20.6 - 27.3(log WP)	0.69
Drought conditioning treatment			
well-watered	366	gl = 21.8 - 40.2(log WP) *	0.73
drought-conditioned	366	gl = 23.2 - 22.4(log WP)	0.62
well-watered	731	gl = 20.5 - 38.3(log WP) *	0.75
drought-conditioned	731	gl = 22.0 - 20.1(log WP)	0.59

1 A photosynthetic photon flux density of 1233 (±139) μmol m⁻² s⁻¹, temperature of 23.5 (±1.2) °C, relative humidity of 36 (±10)% and CO₂ concentration of 366 (±6) ppm or 731 (±18) ppm were provided in the cuvette during gas exchange measurement.

* Indicates a significant difference in intercept or slope estimates between drought conditioning or CO₂ growth treatment at the p≤0.05 level (n=68).

1986). In addition, well-watered, elevated CO₂-grown red spruce seedlings reached a lower water potential sooner during water stress than well-watered, ambient CO₂-grown seedlings. Elevated CO₂-grown seedlings reached a mean water potential of -1.82 MPa after 6 days of water stress whereas well-watered, ambient CO₂-grown seedlings reached a water potential of -1.36 MPa after 6 days. Seedlings grown in elevated CO₂ experienced water stress sooner most likely because of their larger size. Elevated CO₂-grown seedlings were larger in both size and weight than ambient CO₂-grown seedlings at 12 months (please see Chapter III). Any increase in red spruce leaf WUE in elevated CO₂ was most likely offset by an increase in total leaf area, as suggested by Eamus and Jarvis (1989).

The lack of difference in photosynthetic and conductance responses to decreasing water potential between elevated and ambient CO₂-grown seedlings implies that leaf WUE (net photosynthesis/conductance) responses to plant water potential were not influenced by CO₂ growth treatment. Positive increases in WUE in response to growth in elevated CO₂ have been documented for tree species including Pinus echinata, Pinus radiata, Nothofagus fusca and Pseudotsuga menziesii (Hollinger 1987, Norby et al. 1987,). However, most of these studies measured and compared the WUE of well-watered seedlings at their respective CO₂ growth

concentration rather than examine the response of WUE to increasing water stress at varying CO₂ measurement concentrations.

Drought-conditioning did influence the response of photosynthesis and conductance to decreasing water potential. Drought-conditioned red spruce seedlings maintained stomatal opening and photosynthesis at lower water potentials than well-watered seedlings (indicated by the differences in slope estimates in the regression equations for well-watered and drought-conditioned treatments, Tables 5.2 and 5.3, and Figure 5.1). Moisture stress-conditioned Pinus taeda seedlings also maintained photosynthesis to lower water potentials than did well-watered seedlings (Seiler and Johnson 1985). Drought acclimation was reported for drought-preconditioned Picea mariana (Zwiazek and Blake 1989). In this study, photosynthesis at low water potentials appeared to be CO₂ limited rather than mesophyll limited because photosynthesis continued at low leaf conductances when gas exchange was measured at an elevated CO₂ concentration (indicated by the high photosynthetic rates between -1.5 and -2.0 MPa in Figure 5.1). Seiler and Cazell (1990) also found high rates of photosynthesis at low leaf conductances in water-stressed red spruce seedlings when gas exchange was measured at an elevated CO₂ concentration. However, Seiler and Cazell

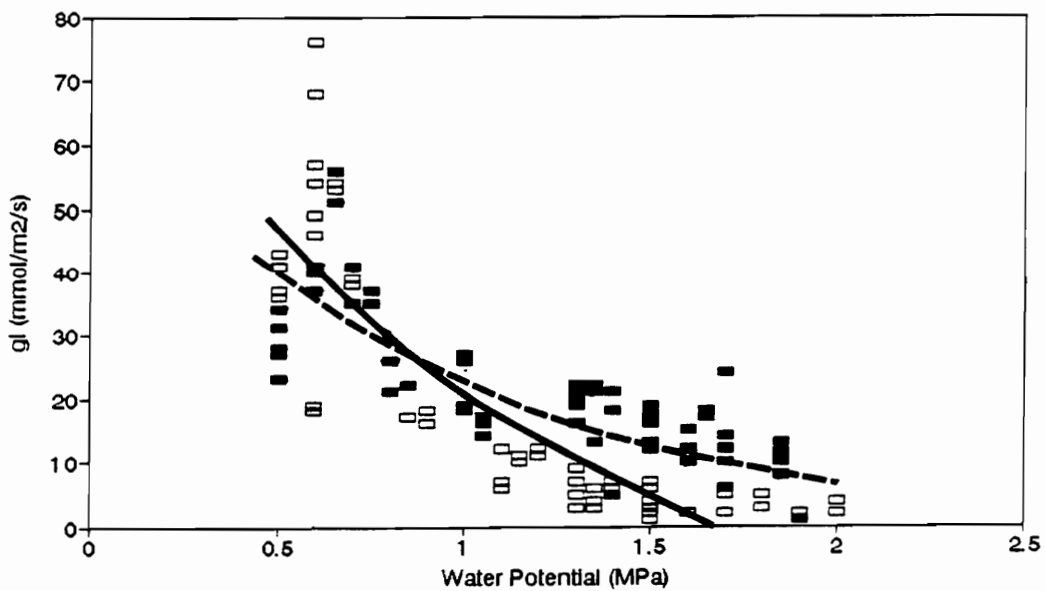
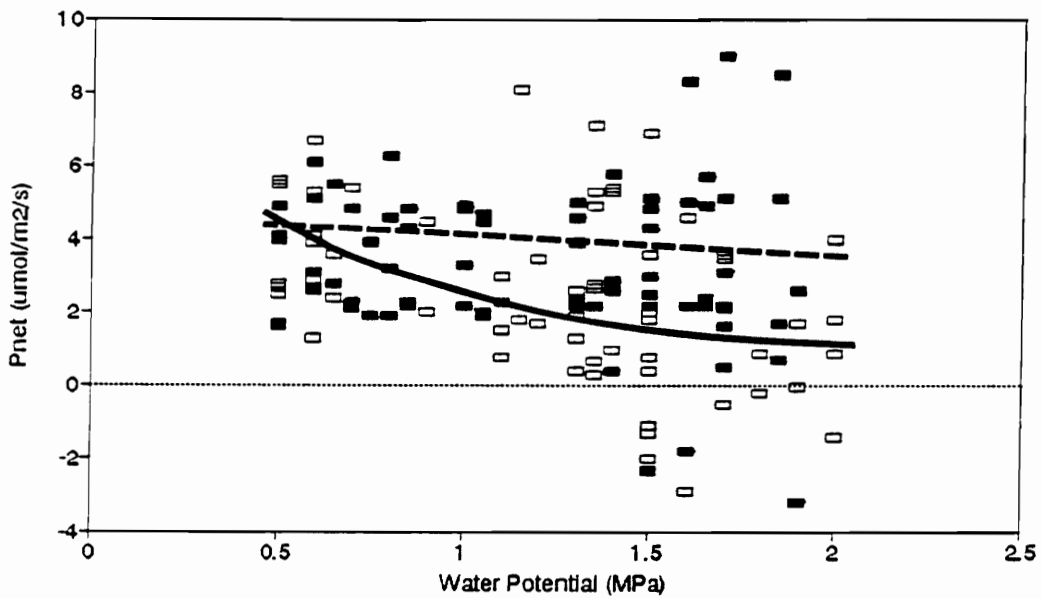


Figure 4.1. Net photosynthesis (Pnet) and leaf conductance (gl) of well-watered (empty squares, solid line) and drought-conditioned (solid squares, dashed line) red spruce seedlings in response to decreasing water potential. Gas exchange was measured at 366 and 731 ppm CO_2 .

(1990) found no influence of drought conditioning on photosynthetic and stomatal responses to decreasing water potential in red spruce seedlings. Results from the two studies may differ because of differences in seedling ages, seed origins and the length of drought conditioning. In the previous study, two-year-old red spruce seedlings from one seed source in New Hampshire were subjected to drought conditioning for 87 days whereas in the current study, seeds from various seed sources in the northeast were grown from seed while subjected to one year of drought conditioning. In addition, the study by Seiler and Cazell (1990) water stressed red spruce seedlings to much lower water potentials (-3.5 MPa) than our study (-2.0 MPa). Drought conditioning possibly improved the drought tolerance of gas exchange processes in red spruce through changes in osmotic potential and subsequent turgor maintenance. A change in osmotic relations in response to drought adaptation has been documented for some woody plant species (Bahari et al. 1985, Seiler and Johnson 1985), but was not found in red spruce (Seiler and Cazell 1990).

In conclusion, growth in elevated CO₂ did not affect the physiological responses of red spruce to water stress. An elevated CO₂ atmosphere did not allow photosynthesis and conductance to lower water potentials. This study suggests that, in red spruce seedlings, an increase in ambient

atmospheric CO₂ concentration may not reduce the effects of increasing drought frequency on leaf gas exchange characteristics.

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CHAPTER VI

PHYSIOLOGICAL RESPONSES OF RED SPRUCE SEEDLINGS TO TEMPERATURE, LIGHT AND CO₂ CONCENTRATION IN COMBINATION WITH WATER STRESS

INTRODUCTION

Recently, considerable effort has focused on determining if and why red spruce decline is occurring in the high elevation mountains of the northeastern and southeastern United States. Declines in growth are possibly a result of a number of diverse influences including pollution, nutrient limitation and drought (Hornbeck et al. 1986, McLaughlin et al. 1990). Some spruce populations may not be declining but rather experiencing natural changes in stand development (Zedaker et al. 1987). The physiological capability of red spruce to adapt to changes in a single environmental resource must first be examined before an understanding of the complex, interactive anthropogenic and natural influences on red spruce growth can be developed. The general objective of this research was to examine the physiological responses of red spruce to light, temperature, and CO₂ concentration in combination with water stress to determine if drought is a relevant factor in red spruce

decline. Light, temperature, and CO₂ response curves and total leaf water and osmotic potential were measured on drought-conditioned and well-watered red spruce seedlings before and during water stress to determine (1) if drought-conditioned seedlings are better adapted to water stress than well-watered seedlings and (2) if water stress changes the pattern of physiological responses to temperature, light and CO₂ concentration.

MATERIALS AND METHODS

Red spruce seedlings of unknown age and similar size along with soil from Whitetop mountain Virginia were transplanted into 1000-cm³ pots. Seedlings were placed in a greenhouse and allowed to grow for two growing seasons. All seedlings were kept well-watered and were fertilized weekly with a 10 ml/seedling solution consisting of 200 µg/g N, 87 µg/g P and 166 µg/g K (Peters General Purpose 20-20-20 fertilizer, Fogelsville, PA, USA).

During the experiment the seedlings were maintained in a growth chamber (Convion E15, Convion Products of America, Pembina, ND, USA) and subjected to a 14 hr photoperiod, a 15 (± 0.5)°C night and 25 (±0.5)°C day. Seedlings received, at the leaf surface, a photosynthetic photon flux density (PPFD) of 150 µmol m⁻² s⁻¹ for 1 hr

between 0600 and 1900 hr, a PPFD of $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ between 0700 and 1600 hr for 3 hr and a PPFD of $600 \mu\text{mol m}^{-2} \text{s}^{-1}$ from 1000 to 1600 hr to simulate a natural day. Average relative humidity (RH) within the chamber was maintained at $70 (\pm 2)\%$.

Due to the limited amount of leaf tissue on a seedling, drought-conditioned seedlings were watered when photosynthesis was reduced by approximately 75%. Photosynthesis was monitored using a PACsys null balancing gas exchange system with an environmentally controlled cuvette (Data Design Group, La Jolle, CA, USA). Monitoring was done at a leaf temperature of 25°C , a cuvette RH of 30%, a PPFD of $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ (supplied by a 400 W sodium vapor lamp suspended over the cuvette), and a CO_2 concentration of 350 ppm. A previous study of red spruce determined that a photosynthetic reduction of 80% occurred at an average leaf water potential of -1.9 MPa (Seiler and Cazell 1990). Leaf tissue used in photosynthetic monitoring was marked so that the same tissue was consistently sampled. The drought-conditioned seedlings were subjected to 12 drought cycles which was equivalent to 4 months of prolonged sublethal water stress. Control seedlings were watered regularly. Seedlings were not fertilized once drought conditioning began to eliminate confounding fertilization and water stress responses.

Response curves were measured on hydrated drought-conditioned and control seedlings (initial water stress stage, average leaf water potential of -0.8 MPa) and after approximately 14 days of water stress when photosynthesis was reduced by approximately 70% (final water stress stage, average leaf water potential of -1.7 MPa). Water potentials and osmotic potentials were measured at the initial and final water stress stage of the dry down using a Scholander pressure chamber and leaf psychrometers (JRD Merrill, 75 series, Logan, Utah), respectively. Water potentials were measured on one branch per seedling while two osmotic potential readings per seedling were made and averaged. An even mixture of current and 1-yr-old foliage was removed from a seedling, placed in a leaf psychrometer and frozen with liquid nitrogen.

Response Curves

Gas exchange responses to light, temperature and CO₂ concentration were measured before water stress initiation and after 14 days of water stress. Response curves were measured on leaf tissue located at the top of the main stem and included both current and 1-year-old foliage. Gas exchange was measured using the PACsys null-balancing system at a PPFD of 100, 350 and 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and a constant relative humidity of 33 (± 5)%, leaf temperature of 25.0

(± 0.5) $^{\circ}\text{C}$ and CO_2 concentration of 350 ppm when physiological responses to light were studied. A sodium vapor lamp was suspended over the cuvette and used as a light source. Shade cloth was placed over the cuvette to alter the PPFD level.

The temperature response of photosynthesis was determined by measuring gas exchange with the PACsystem at a RH of 33 (± 6)%, a temperature of 15, 25 and 35 $^{\circ}\text{C}$ (a vapor pressure deficit of 9.0, 16.1 and 27.7 g m^{-3} , respectively), a PPFD of 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and a CO_2 concentration of 350 ppm. The temperature response may thus include a response to vapor pressure deficit as well.

Leaf physiological response to CO_2 concentration was determined by measuring gas exchange at 200, 400 and 600 ppm CO_2 using a portable photosynthesis system (Licor 6250, Licor Inc., Lincoln, NE, USA). A leaf temperature of 31.0 (± 2.0) $^{\circ}\text{C}$ was maintained by placing a clear water bath between the light source and the cuvette. Magnesium perchlorate was used to scrub the water vapor in the gas exchange system to maintain a cuvette RH of 34 (± 6)%. A PPFD of 1200 $\mu\text{mol m}^{-2} \text{s}^{-1}$ was supplied at the leaf surface with a sodium vapor lamp.

Net photosynthesis, transpiration and leaf conductance were calculated on a leaf dry weight basis. Sample leaf tissue was oven-dried at 60 $^{\circ}\text{C}$ for at least 48 hr before dry

weight determination. Internal CO₂ concentration values (C_i) were not presented because of the consistently low values determined by the PACsys gas exchange system.

Results were analyzed using a 2 factor analysis of variance in a randomized complete block design with a total of 8 blocks. The environmental treatment (PPFD, temperature or CO₂ concentration) was considered a factor along with before or after the final dry down. Because the drought conditioning cycles were staggered through time, each control, drought-conditioned pair was treated as a block. Tukeys test was used to separate treatment means.

RESULTS AND DISCUSSION

Mean net photosynthesis and leaf conductance were significantly ($p \leq 0.05$) lower in the well-watered treatment than in the drought-conditioned treatment in the temperature, PPFD and CO₂ studies most likely because of nutrient stress in the well-watered treatment (0.026 $\mu\text{mol g}^{-1} \text{s}^{-1}$ and 0.16 $\text{mmol g}^{-1} \text{s}^{-1}$ versus 0.032 $\mu\text{mol g}^{-1} \text{s}^{-1}$ and 0.21 $\text{mmol g}^{-1} \text{s}^{-1}$, respectively). Needles were visibly chlorotic in the well-watered treatment. However, drought conditioning did not change the nature of the physiological responses of red spruce seedlings to light, temperature and CO₂ treatment during the final "challenge" dry down. No

significant ($p \leq 0.05$) interactions between drought conditioning treatment and stage of drought stress were observed. Both drought-conditioned and well-watered seedlings responded to light, temperature and CO_2 concentration before and after drought similarly. So, gas exchange response data from drought-conditioned and well-watered seedlings were combined. Physiological responses before and during water stress were compared and presented.

Drought conditioning for 4 months had no significant effect on water potential (WP) and osmotic potential (OP) responses of seedlings "challenged" to a final dry down (Table 6.1). WP and OP were similar between drought-conditioned and well-watered seedlings. The dry down reduced WP and leaf net photosynthesis and conductance but had no effect on OP (Tables 6.1 and 6.2). A lack of osmotic adjustment during water stress has been previously reported for red spruce (Seiler and Cazell 1990). Water use efficiency was greater after approximately 14 days of drought in the temperature and PPFd study (Table 6.2). Lowered photosynthesis during water stress was most likely due to both stomatal limitation (indicated by lower leaf conductance after water stress) and possibly mesophyll limitation. Reduced photosynthesis during water stress may be a result of reductions in protein synthesis, protoplast volume and rubisco activity (Vapaavouri et al. 1981,

Table 6.1. Water potential (WP) and leaf osmotic potential (OP) of drought-conditioned and well-watered red spruce seedlings at the beginning of a final dry down (initial) and after 14 days of drought (final).

Treatment	WP (MPa)	OP (MPa)
Drought conditioning		
Drought-conditioned	-1.21	-2.29
Well-watered	-1.26	-2.16
Water stress		
Initial	-0.91 *	-2.22
Final	-1.56	-2.23

* Indicates a significant difference in WP or OP between drought conditioning or water stress treatments at the $p \leq 0.05$ level.

Table 6.2. Net photosynthesis (Pn), leaf conductance (gl) and water use efficiency (WUE) of red spruce seedlings before drought initiation (initial) and 14 days after drought stress (final) in the temperature, PPFD and CO₂ response studies.

Treatment	Pn ($\mu\text{mol g}^{-1} \text{s}^{-1}$)	gl ($\text{mmol g}^{-1} \text{s}^{-1}$)	WUE ($\mu\text{mol}/\text{mmol}$)
<u>Temperature</u>			
Initial	0.028 *	0.18 *	0.17 *
Final	0.018	0.08	0.24
<u>PPFD</u>			
Initial	⁻¹	0.18 *	0.13 *
Final	-	0.07	0.19
<u>CO₂</u>			
Initial	0.019 *	0.29 *	- ¹
Final	0.013	0.16	-

* Indicates a significant difference between initial and final treatment at the $p \leq 0.05$ level.

¹ Main effect means are not presented when a significant interaction between stage of water stress and temperature, PPFD or CO₂ treatment was observed.

Berkowitz and Kroll 1988, Farquhar et al. 1989). However, separation of stomatal and mesophyll limitations to net photosynthesis is complicated by heterogeneity of stomatal opening (Farquhar et al. 1989).

Only main effect means are presented when no interaction between environmental treatment (temperature, PPF and CO₂ concentration) and stage of water stress (initial, before drought initiation and final, 14 days into the dry down) was observed.

Temperature had no significant effect on net photosynthesis or leaf conductance of red spruce seedlings (Table 6.3). In contrast, a temperature optimum for photosynthesis has been reported for Pinus taeda but no effect of temperature on conductance was observed (Teskey et al. 1986). The challenge dry down did not change the nature of the response of photosynthesis and conductance to temperature. In well-watered plants, stomata tend to open with increasing temperature while water-stressed plants tend to close stomata with rising temperature (Berry and Bjorkman 1980). Red spruce conductance tended to decrease with increasing temperature but this trend was not significant. Plants exposed to high temperatures (30 to 40°C) and water stress may suffer from photoinhibition (Chaves 1991). In red spruce, 35°C did not significantly reduce photosynthesis but did enhance WUE. Leaf WUE was greater at 25 and 35°C

Table 6.3. Net photosynthesis (Pn), leaf conductance (gl) and water use efficiency (WUE) of red spruce seedlings in response to temperature. Pn and gl means are averaged across stage of water stress (initial, before drought initiation and final, 14 days after water was withheld).

Gas exchange variable	Temperature (°C)		
	15	25	35
Pn ($\mu\text{mol g}^{-1} \text{s}^{-1}$)	0.021 A	0.024 A	0.023 A
gl ($\text{mmol g}^{-1} \text{s}^{-1}$)	0.140 A	0.126 A	0.128 A
WUE ($\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$)	0.182 A	0.221 B	0.213 B

Different letters indicate a significant difference between temperature treatment at the $p \leq 0.05$ level.

than at 15°C (Table 6.3). Photoinhibition and reductions in photosynthesis at high temperature may occur with more severe water stress.

PPFD had no significant effect on leaf conductance but WUE was greater at 350 and 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ than at 150 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 6.4) most likely because of enhanced photosynthesis at higher light intensities. An interactive effect between stage of water stress and PPFD on net photosynthesis was found. Net photosynthesis was lower after 14 days of drought when measured at 350 and 600 $\mu\text{mol m}^{-2} \text{s}^{-1}$ (Table 4). This decrease in photosynthesis at higher PPFD may be due to a downward regulation of light reaction centers in the chloroplast during water stress (Bjorkman and Powles 1984) or to a slowing of electron transport (Genty et al. 1987). Sharp and Boyer (1986) found reduced photosynthesis at both low and high PPFD in water-stressed Helianthus annuus. The authors suggest that the reduction in photosynthesis was due to a direct effect of water availability on chloroplast function rather than photoinhibition.

CO₂ concentration had no significant effect on leaf conductance but increased photosynthesis from 200 to 600 ppm CO₂ (Table 6.5). Increased photosynthesis in response to higher CO₂ concentration is a result of increased substrate and activation of rubisco (Eamas and Jarvis 1989). An

Table 6.4. Net photosynthesis (Pn), leaf conductance (gl) and water use efficiency (WUE) of red spruce seedlings in response to photosynthetic photon flux density (PPFD). WUE and gl means are averaged across stage of water stress (initial, before drought initiation and final, 14 days after water was withheld).

Gas exchange variable	PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$)		
	100	350	600
Pn ($\mu\text{mol g}^{-1} \text{s}^{-1}$)			
initial	0.016	0.026 *	0.028 *
final	0.011	0.015	0.016
gl ($\text{mmol g}^{-1} \text{s}^{-1}$)	0.125 A	0.131 A	0.134 A
WUE ($\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$)	0.127 A	0.173 B	0.185 B

Different letters indicate significant differences between PPFD treatment at the $p \leq 0.05$ level.

* Indicates a significant difference between initial and final water stress treatment at the $p \leq 0.05$ level.

Table 6.5. Net photosynthesis (Pn), leaf conductance (gl) and water use efficiency (WUE) of red spruce seedlings in response to CO₂ concentration. Pn and gl means are averaged across stage of water stress (initial, before drought initiation and final, 14 days after water was withheld).

Gas exchange variable	CO ₂ Concentration (ppm)		
	200	400	600
Pn ($\mu\text{mol g}^{-1} \text{s}^{-1}$)	0.004 A	0.016 B	0.280 C
gl ($\text{mmol g}^{-1} \text{s}^{-1}$)	0.216 A	0.219 A	0.230 A
WUE ($\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$)			
initial	0.019	0.074 *	0.121 *
final	0.026	0.088	0.155

Different letters indicate significant differences between CO₂ treatment at the $p \leq 0.05$ level.

* Indicates a significant difference between initial and final water stress treatment at the $p \leq 0.05$ level.

interaction between stage of drought stress and CO₂ concentration on WUE was observed. WUE was greater in seedlings exposed to drought for 14 days than in hydrated seedlings only at 400 and 600 ppm CO₂ (Table 6.5). An elevated CO₂ concentration possibly overcame any existing stomatal limitation to photosynthesis in water-stressed plants. Seiler and Cazell (1990) observed a greater enhancement of photosynthesis in water-stressed red spruce seedlings when measured at 838 ppm CO₂ than at ambient CO₂. In this study, the nature of the response of photosynthesis and conductance to CO₂ concentration was not effected by moderate drought stress.

In summary, drought-preconditioning did not change the physiological responses of red spruce to light, temperature and CO₂ concentration. The challenge dry down did reduce conductance and photosynthesis. Red spruce photosynthetic response to PPF_D was affected by water stress but the pattern of photosynthetic and conductance responses to varying temperature and CO₂ concentration were not influenced by water stress. This study suggests that moderate water stress does reduce leaf conductance and photosynthesis but does not change characteristic physiological responses. More severe water stress or water stress in combination with other stresses such as pollution

may have a greater impact on red spruce physiological responses.

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CHAPTER VII

INFLUENCE OF WATER STRESS ON THE PHYSIOLOGY AND GROWTH OF RED SPRUCE SEEDLINGS GROWING ON WHITETOP MOUNTAIN, VIRGINIA

INTRODUCTION

Red spruce (*Picea rubens* Sarg.) is a dominant forest tree species in the spruce-fir ecosystem which has persisted over the past 18,000 years (Delcourt and Delcourt 1984). Currently, the southern Appalachian spruce-fir forests are restricted to seven high elevation mountains between the Virginia Balsam mountains and the Great Smokey mountains (Ramseur 1960). Growth declines and mortality have been reported for some spruce stands in the southern Appalachians (McLaughlin 1985, McLaughlin et al. 1987). These changes in stand growth may be a result of pollution, natural stand development, climate, nutrient limitations, insects or a combination of the above (Hornbeck et al. 1986, Zedaker et al. 1987, McLaughlin et al. 1990). Predicted changes in global climate, such as increased drought frequency, may push red spruce populations toward extinction in their southern range. The objective of this study was to examine the physiological responses to drought of native red spruce seedlings growing in their natural habitat. The specific

hypothesis tested was that gas exchange characteristics, plant water potentials and leaf osmotic potentials of red spruce seedlings growing on Whitetop mountain, Virginia will not be influenced by subjection to long-term drought.

MATERIALS AND METHODS

Native red spruce seedlings of unknown age and approximately 150 to 250 mm in height growing on a research site on Whitetop Mountain Virginia were separated into a water stress treatment or control treatment. Three blocks were chosen in close proximity to one another and separated into control or water stress plots. Five seedlings of similar size located in the center of each plot were selected for height, biomass, gas exchange and water status measurements. Total height from the root collar was measured on each of the five seedlings. Rain exclusion chambers were placed over each treatment plot to maintain similar environmental conditions between treatments. All plots were left in a "natural condition" except for the presence of the chambers. The rain exclusion chambers were constructed in a "covered wagon" style with Pvc tubing forming a rounded roof that inserted into a wooden base. Chamber dimensions were 1.5m length x 1.2m width x 1.2m height. Clear winter window plastic covered each chamber

down to 30.5 cm above the ground to allow for air mixing and cooling. Gutters were attached to the sides of all chambers to collect rainfall and cloud condensation and channel it to the control plots. Control plots were additionally watered every 2 weeks. The area around each block was not trenched due to high erodability of the shallow organic soil.

The chambers were first installed on 20 June 1990. The site was visited every other week to water the control treatments and check for water stress signs in the stress treatments. Unfortunately, the research site received high amounts of rainfall and cloud condensation during most of the summer. Plots were literally dripping wet when visited. However, by September of 1990 the research site appeared drier and measurements were made. Gas exchange and plant water status measures were taken on 6 September 1990 when the water stress plots were mildly stressed and on 27 September 1990 after the stressed plots were watered. The original goal was to compare responses of water-stressed treatment to the control treatment when the stressed plots were experiencing water stress and when the stressed plots were rewatered to determine if water stress induced permanent changes in seedling physiological characteristics.

On a measurement day, gas exchange was measured on a detached branch from two seedlings from each treatment plot using a portable photosynthesis system (LiCor 6250, Li-Cor

Inc, Lincoln, NE, USA) between 1100 and 1500 hr. Fully-expanded, current year foliage was selected. Gas exchange was measured at approximately 200, 400 and 600 ppm CO₂ to determine if changes in carboxylation efficiency occurred between treatments. Water potential (WP) was measured on a detached branch from 2 seedlings from each chamber using a Scholander pressure chamber. Osmotic potentials were measured by removing needles from a seedling in each chamber and placing them in a leaf psychrometer chamber (J.R.D. Merrill, 75 Series, Logan, UT, USA). Two seedlings per treatment plot were measured. Samples were frozen on site using liquid nitrogen and osmotic potentials were measured 4 hours later. Although this method of measuring osmotic potential is not necessarily a measure of active osmotic adjustment, a difference in osmotic potential between treatments would indicate a change in turgor maintenance, whether actively or passively driven. The plastic covering over each chamber was removed for the winter to prevent snow damage.

On 14 May 1991 new plastic was installed over each chamber. Gas exchange and plant water status measurements were made on 15 July, 22 July and 16 August 1991. Gas exchange was measured at ambient CO₂ (approximately 280 ppm CO₂). No measurements were made after 16 August 1991 because the high storm frequency of the season continually

saturated the site with water. The study was concluded by measuring the height of each of the 5 seedlings in a plot. The seedlings were then dug up and returned to the laboratory, washed and oven-dried for at least 48 hours at 60°C. Organ dry weights were then measured to determine if any water stress induced changes in biomass occurred.

Subsamples within each treatment plot (gas exchange and water and osmotic potentials, 2 subsamples; height and dry weights, 5 subsamples) were averaged and the mean used as the experimental unit. The influence of the rain exclusion treatment on the collected variables was analyzed as a completely randomized block design with three blocks. CO₂ measurement concentration was included as a main effect in the analysis of gas exchange responses for 1990.

RESULTS

On 6 September 1990 water stress reduced leaf conductance and WP and increased leaf water use efficiency (WUE) (Table 7.1). No significant effect of water stress on net photosynthesis or osmotic potential (OP) was observed (Table 7.1). No interactions between CO₂ measurement concentration and water stress treatment on gas exchange responses were found, therefore, means presented are averaged across all 3 CO₂ measurement concentrations.

Table 7.1. Net photosynthesis (Pn), leaf conductance (gl), and water use efficiency (WUE) averaged over three CO₂ measurement concentrations, water potential (WP) and leaf osmotic potential (OP) of red spruce seedlings grown on Whitetop Mountain subjected to control or water-stressed treatment measured on 6 September 1990.¹

Physiological variable	Water stress treatment	
	Control	Water-stressed
Pn ($\mu\text{mol g}^{-1} \text{s}^{-1}$)	0.095	0.096
gl ($\text{mmol g}^{-1} \text{s}^{-1}$)	1.16 *	0.96
WUE ($\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$)	0.081 *	0.100
WP (MPa)	-0.90	-1.30
OP (MPa)	-0.59	-1.21

* Indicates a significant difference between water stress treatment at the $p \leq 0.05$ level.

¹ Gas exchange measurements were made at an average PPFD of 1865 (± 218) $\mu\text{mol m}^{-2} \text{s}^{-1}$, temperature of 29.8 (± 0.8) °C, relative humidity of 44 (± 5)% and CO₂ concentration of 205 (± 10) ppm, 394 (± 7) ppm and 579 (± 13) ppm. Standard deviations are noted in parentheses.

After rewatering, the water-stressed plots had lower leaf conductance, but no significant effect of water stress on net photosynthesis, WUE or WP was found (Table 7.2). Again, no interactions between CO₂ measurement concentration and water stress treatment on gas exchange response variables were observed. On all three measurement days in 1991, water stress treatment had no significant effect on net photosynthesis, leaf conductance, WUE, WP or OP (Table 7.3). Height growth from 1990 to 1991 and final root, shoot, leaf and total dry weights were not influenced by water stress treatment (Table 7.4).

DISCUSSION

During both the 1990 and 1991 growing season, the water stress treatment was not severe enough to test the specified hypothesis of this study. At the end of the 1990 season, water stress did reduce leaf conductance but the reduction in conductance was not enough to lower leaf photosynthesis. Also, mean plant water potential indicated only a mild degree of stress (-1.30 MPa). Osmotic potentials were lower, but not significantly so during both 1990 and 1991

Table 7.2. Net photosynthesis (Pn), leaf conductance (gl), and water use efficiency (WUE) averaged over three CO₂ measurement concentrations and water potential (WP) of red spruce seedlings grown on Whitetop Mountain subjected to control or water-stressed treatment measured on September 27, 1990.¹ Both treatments were watered before measurement.

Physiological variable	Water stress treatment	
	Control	Water-stressed
Pn ($\mu\text{mol g}^{-1} \text{s}^{-1}$)	0.073	0.063
gl ($\text{mmol g}^{-1} \text{s}^{-1}$)	0.79 *	0.60
WUE ($\mu\text{mol CO}_2/\text{mmol H}_2\text{O}$)	0.097	0.102
WP (MPa)	-0.84	-0.72

* Indicates a significant difference between water stress treatment at the $p \leq 0.05$ level.

¹ Gas exchange measurements were made at an average PPFD of 1530 (± 645) $\mu\text{mol m}^{-2} \text{s}^{-1}$, temperature of 25.0 (± 3.0) °C, relative humidity of 49 (± 6)% and CO₂ concentration of 196 (± 8) ppm, 397 (± 12) ppm and 596 (± 14) ppm. Standard deviations are noted in parentheses.

Table 7.3. Net photosynthesis (Pn), leaf conductance (gl), water use efficiency (WUE), water potential (WP) and leaf osmotic potential (OP) of red spruce seedlings grown on Whitetop Mountain subjected to control or water-stressed treatment measured during 1991.

Physiological variable	7-15-1991 ¹		7-22-1991 ²		8-16-1991 ³	
	control	stress	control	stress	control	stress
Pn ($\mu\text{mol g}^{-1}\text{s}^{-1}$)	0.043 ⁴	0.040	0.065	0.068	0.057	0.069
gl ($\text{mmol g}^{-1}\text{s}^{-1}$)	0.94	0.86	0.88	1.03	1.60	1.65
WUE ($\mu\text{mol}/\text{mmol}$)	0.045	0.063	0.074	0.068	0.042	0.049
WP (MPa)	-0.83	-1.17	-0.63	-0.86	-0.78	-0.75
OP (MPa)	-1.24	-1.45	-1.15	-1.53	-1.37	-1.58

¹ On 7-15-1992, gas exchange measurements were made at an average PPFD of 1459 (± 635) $\mu\text{mol m}^{-2}\text{s}^{-1}$, temperature of 28.0 (± 2.8) $^{\circ}\text{C}$, relative humidity of 45 (± 6)% and CO₂ concentration of 274 (± 7) ppm.

² On 7-22-1991, gas exchange measurements were made at an average PPFD of 847 (± 473) $\mu\text{mol m}^{-2}\text{s}^{-1}$, temperature of 27.4 (± 1.9) $^{\circ}\text{C}$, relative humidity of 59 (± 6)% and CO₂ concentration of 278 (± 6) ppm.

³ On 8-16-1991, gas exchange measurements were made at an average PPFD of 732 (± 61) $\mu\text{mol m}^{-2}\text{s}^{-1}$, temperature of 17.9 (± 0.3) $^{\circ}\text{C}$, relative humidity of 63 (± 4)% and CO₂ concentration of 285 (± 15) ppm.

⁴ No significant differences between water stress treatment for any variable were observed at the $p \leq 0.05$ level.

Table 7.4. Height growth from 1990 to 1991 and dry weights of red spruce seedlings grown on Whitetop Mountain and subjected to control or water stress treatment.

Water stress treatment	Height (mm)	(g)				Total
		Root	Stem	Leaf		
control	47 ¹	2.6	3.3	4.3	10.2	
stress	42	3.1	5.0	5.8	14.0	

¹ No significant differences between water stress treatment were observed at the $p \leq 0.05$ level.

growing season. Reductions in leaf osmotic potential during drought have been reported for coniferous species such as Pinus taeda, Juniperus virginiana and Picea mariana (Bahari et al. 1985, Seiler and Johnson 1985, Zwiazek and Blake 1989). But, osmotic potentials were, in general, too high in comparison to plant water potentials. Possibly, water condensed within the leaf psychrometer chambers upon freezing and raised osmotic readings. We have observed this phenomenon with other species using the same psychrometers.

After rewatering of the stressed treatment in 1990, leaf conductance was still lower than the control treatment suggesting a possible long-term reduction in stomatal conductance. However, measurements made during the 1991 season showed no permanent influence of water stress on leaf conductance. No interactive influence of water stress and varying CO₂ measurement concentration on gas exchange was observed in 1991. For example, Seiler and Cazell (1990) found that net photosynthesis of water-stressed red spruce was 15% of well-watered seedlings when measured at ambient CO₂ and 73% of well-watered seedlings when measured at 838 ppm CO₂. Interactions may have occurred had the water stress been greater.

In conclusion, the lack of water stress on the treatment plots was possibly due to a combination of several factors. Rainfall and cloud movement through the site was

high enough both seasons to completely saturate the soil around and sometimes within the chambers. Water often times condensed on the inside of the chambers and dripped onto the soil. Finally, roots of some of the seedlings were long enough to reach toward the edge or out of the chambers. Future studies should consider transplanting the seedlings and the original surrounding soil into large pots and placing them back into the ground to limit root extension and subsurface soil water flow.

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SUMMARY AND CONCLUSIONS

The overall objective of this research was to determine how red spruce seedling growth and physiology would respond to potential changes in the environment. The growth responses of red spruce seedlings grown from seed for 12 months in ambient (374 ppm) or elevated (713 ppm) CO₂ in combination with a high or low soil fertility treatment and well-watered or water-stressed treatment were examined to determine the potential influence of an increase in global CO₂ concentration on growth. No interactions between CO₂, soil fertility and water stress treatment on diameter, height and seedling dry weight accumulation were observed at any time. Red spruce seedlings grown in elevated CO₂ had greater stem diameter, height, specific leaf weight, leaf weight, root weight, stem weight, and total weight than seedlings grown in ambient CO₂ at 12 months of age. Fixed growth potential was also examined in red spruce grown in elevated CO₂. Total shoot growth and total terminal dry weight were greater in seedlings grown and allowed to set bud in elevated CO₂ at 5 months of age. Total leaf weight, total leaf area and stem height originating from the terminal bud were greater in elevated CO₂-grown seedlings than in ambient CO₂-grown seedlings in seedlings subjected to water stress. A subset of seedlings was transplanted

from 175-cm³ pots into 646-cm³ pots at 7 months of age to eliminate possible pot-binding interactions. Seedlings transplanted into the larger pots at 7 months showed greater growth at 12 months than seedlings grown in the smaller pots, but no interaction between CO₂ treatment and pot size treatment was observed. Allometric analysis revealed that growth in elevated CO₂ for 1 year did not change organ dry weight allocation patterns.

Gas exchange characteristics of red spruce grown in the various CO₂, soil fertility and water stress treatments were also examined periodically over 12 months of fumigation. Gas exchange responses of elevated CO₂-grown seedlings grown in low soil fertility with water stress were similar to responses of elevated CO₂-grown seedlings grown under high soil fertility and well-watered conditions. No change in photosynthetic rate was observed during the first 5 months. Growth in elevated CO₂ had no influence on photosynthesis measured at either an ambient CO₂ concentration (approximately 363 ppm) and an elevated CO₂ concentration (approximately 730 ppm). At 9 months of age, elevated CO₂-grown seedlings had lower photosynthetic rates than ambient CO₂-grown seedlings when measured at an ambient CO₂ concentration. At 12 months, seedlings grown in both pot sizes in elevated CO₂ had lower rates of photosynthesis than ambient CO₂-grown seedlings when measured at both an ambient

and elevated CO₂ level. Both undeveloped and fully expanded foliage of elevated CO₂-grown seedlings had lower rates of photosynthesis when compared to ambient CO₂-grown seedlings. Transplanting seedlings to a larger rooting volume increased seedling photosynthetic rate but had no influence on seedling photosynthetic response to growth in elevated CO₂. No influence of growth in an elevated CO₂ atmosphere on dark respiration was observed at 12 months. Elevated CO₂-grown seedlings subjected to water stress at 12 months physiologically responded to decreasing plant water potential as did ambient CO₂-grown seedlings. While growth in elevated CO₂ had no effect on physiological responses to water stress, drought-conditioning maintained leaf conductance and photosynthesis to lower water potentials.

The majority of elevated CO₂ studies have examined the influence of enhanced atmospheric CO₂ on tree growth and physiology on a short-term (days and months) basis. This year long study of red spruce response to elevated CO₂ was one of the few studies that examined a variety of physiological and growth responses on a relatively long-term basis. Red spruce seedlings grew bigger faster in elevated CO₂ most likely through an increase in the substrate CO₂ for photosynthesis, but no long-term influence of elevated CO₂ on the mean relative growth rate and organ allocation was observed. Long-term, elevated CO₂ growth enhancement has

been documented in a few other tree species such as Pinus ponderosa, Pinus radiata and Citrus aurantium, (Tinus 1972, Surano et al. 1986, Conroy et al. 1990a, Idso et al. 1991). Interesting, red spruce growth was reduced by limited nutrient and water availability but growth responses to elevated CO₂ were not influenced by resource availability. Researchers have proposed that the ability of plants to benefit from an elevated CO₂ concentration will depend on the availability of other resources. This dependence is clearly species specific. Red spruce was able to utilize the increase in atmospheric CO₂ possibly by increasing internal nutrient use efficiencies and maintaining the photosynthetic capacity of the mesophyll during water stress. Red spruce seedlings showed a remarkable tolerance of water stress in this study and in others (Seiler and Cazell 1990).

Reduced photosynthetic rate in elevated CO₂-grown red spruce seedlings when compared to ambient CO₂-grown seedlings has been documented for other tree species. Because actively-growing seedlings transplanted into a large rooting volume also expressed this photosynthetic adjustment, an increase in plant source/sink ratio does not appear to be the cause of photosynthetic change. The most feasible explanations for photosynthetic change in red spruce include long-term accumulation of starch in leaf

chloroplasts, feedback inhibition of photosynthesis or a reduction in Calvin cycle enzymes (Madsen 1975, Bowes 1991, Stitt 1991).

This research suggests red spruce seedlings growing in their natural environment will initially benefit when atmospheric CO₂ concentrations rise even when other resources are limited. The observation of photosynthetic acclimation to temperature suggests that if greater global temperatures accompany increases in atmospheric CO₂, red spruce photosynthesis will not be detrimentally affected. In this study, the gain in dry weight acquired through an increase in growth efficiency (RGR) from 3 to 5 months was maintained up to 12 months of age. Whether these initial gains in seedling growth can be compounded until tree maturity is not known. A larger respiring mass may be more costly in carbohydrates and inefficient in terms of tree carbon balance if predicted increases in global temperature take place.

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