Response of Vegetation to Carbon Dioxide

Effects of Long-Term Elevated Atmospheric CO₂ Concentrations on *Pinus ponderosa*

Edited by:
K.A. Surano
J.R. Kercher

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**Effects of Long-Term Elevated
Atmospheric CO₂ Concentrations
on *Pinus ponderosa***

Series: RESPONSE OF VEGETATION TO CARBON DIOXIDE

Report of Research: **EFFECTS OF LONG-TERM ELEVATED
ATMOSPHERIC CO₂ CONCENTRATIONS ON
*PINUS PONDEROSA***

Edited by:
K.A. Surano¹ and J.R. Kercher²

Contributions by:

J.L.J. Houpis²

P.F. Daley¹

P.D. Anderson³

J.S. Fried³

J.H. Shinn²

J.A. Helms³

P.J. Tate¹

J.R. Kercher²

R.J. Palassou²

M.P. Costella¹

S. Cowles²

¹Environmental Protection Department, L-621

²Health and Ecological Assessment Division, L-524

Lawrence Livermore National Laboratory

P.O. Box 808

Livermore, CA 94550

³The Department of Forestry and Resource Management

University of California, Berkeley

Berkeley, CA 94720

Program of:

The U.S. Department of Energy

Environmental Sciences Division

Office of Health and Environmental Research

Office of Energy Research

Washington, DC 20545

EXECUTIVE SUMMARY

Global atmospheric carbon dioxide (CO₂) concentrations have increased significantly during this century, due mostly to fossil fuel emissions. Most scenarios predict CO₂ concentrations will continue to increase at a rate of 1-1.5 μLL^{-1} per year until they double sometime during the next century. Nearly all species of plants are expected to be affected by increased CO₂ levels.

Forests occupy 1/3 of global land area and carry on 2/3 of global photosynthesis, so effects on them may have global impacts. Primary production in forests could potentially increase under elevated CO₂, resulting in increased standing biomass and therefore increased sequestering of carbon. However, forests have long generation times, and will be unable to adapt to increasing CO₂ concentrations as quickly as annual species; trees currently beginning growth will reach their periods of greatest growth under much higher CO₂ concentrations.

Despite the importance of forests, very little information is available on their responses to long-term elevated CO₂ exposures. Therefore, this pilot study, designed to test the usefulness of open-top chambers for long-term elevated CO₂ exposures on sapling-sized trees and to obtain preliminary data on the responses of a woody perennial species, was conducted.

Ponderosa pine (*Pinus ponderosa* Laws.) saplings and seedling populations from both the Sierra Nevada and Rocky Mountains were exposed to continuously enriched CO₂ atmospheres in an outdoor open-top chamber facility for a two-and-one-half-year period. Carbon dioxide was added to ambient concentrations; the following treatments were used: ambient +0 μLL^{-1} , ambient +75 μLL^{-1} , ambient +150 μLL^{-1} , and ambient +300 μLL^{-1} .

Open-top chambers proved to be a useful approach for studies of this type. The ruggedness and ease of installation permitted these chambers to be used with relatively large plants in the field. However, the physical environment of the enclosed trees was modified by the chambers. Air temperatures and relative humidity were increased, and light levels were decreased by the chambers. The CO₂ responses of the trees also affected the physical environment. Trees subjected to the higher levels of CO₂ transpired less, resulting in slightly higher air temperatures and lower relative humidity than those found in the lower CO₂ treatment chambers. Foliar temperatures were also elevated in the chambers, partly due to elevated air temperatures.

However, increased foliar temperatures were also due to CO₂-induced reduction of transpirational cooling.

Seedling growth characteristics (main stem diameter, height, volume) increased with increasing CO₂ concentration, though the two populations exhibited different patterns of response. After two years of treatment, the seedlings of the Rocky Mountain variety showed no significant difference in total stem height or volume, but the basal diameters of those grown at +300 μLL^{-1} CO₂ were significantly greater than those grown at +0 μLL^{-1} and +75 μLL^{-1} . The response of the seedlings of the Sierran variety in these parameters was quite different, with those at +150 μLL^{-1} and +300 μLL^{-1} significantly greater in height than those at +75 μLL^{-1} , and those at +150 μLL^{-1} and +300 μLL^{-1} significantly greater than those at +0 μLL^{-1} and +75 μLL^{-1} in basal diameter and stem volume. However, using a combined analysis based on percent change in height, diameter, or volume, seedlings at +150 μLL^{-1} responded to a significantly greater degree than all other levels. Thus, the beneficial effects of elevated carbon dioxide increase up to +150 μLL^{-1} and begin to decrease between +150 μLL^{-1} and +300 μLL^{-1} .

Saplings under the highest CO₂ levels showed signs of stress, which included accelerated needle abscission, chlorosis, and apparent alteration of tolerance to heat, by the beginning of the last growth season. As a result, sapling above-ground biomass production was reduced by elevated CO₂ levels. The stress response is partly attributable to elevated foliar temperatures resulting from CO₂-induced stomatal closure, which in turn lowered transpirational cooling of needles.

Most plants respond to elevated CO₂ concentrations by increasing production and lowering stomatal conductance. These responses result in increased water-use efficiency (WUE). However, reduced consumptive water use was not demonstrated for the saplings in this study, even though stomatal conductance was decreased. Seedling water use declined with increasing CO₂, but WUE increases were still negligible. Whether the absence of observed WUE increases was due to a lack of a CO₂ response, increased leaf area, increased stomatal density or inadequacies in measurement technique is unknown.

Foliar analyses were conducted to determine the nutrient status of seedlings in the study. Comparisons of our values with those in the literature indicated that the foliar concentrations of nitrogen and sulfur tended to be

greater than normal while the foliar concentrations of potassium were lower than normal. When expressed on both dry weight and surface area bases, the foliar concentration of calcium increased with increasing CO₂ level. Foliar potassium concentration, when expressed on a dry weight basis, decreased with increasing CO₂ level. The effect of foliage-age on nutrient concentration was significant for all macronutrients plus copper and iron. Mobile elements had greater concentrations in current-year foliage while immobile elements had greater concentrations in one-year-old foliage. Foliage-age x CO₂ level interaction effects were significant for phosphorus only. The N/P, Ca/Mg, Ca/K and Ca+Mg/K ratios were greater at high CO₂ levels. The N/P and Mn/Fe ratios of current-year foliage were greater than those in one-year-old foliage. The Ca/Mg, Ca/K, and Ca+Mg/K ratios were greater in one-year-old foliage than in current-year foliage. There was a significant interaction among foliage-age classes and CO₂ level effects for the N/P ratio. The results suggest that exposure to elevated atmospheric CO₂ can result in an alteration of the foliar nutrient status of ponderosa pine seedlings. In general, the changes in nutrient status did not result in deficiencies. With the exception of K, all nutrient elements were present in sufficient concentrations. Current-year foliage from seedlings exposed to +150 μLL^{-1} and +300 μLL^{-1} CO₂ were deficient in K concentration.

At the end of the experiment, chlorophyll a, chlorophyll b, and carotenoid concentrations were measured in two age classes of needles of each variety. In both varieties, exposure to CO₂ concentrations greater than ambient +75 μLL^{-1} resulted in lower chlorophyll a, chlorophyll b, and carotenoid concentrations. Also, after two years of exposure to elevated CO₂, the two varieties and age classes of needles showed different pigmentation content. The pigments in needles of the Sierran variety exposed to elevated CO₂ were lower than those in the Rocky Mountain variety. Also, at elevated CO₂ levels, pigments in the older age class of needles were lower than were those in current needle tissue.

Responses of ponderosa pine, and possibly other woody perennial species, to elevated levels of atmospheric CO₂ may be more complex than those previously reported for annual species, affecting physiology, growth, morphology, water use, nutrient status, and pigmentation in a variety of ways. The magnitude of responses, whether growth is stimulated or retarded, and whether plant physiological rates are increased or decreased are dependent on several factors, including the atmospheric CO₂ concentration, duration of exposure, plant age, genotype, and other conditions of growth. However, the

biomass, physiological, growth, chlorophyll and nutrient data from this pilot experiment suggest that this species, and perhaps other perennial species, may respond positively to elevated CO₂ concentrations up to 150 µLL⁻¹ above ambient levels, but may respond negatively to concentrations approaching double that of present ambient levels. Future field studies are therefore necessary.

Although all species of plants will be affected by increases in atmospheric CO₂, differential species responses are likely. Effects on competitive interactions among coexisting species may cause changes in plant community structure and ecosystem dynamics.

Very little information is available regarding the responses of plant communities and ecosystems to elevated CO₂. Field studies are cost and complexity prohibitive. Simulation modelling may give insight into critical data needs so future research can be directed. Existing models may provide a starting point for simulation research. While accurate predictions using these models are impossible because of the assumptions that must be made in the simulations, the modelling exercises may serve useful purposes.

An existing western coniferous forest simulation model, SILVA was adapted to run under elevated atmospheric CO₂ scenarios using differential species responses. A sensitivity analysis performed on the CO₂ response variable indicated that SILVA was moderately sensitive to CO₂. Two types of scenarios were run, one with CO₂ remaining constant, and the other with CO₂ increasing at 1 µLL⁻¹ per year. Both positive and negative growth responses were incorporated into the scenarios.

Elevated CO₂ concentrations, depending on the scenario, had various impacts on the composition and structure of the forest. Total production either increased, decreased, or remained constant. Differential species responses were demonstrated, causing changes in dominance and community structure. One species exhibited increased production even when negatively impacted at CO₂ concentrations above 500 µLL⁻¹. Another species remained a minor component of the community, but played a significant role in the relative success of another species.

Simulation may be the only method to predict the responses of long-lived species to long-term elevated CO₂ concentrations. However, more experimental and modelling research is required before accurate predictions can be made.

INVESTIGATORS

Areas of Responsibility and Scientists Primarily Responsible

<u>Area</u>	<u>Personnel</u>	
Coordination and Management	K.A. Surano J.A. Helms	J.H. Shinn P.F. Daley
Chambers, CO ₂ Exposures and Monitoring	K.A. Surano	
Meteorology	K.A. Surano	
Water Use	J.H. Shinn	J.S. Fried
Biomass	K.A. Surano P.D. Anderson M.A. Costella	R.J. Palassou J.L.J. Houpis
Morphology	J.L.J. Houpis	M.P. Costella
Net Photosynthesis	K.A. Surano	P.F. Daley
Foliage Temperature	P.F. Daley	K.A. Surano
Stomatal Conductance	K.A. Surano	J.L.J. Houpis
Leaf Water Potential	K.A. Surano	R.J. Palassou
Chlorophyll Fluorescence	P.F. Daley	
Ethylene Production	P.F. Daley	
Nutrient Analyses	P.D. Anderson	R.J. Palassou
Chlorophyll and Carotenoid Concentrations	J.L.J. Houpis	
Modelling	K.A. Surano J.R. Kercher	P.J. Tate J.H. Shinn

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INTRODUCTION

Global atmospheric carbon dioxide (CO₂) concentrations have increased significantly during this century, due mostly to fossil fuel emissions (Strain and Cure, 1985). Because fossil fuel use is projected to increase in the future, there is no longer a question of whether CO₂ concentrations will increase, but rather a question of how rapidly and high they will rise (Hoffman, 1984). Most scenarios predict CO₂ concentrations will continue to increase at a rate of 1-1.5 μLL^{-1} per year until they double sometime during the next century (Strain and Cure, 1985).

All species of plants will be affected by elevated CO₂ concentrations (see reviews by Lemon, 1983; Strain and Cure, 1985). A stated goal of CO₂ research is the quantitative estimation of future responses for both crops and ecosystems (Dahlman, 1985). However, nearly all previous CO₂ studies to date have involved relatively short-term exposures of crop species grown in typical monocultural conditions (Strain and Cure, 1985). While much knowledge has been gained about crop responses, results from crop studies are of little use for prediction of native species and ecosystem responses to long-term increasing CO₂ concentrations (Strain and Cure, 1985; Dahlman, 1985). The dearth of information regarding the responses of plant communities, and of perennial plants in particular, to elevated CO₂ has been mentioned often (Strain and Cure, 1985; Strain and Bazzaz, 1983; Dahlman, 1985; Baker and Enoch, 1983; Lemon 1983). Forests may be a very important community type with respect to the CO₂ question, as they have great economic, ecological, recreational, and aesthetic value. Primary production in forests could potentially increase under elevated CO₂, resulting in increased standing biomass and therefore increased sequestering of carbon. However, forests have long generation times, and will be unable to adapt to increasing CO₂ concentrations as quickly as annual species (Kimball, 1985); trees currently beginning growth will reach their periods of greatest growth under much higher CO₂ concentrations. Instead, the response of long-lived tree species may be determined by their ability to acclimate to elevated CO₂ conditions (Kimball, 1985). However, despite their importance, very little previous work has been conducted regarding CO₂ effects on forest species.

This report details the results from an experiment of the effects of long-term elevated atmospheric CO₂ concentrations on ponderosa pine (*Pinus*

ponderosa Laws.) saplings and seedlings. The research was conducted as part of the U.S. Department of Energy Carbon Dioxide Research Division effort .

The study began in 1983 as a pilot study designed to explore the feasibility of using open-top chambers for continuous multi-year exposures on sapling-sized trees and to examine possible CO₂ responses so that future fully-replicated and detailed research could be adequately designed. However, following the first year of exposure, preliminary results from the study indicated that measurements of CO₂ responses should be intensified.

The original objectives of the pilot study were realized. Open-top chambers proved suitable for use in multiyear exposures of mature trees. With respect to the preliminary examination of CO₂ responses, many interesting observations were made. However, the results from this study should be interpreted with care because of the inadequate statistical design at the outset of experiment. Nevertheless, the nature of the preliminary results suggests that future long-term field CO₂ exposures on perennial species may be critical to our understanding and preparation for future environments.

Other research reported here attempted to adapt an existing western coniferous forest growth and succession model for use in elevated CO₂ scenarios using differential species responses, and assessed the usefulness of the model in that regard. It is stressed that the results from these primitive simulations are not predictive, and should be used with caution. However, the modelling exercises determined the model may be useful for future projections if it can be properly parameterized, once appropriate data become available .

This report is organized into 6 chapters. The chapters are independent papers regarding the various topics explored in the research, and therefore often contain repeated information, particularly with respect to materials and methods. The papers were written by various authors for submission to appropriate scientific journals. Therefore, literary style and paper formats vary. The pages of this volume are numbered consecutively, but the figures and tables are numbered with a prefix, identifying the paper to which they belong.

References

Baker DN , Enoch HZ (1983) Plant growth and development. In: Lemon, ER (ed) CO₂ and plants. Westview Press, Boulder, CO

Dahlman RC (1985a) Preface. In: Strain BR, Cure JD (eds) Direct effects of increasing carbon dioxide on vegetation. DOE/ER-0238, U.S. Department of Energy, Office of Energy Research, Carbon Dioxide Research Division, Washington, DC

Hoffman JS (1984) Carbon dioxide and future forests. J Forestry 82:164-167

Kimball BA (1985) Adaptation of vegetation and management practices to a higher carbon dioxide world. In: Strain BR, Cure JD (eds) Direct effects of increasing carbon dioxide on vegetation. DOE/ER-0238, U.S. Department of Energy, Office of Energy Research, Carbon Dioxide Research Division, Washington, DC

Lemon ER (ed) (1983) CO₂ and plants. Westview Press, Boulder, CO

Strain BR, Bazzaz FA (1983) Terrestrial plant communities. In: Lemon ER (ed) CO₂ and plants. Westview Press, Boulder, CO

Strain BR, Cure JD (eds) (1985) Direct effects of increasing carbon dioxide on vegetation. DOE/ER-0238, U.S. Department of Energy, Office of Energy Research, Carbon Dioxide Research Division, Washington, DC

**OPEN-TOP CHAMBER ENVIRONMENTS
AND FOLIAGE TEMPERATURES OF
PONDEROSA PINE SAPLINGS AND SEEDLINGS
EXPOSED TO LONG-TERM ELEVATED CO₂**

K.A. Surano¹, J.L.J. Houpis², and P.F. Daley¹

¹Environmental Protection Department

²Health and Ecological Assessment Division

Lawrence Livermore National Laboratory

P.O. Box 808, L-524

Livermore, CA 94550

USA

**LAWRENCE LIVERMORE NATIONAL LABORATORY
University of California • Livermore, CA • 94550 • USA**

Introduction

Most all research conducted to date on the response of plant species to increasing atmospheric carbon dioxide (CO₂) concentrations has focused on annual crop species. While this research has yielded valuable results, little effort has been expended towards understanding the effects of CO₂ increases on perennial species. Especially noteworthy by their absence are significant studies on woody perennials, and of forest tree species in particular (Strain and Cure, 1985).

Data are needed on the response of forest tree species to elevated atmospheric CO₂ concentrations because forests have important economic, global carbon balance, ecologic, aesthetic and recreational values. However, forest tree species have large stature and long lifetimes, making them difficult to study, especially when questions are asked about long-term responses.

The few existing elevated-CO₂ studies on forest tree species have generally involved exposure of seedlings to short-term fumigations in greenhouses and controlled environment chambers (Drake et al., 1985). Because of the artificial and short-term nature of these studies, it is critical that future exposures be conducted in the field on mature tissue, over as long a term as possible, and on a year-round basis. However, such studies are both difficult and expensive. Before any intensive long-term field effort can be launched, pilot studies using a variety of refined field exposure methods should be performed (Drake et al., 1985; Olszyk et al., 1986).

Open-top field exposure chambers have been so widely in air pollution research (Heagle et al., 1973, 1979; Mandl et al., 1973; Olszyk et al., 1980; Rogers et al., 1983; Drake et al., 1985) that they have become standardized research tools (Houpis and Surano, 1987). However, results from open-top chambers have sometimes received criticism because the chambers can cause elevated air temperatures, lower light intensities, increased relative humidity and different air-flow patterns when compared to the ambient environment (Heagle et al., 1973; Olszyk, 1980, 1986). Whatever the differences, it has been widely accepted that the meteorological differences caused by open-top chambers do not result in large differences in plant growth and yield (Drake et al., 1985). Therefore, open-top chambers may be suitable candidates for long-term studies with forest species.

Unfortunately, foliar temperatures may also be increased in open-top chambers due to both increased air temperatures in the chambers and elevated

CO₂ levels. One of the effects of transpiration is to cool transpiring tissues through latent heat of vaporization. Because elevated CO₂ causes reduced transpiration, the possibility exists that elevated leaf temperatures may result. If foliar temperatures increase to a high enough degree, serious thermal stress may result (Surano et al., 1986). Conversely, enclosed plant tissue may have effects on the chamber environment. Water vapor released from plants of large surface area can help cool the surrounding air mass. If open-top chambers are to become a standardized tool for elevated CO₂ studies on forest tree species, the effects of both CO₂ and the chambers themselves on foliar temperatures is of vital concern.

This report summarizes chamber meteorological and foliage temperature data from a pilot study conducted on ponderosa pine (*Pinus ponderosa*) at Livermore, CA, from 1983-1985. The study was designed to help identify open-top chamber procedural problems specific to trees that may not have been encountered during previous crop studies. Data taken will hopefully direct future studies in terms of measurements taken and exposure protocols.

Materials and Methods

Open-Top Chambers and CO₂ Treatments

A complete description of the chambers is found in Surano et al. (1986). The chambers were essentially cylindrical aluminum frames, 3 m in diameter by 2.4 m high, covered on the sides with PVC film. Frustra were placed on the tops of the chambers to impede ambient air incursions and thus facilitate control of exposure levels (Davis and Rogers, 1980; Davis et al., 1983). Ambient air was drawn through particle filters by 560-W axial fans, injected with pure CO₂, and delivered to the chambers through plenums. Air speed of approximately 1.0 m s⁻¹ (four chamber-air changes min⁻¹, Heagle et al., 1973, 1979) were designed to keep chamber air temperatures and relative humidity close to ambient and to assure uniform distribution of CO₂ throughout the canopies (Surano et al., 1986).

Carbon dioxide exposures were continuous from May, 1983 until September 1985 on four 10-year old saplings, each centered in an open-top chamber and rooted in the ground. Thirty seedlings, planted in pots, of both Sierran and Coloradan ecotypes were also placed in each chamber. The seedlings were placed in the north shade of the sapling to minimize the thermal

stress often seen in potted plants. Saplings rooted in the soil nearby served as controls to quantify chamber effects.

The treatment levels of CO₂ were ambient +0 μLL^{-1} (no added CO₂), ambient +75 μLL^{-1} , ambient +150 μLL^{-1} and ambient +300 μLL^{-1} , or approximately 350, 425, 500 and 650 μLL^{-1} , and were monitored continuously. This method of fumigation took into account both diurnal and seasonal fluctuations in ambient atmospheric CO₂ concentrations and tracked them accordingly. All trees were kept well-watered throughout the experiment.

Meteorological Measurements

In most previous studies, meteorological conditions of a single chamber were taken as representative for comparison to the ambient environment. The assumption was that all chambers exhibited similar conditions. This experiment sought to survey all chambers to see if there was between-chamber variation in conditions and to see if any variation could be attributable CO₂ treatment levels. Two types of survey were conducted. Measurements were taken daily near solar noon for seasonal mean estimations of midday chamber conditions. Diurnal surveys, with measurements made in all chambers every two hours between 0800 and 1600 hours (PST), were conducted once per week.

Ambient and chamber air temperatures were measured with Fe-constantan thermocouples. Readings were taken in the shade at a height of 1 m at mid-chamber after the instrument had completely stabilized.

Measurements of chamber relative humidity were made with a Vaisala relative humidity sensors incorporated into a steady-state porometer (Model LI-1600; LI-COR Inc., Lincoln, NE). The relative humidity measurements were taken in the shade at a height of 1-m with the cuvette head of the porometer kept open.

For the purposes of this paper, the term "light" refers to photosynthetically active radiation (PAR; that portion of the visible spectrum which is important to photosynthesis (400-700 nm)). Ambient and individual chamber light levels were also measured daily at mid-day, with the steady-state porometer quantum sensor. Diurnal surveys were also conducted once per week. Maximum light levels were recorded from the continuously-exposed south-facing aspect of saplings, both directly and through each set of chamber panels. Shaded light readings were taken in the north shade of the chambered saplings, above the potted seedlings. A simple additional experiment was

conducted on the effects of chamber cleanliness on reductions of PAR. Diurnal measurements were taken in three chambers. One chamber had been thoroughly dusted and washed, and thus represented a best-case chamber for light transmission. Another chamber had been allowed to accumulate dust for 2 weeks, and the third chamber was allowed to accumulate dust for 4 weeks.

Leaf Temperatures

We measured foliage temperatures on all the trees in this study on a regular basis, with an infrared thermometer (model 210, Everest Interscience, Tustin, CA), using techniques described by Idso et al. (1984) and Jackson et al. (1981). Conifer tree and needle morphology presented problems for measurements. Individual needles were too small for individual temperature measurements using infrared thermometers. Whole canopy temperatures could have been obtained, but background reflectance may have caused erroneous readings. However, limited canopy temperature measurements, taken utilizing procedures to minimize background effects, provided integrated measurements of needles held at all angles, both in the shade and in the sun. In order to get data on the effects of CO₂ concentrations and the open-top chambers themselves on leaf temperatures detailed measurements on both small groups of needles and the total canopy were made. Standardized procedures designed to minimize the effects of background interference and small needle sizes were employed. Like the surveys for meteorological parameters, daily measurements of foliage temperatures were made at mid-day (1315 PDT), and diurnal surveys are made at least once per week. Measurements were made of sapling canopy temperatures by holding the infrared thermometer level at a distance of 0.5 m at three levels (apical, mid-canopy and lower canopy) from each of four compass directions. These temperatures were averaged to determine sapling canopy temperature.

Results

Results of seasonal mean midday meteorological conditions are presented in Table 1. Analyses of variance and multiple range tests indicated that there were significant effects of the chambers on all meteorological parameters.

Seasonal mean midday air temperature was lowest in the ambient environment, and increased nearly 5°C in one chamber. A gradual, but

Table 1. Seasonal mean midday meteorological conditions ($\pm$ SD), Livermore, CA, 31 May-5 September, 1985. Values followed by the same letter are not significantly different (Fischer's LSD test).

Chamber	Treatment	Air Temperature C ($p < 0.0001$)	Relative Humidity % ($p < 0.0170$)	PAR ($\mu\text{mol/m}^2/\text{s}$) ($p < .0001$)
-	ambient	$27.4 \pm 3.3a$	$30.6 \pm 9.5 a$	$2026 \pm 270a$
17	+0 $\mu\text{L/L}$	$29.5 \pm 3.7b$	$33.4 \pm 9.3a$	$1696 \pm 321b$
18	+75 $\mu\text{L/L}$	$30.9 \pm 3.8bc$	$29.0 \pm 8.6b$	$1671 \pm 282b$
20	+150 $\mu\text{L/L}$	$32.1 \pm 4.1cd$	$27.7 \pm 7.9b$	$1750 \pm 254b$
19	+300 $\mu\text{L/L}$	$32.8 \pm 4.2d$	$27.7 \pm 8.0b$	$1679 \pm 311b$

Figure 1. Diurnal open-top chamber air temperatures of various CO₂ treatments compared to ambient air temperatures. Points are means of weekly data taken May-September, 1985. Units for the +0, +75, +100, and +300 treatments are ppm.

significant trend of increasing air temperatures with increasing CO₂ concentration was also apparent (Table 1). This trend is also observable in the seasonal diurnal plot (Figure 1). Ambient air temperatures are much lower than chamber temperatures throughout the day. Diurnal air temperatures followed CO₂ concentrations, with the ambient +0 treatment closest to ambient conditions, and the higher CO₂ treatments being elevated.

Seasonal mid-day relative humidity was lowest at the two highest CO₂ levels and was significantly higher in the ambient environment and in the ambient+0 CO₂ treatment (Table 1). Diurnally (Figure 2), ambient and ambient+0 treatment relative humidity are highest and grouped away from other CO₂ treatment humidities throughout the day. Once again, reductions in relative humidity follow CO₂ treatments in an orderly progression. This is attributed to direct CO₂ effects of reduced stomatal conductance, which reduced transpiration.

Chamber panels significantly reduced chamber-interior PAR levels when compared to ambient values (Table 1, Figure 3). Although not statistically significant, there is an indication that the ambient +300 CO₂ treatment chamber

Figure 2: Diurnal open-top chamber relative humidity of various CO₂ treatments compared to ambient relative humidity. Points are means of weekly data taken May-September, 1985. Units for the +0, +75, +150, and +300 treatments are ppm.

Figure 3: Diurnal open-top chamber photosynthetically-active radiation (PAR) of various CO₂ treatments compared to ambient PAR. Points are means of weekly data taken May-September, 1985. Units for the +0, +75, +150, and +300 treatments are ppm.

had slightly higher light levels both seasonally and diurnally than the other chambers (Table 1, Figure 3). This may be attributed to the open architecture of the sapling in that chamber which responded to elevated CO₂ levels with much needle abscission (Surano et al., 1986). The slightly elevated light may be the result of more scattering in the chamber and less absorption of light by tissues.

The results of the chamber cleanliness experiment are shown in Table 2. The clean chamber panels allowed transmission of over 90% of PAR. The middle chamber, with 2 weeks of dust, allowed approximately 85% of PAR. But the dirty chamber, with 4 weeks of dust loading allowed just 70% of PAR to reach trees in that chamber.

Figure 4 indicates that problems may exist with fixed-in-place sensors. The chamber framework and overhead power lines interfere with light measurements because they shade the sensor for part of the day. It is obvious that the drops in light are due to some outside agency, and if appropriate corrections are made, data can be retrieved.

The results of the seasonal mean midday foliar temperature surveys are shown in Table 3. Chambers have a definite effect on foliar temperatures. Diurnal evidence of these effects is also presented in Figure 5. For all

Table 2. Diurnal reductions of ambient photosynthetically-active radiation (PAR) through open-top chamber panels. Readings were taken just after the chambers were dusted and washed (Clean), or after dust was allowed to accumulate on the panels for 2-4 weeks.

Time of Day	(PAR) ambient	(PAR) Clean		(PAR) 2-weeks dust		(PAR) 4-weeks dust	
	$\mu\text{mol}/\text{m}^2/\text{s}$	$\mu\text{mol}/\text{m}^2/\text{s}$	% reduction	$\mu\text{mol}/\text{m}^2/\text{s}$	% reduction	$\mu\text{mol}/\text{m}^2/\text{s}$	% reduction
900	1600	1540	4	1350	16	1130	29
1000	1810	1690	7	1560	14	1290	29
1100	1920	1740	9	1620	16	1360	29
1200	2000	1830	9	1700	15	1440	28
1300	1970	1800	9	1680	15	1400	29
1400	1870	1690	10	1550	17	1250	33
1500	1540	1410	8	1280	17	1070	31

Table 3. Seasonal mean midday foliar temperatures ($\pm$ SD), Livermore, CA, at various aspects, 31 May-5 September, 1985. Values followed by the same letter are not significantly different (Fischer's LSD test).

		South Foliage Temperature $^{\circ}\text{C}$ ($p < 0.0001$)	North Foliage Temperature $^{\circ}\text{C}$ ($p < 0.0001$)	East Foliage Temperature $^{\circ}\text{C}$ ($p < 0.0003$)	West Foliage Temperature $^{\circ}\text{C}$ ($p < 0.0001$)
Chamber	Treatment				
-	ambient	$30.1 \pm 4.9\text{a}$	$29.3 \pm 4.8\text{a}$	$29.8 \pm 4.9\text{a}$	$29.8 \pm 4.6\text{a}$
17	+0 $\mu\text{L}/\text{L}$	$33.6 \pm 4.7\text{b}$	$32.7 \pm 4.6\text{b}$	$33.1 \pm 4.7\text{b}$	$33.3 \pm 4.5\text{b}$
18	+75 $\mu\text{L}/\text{L}$	$33.9 \pm 5.1\text{b}$	$33.6 \pm 4.9\text{b}$	$34.2 \pm 5.0\text{b}$	$33.1 \pm 4.8\text{b}$
20	+150 $\mu\text{L}/\text{L}$	$34.1 \pm 5.3\text{b}$	$32.2 \pm 5.0\text{b}$	$33.5 \pm 5.4\text{b}$	$33.4 \pm 5.3\text{b}$
19	+300 $\mu\text{L}/\text{L}$	$35.7 \pm 5.2\text{b}$	$34.2 \pm 4.8\text{b}$	$34.5 \pm 5.0\text{b}$	$35.6 \pm 5.6\text{c}$

Figure 4: Diurnal plot of open-top chamber vs. ambient photosynthetically-active radiation (PAR), 19 August 1985, using data from a fixed-in-place PAR sensor. Note drops in measured chamber levels due to chamber framework and overhead power line.

Figure 5: Diurnal temperatures of southerly-exposed ponderosa pine sapling foliage from various CO₂ treatments in open-top chambers compared to foliage temperatures of a non-chambered control sapling. Points are means of weekly data taken May-September, 1985. Units for the +0, +75, +150, and +300 treatments are ppm.

Figure 6: Diurnal temperatures of shaded ponderosa pine seedling foliage from various CO₂ treatments in open-top chambers compared to ambient air temperatures. Points are means of weekly data taken May-September, 1985. Units for the +0, +75, +150, and +300 treatments are ppm.

chambers, and independent of aspect, foliar temperatures were elevated in the chambers when compared to ambient foliar temperatures. Additionally, the western aspect of the ambient+300 treatment exhibited higher temperatures than all other treatments at that same aspect. In general, although not statistically significant, the higher CO₂ treatments had higher foliar temperatures than the lower treatments. Shaded seedlings (Figure 6) had diurnal foliar temperatures that did not deviate from air temperatures. Apparently, the lack of direct sun allowed the seedlings to maintain normal foliar temperatures.

Discussion

Open-top chambers have been criticized as a method for exposing plants in atmospheric studies because the chamber environment is not identical to that outside the chamber. While some investigators have reported that air temperatures inside the chambers do not differ appreciably from those outside (Kats et al., 1976; Heagle et al., 1979; Olszyk et al., 1980; Mandl et al., 1973) we found that conditions inside chambers are definitely different. Additionally, we found that there may be variations in environment from chamber to chamber. Although the observed difference may have been due to CO₂ effects on the plants, it may not be wise to assume all open-top chambers behave the same. We do not mean to suggest that open-top chambers are an invalid research tool. Quite the contrary, if investigators monitor all individual chamber environments, and use companion plants in the ambient environment to delimit chamber effects, meaningful results can be obtained with open-top chambers. Even if it does not track ambient conditions exactly, the environment within the chamber represents a valid set of conditions for the experiment that could be possible in the natural environment.

It should be stressed that this was pilot study, designed to help identify meaningful questions for future studies and to work out procedural details. Therefore, all results should be treated as preliminary. Future studies should be designed with adequate replication so that the direct effects of CO₂ on foliage temperature can be examined. Those studies should incorporate various amount of foliage within chambers, as well as chambers without any foliage.

Open-top chambers may be the most effective trade-off when compared with the cost, logistical problems, and uncertainty of other methods (Olszyk, 1986; Drake, 1985). Their ease of installation and use, as well as their ruggedness, permits them to be used with relatively large plants in the field for extended periods. Their use can yield valuable information about plant

responses, as long as investigators understand and deal with their limitations, under the most realistic simulations of future environments that are currently economically and technically feasible.

Summary

Research that has been conducted on the response of forest trees to elevated carbon dioxide (CO₂) concentrations has mostly involved exposure of seedlings to short-term exposures in greenhouses and growth chambers. A need exists for long-term field exposures of mature forest species to elevated CO₂. Open-top chamber experiments may provide the only feasible research method currently economically and technically feasible.

This study examined the environmental conditions and foliar temperatures of ponderosa pine saplings and seedling in a long-term field CO₂ exposure in open-top chambers. Midday measurements of air temperature, foliage temperatures, relative humidity and light were taken daily and diurnal profiles were conducted weekly. There were significant differences between the ambient environment and that inside the chambers. Air temperatures, foliar temperatures and relative humidity were elevated, and light was reduced, in the chambers, when compared to ambient conditions. The highest CO₂ concentrations caused increased air and foliar temperatures, but reduced relative humidity. These responses were attributed to direct CO₂ effects on transpiration.

If investigators monitor the environments of individual chambers and use non-chambered controls to delimit chamber effects, meaningful results can be obtained in studies of long-term elevated CO₂ on woody perennial species using open-top chambers.

References

- Davis, J.M., A.J. Riordan, and R.E. Lawson, Jr. 1983. A wind tunnel study of the flow field within and around open-top chambers used for air pollution studies. *Boundary-Layer Meteorol.* 25: 193-214.
- Davis, J.M., and H.H. Rogers. 1980. Wind tunnel testing of open top field chambers for plant effects assessment. *J. Air Poll. Cont. Assoc.* 30: 905-907.
- Drake, B.G., H.H. Rogers, and L.H. Allen. 1985. Methods of exposing plants to elevated carbon dioxide. In: Strain, B.R. and J.D. Cure, Eds. 1985. *Direct effects of increasing carbon dioxide on vegetation.* DOE/ER-0238, U.S. Department of Energy, Carbon Dioxide Research Division, Washington, DC

- Heagle, A.S., D.E. Body,, and W.W. Heck. 1973. An open-top field chamber to assess the impact of air pollution on plants. *J. Environ. Qual.* 2:365-368.
- Heagle, A.S., R.B. Philbeck, H.H. Rogers, and M.B. Letchworth. 1979. Dispensing and monitoring ozone in open-top field chambers for plant effects studies. *Phytopathology* 69:15-20.
- Houpis, J.L.J. and K.A. Surano. 1987. Standardization of branch exposure chambers: Basic performance specifications, test procedures, and field implementation. pp. 75-86. *In* Response of Trees to Air Pollution, The Role of Branch Studies, Workshop Proceedings (Western Conifers Research Cooperative/National Forest Response Program.)
- Idso, S.B., B.A. Kimball, and K.L. Clawson. 1984. Quantifying effects of atmospheric CO₂ enrichment on stomatal conductance and evapotranspiration of water hyacinth via infrared thermometry. *Agric. Forest Meteorol.* 33:15-22.
- Jackson, R.D., S.B. Idso, R.J. Reginato, and P.J. Pinter, Jr. 1981. Canopy temperature as a crop water stress indicator. *Water Res. Res.* 17:1133-1138.
- Kats, G., C.R. Thompson, and W.C. Koby. 1976. Improved ventilation of open-top chambers. *J. Air Poll. Control Assoc.* 26:1089-1090.
- Mandl, R.H., L.H. Weinstein, D.C. McCume, and M. Kevemeg. 1973. A cylindrical, open-top chamber for the exposure of plants to air pollutants in the field. *J. Environ. Qual.* 2:371-376.
- Olszyk, D.M., G. Kats, P.J. Dawson, A. Bytnerowicz, J. Wolf, and C.R. Thompson. 1986. Characteristics of air exclusion systems vs. chambers for field air pollution studies. *J. Environ. Qual.* 15:326-334.
- Olszyk, D.M., T.W. Tibbitts, and W.M. Hertsberg. 1980. Environment in open-top field chambers utilized for air pollution studies. *J. Environ. Qual.* 9:610-615.
- Rogers, H.H., W.W. Heck, and A.S. Heagle. 1983. A field technique for the study of plant responses to elevated CO₂ concentration. *JAPCA.* 33:42-44.
- Strain, B.R. and J.D. Cure, Eds. 1985. Direct effects of increasing carbon dioxide on vegetation. DOE/ER-0238, U.S. Department of Energy, Office of Energy Research, Carbon Dioxide Research Division, Washington, DC
- Surano, K.A., P.F. Daley, J.L.J. Houpis, J.H. Shinn, J.A. Helms, R.J. Palassou, and M.P. Costella. 1986. Growth and physiological responses of *Pinus ponderosa* Dougl. ex P. Laws to long-term elevated CO₂ concentrations. *Tree Phys.* 2: 243-259.

**GROWTH AND PHYSIOLOGICAL RESPONSES OF
PINUS PONDEROSA TO LONG-TERM
ELEVATED CO₂ CONCENTRATIONS**

K. A. Surano¹, P.F. Daley¹, J.L.J. Houpis², J.H. Shinn²,
J.A. Helms³, R.J. Palassou², and M.P. Costella¹

¹Environmental Protection Department

²Health and Ecological Assessment Division

Lawrence Livermore National Laboratory

P.O. Box 808, L-524

Livermore, CA

³Department of Forestry

University of California

Berkeley, California 94720

**LAWRENCE LIVERMORE NATIONAL LABORATORY
University of California • Livermore, CA • 94550 • USA**

Introduction

The increase in global atmospheric carbon dioxide (CO₂) concentrations from a pre-industrial level of approximately 290 μLL^{-1} to present levels of 340 to 350 μLL^{-1} is a well-established phenomenon (Pearman, 1980; Clark et al., 1982; Keeling, 1983; Dahlman et al., 1985). Projections of future CO₂ levels include accelerated increases with a doubling of present concentrations sometime during the next century (Bacastow and Keeling, 1973; Clark et al., 1982). While there is limited evidence that past increases have directly affected plants (LaMarche et al., 1984), it is generally accepted that future increases will affect nearly all species of terrestrial plants (Dahlman et al., 1985). However, responses will probably be species specific with respect to both CO₂ concentration and duration of exposure (Strain, 1978; Lemon, 1983; Strain and Bazzaz, 1983; Tolley and Strain, 1985). Because plants form the support system for the rest of the biosphere and are very important in the global carbon system, their responses to increased CO₂ concentrations should be quantified.

Several reviews broadly summarize previous experiments conducted to determine individual species' responses to elevated atmospheric CO₂ levels (Wittwer, 1979; Kramer, 1981; Kimball, 1983; Lemon, 1983). In general, past studies have focused on annual species and have demonstrated that elevated CO₂ levels can increase photosynthetic rates, production and yield while reducing water-use.

While research with annual species has yielded valuable results, relatively few studies have addressed the effects of elevated CO₂ concentrations on perennial species. Moreover, there is a paucity of long-term studies dealing with woody plants; few have concerned forest trees. It is vital that the responses of forest-tree species to elevated CO₂ be studied adequately because of the ecological, economic, recreational and aesthetic importance of these species. In addition, because forests occupy approximately one-third of global land area and carry on approximately two-thirds of global terrestrial photosynthesis (Kramer, 1981), effects on them may have local, regional and/or global consequences.

Forest species have large stature and long lifetimes, making them difficult to study and model. But attempts to understand their responses to elevated CO₂ must be made directly without assuming they will respond as have annual species. Forest trees differ from annual species with respect to some aspects of morphology, phenology, physiology and longevity, and frequently are managed

quite differently, so their responses to elevated CO₂ may be completely unlike those observed for annuals. In addition, they usually occur in complex plant communities composed of mixtures of annuals and other perennials, all with potentially differential responses to CO₂.

Previous studies regarding forest tree species' responses to elevated CO₂ concentrations have used leafy shoots enclosed in cuvettes for short periods (Wright, 1974; Bryan and Wright, 1976; Hurt and Wright, 1976; Green and Wright, 1977), greatly elevated concentrations between 1000 and 3600 μLL^{-1} (Hardh, 1966; Molnar and Cumming, 1968; Funsch et al., 1970; Siren and Alden, 1972; Tinus, 1972), controlled environment exposures of potted seedlings (Tolley and Strain, 1984, 1985; Higginbotham et al., 1985; Sionit et al., 1985), or potted seedlings in open-top chambers (Rogers et al., 1980, 1983a, 1983c). Under no known circumstances prior to this study have elevated CO₂ exposures on forest tree species been performed in situ on entire plants or for longer than a single growing season.

Results obtained from these studies have generally indicated that forest tree species respond to elevated CO₂ levels in many of the same ways as have been reported for annual species. Increases in height, stem basal diameter, leaf area, photosynthetic rates and biomass production as well as reduced stomatal conductance and transpiration have been reported. However, any deleterious effects of elevated CO₂ may be more likely to occur after long-term exposure of perennial species. In addition, forest tree species may become acclimated to elevated CO₂ concentrations after initial changes in various physiological functions (Kramer, 1981), so it may be difficult to predict their long-term performance by examination of short-term responses.

Reported here are results from a CO₂-exposure study conducted with ponderosa pine (*Pinus ponderosa* Laws.), a species of widespread distribution and great economic value and ecological importance in the Western U.S. By conducting this study in situ on a single representative tree species, systematic problems specific to trees, not encountered using techniques developed for previous field-crop studies, were identified. Results can direct future studies in terms of formation of hypotheses, fumigation concentrations selected, measurements taken, interpretation of data and modeling.

The experiment was designed to (1) test the usefulness of open-top chambers in long-term studies with large-stature vegetation, (2) give a first-order estimate of the effects of long-term elevated atmospheric CO₂

concentrations on ponderosa pine primary production in situ, (3) identify physiological processes apparently sensitive to elevated CO₂, and (4) aid the design and development of future large-scale studies. The study involved continuous exposures of seedling populations of both Sierra Nevada and Rocky Mountain origin as well as established saplings. The use of different geographic populations and ages allowed the exploration of the possibilities that CO₂ responses may differ due to plant age or that intraspecific variation in responses may exist.

Materials and Methods

Treatments. Elevated CO₂ exposures were begun in July 1983 and terminated in October 1985. Exposures were conducted year-round, 24 hours per day, since initiation on four 7-year-old saplings, each centered in an open-top chamber (described below) and rooted in the ground. The soil was a Rincon silty clay loam (a fine, montmorillinitic, thermic Mollic Haploxeralfs). The saplings were chosen from an established plantation of San Bernardino Mountain lineage. Selection was based on initial morphological similarity, close proximity to each other to minimize any soil difference effects, and uniformity of environmental conditions. Twenty seedlings from each population, planted in 20-cm pots, were placed in the north shade of each of the saplings to minimize thermal stress often seen in potted plants and to emulate understory conditions. The Sierra seedlings were 1-year old and the Rocky Mountain seedlings were 2-years old when the exposures began. Nearby saplings, unenclosed by chambers, served as controls. Use of data from the control trees in comparison with that from trees in the "ambient" chamber (no added CO₂) allowed quantification of chamber effects.

Exposures were conducted in four standard open-top fumigation chambers as described by Heagle et al. (1973), Rogers et al. (1983a, 1983b) and Dahlman et al. (1985). The chambers were cylindrical aluminum frames, 3 m in diameter by 2.4 m in height, covered on the sides by clear polyvinyl chloride (PVC) film. Cone-top baffles (frustra), also covered with PVC film, were placed on the tops of the chambers to facilitate control of CO₂ levels, after the studies of Davis and Rogers (1980), and Davis et al. (1983). The bottom half of the chamber covers consisted of a double wall with the inner surface perforated by 2.5-cm holes through which chamber ventilating air and CO₂ were introduced. Ambient air was drawn through particle filters by 560-W axial

fans and delivered into the perforated portions of the inner-chamber wall through plenums. Pure CO₂ from a 12.7-Mg liquid CO₂ receiver was vaporized and delivered to the fan housings through a custom-made high-volume dispensing manifold consisting of heated regulators, flowmeters and Teflon[®] tubing. Mixing took place in the fan housings, plenums and perforated chamber covers prior to injection. Control of CO₂ levels was performed manually by adjustment of each chamber's flowmeter. Air speeds of approximately 1.0 m s⁻¹ (four chamber-air changes min⁻¹, Heagle et al. 1973, 1979) were designed to hold chamber-air temperatures close to ambient and to assure well mixed CO₂ concentrations throughout the canopies.

The levels of CO₂ were ambient, ambient + 75 µLL⁻¹, ambient + 150 µLL⁻¹, and ambient + 300 µLL⁻¹, or approximately 350, 425, 500, and 650 µLL⁻¹ CO₂. This method of fumigation took into account both diurnal and seasonal fluctuations of ambient atmospheric CO₂ concentrations and tracked them accordingly.

Samples of ambient and chamber atmospheres were drawn continuously at 3 L min⁻¹ through Teflon[®] tubing capped with 0.5 µm Teflon[®] particle filters (Nuclepore, Pleasanton, CA) located 1 m above the ground in the sapling canopy and adjacent to the main stem. Sampling intakes were placed inside inverted Erlenmeyer flasks to buffer short-term CO₂ concentration fluctuations. Chamber CO₂ concentrations were sampled for a two-min period every 20 min. Mean concentrations were computed for each sampling period. A multiple valve system (Samplivalve, Scanivalve Corp., San Diego, CA) made one sample available for analysis while others were purged. CO₂ concentrations were analyzed with an infrared gas analyzer (Model PIR-2000, Horiba Instruments, Irvine, CA) and data acquisition was provided by a desktop-calculator data-acquisition unit (Model 9830, Hewlett-Packard, Palo Alto, CA). Ambient CO₂ concentrations were monitored continuously and averaged every 10 min. Data were stored every hour on cassette tape with strip-chart recorder and computer printouts as backups. The printouts also facilitated manual control of chamber CO₂ concentrations. Zero-span calibrations were conducted every three hours to correct for analyzer drift, and multipoint calibrations over the entire sampling range were conducted daily to assess analyzer linearity and general performance. All calibration gases were referenced to standards traceable to the Scripps Institution of Oceanography (La Jolla, CA).

Conditions of Growth. All trees were kept well-watered. Accurate measurements of both applied water (using a metering-valve system) and precipitation were conducted. The seedling pots were saturated three times per week and the saplings flood irrigated with 7.6 cm of water every two weeks during the growing season. Foliage water status was determined twice weekly by pre-dawn needle water-potential surveys, using a modified Scholander pressure chamber (Squire et al., 1981). Seedlings were fertilized each month during the growing season with commercial horticultural fertilizer (10-8-6). Surveys of meteorological conditions both within the chambers and of the ambient environment were made throughout the final growing season. Midday measurements of photosynthetically active radiation (PAR), relative humidity and air temperatures were made daily along with those of foliage temperature (discussed below). Diurnal measurements of the same parameters were conducted at least once per week, with data taken every two hours throughout the daylight hours.

Morphology. Morphological measurements of all trees were made on a monthly basis throughout the study. Main stem heights and basal diameters were measured non-destructively on all saplings and seedlings, and stem volumes were calculated. All trees were destructively harvested and dissected at the completion of the exposures. The final harvests allowed direct determination of stem volumes, internode lengths, diameters at all nodes and branch lengths, as well as needle and bud characteristics. These detailed dissections also were useful in other parts of the study, including biomass determinations, partitioning studies and growth analyses.

Stomatal Conductance. Carbon dioxide is known to cause stomatal closure, with possible subsequent effects on transpiration, consumptive water-use, water-use efficiency and tolerance of drought. Therefore, weekly surveys of both seedling and sapling stomatal conductance and transpiration were made throughout the course of the study using a steady-state porometer (Model LI-1600, Licor, Inc., Lincoln, NB). Sampling was conducted on both current and 1-year-old needles. Measurements were taken on saplings immediately prior to and one day after irrigation as well as during the middle of the watering cycle. Seedlings were measured immediately prior to irrigation and one day following application of water. Seedlings were moved to the south side of the sapling in direct sunlight for these measurements. All needles in a fascicle were enclosed in the porometer chamber and kept in full light until steady-states were

achieved. As the porometer uses a fixed area in its calculations, actual needle areas were measured and data were corrected accordingly.

Foliage temperature. Foliage temperatures were measured in conjunction with daily midday and weekly diurnal surveys of chamber meteorological conditions, with the use of an infrared thermometer (Model 210, Everest Interscience, Tustin, CA) following procedures outlined in Idso et al. (1984) and Jackson et al. (1981). Together, these measurements determined if elevated foliar temperatures were related to the CO₂ exposures or were a chamber effect. Foliar temperature measurements of groups of current and 1-year-old needles were made with the infrared thermometer measurement area confined to 0.5 cm². As a simple test to determine whether elevated foliar temperatures could be attributed to CO₂ effects on transpiration, CO₂ exposures were suspended for a three-day period during midsummer of the final growing season. Daily diurnal measurements of foliar temperatures were taken both during the absence of elevated CO₂ levels and during the three days immediately following the resumption of exposures.

Temperature Dependent Chlorophyll Fluorescence. The physiological nature of the CO₂ response was examined using temperature dependent chlorophyll fluorescence (Chl-FT) yield, a ready means for determining the upper thermal limit for orderly energy transfers necessary for photosynthesis. Both Schreiber and Berry (1977) and Seemann et al. (1984) have shown that the thermal transition point in the Chl-FT plot coincides with the upper temperature limit for stable photosynthetic rates by gas-exchange techniques. This upper temperature limit can show acclimation when plants of the same species are grown at different temperatures (Seeman et al., 1984). Assays for Chl-FT were performed in late September of the final growing season on current and 1-year-old needles taken from the pine saplings, with three replicates for each age class, from each CO₂ treatment.

Needles were sampled near midday on each of three days, enclosed in plastic bags with moist toweling, and transported to the laboratory for the fluorescence assays. Fluorescence was stimulated using exciting light at 480 nm (0.1 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) and measured at 690 nm (12-nm bandwidth). Measurements started at 25° C with temperature transitions in fluorescence yield at 47° to 51° C.

Thermal Limit for Photosynthetic Gas Exchange. Direct measurement of the thermal limits of photosynthetic gas-exchange also explored the

physiological nature of the CO₂ response during 1985. The upper limits for stable photosynthetic gas exchange (Seemann et al., 1984) was determined with a null-point compensating minicuvette system (Bingham et al., 1980), which was operated within the open-top chambers. Steady state photosynthetic CO₂ exchange was measured from 20° C to about 40° C in 5° steps; further increases were made in smaller increments, so that near the thermal limit the steps were in fractions of a degree. Trees in the +150- and +300-μLL⁻¹ treatments, representing the highest and lowest thermal transition points respectively, as measured by chlorophyll fluorescence, were measured, with three replicates per tree.

Ethylene Production. Ethylene production, usually an indication of stress in tissues not expected to be undergoing senescence, was measured to determine if stress occurred in the high CO₂ treatments. Ethylene production by detached fascicles was determined with headspace gas analysis by gas-solid chromatography. Five fascicles were collected in the afternoon and placed in a 40-ml centrifuge tube with a foil-wrapped stopper. A stainless steel tube mounted in the stopper was capped with a septum seal, so that a syringe sample of the headspace gas could be withdrawn after piercing the foil. About 300 μl of distilled water were added to the tubes to prevent dessication. The tubes were incubated in the dark overnight (Peiser and Yang, 1979) at 25° C in a stirred bath. On each sampling date, three samples of five fascicles each were collected from the south-facing side of each tree.

Gas analysis was performed on a gas chromatograph (Model 5880A, Hewlett-Packard, Palo Alto, CA) equipped with a 6 ft x 2 mm (ID) glass column packed with 100/120 Porapak Q (Waters Assoc., Framingham, MA), and a six-port external volume-sampling valve (Valco Instruments Co., Houston TX). The column was pre-conditioned at 200° C, with nitrogen as carrier gas. Analyses were performed with temperature programming between 90° and 120° C, which was found necessary to elute other, more highly retained, volatile hydrocarbons. Detection was by flame ionization; calibration was performed at regular intervals by injecting various volumes of a dilute ethylene standard (Scott Specialty Gases, San Bernardino, CA).

Results

Conditions of Growth. Atmospheric CO₂ concentrations within the chambers were usually maintained within ± 15 μLL⁻¹ of desired values even

though control was manual. Some deviations arose when high wind conditions caused dilutions by influx through the chamber openings, but the cone-top baffles helped to reduce these problems. Ambient CO₂ levels were higher in winter, as is expected in the Northern Hemisphere due to a decrease in photosynthesis and an increase in fossil-fuel burning. Atmospheric CO₂ concentrations were also higher year-round at night with daytime photosynthetic drawdown of 5 to 10 μLL^{-1} observable during the growing season, especially within the ambient chambers (no added CO₂). Chamber CO₂ concentrations tracked ambient conditions on both a seasonal and diurnal basis.

The saplings within the chambers received direct sunlight through the open tops of the chambers during a portion of every day, the period of which depended on the time of year and the position of the solar track. However, most light was filtered through the chamber panels. By keeping the panels clean, light readings in the chambers were maintained at 80-90% of full sunlight, well above the light saturation point for pine-needle photosynthesis. The light levels for the seedlings, kept in the north shade of the saplings, were not appreciably different than those in the shade of unchambered saplings.

Midday air temperatures were elevated in all chambers by as much as 5 °C, depending on ambient conditions. Early morning and late afternoon chamber air temperatures were closer to ambient temperatures, with nighttime values being very similar. However, no differences were observed in air temperatures between chambers. The chambers themselves acted as greenhouses, with the panels being partially opaque to re-radiated infrared radiation. Air flow through the chamber was not sufficient to keep air temperatures at ambient levels.

No differences were found between chambers or between the chambers and the ambient environment with respect to humidity. Relative humidity values varied throughout the year, but were as low as 10% at midday during mid-summer, as is common in California.

Gross Morphology of Chambered Trees. Seedlings exposed to elevated levels of CO₂ showed significant increases in basal stem volume (Figure 1) and diameter, although trends for total height and total main stem volume were significant only in the Sierra population. It can also be seen that the trends of the measurements differ between the two populations. While the Sierra seedlings appear to have reached saturation of response, the Rocky Mountain

Figure 1. Seedling gross morphology. Means for ten seedlings at each CO₂ level, with standard error bars, are shown for the Rocky Mountain (a- d) and Sierra (e- h) seedling morphological measures: total height (a, e), basal stem diameter (b, f), basal segment volume (c, g), and main stem volume (d, h).

seedlings appear to be steadily gaining in these parameters, even at +300 μLL⁻¹.

However, while gains in seedling volume were significant, signs of stress were observed in both populations. By the termination of the experiment, both seedling types had relatively poor bud development in the +300-μLL⁻¹ treatment; the Rocky Mountain seedlings also showed poor bud development in the +150-μLL⁻¹ treatment. In addition, there was a marked increase in needle abscission. By the experiment's end, no two-year-old needles were present on

the Rocky Mountain seedlings in the +150- and +300- μLL^{-1} treatments, and relatively few 1-year-old needles were retained. In the Sierra seedlings at +150 and +300 μLL^{-1} , many needles had extensive dieback, with abscission of the necrotic portions at mid-needle. Also, many of the current-year needles were twisted and distorted in shape.

Observations of the saplings after 1.5 years of exposure indicated that the trees at the highest levels of exposure to CO_2 were under either water- or thermal-stress. Measurements of needle water-potential indicated that water-stress was not present. While the tree in the ambient chamber closely resembled unenclosed companion trees, the trees exposed to enriched CO_2 atmospheres showed increasing signs of stress with each increment of added CO_2 . However, the +150- μLL^{-1} treated tree was the tallest, and had grown out of the top of its chamber by early summer during the final growing season, indicating some positive growth response to elevated CO_2 , at least to this level. At ambient CO_2 , the treated sapling had retained current, 1-, and 2- year-old needles at the termination of exposures. However, in the +75- μLL^{-1} treatment, while the same age classes of needles were retained, 2-year old needles showed general tip necrosis and 1-year-old needles showed signs of chlorosis; the tree exposed to the +150- μLL^{-1} treatment had lost some 1-year-old needles, and many of its 2-year-old needles. Most of the 1-year-old needles from the +150- μLL^{-1} treatment showed advanced necrosis.

The sapling receiving the highest enrichment level was in an obviously deteriorated condition by the end of this study. Few 2-year-old needles remained, and many of the 1-year-old needles had abscised, or were markedly necrotic. In addition, the current-year's needles were only about half the length of companion tree needles of equivalent age by the end of the final growing season. However, the condition of this tree did not rapidly diverge from the others; its unusual state was not obvious until the beginning of the final growing season. An additional phenomenon observed in the final season was a decrease of the angle of divergence between needles and branches. This effect was most pronounced in the +150- and +300- μLL^{-1} treatments. The physiological characteristics of the stressed state of the trees at the higher CO_2 exposure levels are described below.

Stomatal Conductance. As expected, foliage exposed to the elevated CO_2 treatments generally exhibited lower stomatal conductance than control

Table 1. Means and standard deviations of diurnal stomatal conductance ($10^{-1} \text{ cm s}^{-1}$) values for Rocky Mountain ponderosa pine seedling needles under varying CO_2 treatments, measured with a LiCor 1600 steady-state porometer on 19 June 1984; $n=10$ for each value. Values followed by the same letter at a given time are not significantly different as determined by Duncan's Multiple Range Test.

Treatment (μLL^{-1})	Time of day					
	0700	0900	1100	1300	1500	1800
+0	$0.47 \pm 0.17 \text{ b}$	$1.04 \pm 0.13 \text{ a}$	$0.98 \pm 0.19 \text{ a}$	$0.47 \pm 0.17 \text{ b}$	$0.72 \pm 0.19 \text{ a}$	$0.63 \pm 0.23 \text{ a}$
+75	$0.52 \pm 0.12 \text{ ab}$	$0.81 \pm 0.20 \text{ b}$	$0.94 \pm 0.30 \text{ a}$	$0.52 \pm 0.12 \text{ ab}$	$0.84 \pm 0.28 \text{ a}$	$0.74 \pm 0.26 \text{ a}$
+150	$0.63 \pm 0.17 \text{ a}$	$0.71 \pm 0.21 \text{ b}$	$0.55 \pm 0.20 \text{ a}$	$0.63 \pm 0.17 \text{ a}$	$0.44 \pm 0.15 \text{ b}$	$0.29 \pm 0.17 \text{ b}$
+300	$0.61 \pm 0.18 \text{ ab}$	$0.77 \pm 0.15 \text{ b}$	$0.81 \pm 0.17 \text{ a}$	$0.61 \pm 0.18 \text{ ab}$	$0.46 \pm 0.15 \text{ b}$	$0.24 \pm 0.12 \text{ b}$

foliage (Figure 2). The conductance values could be grouped into two distinct classes of low CO_2 (+0 / +75 μLL^{-1}) and high CO_2 (+150 / +300 μLL^{-1}) (Table 1). Differences between the groups were small in the early morning hours, but stomatal conductance was reduced in the high treatments by mid-morning. At midday the lower treatments exhibited stomatal closure to a point where conductances were actually lower than that of the higher treatments, but their conductance values recovered quickly in the early afternoon. In contrast, the conductance values for the higher treatments fluctuated to a much lesser degree, remaining nearly constant through the midday period. By mid-afternoon the average conductance level in the two highest treatment levels was less than half the value for the lower treatments.

Foliage Temperature. Foliar temperatures were elevated with increasing CO_2 concentration (Figure 3). The top trace is an example of a typical, warm summer day with highly significant increases in foliar temperatures occurring between the treatments. Foliar temperatures generally tracked ambient temperatures, as can be expected for conifers (Vanderwaal and Holbo, 1984; Choudhury, 1985), with foliar temperatures in all treatments usually lower than ambient air temperatures, with the notable exception of those in the +300- μLL^{-1} treatment. The foliar temperatures in the +150- μLL^{-1} treatment were usually

Figure 2. Stomatal conductance of ponderosa pine during summer, 1984. The "ambient" points (closed circles) are the means, with standard error bars, of ten readings from the ambient ($+0 \mu\text{L L}^{-1}$) and ten from the $+75 \mu\text{L L}^{-1}$ treatments. Similarly, the "high CO_2 " points (open circles) are the means, with standard error bars, of ten readings from the $+150 \mu\text{L L}^{-1}$ and ten from the $+300 \mu\text{L L}^{-1}$ treatments.

close to, but less than, ambient air temperatures; those in the $+75\text{-}\mu\text{LL}^{-1}$ treatment were always lower than those of the $+150\text{-}\mu\text{LL}^{-1}$ treatment; and those of the $+0\text{-}\mu\text{LL}^{-1}$ treatment lower still. Highest foliar temperatures were recorded during the middle of the final season, when foliage in the $+300\text{-}\mu\text{LL}^{-1}$ treatment exceeded 45°C during the afternoon hours.

The results of the short experiment in which additional CO_2 was withheld from all treatments for a three-day period, an effort to determine the extent to which the temperature elevation could be attributed to CO_2 -induced stomatal closure, are also shown in Figure 3. The two lower traces show the midday temperature averages at the end of the period without additional CO_2 ("CO₂ off, cool day" in Figure 3) and after flow was resumed ("CO₂ on, cool day"). There were no significant differences between foliage temperatures of trees in any of the chambers when CO_2 was off, yet only three days later, with treatments resumed, there were significant temperature increases directly related to CO_2 concentration. The results suggest that at least a part of the foliar temperature elevation can be attributed to CO_2 -induced reduction of transpiration.

Heat Tolerance. To investigate the possibility that the higher foliar temperatures experienced by these trees might have influenced their overall

Figure 3. Midday foliar temperatures measured by infrared thermometry during the summer of 1985. Points are means of south-facing needles in direct sunlight with age classes combined.

Figure 4. Chlorophyll fluorescence transition temperatures of ponderosa pine sapling needles. Points are means and standard error bars for current year (closed circles) and year old needles (open circles).

Figure 5. Ethylene production by ponderosa pine sapling needles. Points are means with standard error bars for measurements on 23 July 1985 (closed circles), and for 19 August 1985 (open circles).

tolerance to heat, two independent approaches were applied to determine the upper thermal limits of the photosynthetic apparatus. This was appropriate considering the high foliar temperatures that had been recorded, since damage of photosynthetic membrane systems by high temperatures can be detected before other types of injury (Björkman, 1975).

Transition temperatures did increase up to the +150-μLL⁻¹ level of CO₂ enrichment (Figure 4). At +300 μLL⁻¹, the limit was depressed, even though this sapling had experienced chronically higher foliage temperatures for the entire preceding season. The simple (one-way) ANOVA for CO₂-treatment effects on chlorophyll fluorescence transition temperature (when both current- year and one-year-old needles were taken together) was significant.

Depressed tolerance to heat at the highest CO₂ level was corroborated by running temperature-response curves for net photosynthesis with the steady-state minicuvette (Table 2). Both the high correlation with the thermal limits estimated with the Chl-FT technique, and the depressed value at +300 μLL⁻¹ can be seen from this table.

Table 2. Means, standard deviations, and t-statistic for estimates of the upper thermal limit for stable photosynthetic gas exchange of ponderosa pine sapling needles. The hypotheses tested were: $H_0: \mu_1 = \mu_2$, and $H_a: \mu_1 \neq \mu_2$, with three observations at each level, for four degrees of freedom.

	CO ₂ Concentration		t-statistic	p
	+150	+300		
Mean Limit (°C ± Std.Dev.):	50.3 ± 0.1	48.8 ± 0.6	-3.672	< 0.022

As an additional indication of the degree of stress, ethylene production by foliage from the saplings was also determined by gas chromatography. Figure 5 shows increasing ethylene evolution rates by needles in the highest CO₂ treatments. The effect of CO₂ treatments on ethylene production, as determined by a simple one-way ANOVA on data from two days' measurements was statistically significant. The analysis was performed on ethylene production rates, which were converted to nL ethylene · gram dry matter⁻¹ · hour⁻¹.

Discussion and Conclusions

The open-top chamber technique offers a useful approach to studies involving modifications of the atmospheric environment of plants. Their ruggedness and ease of installation permit them to be used in field situations with relatively large plants, as was the case in the work described here. However, the physical environment of enclosed trees is definitely modified by the chambers. Air temperatures are elevated, as compared with ambient conditions, due to the chamber walls, which reduce re-radiation of infrared from the enclosed trees. We observed elevated air-temperatures within the open-top chambers, which ranged from fractions of a degree during cool days, to as much as 5° C under hot, clear sky conditions. These are greater chamber-induced elevations of air-temperature than have been previously reported (Heagle et al., 1973; 1979; Rogers et al., 1983b), which may be due to lower humidities experienced in California. However, no air-temperature differences were detected between chambers. The chamber air flow, while inadequate to

maintain chamber air temperatures at ambient levels, is sufficient to compensate for direct effects of CO₂ on air-temperature increases, if any exist.

In addition, the chambers alter boundary-layer conditions of enclosed foliage. Heagle et al. (1979) reported maximum air velocities of 8.6 m s⁻¹ near the perforations in the plenum panels that provided forced ventilation of the chambers, but these fell off toward the center of the chamber, to between 0.4 and 0.8 m s⁻¹. In our ambient chambers, foliage temperatures were also elevated as much as 2 to 3° C above unenclosed companion-tree foliage. This was likely due to a combination of altered boundary-layer conditions and elevated air temperatures.

Despite these microclimatic effects, we believe chamber environments represent valid sets of conditions for experimentation, because they represent possible natural environments, even if ambient conditions are not tracked exactly. Because there appear to be no microclimatic differences between chambers, any effects of elevated CO₂ levels should be apparent. In addition, because both ambient and chamber environments are carefully monitored and parallel measurements from unenclosed companion trees are made, the direct effects of the chambers themselves can be quantified. We feel that the use of open-top chambers can yield valuable information regarding plant responses under realistic future environmental conditions. This information may not be readily obtainable by conducting studies in growth chambers or greenhouses that impose higher degrees of artificiality.

The gains in height, basal diameter and volume for ponderosa pine seedlings resemble previous results from other workers (Funsch et al., 1970; Tinus, 1972), although there was some divergence of response with respect to the two study populations. The Sierra seedlings' gains appeared to be leveling off, while the Rocky Mountain seedlings seemed to have a more gradual response that may still have been increasing, even at the +300-μLL⁻¹ enrichment level. Genotypic variation in response to elevated CO₂ has been reported elsewhere (Wulff and Alexander 1985).

Stress effects of elevated CO₂ have been previously reported (Gates et al., 1983; Madsen 1973) in herbaceous species exposed to very high CO₂ concentrations. These included distortion of chloroplasts by excessively large starch grains, and other cytological changes. We cannot completely rule out these types of causes for the stress observed during this study. Ultrastructural studies are currently underway to search for starch grain induced injury and

other possible cytological causes of the stress response. We do, however, suspect that foliar heating was the probable cause of several stress symptoms, including terminal-needle necrosis, increased ethylene production, and premature needle abscission. Relatively few reports of foliar heating arising from CO₂ induced transpiration changes exist, although the theoretical basis for this effect has been developed (Kimball et al., 1984), and preliminary measurements have been made in other plant types (Idso et al., 1984).

The placement of the seedlings in the shade of the saplings probably allowed them to avoid extreme foliar heating. Any CO₂-induced foliar heating was probably reduced by lack of direct insolation. The saplings, on the other hand, received direct insolation throughout the day and therefore were more likely to exhibit temperature-stress symptoms. In addition, it should be noted that as CO₂ levels increased, the seedlings did exhibit symptoms indicating that next year's growth may have been impacted by the CO₂ treatments. These symptoms included needle abscission, poor bud development and foliar necrosis.

We conclude that the substantial reduction in transpiration at the higher levels of CO₂ enrichment (Figure 2) caused foliar temperatures to reach or exceed air temperatures for at least several afternoon hours each day during summer conditions. Reduced stomatal conductance and transpiration in the morning hours was probably not a thermal-stress-inducing factor since air temperatures at these times were not high. And while midday conductance values were similar between all treatments, afternoon conductances, and therefore transpiration and transpirational cooling, were much reduced in the higher CO₂ treatments, at the time when air temperatures were greatest. On warm days, this translated into needle temperatures close to 45° C, which although lower than the lethal temperatures reported for pine seedlings (Baker, 1929), were probably sufficiently high to adversely affect the integrity of intercellular membranes. The elevated ethylene-production rates observed in the sapling needles may reflect decompartmentalization of organelles resulting from primary heating effects on membranes, which should be an auto-catalyzing phenomena, as has been demonstrated in a variety of other plant tissues (Hanson and Kende, 1975; Kende and Hanson 1976; Suttle and Kende, 1978). Furthermore, the elevated ethylene within the needles may have started to compromise the physical structure of the membrane-bound-photosystem complex, leading to reduced tolerance of this system to heat (Figure 4, Table 2).

The decrease in observed needle angles may have been related to direct effects of ethylene, resulting in needle angles that reduce heat loading and subsequent direct heat damage (Dhawan et al., 1981; Ludlow and Björkman, 1984).

Future studies should take into account several issues raised by this study. The responses of ponderosa pine, and perhaps other woody, long-lived perennial species, to elevated levels of atmospheric CO₂ may be more complex than responses previously reported for annual species. It appears that CO₂ can affect the growth, morphology and physiology of ponderosa pine in a variety of ways. The magnitude of responses, whether growth is stimulated or retarded, and whether plant physiological rates are increased or decreased are dependent on several factors, including CO₂ concentration, duration of exposure, plant age, genotype, and other conditions of growth.

Studies of plant-community and ecosystem responses should address intraspecific variation in responses, as shown in this study, as well as differential species responses. Ecosystem studies should attempt to study local populations, in situ, for most accurate determinations of effects. Studies of any particular species should include surveys of the responses of several populations before definitive statements can be made regarding that species' responses to elevated CO₂ levels. For example, while data obtained from exposures of Sierra ponderosa pine to elevated CO₂ can be used to predict the response of ponderosa pine in the Rocky Mountains, additional uncertainty must be accepted.

Carbon dioxide concentration is only a single parameter in the complex environment of plants and emphasis should be made accordingly, especially when modeling is attempted. For example, shrub competition can impact ponderosa pine production in some areas (Oliver, 1984). Such interactions may or may not be exacerbated under elevated CO₂ owing to differential responses by the species involved, and other limiting factors.

Furthermore, since foliar temperatures are largely dependent on ambient air temperatures, regardless of CO₂ concentrations, the foliar heating stress shown in this study may only be important in geographical areas with air temperatures at the upper limits of the temperature range for ponderosa pine. Gymnosperms may already be at a competitive disadvantage under elevated CO₂ levels (Wright, 1974), and CO₂-induced foliar heat stress, in addition to other stresses present, may limit the success of these species in such marginal

areas. In regions of less extreme air temperatures, increased foliar temperatures due to elevated CO₂ might not induce stress responses.

REFERENCES

- Bacastow, R., and C.D. Keeling. 1973. Atmospheric carbon dioxide and radiocarbon in the natural carbon cycle. II. Changes from AD 1700 to 2070 as deduced from a geochemical model. *in* : Carbon and the Biosphere. Edited by G. Woodwell and E. Pecan. U.S.A.E.C., Washington, D.C. pp. 86-134.
- Baker, F.S. 1929. Effect of excessively high temperatures on coniferous reproduction. *J. Forest.* 27: 949-975.
- Bingham, G.E., P.I. Coyne, R.B. Kennedy, and W.L. Jackson. 1980. Design and fabrication of a portable minicuvette system for measuring leaf photosynthesis and stomatal conductance under controlled conditions. UCRL-52895. University of California Lawrence Livermore National Laboratory, Livermore, CA.
- Björkman, O. 1975. Thermal stability of the photosynthetic apparatus in intact leaves. *Carnegie Inst. Wash. Yr. Bk.* 74: 748-751.
- Bryan, W., and R. Wright. 1976. The effect of enhanced CO₂ levels and variable light intensities on net photosynthesis in competing mountain trees. *Am. Midland Nat.* 95: 446-450.
- Choudhury, B. 1985. A note on canopy and air temperature equality. *Ag. Forest. Meteorol.* 34: 333-336.
- Clark, W.C., K.H. Cook, G. Marland, A.M. Weinberg, R.M. Rotty, P.R. Bell, L.J. Allison, and C.L. Cooper. 1982. The carbon dioxide question: perspectives for 1982. *in* : Carbon Dioxide Review : 1982. Edited by W.C. Clark. Oxford University Press, New York. pp. 3-44.
- Dahlman, R.C., B. R. Strain, and H.H. Rogers. 1985. Research on the response of vegetation to elevated atmospheric carbon dioxide. *J. Environ. Qual.* 14:1-8.
- Davis, J.M., A.J. Riordan, and R.E. Lawson, Jr. 1983. A wind tunnel study of the flow field within and around open-top chambers used for air pollution studies. *Boundary-Layer Meteorol.* 25: 193-214.
- Davis, J.M., and H.H. Rogers. 1980. Wind tunnel testing of open top field chambers for plant effects assessment. *J. Air Poll. Cont. Assoc.* 30: 905-907.
- Dhawan, K.R., P.K. Bassi, and M.S. Spencer. 1981. Effects of carbon dioxide on ethylene production and action in intact sunflower plants. *Plant Physiol.* 68: 831-834.

- Funsch, R.W., R.H. Mattson, and G.R. Mowry. 1970. CO₂ supplemented atmosphere increases growth of *Pinus strobus* seedlings. *Forest Sci.* 16: 459-460.
- Gates, D.M., B.R. Strain, and J.A. Weber. 1983. Ecophysiological effects of changing atmospheric CO₂ concentration. *in*: *Physiological Plant Ecology IV. Ecosystem processes: mineral cycling, productivity and man's influence.* Edited by O.L. Lange, P.S. Nobel, C.B. Osmond, and H. Ziegler. Springer-Verlag, New York. pp. 503-526.
- Green, K., and R. Wright. 1977. Field response of photosynthesis to CO₂ enhancement in ponderosa pine. *Ecology* 58: 687-692.
- Hanson, A.D., and H. Kende. 1975. Ethylene-enhanced ion and sucrose efflux in morning glory flower tissue. *Plant Physiol.* 55: 663-669.
- Hardh, J.E. 1966. Trials with carbon dioxide, light and growth substances on forest tree plants. *Acta Forest Fenn.* 81: 1-10.
- Heagle, A.S., D.E. Body, and W.W. Heck. 1973. An open-top chamber to assess the impact of air pollution on plants. *J. Environ. Qual.* 2:365-368.
- Heagle, A.S., R.B. Philbeck, H.H. Rogers, and M.B. Letchworth. 1979. Dispensing and monitoring ozone in open-top field chambers for plant-effects studies. *Phytopathology* 69: 15-20.
- Higginbotham, K.O., J.M. Mayo, S. L'Hirondelle, and D.K. Krystofiak. 1985. Physiological ecology of lodgepole pine (*Pinus contorta*) in an enriched CO₂ environment. *Can. J. For. Res.* 15: 417-421.
- Hurt, P., and R. Wright. 1976. CO₂ compensation point for photosynthesis: Effect of variable CO₂ and soil moisture levels. *Am. Midland Nat.* 95: 450-455.
- Idso, S.B., B.A. Kimball, and K.L. Clawson. 1984. Quantifying effects of atmospheric CO₂ enrichment on stomatal conductance and evapotranspiration of water hyacinth via infrared thermometry. *Ag. Forest Meteorol.* 33:15-22.
- Jackson, R.D., S.B. Idso, R.J. Reginato, and P.J. Pinter, Jr. 1981. Canopy temperature as a crop water stress indicator. *Water Resources Res.* 17:1133-1138.
- Kende, H., and A.D. Hanson. 1976. Relationship between ethylene evolution and senescence in morning-glory flower tissue. *Plant Physiol.* 57: 523-527.
- Keeling, C.D. 1983. The global carbon cycle: What we know and could know from atmospheric, biospheric, and oceanic observations. *Proceedings CO₂*

Research Conference, Berkeley Springs, W. Va., DOE/CONF-820970. U.S. Department of Energy, Washington, DC.

Kimball, B.A. 1983. Carbon dioxide and agricultural yield: An assemblage and analysis of 430 prior observations. *Agron. J.* 75:779-788.

Kimball, B.A., J.R. Mauney, G. Gwinn, F.S. Nakayama, P.J. Pinter, Jr., K.L. Clawson, S.B. Idso, G.D. Butler, and J.R. Radin. 1984. Responses of Vegetation to Carbon Dioxide: Effects of Increased Atmospheric CO₂ on the Yield and Water-Use of Crops. Report 023, U.S. Department of Energy, Carbon Dioxide Research Division, Office of Energy Research, Washington, D.C.

Kramer, P.J. 1981. Carbon Dioxide concentration, photosynthesis, and dry matter production. *BioScience* 31: 29-33.

LaMarche, V.C., Jr., D.A. Graybill, H.C. Fritts, and M.R. Rose. 1984. Increasing atmospheric carbon dioxide: Tree ring evidence for growth enhancement in natural vegetation. *Science* 225: 1019-1021.

Lemon, E.R. (ed.). 1983. CO₂ and Plants: The response of plants to rising levels of atmospheric carbon dioxide. American Association for the Advancement of Science Selected Symposium 84. Westview Press, Boulder, CO.

Ludlow, M.M., and O. Björkman. 1984. Paraheliotropic leaf movement in *Siratro* as a protective mechanism against drought-induced damage to primary photosynthetic reactions: damage by excessive light and heat. *Planta* 161: 505-518.

Madsen, E. 1973. Effect of CO₂-concentration on the morphological, histological and cytological changes in tomato plants. *Acta Agric. Scand.* 23: 241-246.

Molnar, J.M., and W.A. Cumming. 1968. Effect of carbon dioxide on propagation of softwood, conifer and herbaceous cuttings. *Can. J. Plant Sci.* 48: 595-599.

Oliver, W.W. 1984. Brush reduces growth of thinned ponderosa pine in Northern California. Research Paper PSW-172, U.S.D.A. Forest Service Pacific Southwest Forest and Range Experiment Station, Berkeley, CA.

Pearman, G. I. 1980. The global carbon cycle and increasing levels of atmospheric carbon dioxide. In *Carbon Dioxide and Climate: Australian Research*. Edited by G.I. Pearman. Australian Academy of Science, Canberra, pp. 11-20.

Peiser, G.D., and S.F. Yang. 1979. Ethylene and ethane production from sulfur dioxide-injured plants. *Plant Physiol.* 63: 142-145.

- Rogers, H.H., G.E. Bingham, J.D. Cure, W.W. Heck, A.S. Heagle, D.W. Israel, J. M. Smith, K.A. Surano, and J.F. Thomas. 1980. Responses of Vegetation to Carbon Dioxide: Field Studies of Plant Responses to Elevated Carbon Dioxide Levels. Report 001, U.S. Department of Energy, Carbon Dioxide Research Division, Office of Energy Research, Washington, D.C.
- Rogers, H.H., G.E. Bingham, J.D. Cure, J.M. Smith, and K.A. Surano. 1983a. Responses of selected plant species to elevated CO₂ in the field. *J. Env. Qual.* 12:569-574.
- Rogers, H.H., W.W. Heck, and A.S. Heagle. 1983b. A field technique for the study of plant responses to elevated carbon dioxide in the field. *J. Air Poll. Control. Assoc.* 33:42-44.
- Rogers, H.H., J.F. Thomas, and G.E. Bingham. 1983c. Responses of agronomic and forest species to elevated CO₂. *Science* 220:428-429.
- Schreiber, U. and J.A. Berry. 1977. Heat-induced changes in chlorophyll fluorescence in intact leaves correlated with damage of the photosynthetic apparatus. *Planta* 136:233-238.
- Seemann, J.R., J.A. Berry, and W.J.S. Downton. 1984. Photosynthetic response and adaptation to high temperature in desert plants. *Plant Physiol.* 75: 364-368.
- Sionit, N., B.R. Strain, H. Hellmers, G.H. Riechers, and C.H. Jaeger. 1985. Long-term CO₂ enrichment affects growth and development of *Liquidambar styraciflua* and *Pinus taeda* seedlings. *Can. J. For. Res.* 15:468-471.
- Siren, G., and T. Alden. 1972. CO₂ supply and its effects on the growth of conifer seedlings grown in plastic greenhouses. Royal College of Stockholm Department of Reforestation Research Note Number 37, Stockholm, Sweden. pp. 1-15.
- Squire, G.R., C.R. Black, and P.J. Gregory. 1981. Physical measurements in crop physiology, II. Water relations. *Exp. Agricul.* 17:225-242.
- Strain, B. R. (Ed.). 1978. Report of the Workshop on Anticipated Plant Responses to Global Carbon Dioxide Enrichment. Duke University Environmental Center, Durham, NC.
- Strain, B.R., and F. A. Bazzaz. 1983. Terrestrial plant communities. *in*: CO₂ and Plants: The response of plants to rising levels of atmospheric carbon dioxide. Edited by E.R. Lemon. American Association for the Advancement

of Science Selected Symposium 84. Westview Press, Boulder CO. pp. 177-222.

Suttle, J.C., and H. Kende. 1978. Ethylene and senescence in petals of *Tradescantia*. *Plant Physiol.* 62: 267-271.

Tinus, R.W. 1972. CO₂ enriched atmosphere speed growth of ponderosa pine and blue spruce seedlings. *Tree Planters' Notes* 23: 12-15.

Tolley, L.C., and B.R. Strain. 1984. Effects of CO₂ enrichment on growth of *Liquidambar styraciflua* and *Pinus taeda* seedlings under different irradiance levels. *Can. J. For. Res.* 14: 343-350.

Tolley, L.C., and B.R. Strain. 1985. Effects of CO₂ enrichment and water stress on gas exchange of *Liquidambar styraciflua* and *Pinus taeda* seedlings grown under different irradiance levels. *Oecologia (Berlin)* 65:166-172.

Vanderwaal, J.A., and H.R. Holbo. 1984. Needle-air temperature differences of Douglas-fir seedlings and relation to microclimate. *Forest Sci.* 3: 635-644.

Wittwer, S. H. 1979. Future technological advances in agriculture and their impact on the regulatory environment. *BioScience* 29: 603-610.

Wright, R. D. 1974. Rising atmospheric CO₂ and photosynthesis of San Bernardino mountain plants. *Am. Midland Nat.* 91: 360-370.

Wulff, R.D., and H.M. Alexander. 1985. Intraspecific variation in the response to CO₂ enrichment in seeds and seedlings of *Plantago lanceolata* L. *Oecologia (Berlin)* 66: 458-460.

**BIOMASS AND MORPHOLOGY OF
PINUS PONDEROSA EXPOSED TO LONG-TERM
ELEVATED ATMOSPHERIC CO₂ CONCENTRATIONS**

J.L.J. Houpis¹, K. A. Surano², J.S. Fried³, P.F. Daley², P.D. Anderson³
and J.H. Shinn¹

¹Health and Ecological Assessment Division

²Environmental Protection Department

Lawrence Livermore National Laboratory

P.O. Box 808

Livermore, CA 94550

³Department of Forestry and Resource Management

University of California

Berkeley, California 94720

**LAWRENCE LIVERMORE NATIONAL LABORATORY
University of California • Livermore, CA • 94550 • USA**

Introduction

A long-term exposure study of the effects of elevated atmospheric carbon dioxide (CO_2) on ponderosa pine (*Pinus ponderosa*) was conducted at the Lawrence Livermore National Laboratory, Livermore, California. Ponderosa pine was chosen because of its economic importance to the western United States and also because of the scarcity of long-term physiological and morphological studies of the effects of elevated CO_2 on long-lived woody species. This paper examines the growth, biomass production and morphological responses of both saplings and seedlings in the study.

Several studies have been conducted to determine individual plant species' responses to elevated CO_2 concentrations (Lemon, 1983; Kimball, 1983). In general, forest tree species have responded to short-term exposures by increasing height growth, stem basal diameter growth, leaf area, photosynthetic rates and biomass production and by reducing stomatal conductance and transpiration (Tolley and Strain, 1984; Higginbotham et al., 1985; Sionit et al., 1985; Rogers et al., 1980, 1983). This study on the effects of elevated atmospheric CO_2 concentrations on ponderosa pine is the first to be performed in situ on entire trees for longer than one growing season, and was designed to determine whether previously reported responses to short-term exposures would continue, or whether acclimation might occur. The growth conditions were unique among related studies because we used open-top chambers and levels of CO_2 that can realistically be expected to prevail globally within the next 80 to 100 years.

Increases in biomass of Virginia pine seedlings, particularly in the roots, have been demonstrated with short-term exposures (Luxmoore et al., 1986). In our long-term study, biomass of needles, buds, branches, boles and roots was measured at the end of the experiment, after 27 months of continuous exposures. Changes in needle biomass and leaf area could have significant effects on water and nutrient use and these changes could affect the productivity of marginal sites. Though a small contribution to total biomass, the weight of buds is an excellent indicator of future growth.

Materials and Methods

Exposure with elevated CO_2 began in July 1983 and was terminated in September 1985. Exposures were continuous and were conducted in four standard open-top field exposure chambers as described by Heagle et al.

(1973) and Rogers et al. (1983a, 1983b). The levels of CO₂ exposure were ambient (approximately 350 μLL^{-1} ; +0 μLL^{-1}), ambient + 75 μLL^{-1} (+75 μLL^{-1}), ambient + 150 μLL^{-1} (+150 μLL^{-1}), and ambient + 300 μLL^{-1} (+300 μLL^{-1}). A monitoring and control system was developed to monitor and maintain these levels (Surano et al. 1986).

The experiments examined seedlings of two varieties of ponderosa pine, one being the Sierran variety (*P. ponderosa* var *ponderosa*) and the other the Rocky Mountain variety (*P. ponderosa* var *scopulorum*). Saplings of San Bernardino Mountain lineage were also used. At the initiation of the exposures, the Sierran seedlings were one-year old, the Rocky Mountain seedlings were three-years old, and the saplings were 7-years old. Both varieties of seedlings were potted in 4-L containers using a soil mixture containing equal parts of cohasset clay loam, redwood compost, #4 Monterey sand, and perlite. Throughout the study, all trees were maintained under well-watered conditions, and fertilized regularly. The seedlings were grown under the shade of the saplings. There were ten seedlings and 1 sapling for each treatment.

Growth measurements were taken from April 1984 to August 1985. Basal diameter was determined with a micrometer placed at the cotyledon whorl. Two perpendicular measurements were taken and were averaged. Height measurements were taken from the cotyledon whorl to the top of the apical meristem using a tape measure. Volume was calculated from the diameter and height measurements using a parabolic formula (Husch et al. 1972).

Morphological data were collected at the conclusion of the exposures, when both seedlings and saplings were dissected. Morphological measurements included biomass, volume, specific gravity, height, diameter, needle dry weight and surface area, and the number of buds. Biomass data included dry weight of main stem and lateral stems by age class, roots, needles, and buds. Specific gravity was calculated as the ratio of dry weight per volume of the main stem segment. Height and diameter measurements were taken for each stem segment. The amount of mid-needle abscission was observed as a percent of total needles present.

Additionally, a 5-mm-thick "round" was removed from the basal end of each sapling bole segment for use in densitometric analysis. Tree-ring densitometry was conducted with a scanning x-ray densitometer of the type described by Echols (1970) to determine wood density and volume for each

Figure 1. X-ray densitometry scans of stem cross sections from the base of saplings growing under +150 and +300 $\mu\text{L L}^{-1}$ above ambient CO_2 . Numbers next to the steps of the calibration wedge represent densities of wood (g cm^{-3}) that correspond to those steps. The arrows point to earlywood and latewood produced in 1984. Note the difference in size of the most recent ring widths of these trees.

age class of wood (Figure 1). The earlywood and latewood widths and average earlywood and latewood densities were calculated for each annual ring for each stem segment of each sapling. The volume increment of both early- and latewood from each age class of wood was calculated by difference using Huber's formula (Husch et al., 1972) under the assumption that conic frustrums best described the volumes of these stem segments. Biomass was calculated as the product of density and volume for each wood type from each wood age class and was aggregated by wood age class over the entire stem. The

estimated biomass of the vertical stem section was aggregated to permit comparison with the corresponding biomass values determined directly by weighing. To estimate the biomass in each conic sheath of wood on each stem segment, we adjusted our densitometrically determined biomass values for each section by the actual weight for that stem section.

Statistical analysis was conducted using analysis of variance, but is not presented here. Comparisons among treatments were made using the Newman-Kuels Multiple Comparison Test (Neter and Wasserman, 1974). A two factor ANOVA was carried out with variety and morphological or growth trait as the two factors (this is referred to as the combined analysis). The varieties were also analyzed independently (Neter and Wasserman, 1974).

Results

Seedling Biomass. The two varieties, analyzed separately, showed similar trends, with the seedlings from the two lower CO₂ levels lower in biomass than those from the two higher levels. Allocation patterns between roots and shoots showed no significant differences. The combined root/shoot ratios were 0.689, 0.672, 0.708, and 0.776 for the +0 μLL^{-1} , +75 μLL^{-1} , +150 μLL^{-1} , and +300 μLL^{-1} exposures, respectively. Roots were the largest fraction of biomass, accounting for approximately 40% of the total dry weight for all treatments.

There was a statistical difference between treatments with respect to the dry weight of buds, but this was complicated by the number of buds produced in each treatment. For example, the Rocky Mountain variety produced 18, 21, 34, and 38 buds per plant for the +0 μLL^{-1} , +75 μLL^{-1} , +150 μLL^{-1} , and +300 μLL^{-1} exposures, respectively. The dry weights per bud for the Rocky Mountain variety were 34, 42, 34, and 72 mg for the +0 μLL^{-1} , +75 μLL^{-1} , +150 μLL^{-1} , and +300 μLL^{-1} exposures, respectively.

Sapling Biomass. Wood density varied somewhat among trees but remained essentially unchanged within each tree over the 4-year period from 1982 to 1985 in both treatments and controls. Average earlywood density for all trees during this period was 0.36 g cm⁻³, range 0.30-0.44, and average latewood density was 0.56 g cm⁻³, range 0.46-0.64. Absolute stemwood biomass increment over the two-year period 1984-85 varied considerably among treatments, from 2.6 to 5.1 kg; however, since the initial sizes of these trees were not equal, relative growth (current year's stemwood biomass

Figure 2. Stemwood biomass increment as a proportion of the previous year's increment for 1984 and 1985, normalized to relative stemwood biomass increment of the control tree.

increment/previous year's stemwood biomass increment) was a more appropriate index of comparison. Relative growth for all trees increased from 1982 to 1983, most likely in response to irrigation, and then declined in both 1984 and 1985 as the previous stemwood biomass increment term became large. When the effects of the treatments on relative stem growth were normalized to the control tree (Figure 2), it became apparent that in 1984, the +75 and +150 μLL^{-1} treatments showed greater relative growth than the control, but the tree at +300 μLL^{-1} grew relatively less. In 1985, the trees at the intermediate treatment levels performed more poorly than the control and the tree at +300 μLL^{-1} again grew even less.

Without densitometric analysis, determination of total branch biomass increment is impossible; however, since the branch biomass accumulated during the experiment is large relative to any initial differences in branch biomass, total branch biomass was used as an index of the actual increment, as was the weight of the last two branch segments of each branch, since they were formed entirely from wood produced in 1984 and 1985. Both indices indicated an increase up to +75 μLL^{-1} and decreases in branch biomass at higher

Figure 3. (a) Total biomass of all branches and of the outer-most 2 branch segments at different concentrations of CO₂. (b) Total biomass of buds at different concentrations of CO₂.

concentrations of CO₂ (Figure 3a). About half of the total woody biomass of the saplings was in the branches. Total dry weight of terminal and lateral buds formed in 1985 was greatest at +75 and +150 μLL^{-1} and least at +0 and +300 μLL^{-1} (Figure 3b).

Needle biomass of both 1984 and 1985 age classes at the conclusion of the experiment was greatest on the sapling grown at ambient CO₂ concentrations and was dramatically reduced on the sapling grown at +300 μLL^{-1} , particularly in the 1985 needles (Figure 4). Litterfall was not collected; therefore, the reduction in needle biomass may be partially explained by the premature needle abscission that was observed. However, shorter needle lengths were observed in the +300 μLL^{-1} sapling during the final year and may have also contributed to the reduction in needle biomass. Specific leaf area ranged from 83 to 113 cm g^{-1} for the 1985 needles and from 78 to 97 cm g^{-1} for the 1984 needles. There were no apparent trends related to CO₂ concentration.

Fine and very fine root biomass followed the trend of increased production to +150 μLL^{-1} followed by a reduction at +300 μLL^{-1} . In the top 35 cm of soil, the densities of roots 0-5 mm in diameter were 0.55, 1.36, 1.90 and

Figure 4. Biomass of two age classes of needles on pine saplings at different concentrations of CO₂.

0.70 cm root g soil⁻¹ for the +0, +75, +150, and +300 μLL^{-1} CO₂ treatments respectively.

Seedling Stem Volume. The volumes of the seedling stem segments in the Sierran variety showed similar trends to that of biomass with a statistical grouping of both the two lower and two higher CO₂ levels. In the Rocky Mountain variety and in the combined analysis only the volumes of the stem segments at +300 μLL^{-1} were greater than all other treatments. Total volume of the main stem as determined by the combined analysis is shown in Figure 5. Analysis of percent change in volume indicated that the higher volume at +300 μLL^{-1} for the Rocky Mountain variety may have been in part due to a statistically larger initial volume. Percent change in volume of the seedlings at +300 μLL^{-1} was only statistically greater than those at +0 μLL^{-1} (rather than +0 μLL^{-1} , +75 μLL^{-1} , and +150 μLL^{-1} as indicated by the ending volume). The percent change in volume for the Sierran variety at +150 μLL^{-1} was greater in percent change than all other treatments, whereas at +300 μLL^{-1} the volume was not statistically different from those at either of the lower CO₂ levels (Figure 6). In the combined

Figure 5. Volume of the main stem at the various CO₂ treatment levels.

analysis, the seedlings at +150 μLL^{-1} were also statistically the largest value in percent change in volume (Figure 7).

Seedling Specific Gravity. Specific gravities, using the combined analysis, were 0.485, 0.529, 0.603, and 0.602 g cm⁻³ for +0 μLL^{-1} , +75 μLL^{-1} , +150 μLL^{-1} , and +300 μLL^{-1} treatments respectively. Statistically, only the seedlings at +150 μLL^{-1} and +300 μLL^{-1} were greater than those at +0 μLL^{-1} . The trend observed in specific gravity was due to variations in the Rocky Mountain variety.

Seedling Stem Height. At the end of the study, the Sierran variety's seedling heights at +0 μLL^{-1} , +150 μLL^{-1} , and +300 μLL^{-1} were significantly greater than those at +75 μLL^{-1} . This was also true when applying the

Figure 6. Percent change in volume over the course of the study for (a) Rocky Mountain and (b) Sierra seedlings.

Figure 7. Percent change in growth of height, diameter, and volume by the end of the experiment, using the combined analysis.

Figure 8. Height from the cotyledon whorl to the tip of the apical bud for seedlings in the combined analysis plotted against treatment level.

combined analysis. However, there were no significant differences between any of the treatments for the Rocky Mountain variety (Figure 8).

In the independent analysis of total percent change of height for each variety, only the Sierran variety showed a significant difference (those at +150 μLL^{-1} were greater than those at +0 μLL^{-1} ; Figure 9). However, using the combined analysis, the seedling heights at +75 μLL^{-1} , +150 μLL^{-1} , and +300 μLL^{-1} were significantly greater than those at +0 μLL^{-1} , and those at +150 μLL^{-1} were statistically greater than those at +75 μLL^{-1} and +300 μLL^{-1} (Figure 7). Differences observed in final height in absolute terms and with regard to the seedlings at +75 μLL^{-1} being lowest, can be attributed to a lower initial height.

Seedling Stem Diameter. Seedling stem diameter measurements were taken at each successive whorl up the main stem (Figure 10). In the combined analysis, the basal diameters of the seedlings at +0 μLL^{-1} and those at +75 μLL^{-1} were statistically less than those at +150 μLL^{-1} and +300 μLL^{-1} , and those at +150 μLL^{-1} were less than those at +300 μLL^{-1} . However, when comparing percent change of basal diameter, the seedlings at +150 μLL^{-1} were statistically the largest, followed by those at +300 μLL^{-1} , +75 μLL^{-1} , and +0 μLL^{-1} respectively (Figure 7). One reason for this difference was that the seedlings in the +300 μLL^{-1} treatment had a statistically larger initial basal diameter.

Seedling Needle Surface Area. The differences observed in surface area as determined by the combined statistical analysis were due to the Rocky Mountain variety. For the seedlings of the Sierran variety, the only significant difference in total surface area was that those at +300 μLL^{-1} were greater than those at +75 μLL^{-1} , and in the Rocky Mountain variety those at +150 μLL^{-1} were greater than those at +75 μLL^{-1} .

In Table 1 there are indications of a discontinuous needle surface area phenomenon that occurred between +150 μLL^{-1} and +300 μLL^{-1} . Mid-needle abscission was observed in the +300 μLL^{-1} treatment and this is partially responsible for the high standard deviation and large range for that treatment.

Discussion

The changes in sapling stemwood biomass increment indicate that elevated CO_2 at all levels including +300 μLL^{-1} may initially favor wood growth; however, this increase does not seem to persist over multiple growing seasons. By the end of the third growing season, wood production of the +300 sapling was considerably reduced below that of the control tree.

Figure 9. Percent change in total height over the course of the study for (a) Rocky Mountain and (b) Sierra seedlings.

Table 1. Needle surface area for the Sierra seedlings by CO₂ level.

CO ₂ Level (μLL^{-1})	Mean	Standard Deviation	Minimum	Maximum
+0	450	260	143	869
+75	370	240	149	903
+150	590	270	250	1062
+300	770	590	48	1925

Figure 10. Stem basal diameter from the combined analysis.

Based upon absolute measures of seedling morphology, there was the appearance of a beneficial effect of increasing CO₂. Even though the seedlings were randomized at the start of the study, the seedlings at the +300 treatment were statistically larger in basal diameter and volume. Percent change is a more appropriate measurement because it eliminates the possible bias of initial size. Based upon percent change there were maximum benefits at +150 μLL^{-1} , with a reduction in growth at +300 μLL^{-1} . This was found in height, volume, and diameter growth. How other factors were affected by initial values, such as

Figure 11. Percent change in diameter over the course of the study for (a) Rocky Mountain and (b) Sierra seedlings.

biomass and leaf surface area are unknown. Furthermore, the response to elevated CO₂ levels appears to be different between varieties of the same species. In particular, at +300 μLL^{-1} the Sierran variety had a reduction in percent growth, whereas the Rocky Mountain variety did not have a corresponding reduction.

Conclusions

Although it is generally accepted that increasing CO₂ is beneficial to annual plants, it may be premature to extend this conclusion to naturally occurring long-lived plants such as ponderosa pine. While beneficial effects may be seen initially, there is no evidence that they continue over extended periods. In fact, this study indicates that there may be a detrimental effect beginning at CO₂ concentrations greater than 500 μLL^{-1} (150 μLL^{-1} above present ambient concentrations). Such morphological evidence includes a decrease in percent growth and a reduction in needle surface area through mid-needle abscission. This study emphasizes the need for more long-term research on the effects of elevated CO₂, particularly with respect to effects on long-lived woody perennial species and natural ecosystems.

References

- Fried, J.S., K.A. Surano, P.F. Daley, J.H. Shinn and P. Anderson. 1986. Biomass production and nutrient responses of ponderosa pine to long-term elevated CO₂ concentrations. Proceedings of the Ninth N. Amer. For.Bio. Wkshp. Stillwater, OK.
- Heagle, A.S., D.E. Body, and W.W. Heck. 1973. An open-top chamber to assess the impact of air pollution on plants. J. Environ. Qual. 2:365-368.
- Higginbotham, K.O., J.M. Mayo, S. L'Hirondelle, and D.K. Krystofiak. 1985. Physiological ecology of lodgepole pine (*Pinus contorta*) in an enriched CO₂ environment. Can. J. For. Res. 15:417-421.
- Husch, B., C.I. Miller, T.M. Beers. 1972. Forest mensuration. John Wiley and Sons, New York, New York, 410pp.
- Kimball, B.A. 1983. Carbon dioxide and agricultural yield: An assemblage and analysis of 430 prior observations. Agron. J. 75:779-788.

- Lemon, E.R. (Ed.) 1983. CO₂ and Plants: The response of plants to rising levels of atmospheric carbon dioxide. AAAS Selected Symposium 84. Westview Press, Boulder, CO.
- Luxmoore, R.J., E.G. O'Neill, J.M. Ells, and H.H. Rogers. 1986. Nutrient uptake and growth responses of Virginia pine to elevated atmospheric CO₂. J. Environ. Qual. 15:244-251.
- Neter, J., W. Wasserman. 1974. Applied linear statistical models. Richard D. Irwin, Inc., Homewood, Illinois, 842pp.
- Rogers, H.H., G.E. Bingham, J.D. Cure, W.W. Heck, A.S. Heagle, D.W. Israel, J.M. Smith, K.A. Surano and J.F. Thomas. 1980. Responses of Vegetation to Carbon Dioxide: Field Studies of Plant Responses to Elevated Carbon Dioxide Levels, Report 001. U.S. Department of Energy, Carbon Dioxide Research Division, Office of Energy Research, Washington, DC.
- Rogers, H.H., G.E. Bingham, J.D. Cure, J.M. Smith, and K.A. Surano. 1983a. Responses of selected plant species to elevated CO₂ in the field. J. Environ. Qual. 12:569-574.
- Rogers, H.H., W.W. Heck, and A.S. Heagle. 1983b. A field technique for the study of plant responses to elevated carbon dioxide in the field. J. Air Poll. Cont. Assoc. 33:42-44.
- Sionit, N., B.R. Strain, H. Hellmers, G.H. Riechers, and C.H. Jaeger. 1985. Long-term CO₂ enrichment affects growth and development of *Liquidambar styraciflua* and *Pinus taeda* seedlings. Can J. For. Res. 15:468-471.
- Surano, K.A., P.F. Daley, J.L.J. Houppis, J.H. Shinn, J.A. Helms, R.J. Palassou, and M.P. Costella. 1986. Growth and physiological responses of *Pinus ponderosa* to long-term elevated CO₂ concentration. Tree Physiology (in press).
- Tolley, L.C. and B.R. Strain. 1984. Effects of CO₂ enrichment on growth of *Liquidambar styraciflua* and *Pinus taeda* under different irradiance levels. Can. J. For. Res. 14:343-350.

**WATER USE AND WATER-USE EFFICIENCY OF
PONDEROSA PINE EXPOSED TO LONG-TERM
ELEVATED ATMOSPHERIC CO₂ CONCENTRATIONS**

J.S. Fried¹ and J.H. Shinn²

¹Department of Forestry
University of California
Berkeley, California 94720

²Health and Ecological Assessment Division, L-453
Lawrence Livermore National Laboratory
P.O. Box 5507, L-524
Livermore, CA 94550

**LAWRENCE LIVERMORE NATIONAL LABORATORY
University of California • Livermore, CA • 94550 • USA**

Introduction

The effect of elevated CO_2 concentrations on the water-use efficiency (WUE) of plants is thought to be the most significant effect yet demonstrated (Wittwer, 1984). Increases in yield have been demonstrated for numerous crop species and some tree seedlings (Kimball, 1983; Wittwer, 1984, Tolley and Strain, 1984), and reduced transpiration rates (and stomatal conductance) have been linked with elevated CO_2 concentrations (Oberbauer, et al., 1985). Increases in WUE result from both yield increases and reduced stomatal conductance and the effects are in some sense additive. Although C_4 plants, such as *Zea mays*, may show no yield responses to increase CO_2 , they may still exhibit increased WUE as a result of reduced transpiration (King and Greer, 1986). C_3 plants, like ponderosa pine (*Pinus ponderosa*), often respond with both increased yields and lower stomatal conductance when exposed to elevated CO_2 and thus substantially increase WUE. However, despite more efficient water use per unit biomass or per unit leaf area, total plant water use may increase because of larger plant sizes and leaf areas associated with plants grown at elevated concentrations of CO_2 .

There are two distinctly different approaches to calculating WUE. Physiologists define WUE as mg or μmol of CO_2 fixed per g or mol of water transpired (Fischer and Turner, 1978) to obtain estimates from gas-exchange measurements. This type of WUE can be called "process WUE." Rogers et al. (1983) reported substantial gains in pine, maize, sweetgum and soybean process WUE under elevated CO_2 concentrations. Akita and Moss (1972) compared the process WUE of a C_4 and a C_3 species and determined that C_4 plants had higher WUE because they exhibited better control of stomatal apertures in response to environmental changes. While process WUE provides useful information at the physiological level, it is difficult to interpret this type of information in practical terms. For example, process WUE is not appropriate for determining the potential production performance of a plant growing in an environment with a known availability of soil moisture. Translation of CO_2 uptake to photosynthesis to biomass production requires knowledge of several variables and uncertain relationships. Thus, other types of WUE are sometimes used. A useful type of WUE that is measured directly, and is expressed as weight of dry matter or yield per unit of water use, is called "production WUE". This is the type of WUE addressed in this report.

Materials and Methods

The environment where the pines in this study were grown, the east end of California's Livermore Valley, is characterized by little annual rainfall (29 cm between 8 September 1984 and 7 September 1985) and no summer precipitation (early May through mid-September). All precipitation comes in the form of rain or mist and the climate is temperate, although winter temperatures typically drop to 29-32 °F and occasionally exceed 40 °F.

The environments in which ponderosa pine grows naturally are considerably cooler and more mesic. While these area also receive little or no summer precipitation, at least some of the winter precipitation arrives as snow, which when stored as a snow-pack enters the soil profile during the spring run-off. Even after the snow-pack melts completely, sub-surface moisture is considerably more abundant and available in the loamy, granitic/volcanic-derived Sierran and Rocky Mountain soils than in the clayey, hard-pan underlain, alluvial-originated soils of the Livermore Valley.

Consequently, during the droughty period (April through September), frequent irrigation of the trees in this study was essential to support growth rates high enough to be comparable with those of ponderosa pine in its natural habitat and to permit the identification of CO₂-induced changes in biomass accumulation.

Water use was measured for the ten-year-old saplings that were grown in open-top chambers and for 5 seedlings from each of the two seedling varieties (Sierran and Rocky Mountain). Two different techniques for measuring water use were employed in this study. Seedling water use was measured gravimetrically, by weighing pots before and after watering. Sapling water use was measured indirectly, with a neutron probe.

Sapling Water Use Measurement. All six saplings were irrigated whenever neutron probe measurements indicated substantial drawdown of soil water, about every two weeks between April and October. At each irrigation, 7.75 cm of equivalent precipitation was applied to the 7.3 m² circular area surrounding each sapling. Precipitation was monitored daily during the two year experiment with a 2-cm diameter rain gauge. Pre-dawn leaf water potential measurements, always greater than -1.0 MPa, confirmed that our irrigation schedule precluded these saplings from ever being subjected to water stress during the course of this experiment.

Because of the length of the experiment, the small volume of soil in each plot, and the requirement for replicated measurements, we rejected direct gravimetric measurement of sapling soil moisture (Kramer 1983). Instead, we employed a neutron moderation approach, which resulted in less disturbance to the plots, repeated measurements of the same soil volume, and allowed measurement of soil moisture in a large volume that was more likely to be heterogeneous than typical gravimetric samples (Kramer, 1983). The neutron moderation technique is based on the capability of hydrogen atoms to slow down and scatter fast neutrons so that a count of slow neutrons in the vicinity of a fast neutron source allows a good estimation of hydrogen content. Since water accounts for the great bulk of soil hydrogen, this method provides an indirect estimation of volumetric soil water content of a 20-cm radius sphere of soil (Kramer, 1983; Rawlins, 1976). The neutron probe consists of a source of fast neutrons which is lowered into a previously installed, reusable access tube along with a counter tube for detecting slow neutrons. The counter is connected to an amplifier and scaler and the count rates are adjusted for background and calibrated against direct determinations of soil water content.

Regular monitoring of moisture content of the soil profile to a depth of 122 cm began in summer, 1984 and continued to the end of the experiment. Volumetric moisture content was estimated with the neutron probe at depths of 10, 25, 45, 60, 90 and 120 cm via an aluminum access tube located in the center of each plot. A soil moisture profile was thus obtained for each chamber on each measurement date. We measured soil moisture immediately prior to each irrigation, two days after each irrigation, and midway between irrigations during the droughty period from May through September and at least monthly during the winter.

The soil moisture content profiles were transformed into soil moisture deficit profiles by subtracting the measured volumetric moisture contents from field capacity moisture contents at each depth. We estimated field capacity for each soil layer as the moisture content two days after the end of a series of wet, winter storms in 1984. We calculated the total soil moisture deficit for each profile for each measurement date by applying the following box-sum formula:

$$profile\ deficit = \sum_{n=1}^6 (deficit @ depth\ x_n) \left\{ \left[x_{n+1} - \frac{x_{n+1} + x_n}{2} \right] - \left[x_{n-1} + \frac{x_{n-1} + x_n}{2} \right] \right\}$$

In essence, we applied each soil layer moisture deficit value to the depth corresponding to the zone of soil that was assessed for soil moisture by neutron probe to obtain that moisture deficit value, and summed these weighted values to yield profile deficit.

Initially, we attempted to use the classic water balance approach (Kramer, 1983) to calculate water use where:

$$\text{change in water storage} = \text{precipitation} + \text{irrigation} - (\text{runoff} + \text{deep drainage} + \text{evapotranspiration})$$

Theoretically, by accounting for precipitation and irrigation and by assuming runoff and deep drainage to be negligible, one can easily estimate evapotranspiration (ET) from the changes in soil moisture deficit for each period spanned by neutron probe measurements. In this case, the guiding assumptions proved invalid, primarily because much of the water applied by irrigations could not be accounted for by changes in the moisture profiles of the plots. Instead, much of the water must have moved away from the plot, either by lateral diffusion after penetrating hardpan layers in the soil profiles or through rodent tunnels and root channels. Some water may have been withdrawn by adjacent saplings with roots extending into the study plots. In any event, most of the irrigation water was gone from the zone of measurement before neutron probe readings were taken. Evapotranspiration values obtained during periods in which irrigation or precipitation had occurred were up to ten times greater than during periods without irrigation or precipitation. Therefore, we modified the calculations to exclude from consideration those periods in which irrigation plus precipitation exceeded 1 cm.

We calculated average daily ET as the change in profile moisture deficit divided by the number of days for periods without irrigation and less than 1 cm of precipitation/irrigation. Seasonal average daily ET values were computed as the mean of the periodic average daily ET values multiplied by the number of days in the season to yield seasonal ET values, expressed in cm.

Water use efficiency was calculated three ways. We estimated total production WUE (WUE_{tp}) as the summed weight of stemwood formed in 1985, outer-most branch segments (consisting of wood formed entirely during 1985), and buds and needles produced in 1985 divided by the total amount of water used over one year. A wood production WUE (WUE_{wp}) was calculated from the total weight of the 1985 stemwood biomass increment and the outer-most

branch segments. To provide a check against gas-exchange determined transpiration rates, we also calculated WUE based on water used per unit sapling leaf area (WUE_{la}).

Seedling Water Use Measurements. Half of the 20 seedlings in each chamber were selected for measurement of water use; 5 seedlings of the Sierra variety and 5 of the Rocky Mountain variety. All seedlings were watered to saturation three times weekly so that pre-dawn water potentials were never less than -0.6 MPa, indicating that the seedlings were never water-stressed. Seedling water use was monitored from 1 May 1985 to 16 September 1985 by weighing each pot prior to watering and again four hours following irrigation. Evapotranspiration was calculated as the difference between the field capacity weight and the subsequent pre-irrigation weight. Although there were undoubtedly some changes in weight caused by carbon fixation and growth, they were assumed to be negligible for the two to three-day periods between weighings.

The calculated ET for each period was summed to obtain total seedling water use over the growing season. Production WUE for the seedlings was calculated as the quotient of above-ground seedling biomass increment, estimated as the sum of 1985 needles, buds and the outermost branch segments and main stem leader, and seedling water use over the period that we measured it. Our estimate of 1985 aboveground biomass increment for all seedlings is low because we had no way to measure the biomass of the 1985-formed wood in sheaths surrounding main stems and branches formed in earlier years.

Analyses of variance were performed on seedling water use and WUE and the Tukey test was used with a $p=0.5$ level to test for significant differences among treatments.

Results

Saplings. Sapling water use varied greatly with season and from tree to tree (Table 1). We measured ET of 6 to 11 cm in the winter, and 17 to 43 cm in spring. Both annual water use (23 September 1984 to 23 September 1985) and Spring-Summer (the period of greatest moisture stress) water use were greatest for the trees in the +75 and +150 μLL^{-1} treatments (Table 1). WUE_{la} increased with CO_2 concentrations up to +150 μLL^{-1} then leveled off somewhat at +300 μLL^{-1} . However, since the leaf area component of

Table 1. Ponderosa pine sapling water use (cm), by season and for the one year period Fall 1984 - Summer 1985 as measured by neutron probe.

Season	+0 ppm	+75 ppm	+150 ppm	+300 ppm
Summer 1984	27.29	29.18	19.22	24.53
Fall 1984	23.75	27.86	28.7	21.62
Winter 1985	7.95	11.18	6.23	8.59
Spring 1985	19.59	43.4	27.57	17.06
Summer 1985	11.74	15.77	30.73	19.26
Fall 1984 - Summer 1985	63.03	98.2	93.22	66.53

the estimate for the +300 μLL^{-1} treatment is smaller than was actually the case during part of the season due to premature needle abscission, these estimates of WUE_{la} are probably artificially high. Both WUE_{tp} and WUE_{wp} were greatest in the control chamber and least in the +300 μLL^{-1} treatment (Table 2). However, because of premature needle abscission by the tree in the +300 μLL^{-1} treatment, our estimate of total aboveground biomass production for this sapling is probably low. WUE_{wp} does not appear to be dependent on CO_2 concentration (Table 2).

Seedlings. Water use by both varieties of seedlings increased dramatically in May to a peak in early July, the hottest part of the growing season, and declined sharply in September, along with temperatures (Figure 1). Water use by the older, Rocky Mountain seedlings was always greater than that of the Sierra seedlings, probably due to greater leaf area. The ranking of water use by chamber matched that of air temperatures, with greatest water use and temperatures occurring in the +150 μLL^{-1} chamber. Water use and temperatures diminished in the +300, +75 and +0 chambers (Figure 2).

Both Rocky Mountain and Sierran seedlings used more water in the +150 treatment, than in any other treatment (Table 3). However, the differences in water use were not statistically significant for either variety. WUE of the Sierran seedlings did not vary significantly with CO_2 treatment; however, the Rocky Mountain seedlings were significantly more water efficient at +150 μLL^{-1} than at +0 or +75 μLL^{-1} (Table 4). Since there was little variation in total water

Figure 1. 15-day averages of water use by (a) Rocky Mountain and (b) Sierra seedlings.

Figure 2. Mean cumulative water use by (a) Rocky Mountain and (b) Sierra seedlings.

Table 2. Sapling leaf area, 1985 biomass production, water use per unit leaf area, and water use efficiency.

	+0 ppm	+75 ppm	+150 ppm	+300 ppm
Leaf area (m ²)	51	55.3	48.1	32.2
1985 Biomass production (g)	4953	4860	5238	2672
1985 Wood biomass (g)	2173	2283	2929	1462
Water use / Leaf area (cm/m ²)	1.24	1.78	2.07	1.94
Water use efficiency (total) (g biomass cm ⁻¹ water)	78.58	49.49	56.19	40.16
Water use efficiency (wood) (g woody biomass cm ⁻¹ water)	34.48	23.25	31.42	21.98

Table 3. Average water use (kg) by pine seedlings for the period May 1 to September 16, 1985. Numbers in parentheses are standard errors.

	+0 ppm	+75 ppm	+150 ppm	+300 ppm
Sierran variety	20.15 (1.10)	20.95 (0.67)	25.06 (1.12)	22.31 (1.93)
Colorado variety	29.94 (0.82)	30.62 (1.36)	31.91 (0.50)	31.52 (0.59)

use by the Rocky Mountain seedlings, the differences in WUE are entirely attributable to the differences in biomass production among treatments.

Discussion

The absence of a trend towards increased WUE at elevated concentrations of CO₂ in the pine saplings may be the result of error in our estimates of water use caused by the presence of tree roots of adjacent trees in the rooting zones on some of the plots and idiosyncrasies in the soil profile (ie. clay pans) which act as barriers to the movement of water. In addition, our inability to accurately estimate total needle biomass of the sapling exposed to +300 μLL^{-1} CO₂ introduces error into our estimates of both WUE_{la} and WUE_{tp}. The differences in leaf area among the saplings in the +0, +75, and +150 μLL^{-1} treatment are slight and do not account for the substantially higher water use by

Table 4. Average water use efficiency and 1985 biomass production for pine seedlings. Numbers in parentheses are standard errors.

	+0 ppm	+75 ppm	+150 ppm	+300 ppm
<u>Sierran variety</u>				
1985 Biomass (g)	10.31 (2.15)	8.67 (1.49)	15.99 (2.19)	16.18 (5.51)
Water-use efficiency (g biomass kg ⁻¹ water)	0.50 (0.08)	0.41 (0.06)	0.63 (0.06)	0.62 (0.19)
<u>Colorado variety</u>				
1985 Biomass (g)	20.88 (1.16)	24.96 (2.46)	40.46 (3.87)	32.29 (1.74)
Water-use efficiency (g biomass kg ⁻¹ water)	0.70 (0.02)	0.81 (0.05)	1.27 (0.13)	1.02 (0.06)

the trees at +75 and +150 μLL^{-1} . These increased water usages could be caused by greater stomatal densities or to slightly higher air temperatures in these chambers.

The genus *Pinus* in general, and ponderosa pine in particular, is xeric-adapted. Therefore, these trees already have the capacity to limit water loss during periods of stress by control of stomatal apertures. Since part of the CO_2 response results from smaller stomatal apertures, this species may not benefit from CO_2 -induced stomatal closure. While the WUE of these trees may be expected to increase if photosynthetic efficiency is increased (making the biomass component of the WUE equation larger), the absence of strong changes in stomatal conductance and transpiration may explain the lack of the type of clear CO_2 response that has been demonstrated for so many crop species, as well as tree seedlings. In short-term experiments with the broad-leaved species *Populus deltoides*, *Larrea divaricata*, *Narium oleander* (Downton et al., 1980) and *Liquidambar styraciflua* (Rogers et al., 1983), a doubling of CO_2 concentration brought about a doubling of WUE. However, a similar experiment with *Pinus taeda* (Tolley, 1982) demonstrated little, if any,

WUE response to elevated CO₂. Furthermore, WUE effects have been shown to be greater in water-stressed than in non-water-stressed plants (Tolley, 1982).

The water use trend in the seedlings may well have been caused by the temperature gradient among chambers and because values are so tightly grouped, it seems likely that evaporation directly from the soil surface in the pots accounts for a large fraction of the calculated ET values. The higher WUE of seedlings in the higher CO₂ concentrations may reflect smaller stomatal apertures that reduce water loss without affecting the uptake of CO₂.

A replicated study with older trees should be conducted to determine whether the improvements in WUE at elevated CO₂ concentrations observed in the seedlings would persist.

References

- Akita, S. and D.N. Moss. 1972. Differential stomatal response between C3 and C4 species to atmospheric CO₂ concentration and light. *Crop Sci.* 12:789-793.
- Downton, W.J.S., O. Bjorkman, and C.S. Pike. 1980. Consequences of increased atmospheric concentrations of carbon dioxide for growth and photosynthesis of higher plants. In: CO₂ and Climate, Australian Research, G.I. Pearman, Ed. CSIRO, Aust. Acad. Sci., Canberra.
- Fischer, R.A. and N.C. Turner. 1978. Plant production in the arid and semi-arid zones. *Ann. Rev. Plant Physiol.* 29:277-317.
- Kimball, B.A. 1983. Carbon dioxide and agricultural yield: An assemblage and analysis of 430 prior observations. *Agron. J.* 75:779-788.
- King, K.M., and D.H. Greer. 1986. Effects of carbon dioxide enrichment and soil water on maize. *Agron. J.* 78:515-521.
- Kramer, P.J. 1983. *Water Relations of Plants*. Academic Press, New York.
- Oberbauer, S.F., B.R. Strain and N. Fletcher. 1985. Effect of CO₂ enrichment on seedling physiology and growth of two tropical tree species. *Physiol. Plant.* 65:352-356.
- Rawlins, S.L. 1976. Measurement of water content and the state of water in soils. In: *Water Deficits and Plant Growth Vol. 4*, T.T. Kozlowski, Ed. Academic Press, New York.
- Rogers, H.H., J.F. Thomas, and G.E. Bingham. 1983. Response of agronomic and forest species to elevated atmospheric carbon dioxide. *Science* 220:428-429.

Tolley, L.C. 1982. The effects of atmospheric carbon dioxide enrichment, irradiance, and water stress on seedling growth and physiology of *Liquidambar styraciflua* and *Pinus taeda*. PhD. Dissertation, Duke University, Durham, NC.

Tolley, L.C. and B.R. Strain. 1984. effects of CO₂ enrichment on growth of *Liquidambar styraciflua* and *Pinus taeda* seedlings under different irradiance levels. Can. J. For. Res. 14:343-350.

Wittwer, S.H. 1984. Carbon dioxide levels in the biosphere: Effects on plant productivity. CRC Critical Reviews in Plant Sciences 2:171-198.

**NUTRIENT STATUS RESPONSE OF
PINUS PONDEROSA SEEDLINGS EXPOSED TO LONG-TERM
ELEVATED ATMOSPHERIC CO₂ CONCENTRATIONS**

P.D. Anderson¹, J.L.J. Houpis², R.J. Palassou², K. A. Surano³
and Jeremy Fried¹

¹Department of Forestry and Resource Management
University of California
Berkeley, California 94720

Health and Ecological Assessment Division²
Environmental Protection Department³
Lawrence Livermore National Laboratory
P.O. Box 808
Livermore, CA 94550

**LAWRENCE LIVERMORE NATIONAL LABORATORY
University of California • Livermore, CA • 94550 • USA**

Introduction

The current global concentration of atmospheric carbon dioxide (CO₂) of approximately 350 μLL^{-1} has been projected to double sometime in the next century (Bacastow and Keeling, 1973; Clark et al., 1982; Dahlman et al., 1985). Such an increase will likely have an effect on terrestrial plants (Dahlman et al., 1985). Past studies using annual plants have shown that photosynthetic rates can be increased, and water use decreased, in the presence of elevated atmospheric CO₂ (Kramer, 1981; Kimball, 1983; Lemon, 1983). However, it can be expected that response to elevated CO₂ will vary among species (Strain, 1978; Strain and Bazzaz, 1983). Because there is a potential for long-term exposure of vegetation to elevated CO₂, there is a need to determine whether the physiological response of perennial species to elevated atmospheric CO₂ is different from the response of annual species (Wrey and Strain, 1987).

Physiological changes in response to elevated atmospheric CO₂ will likely be associated with changes in plant nutrient status. Nutrient elements have a wide variety of roles related to physiological processes and, therefore, changes in process rates will be accompanied by changes in nutrient demand. Several studies have shown that production response to elevated CO₂ is limited by low nutrient supply (Zangerl and Bazzaz, 1984; Sionit, 1983; Conroy et al., 1986).

Analysis of foliar nutrient status has been previously utilized in research regarding the response of conifers to elevated atmospheric CO₂. Luxmoore et al. (1986) found that for *P. virginiana* Mill., grown in nutrient-poor soil, increases in the content and uptake of N, Ca, Al, Fe, Zn, and Sr occurred with CO₂ enrichment. Uptake of P and K was not increased with increasing CO₂.

In this study we investigated how the foliar nutrient status of ponderosa pine (*Pinus ponderosa* var. *scopulorum*) seedlings was affected by long-term exposure to elevated atmospheric CO₂.

Materials And Methods

Seedlings of *P. ponderosa* var. *scopulorum* were selected for exposure to elevated CO₂ in standard open-top exposure chambers. The chambers were cylindrical aluminum frames, 3 m in diameter and 2.4 m in height, covered on the sides by clear polyvinyl chloride (PVC) film (Heagle et al., 1973; Rogers et al., 1983; Surano et al., 1986). The seedlings were two years old at the onset of exposure. The seedlings were grown in 20-cm diameter, 4-l volume pots in a

soil mixture of equal parts of clay-loam soil, perlite, redwood compost, and Monterey #4 sand. Through the course of the study, all pots were watered to capacity three times per week. Seedlings were fertilized each month during the growing season ($0.55 \mu\text{g N}$, $0.44 \mu\text{g P}$, $0.33 \mu\text{g K}$, $0.33 \mu\text{g S}$, $0.16 \mu\text{g Ca}$, $0.055 \mu\text{g Fe}$ per seedling per month). The outdoor exposures to elevated CO_2 were continuous 24 hours per day from July 1983 to October 1985. Levels of CO_2 were ambient ($+0 \mu\text{LL}^{-1}$), ambient + $75 \mu\text{LL}^{-1}$ ($+75 \mu\text{LL}^{-1}$), ambient + $150 \mu\text{LL}^{-1}$ ($+150 \mu\text{LL}^{-1}$), and ambient + $300 \mu\text{LL}^{-1}$ ($+300 \mu\text{LL}^{-1}$), or approximately 350, 425, 500, and $650 \mu\text{LL}^{-1} \text{ CO}_2$. This fumigation regime accounted for both diurnal and seasonal fluctuations of ambient atmospheric CO_2 concentrations and tracked them accordingly. A detailed description of the experimental site, exposure methods and monitoring protocols is given in Surano et al. (1986).

On September 22, 1985, following twenty-eight months of continuous fumigation, 6-g samples of both current-year and one-year-old foliage were collected from each of four seedlings per CO_2 treatment. The samples were immersed in liquid nitrogen upon collection, stored on dry ice, freeze-dried, and ground to pass a #20-mesh screen. A subsample was analyzed for total N by the micro-Kjeldahl method (Bremner, 1965). Another was ashed and analyzed for P, K, S, Ca, Mg, Mn, Fe, Cu, B, and Zn by plasma emission spectroscopy (Jones, 1977). We calculated elemental concentrations on both dry weight (mg g^{-1} or $\mu\text{g g}^{-1}$) and surface area (mg cm^{-2} or $\mu\text{g cm}^{-2}$) bases to elucidate differences in results that may arise due to the units of expression.

Many fertilization studies have revealed that plant nutrient status depends not only on the concentrations of individual elements but also on the balance among nutrient elements (Beaufils, 1973; Sumner, 1979; van den Driessche, 1974). To assess the effect of elevated atmospheric CO_2 on the nutrient balance of the seedlings, we evaluated several elemental ratios. Ratios of N to P (N/P) and Mn to Fe (Mn/Fe) were calculated using concentrations based on dry weight. Because surface area concentrations were calculated by multiplying dry weight concentrations by specific leaf area, ratios based on surface area concentrations have the same values as ratios based on dry weight concentrations. Ca to Mg (Ca/Mg), Ca to K (Ca/K), and Ca plus Mg to K (Ca+Mg/K) ratios were calculated using equivalents of element, rather than weight of element, per unit foliage dry weight. This was done because these ratios among cations may be relevant to cation exchange types of reactions

and, therefore, balance of valence charges may be of greater interest than ratios of elemental mass.

We used three established guidelines to determine if nutrients were present in deficient or excessive concentrations. We compared our elemental concentration values to minimum threshold levels (Powers, 1974), to percentile rankings based upon Weibull probability distributions derived from natural populations (Zinke and Stangenberger, 1979), and to a range of observed values in nursery-produced seedlings (Wilde et al., 1972). Elemental ratios were compared to percentile rankings for natural populations (Zinke, unpublished data).

We evaluated the significance of CO₂ level, foliage-age, and CO₂ x foliage-age interaction effects for elemental concentrations and elemental ratios using a two-factor analysis of variance (ANOVA). If the ANOVA proved significant, treatment means were then subjected to the Student-Newman-Keuls multiple range test to determine the significance of differences among means.

Results

Elemental Concentrations. Relative to critical concentrations defined by Powers (1984), K concentrations in one-year-old foliage were deficient for all CO₂ levels. The concentrations of Cu in one-year-old foliage were deficient in the +75 μLL^{-1} and +300 μLL^{-1} CO₂ treatments. The concentrations of all other elements exceeded minimum threshold values. With the exception of S, similar results were obtained when comparing elemental concentrations to ranges compiled by Wilde et al. (1972) from pine nursery stock production. The concentration of S in the current-year foliage was in excess in all CO₂ treatments except the +150 μLL^{-1} CO₂ treatment. The Weibull distribution percentile rankings (Zinke and Stangenberger, 1979) for the foliar concentrations of N, Ca, Mg, Mn, Fe, and Zn were 50 or greater indicating above average concentrations relative to natural populations of *Pinus ponderosa*. The percentile rankings for N concentration of one-year-old foliage were very high as they exceeded 99 for all CO₂ levels. The percentile rankings for K concentrations were below 50 except for one-year-old foliage in the +0 μLL^{-1} and the +75 μLL^{-1} levels. The percentile rankings for K concentration of current-year foliage in the +150 μLL^{-1} and +300 μLL^{-1} levels were less than 25, indicating a deficiency (Zinke and Stangenberger, 1979).

Figure 1. a) Elemental concentration of nitrogen and phosphorus in current-year and one-year-old foliage of *Pinus ponderosa* seedlings grown at different levels of CO₂. Elemental concentrations are expressed as percent difference from the elemental concentration of seedlings grown at +0 $\mu\text{L L}^{-1}$ CO₂. b) N/P ratio of current-year and one-year-old foliage of *Pinus ponderosa* seedlings grown at different levels of CO₂.

Results from the ANOVA indicated a significant effect of CO₂ treatment on the foliar dry weight concentration of K, Ca, B, Fe, and Zn (Table 1). The effect of CO₂ concentration varied among these elements. Foliar K concentrations of seedlings grown at +0 μLL^{-1} and +75 μLL^{-1} were greater than those of seedlings grown at +150 μLL^{-1} and +300 μLL^{-1} . Ca concentration increased with increased CO₂ concentration from 2.72 mg g⁻¹ at +0 μLL^{-1} to 3.35 mg g⁻¹ at +300 μLL^{-1} . There were no discernible trends in the foliar concentrations of B, Fe, and Zn with respect to CO₂ treatment.

CO₂ treatments had a statistically significant effect on the concentration per unit foliage surface area of Ca, N, Mg, and B (Table 1). The CO₂ levels in which maximum and minimum mean concentration values occurred varied among the elements. With the exception of Ca, trends in elemental concentrations with increased CO₂ levels were not found. Ca concentration increased with increased CO₂ concentrations from 0.056 mg cm⁻² at +0 μLL^{-1} to 0.095 mg cm⁻² at +300 μLL^{-1} .

The effects of foliage-age class on elemental concentrations, on both dry weight and surface area bases, were significant for all macronutrients plus Cu and Fe (Table 2). Nutrients having greater concentrations in current-year foliage were N, P, K, and S. Nutrients having greater concentrations in one-year-old foliage were Ca, Mg, Fe, and B. Al concentrations were significantly higher in current-year foliage only when expressed on a dry weight basis. Zn concentrations were significantly higher in one-year-old foliage only when expressed on a surface area basis.

Significant CO₂ level x foliage-age interaction effects occurred only for P concentration expressed on a dry weight basis (Figure 1a). The dry weight concentration of P tended to decrease with increased CO₂ levels in current-year foliage while there was no discernible trend with increased CO₂ levels in one-year-old foliage.

Elemental Ratios. The N/P ratio increased with increased CO₂ concentrations from 10.5 at +0 μLL^{-1} to 12.8 at +300 μLL^{-1} (Figure 2). However, only the N/P ratio for seedlings growing at +300 μLL^{-1} was statistically greater ($0.05 < p < 0.1$) than the N/P ratio for seedlings grown at +0 μLL^{-1} . Foliage-age effects were highly significant as the N/P ratio was greater in current-year foliage than in one-year-old foliage. There was also a highly significant interaction ($0.01 < p < 0.05$) among foliage-age and CO₂ level (Figure 1b). The ratio was relatively constant for one-year-old foliage among CO₂ levels. In

Table 1. Concentrations of nutrient elements per unit foliar biomass and per unit surface area in *Pinus ponderosa* seedlings exposed to different levels of atmospheric CO₂. Values are CO₂ treatment means pooled over foliage-age classes.

Element	CO ₂ Concentration (μL L ⁻¹)				Signif. Level
	+0	+75	+150	+300	
-----Biomass basis (mg g ⁻¹) -----					
N	19.0	18.5	19.6	21.0	0.226
P	1.7	1.6	1.7	1.6	0.127
K	6.5	6.2	4.7	4.8	0.007
Ca	2.7	2.9	3.3	3.4	0.071
Mg	1.8	1.9	1.6	1.6	0.004
-----Biomass basis (μg g ⁻¹) -----					
Cu	4.2	3.1	3.6	2.8	0.386
B	46.9	45.3	40.8	48.8	0.093
Zn	53.0	39.5	44.1	40.9	0.009
Fe	140.9	100.5	120.8	102.9	0.084
Mn	164.5	140.4	126.4	171.4	0.177
Al	118.6	111.5	123.6	102.9	0.308
-----Area basis----- (mg cm ⁻²) -----					
N	0.391	0.503	0.468	0.581	0.033
P	0.040	0.043	0.040	0.045	0.640
K	0.132	0.168	0.112	0.134	0.101
Ca	0.056	0.082	0.095	0.082	0.025
Mg	0.037	0.052	0.040	0.045	0.036
-----Area basis----- (μg cm ⁻²) -----					
Cu	0.09	0.08	0.09	0.08	0.956
B	0.95	1.26	1.01	1.38	0.040
Zn	1.10	1.09	1.08	1.16	0.971
Fe	3.00	2.82	3.02	2.78	0.977
Mn	3.54	3.94	3.04	4.90	0.177
Al	2.46	3.05	3.03	2.84	0.535

contrast, the N/P ratio in current-year foliage increased with increased levels of CO₂.

There was a tendency for greater Ca/Mg concentration ratios with increased CO₂ concentrations (Figure 2). The ratio ranged from 0.91 at +0 μLL⁻¹ to 1.27 at +300 μLL⁻¹. The ratio for seedlings grown at +0 μLL⁻¹ and +75 μLL⁻¹ differed significantly from the ratio for seedlings grown at +150 μLL⁻¹ and +300 μLL⁻¹. Foliage-age effects were highly significant as the ratios were 1.20 and 1.00 in the one-year-old and current-year foliage, respectively. There was no significant interaction among CO₂ levels and foliage- age effects.

Table 2. Concentrations of nutrient elements per unit foliar biomass and per unit surface area of current-year and one-year-old foliage of *Pinus ponderosa* seedlings exposed to different levels of atmospheric CO₂. Values are foliage-age class means pooled over CO₂ levels.

Element	Foliage Age Class		Signif. Level
	Current-year	One-year-old	
---Biomass basis (mg g ⁻¹)-----			
N	24.8	14.2	0.000
P	6.0	3.8	0.000
K	7.0	4.1	0.000
Ca	2.5	3.4	0.000
Mg	1.6	1.9	0.004
---Biomass basis (µg g ⁻¹)-----			
Cu	3.8	3.1	0.228
B	43.8	47.1	0.147
Zn	42.5	46.3	0.185
Fe	90.1	142.4	0.000
Mn	145.8	155.6	0.530
Al	124.3	104.0	0.019
---Foliar basis (mg cm ⁻²)-----			
N	0.596	0.376	0.000
P	0.207	0.130	0.000
K	0.699	0.413	0.000
Ca	0.061	0.097	0.000
Mg	0.037	0.050	0.010
---Foliar basis (µg cm ⁻²)-----			
Cu	0.09	0.08	0.723
B	1.05	1.25	0.092
Zn	0.99	1.22	0.096
Fe	2.11	3.70	0.003
Mn	3.51	4.21	0.248
Al	2.93	2.76	0.608

Both CO₂ level and foliage-age were very highly significant ($p < 0.01$) in affecting the Ca/K ratio (Figure 2). The Ca/K ratio was 0.95 at +0 µLL⁻¹ and approximately 1.5 at +150 µLL⁻¹ and +300 µLL⁻¹ (Table 2). The Ca/K ratio for seedlings grown at +0 µLL⁻¹ and +75 µLL⁻¹ differed significantly from the ratio for seedlings grown at +150 µLL⁻¹ and +300 µLL⁻¹ CO₂. The ratio for one-year-old foliage was 1.77 versus 0.77 for current-year foliage.

There was a significant effect of CO₂ treatment on the Ca+Mg/K ratio. The ratio was greater with increased CO₂ concentrations to +150 µLL⁻¹, then was slightly lower at +300 µLL⁻¹. The ratios at +150 µLL⁻¹ and +300 µLL⁻¹ CO₂

Figure 2. Ratios of foliar nutrient concentrations for *Pinus ponderosa* seedlings grown at different levels of atmospheric CO₂. For each ratio, means marked with the same letter do not differ significantly ($p < 0.10$).

were significantly greater than the ratio at +0 $\mu\text{L L}^{-1}$ CO₂. The ratio was significantly greater for one-year-old foliage than for current-year foliage. Significant CO₂ level x foliage-age interaction was not detected.

Differences in the Mn/Fe ratio among CO₂ levels and CO₂ x foliage-age interaction were not significant. However, the difference in the Mn/Fe ratio among foliage-age classes was highly significant. Ratios for one-year-old and current-year foliage were 1.20 and 1.69, respectively.

Discussion

In our study we collected foliage samples of two age classes on a single day in mid-September. Sampling at this time provided information most suitable for assessing the adequacy of immobile elements such as Ca, Mg, and Al. The concentrations of these elements increase during the growing season and stabilize in the fall (Mead and Pritchett, 1974; Mead and Will, 1976; van den Driessche, 1974). Deficiencies of mobile elements such as N, P, Mg, and Zn may be more difficult to determine from our data. Sampling for these elements is best done during peak demand at midgrowing season (Mead and Pritchett, 1974; Mead and Will, 1976; Powers, 1984). The assessment of K is regarded as difficult in any season because high between- and within-tree variability may prevent detection of shortages (Mead and Pritchett, 1974; Mead and Will, 1976). Our results contradict the literature in that we were able to detect significant differences in K concentration based on a sample of only four replicates per CO₂ treatment.

The significant effect of foliage-age on dry weight nutrient concentrations found in this study generally agrees with results found throughout the literature. The mobile elements, N, P, K, and S, had greater concentrations in current-year foliage than in one-year-old foliage. Whereas, concentrations of immobile elements, Ca and Fe, were greater in one-year-old foliage than in current-year foliage (Mead and Pritchett, 1974; Mead and Will, 1976; van den Driessche, 1974; Powers, 1984). In our study, Mg concentration increased with foliage-age, suggesting that it is an immobile element. In contrast, it has been shown that foliar Mg concentration in slash pine (Mead and Pritchett, 1974) and radiata pine (Mead and Will, 1976) changes with season in a pattern similar to the mobile elements. This disparity in results may be due to our sampling at a single point in time. As a mobile element, Mg may vary in concentration seasonally and show a net annual change in concentration similar to that of an immobile element.

Foliar nutrient status has been expressed in many ways. Our results indicate that the basis of expression may be important in determining whether statistically significant differences among CO₂ treatments are obtained. When concentrations of elements were expressed on a dry weight basis, significant differences among CO₂ treatments were obtained for Ca, K, Fe, B, and Zn. By expressing concentration on a surface area basis, additional significant differences were detected for N and Mg while treatment differences for Fe and

Zn were not significant. Concentrations expressed on a dry weight basis are more common in the literature and, therefore, our results expressed on this basis are more easily compared to those from previous research. Concentrations expressed per unit surface area may be preferable when the elements under consideration have metabolic roles associated with light- and temperature-moderated processes (Smith et al., 1981; van den Driessche, 1974).

Unpublished data (Houpis and Surano) from our study indicate that stomatal conductance values were lower for pine seedlings exposed to higher levels of atmospheric CO₂. The observed reduction in stomatal conductance may be due to a direct effect of high CO₂ concentration on stomatal guard cells (Patterson and Flint, 1982). Alternatively, reduced K concentrations observed at high levels of CO₂ may contribute to lower rates of water use. K serves as an osmoticum for the turgor-mediated process of stomatal opening. Low foliar K concentrations may be associated with lower guard cell turgor and, therefore, with reduced stomatal aperture.

Our results show a possible inverse relationship between Ca and K concentrations similar to that observed by Wells (1965) in loblolly pine. When measured as individual elements, Ca concentration increased and K concentration decreased with increased CO₂ levels. Thus, the Ca/K ratio tends to increase with elevated CO₂ (Figure 2). Also, the Ca/K ratio was significantly greater in one-year-old foliage, relative to current-year foliage, indicating that accumulation of Ca as a structural component of tissue may be involved in this inverse relationship. Given the greater needle mass and greater specific leaf weight at high levels of CO₂ (Table 3), it is reasonable to assume that there will be a higher Ca concentration associated with these increases in structural carbon. In addition, there was a large decrease in K concentration, relative to the increase in Ca concentration, at higher CO₂ levels. This would cause an increase in Ca concentration relative to K concentration with increased CO₂.

The ratio of divalent cations to monovalent cations, Ca+Mg/K, increased with increased CO₂ levels. This can be expected given that Ca/K ratios and Ca/Mg ratios were greater at high levels of CO₂ (Figure 2). The values of Ca+Mg/K observed in this study greatly exceed the 99 percentile ranking of Zinke (unpublished data). The reason for a predominance of divalent cations in the foliage is unknown. One possibility is that long-term irrigation coupled with

Table 3. Needle weight, needle surface area, and specific leaf area for *Pinus ponderosa* seedlings exposed to different levels of atmospheric CO₂.

CO ₂ Conc. (μLL^{-1})	Foliage Age (yr)	Sample Size (n)	Weight (gm)	Surface Area (cm ²)	Specific Leaf Weight (gm cm ⁻²)
+0	1985	4	15.8	814	0.0169
	1984	4	4.8	293	0.0161
	All Ages	4	21.6	1149	0.0166
+75	1985	4	21.9	894	0.0255
	1984	4	6.3	229	0.0290
	All Ages	4	31.5	1245	0.0259
+150	1985	4	24.3	1098	0.0222
	1984	4	12.2	464	0.0264
	All Ages	4	37.3	1590	0.0236
+300	1985	4	25.1	956	0.0263
	1984	4	7.8	262	0.0291
	All Ages	4	34.5	1293	0.0268

fertilization resulted in the cation exchange sites of the soil being dominated by the more strongly held Ca and Mg.

Our analyses indicate that the N/P dry weight ratio increased with elevated CO₂ from 10.0 at +0 μLL^{-1} to 12.5 at +300 μLL^{-1} (Figure 2). For natural stands of ponderosa pine, N/P ratios of 10.0 and 12.5 correspond to percentile rankings of 47 and 63, respectively (Zinke, unpublished data). This greater ratio may indicate improved nitrogen status for seedlings at high CO₂ concentrations. Comerford and Fischer (1984) found that slash pine with a foliar N/P ratio less than 12 were more responsive to fertilization. Alternatively, it may be argued that seedlings grown at high CO₂ levels may have a greater demand for N and, therefore, require a greater N/P ratio. In our study, all seedlings, regardless of CO₂ level, had high foliar concentrations of N and thus, N deficiencies were unlikely.

Significant differences among foliar-age classes were observed in N/P ratios. The greater N/P ratios in current-year foliage may have been the result

of differential rates of N and P translocation. The samples were collected near the end of September when mobile elements could be expected to be moving out of older, senescing foliage into younger foliage.

Differential translocation may also be evident as CO₂ level x foliage-age interaction effects, which were highly significant for N/P ratios. With increased CO₂ concentration, the N/P ratio for one-year-old foliage remains relatively constant while the N/P ratio for current-year foliage increases. The concentrations of both N and P in one-year-old foliage were relatively constant among CO₂ treatments (Figure 2). The concentration of N in current-year foliage increased only slightly with increased CO₂ concentrations. In contrast, the P concentration of current-year foliage decreased significantly at higher concentrations of CO₂. Therefore, the increased N/P ratio was predominantly the result of lower P concentrations rather than higher N concentrations, at high levels of CO₂.

We suggest two possible explanations for the increased N/P ratio of current-year foliage at high CO₂ levels. The higher N/P ratios of current-year tissue at the +150 μLL^{-1} and +300 μLL^{-1} CO₂ levels may be associated with high rates of needle abscission observed in these treatments (Houpis et al., 1986). The seedlings in the high-CO₂ treatments retained few needles older than 1984. As the older needles senesced, the P they contained may have been translocated to younger tissue in a lower proportion than N. A second possible explanation is there may be a differential uptake for P and N associated with CO₂ level, with N uptake increasing at elevated CO₂ and P uptake being reduced or unchanged (Luxmore et al. 1986).

In our investigation, elevated atmospheric CO₂ resulted in changes in the foliar nutrient status of fertilized *Pinus ponderosa* seedlings. The reasons for, and the physiological consequences of, these changes cannot be fully determined from our data. As dependent variables of ANOVA, nutrient ratios increased our ability to detect CO₂ treatment effects. Relative to the results for individual elements, the nutrient ratio results provided a stronger basis for inferring possible physiological and morphological consequences of altered nutrient status. Future investigations should include seasonal foliar analyses to allow better assessment of foliar nutrient dynamics. Attempts should be made to quantitatively relate the status of element concentrations and ratios to specific physiological processes.

References

- Bacastow, R. and C.D. Keeling. 1973. Atmospheric carbon dioxide and radiocarbon in the natural carbon cycle. II Changes from AD 1700 to 2070 as deduced from a geochemical model. In Carbon and the Biosphere. Eds. G. Woodwell and E. Pecan. USAEC, Washington, D.C. pp.86-134.
- Beaufils, E.R. 1973. Diagnosis and recommendation integrated system (DRIS). A general scheme of experimentation and calibration based on principles developed from research in plant nutrition. Soil Sci. Bull. 1, Univ. of Natal, Pietermaritzburg, South Africa.
- Bremner, J.M. 1965. Total nitrogen, Methods of Soil Analysis, Part 2, (Agron. Monogr. 9), Amer. Soc. Agron., Madison, Wisconsin, pp. 1149-1176.
- Clark, W.C., K.H. Cook, G. Marland, A.M. Weinburg, R.M. Rotty, P.R. Bell, L.J. Allison and C.L. Cooper. 1982. The carbon dioxide question: perspectives for 1982. In Carbon Dioxide Review: 1982. Ed. W.C. Clark. Oxford Univ. Press, New York. pp.3-44.
- Comerford, N.B. and R.F. Fisher. 1984. Using foliar analysis to classify nitrogen-deficient sites. Soil Sci. Soc. Am. J. 48:910-913.
- Conroy, J., E.W. Barlow and D.I. Bevege. 1986. Response of *Pinus radiata* seedlings to carbon dioxide enrichment at different levels of water and phosphorus: growth, morphology and anatomy. Annals of Botany 57:165-177.
- Dahlman, R.C., B.R. Strain and H.H. Rogers. 1985. Research on the response of vegetation to elevated atmospheric carbon dioxide. J. Environ. Qual. 14(1):1-8.
- Heagle, A.S., D.E. Body and W.W. Heck. 1973. An open-top field chamber to assess the impact of air pollution on plants. J. Environ. Qual. 2(3):365-368.
- Houpis, J.L.J., K.A. Surano, P.F. Daley and J.H. Shinn. 1986. Growth and morphology of *Pinus ponderosa* seedlings fumigated for two years with elevated CO₂. Proc. Physiological and Genetic Basis of Forest Decline. Ninth North American Forest Biology Workshop, Stillwater, Oklahoma. pp.19-26.
- Jones, J.B. Jr. 1977. Elemental analysis of soil extracts and plant tissue ash by plasma emission spectroscopy. Comm. Soil Sci. Plant Anal. 8(4):349-365.
- Kimball, B.A. 1983. Carbon dioxide and agricultural yield: An assemblage and analysis of 430 prior observations. Agron. J. 75:779-788.
- Kramer, P.J. 1981. Carbon dioxide concentration, photosynthesis, and dry matter production. BioScience 31:29-33.

- Lemon, E.R. 1983. CO₂ and plants: the response of plants to rising levels of atmospheric carbon dioxide. Ed. E.R. Lemon. Amer. Assoc. Adv. Sci. Selected Symp. 84 Westview Press, Boulder, CO.
- Luxmoore, R.J., E.G. O'Neill, J.M. Ellis, and H.H. Rogers. 1986. Nutrient uptake and growth responses of Virginia pine to elevated atmospheric carbon dioxide. J. Environ. Qual. 15(3):244-251.
- Mead, D.J. and W.L. Pritchett. 1974. Variation of N, P, K, Ca, Mg, Mn, Zn, Al in slash pine foliage. Comm. Soil Sci. Plant Anal. 5(4):291-301.
- Mead, D.J. and G.M. Will. 1976. Seasonal and between-tree variation in the nutrient levels in *Pinus radiata* foliage. N. Z. J. For. Sci. 6(1):3-13.
- Patterson, D.T. and E.P. Flint. 1982. Interacting effects of CO₂ and nutrient concentration. Weed Sci. 30:389-394.
- Powers, R.F. 1974. Evaluating fertilizer programs using soil analysis, foliar analysis, and bioassay methods. Proc. 5th Calif. Forest Soil Fert. Conf. 1974:124-162. USDA Forest Serv., Washington, D.C.
- Powers, R.F. 1984. Estimating soil nitrogen availability through soil and foliar analysis. In Earl L. Stone (ed.) Forest Soils and Treatment Impacts (1984). Proc. 6th N. Amer. For. Soils Conf., June 1983.
- Rogers, H.H., W.W. Heck, and A.S. Heagle. 1983. A field technique for the study of plant responses to elevated carbon dioxide concentrations. J. Air Pollution Control Assoc. 33(1):42-44.
- Sionit, N. 1983. Response of soybean to two levels of mineral nutrition in CO₂-enriched atmosphere. Crop Sci. 23:329-333.
- Smith, R.B., R.H. Waring, and D.A. Perry. 1981. Interpreting foliar analyses from Douglas-fir as weight per unit of leaf area. Can. J. For. Res. 11:593-598.
- Strain, B.R. 1978. Report of the workshop on anticipated plant responses to global carbon dioxide enrichment. Ed. B.R. Strain. Duke Univ. Environ. Cen. Durham, N.C.
- Strain, B.R. and F.A. Bazaaz. 1983. Terrestrial plant communities. In CO₂ and Plants: The Response of Plants to Rising Levels of Atmospheric Carbon Dioxide. Ed. E.R. Lemon. Amer. Assoc. Adv. Sci. Selected Symp. 84. Westview Press, Boulder, Co. pp177-222.
- Sumner, M.E. 1979. Interpretation of foliar analyses for diagnostic purposes. Agron. J. 71:343-348.

- Surano, K.A., P.F. Daley, L.J.L. Houpis, J.H. Shinn, J.A. Helms, R.J. Palassou and M.P. Costella. 1986. Growth and physiological responses of *Pinus ponderosa* Dougl ex P. Laws. to long-term elevated CO₂ concentrations. *Tree Physiology* 2:243-259.
- van den Driessche, R. 1974. Prediction of mineral nutrient status of trees by foliar analysis. *Bot. Rev.* 40:347-393.
- Wells, C.G. 1965. Nutrient relationships between soils and needles of loblolly pine (*Pinus taeda* L.). *Soil Sci. Soc. Am. Proc.* 29:621-624.
- Wilde, S.A., G.K. Voigt, and J.G. Iyer. 1972. Soil and plant analysis for tree culture. Oxford and IBH Publishing Co., New Dehli, Bombay, and Calcutta, 4th edition.
- Wrey, S.M. and B.R. Strain. 1987. Competition in old-field perennials under CO₂ enrichment. *Ecology* 68(4):1116-1120.
- Zangerl, A.R. and F.A. Bazaaz. 1984. The response of plants to elevated CO₂. II. Competitive interactions among annual plants under varying light and nutrients. *Oecologia* 62:412-417.
- Zinke, P.J. and A.G. Stangenberger. 1979. Ponderosa pine and Douglas-fir foliage analyses arrayed in probability distributions. *Proc. Forest Fertilization Conference*. Sept. 25-27, 1979. Union, Wa.

**CHLOROPHYLL AND CAROTENOID CONCENTRATIONS
IN TWO VARIETIES OF *PINUS PONDEROSA* SEEDLINGS
SUBJECTED TO LONG-TERM ELEVATED CARBON DIOXIDE**

J.L.J. Houpis¹, K. A. Surano², S.Cowles¹, and J.H. Shinn¹

¹Health and Ecological Assessment Division

²Environmental Protection Department

Lawrence Livermore National Laboratory

P.O. Box 808

Livermore, CA 94550

**LAWRENCE LIVERMORE NATIONAL LABORATORY
University of California • Livermore, CA • 94550 • USA**

Introduction

Global atmospheric CO₂ concentrations have been steadily increasing. If CO₂ concentrations continue to increase as projected, early in the next century the global concentrations should double from the present level of approximately 350 μLL^{-1} (Bacastow and Keeling, 1973; Clark et al., 1982). It is generally acknowledged that such an increase will have an effect upon almost all terrestrial plants. While it is generally accepted that most plants will benefit from elevated CO₂ levels (Carter and Peterson, 1983; Wray and Strain, 1987), there have been reports that increasing CO₂ concentrations will not be beneficial to all species (Cave et al., 1981; Surano et al., 1986).

If certain plant species will be subjected to stress due to increasing CO₂, it becomes important to characterize the effect and mechanisms of the response to stress. Such stress could result in potential decreases in productivity or changes in plant distribution. In previous studies, pigmentation was examined as a means to determine the level of air-pollution-induced stress, even though no visible damage was observed (Gilbert, 1968; Knudson et al., 1977; Berkerson and Hofstra, 1979; Lauenroth and Dodd, 1981). In our study we examined pigmentation of *P. ponderosa* grown under elevated CO₂ concentrations and determined if there were alterations in pigmentation that paralleled other reported observations, including differing varietal and age responses (Surano et al., 1986; Houppis et al., 1986; Houppis et al., 1987).

Materials and Methods

Two varieties of *P. ponderosa* were selected for exposure to elevated CO₂ in standard open-top chambers. The open-top chambers were cylindrical aluminum frames, 3 m in diameter and 2.4 m in height, covered on the sides by clear polyvinyl chloride (PVC) film (Heagle et al., 1973; Rogers et al., 1983; Surano et al., 1986). The varieties were *P. ponderosa* var. *scopulorum* (Rocky Mountain variety) and *P. ponderosa* var. *ponderosa* (Sierran variety). At the onset of exposure, the Sierran and Rocky Mountain varieties were one and two years old, respectively. The seedlings were grown in 20-cm diameter, 4-l volume pots in a soil mixture of equal parts of clay-loam soil, perlite, redwood compost, and Monterey #4 sand. Through the course of the exposure all seedling pots were watered to capacity three times per week. Seedlings were fertilized each month during the growing season (0.55 μg N, 0.44 μg P, 0.33 μg K, 0.33 μg S, 0.16 μg Ca, 0.055 μg Fe per seedling per month). The exposures

to elevated CO₂ were continuous 24 hours per day from July 1983 to October 1985. Levels of CO₂ were ambient (+0 μLL⁻¹), ambient + 75 μLL⁻¹ (+75 μLL⁻¹), ambient + 150 μLL⁻¹ (+150 μLL⁻¹), and ambient + 300 μLL⁻¹ (+300 μLL⁻¹), or approximately 350, 425, 500, and 650 μLL⁻¹ CO₂. This fumigation regime accounted for both diurnal and seasonal fluctuations of ambient atmospheric CO₂ concentrations and tracked them accordingly. A detailed description of the experimental site and exposure set up is given in Surano et al. (1986).

In October 1985, needle samples were collected for analysis of chlorophyll a, chlorophyll b, and carotenoid concentrations. Concentrations are expressed on a per unit leaf surface area basis. Five seedlings from each variety in each CO₂ treatment were sampled and processed immediately. One fascicle was collected off each seedling from the 0-year-old age class of needles, and one fascicle was collected off each seedling from the 1-year-old age class of needles. Thus, there was a total of 80 samples. For each fascicle, the leaf surface area was determined geometrically by measuring the length and radii of each needle of each fascicle. Next, the fascicles were cut into 1-cm segments and placed into vials containing 25 ml of dimethylformamide. The samples were then brought back to the laboratory for pigment extraction. The samples were kept in darkness and at 4 °C for 48 h for completion of pigment extraction (Moran and Porath, 1980). All extractions and measurements were carried out under a green safe light. A spectrophotometer (Beckman Inc., Fullerton, CA) was used to measure pigment concentrations of the extraction solutions.

Final data on pigment concentrations were analyzed using the Statistical Analysis System (SAS Institute Inc., Cary, NC). Analysis initially involved a three factor analysis of variance. The main factors were CO₂ level, variety, and needle age. The dependent variables were chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid concentrations. Multiple range comparisons of main effects and interactions were conducted using the Student-Newman-Keuls method. Finally, a multivariate analysis of variance (MANOVA) was performed. In this case, the dependent variable was total pigment concentration, and the independent variables were CO₂ level, variety, and age. Again analyses of main effects and interactions were performed.

Figure 1. The results of the three factor ANOVA with chlorophyll a concentration as the dependent variable. The three main factors are CO₂ level, variety, and needle age. Within each of the three main factors, values followed by the same letter are not statistically different. (Student-Newman-Keuls Multiple Range Test. See also, "Materials and Methods").

Results

Chlorophyll a. The seedlings exposed to CO₂ concentrations of +150 µLL⁻¹ and +300 µLL⁻¹ had a distinctly lower chlorophyll a concentration (Table 1). This reduced level of chlorophyll a concentration occurred regardless of age class of needles or variety. However, the reduction in chlorophyll a concentration was higher in the Sierran variety than in the Rocky Mountain variety, and was higher in the 0-year-old age class of needles than in the 1-year-old age class of needles. Chlorophyll a concentrations were lowest in the 1-year-old age class of the seedlings of the Rocky Mountain variety.

The ANOVA showed that needle chlorophyll a concentration was significantly affected by all main factors (needle age, variety, and CO₂ level). In Figure 1 are shown the results of the multiple range test for each main factor as

Figure 2. The results of the three factor ANOVA with chlorophyll b concentration as the dependent variable. The three main factors are CO₂ level, variety, and needle age. Within each of the three main factors, values followed by the same letter are not statistically different. (Student-Newman-Keuls Multiple Range Test. See also, "Materials and Methods").

they affect chlorophyll a concentration. There was a lower chlorophyll a concentration in the treatments exposed to elevated CO₂. This reduction in chlorophyll a concentration occurred at the +150 µLL⁻¹ and +300 µLL⁻¹ CO₂ treatments. The ANOVA also showed that the 0-year-old needles had a significantly larger concentration of chlorophyll a than 1-year-old needles. Additionally, leaf tissue from seedlings of the Sierran variety had more chlorophyll a than leaf tissue from those of the Rocky Mountain variety. Of the three first order interactions, only the CO₂ level x variety interaction showed any tendency towards significance ($p < 0.1$). At the higher CO₂ concentrations, leaf tissue from seedlings of the Sierran variety showed a larger decrease in its chlorophyll a concentration than leaf tissue from seedlings of the Rocky Mountain (Table 2 and 3). The CO₂ level x needle age interaction and the

Table 1. Resultant mean value pigment concentrations ($\mu\text{g cm}^{-2}$) in needles of *P. ponderosa* seedlings exposed for 2.5 years to different concentrations of CO_2 . The two varieties of seedlings exposed were Sierra (S) and Rocky Mountain (RM). Both 0-year-old and 1-year-old age classes of needles were analyzed.

		Pigment Concentrations ($\mu\text{g cm}^{-2}$)							
		CO_2 Concentration (μLL^{-1} above ambient)							
		+0		+75		+150		+300	
Pigment Class	Age Class	Variety							
		S	RM	S	RM	S	RM	S	RM
chlorophyll a	0	34.3	26.0	32.9	22.2	27.9	20.4	25.3	22.9
	1	34.0	18.2	31.6	21.9	18.1	16.8	20.9	14.9
chlorophyll b	0	18.9	10.3	16.9	15.9	16.6	13.6	12.5	14.3
	1	27.5	11.0	22.9	14.5	3.9	8.7	12.5	11.0
total chloro.	0	53.1	36.3	49.8	38.1	44.5	34.0	37.8	37.2
	1	61.4	29.2	54.5	36.4	22.1	25.6	33.4	25.8
carotenoids	0	10.3	9.6	10.2	6.0	7.7	6.0	8.0	6.8
	1	8.0	6.0	7.6	5.6	8.2	5.4	6.5	4.2

variety x needle age interaction were not significant in determining observed variations in chlorophyll a.

Chlorophyll b. The 1-year-old age class of needles from seedlings of both varieties that were exposed to CO_2 concentrations of $+150 \mu\text{LL}^{-1}$ showed a distinctly lower chlorophyll b concentration (Table 1). Also, the seedlings of the Sierran variety of either age class of needles that were exposed to CO_2 concentrations of $+300 \mu\text{LL}^{-1}$ showed a distinctly lower chlorophyll b concentration. Unlike chlorophyll a concentration, there were no discernible trends due to age class.

The ANOVA was used to determine that two main factors (variety and CO_2 level) significantly affected needle chlorophyll b concentration (Figure 2). Needle age was clearly insignificant in affecting the concentration of chlorophyll b. Using the multiple range test, we determined that the chlorophyll b concentration of leaf tissue from the $+0 \mu\text{LL}^{-1}$ and the $+75 \mu\text{LL}^{-1}$ treatments were significantly greater than the chlorophyll b concentration found in leaf tissue

Table 2. Resultant mean value pigment concentrations ($\mu\text{g cm}^{-2}$) for CO_2 level x variety interaction (data was pooled across both age classes). Within each pigment class, values followed by the same superscript letter are not statistically different (Student-Newman-Keuls Multiple Range Test. See also "Materials and Methods"). Multiple range results only apply to those pigment classes that showed a significant ANOVA interaction. S = Sierra variety; RM = Rocky Mountain variety.

Pigment Class	Pigment Concentrations ($\mu\text{g cm}^{-2}$)							
	CO ₂ Concentration (μLL^{-1} above ambient)							
	<u>+0</u>		<u>+75</u>		<u>+150</u>		<u>+300</u>	
	Variety							
	S	RM	S	RM	S	RM	S	RM
chlorophyll a	34.1 ^a	22.1 ^b	32.2 ^a	22.1 ^b	23.0 ^b	18.6 ^b	22.8 ^b	18.9 ^b
chlorophyll b	23.2 ^a	10.7 ^b	19.9 ^{ab}	15.4 ^{bc}	10.3 ^c	11.2 ^c	12.5 ^c	12.6 ^c
total chlorophyll	57.3 ^a	32.8 ^b	52.1 ^a	37.5 ^b	33.3 ^b	29.8 ^b	35.3 ^b	31.5 ^b
carotenoids	9.1	7.8	8.9	5.9	7.9	5.6	7.2	5.5

from the +150 μLL^{-1} and the +300 μLL^{-1} treatments. The multiple range test was also used to determine that the chlorophyll b concentration in leaf tissue from seedlings of the Sierran variety were higher than those found in seedlings of the Rocky Mountain variety (Figure 2). As determined by the multiple range test, the statistical trends in chlorophyll b concentration due to CO_2 level or variety were similar to those trends found in chlorophyll a concentrations. There was also a strong interaction between CO_2 level and variety (Table 3). At the higher CO_2 concentrations, leaf tissue from seedlings of the Sierran variety showed a larger decrease in chlorophyll b concentration than leaf tissue from seedlings of the Rocky Mountain variety. However, unlike the results found for chlorophyll a, there was also a strong interaction between CO_2 level and needle age. In Table 2, the CO_2 level x needle age class interaction is shown to be similar to the CO_2 level x variety interaction. The chlorophyll b concentration of the 1-year-old age class of needles was reduced to a greater extent at the higher CO_2 concentrations than was the chlorophyll b concentration of the 0-year-old age

Figure 3. The results of the three factor ANOVA with total chlorophyll concentration as the dependent variable. The three main factors are CO₂ level, variety, and needle age. Within each of the three main factors, values followed by the same letter are not statistically different. (Student-Newman-Keuls Multiple Range Test. See also, "Materials and Methods").

class of needles, with the lowest chlorophyll b concentration occurring at +150 µLL⁻¹.

Total Chlorophyll. Due to the predominance of chlorophyll a concentration in needle pigmentation, the resultant total chlorophyll concentrations reflect those results determined for chlorophyll a (Table 1). The results from the statistical analysis of total chlorophyll concentration are presented in Figure 3 and Tables 2 and 3. Regarding the main factors (needle age, variety, CO₂ level), these results paralleled the statistical results of the analysis of chlorophyll a. These include: (1) distinctly lower total chlorophyll concentration at the elevated CO₂ levels of +150 µLL⁻¹ and +300 µLL⁻¹ CO₂; (2) higher total chlorophyll concentration in leaf tissue from seedlings of the Sierran variety than in leaf tissue from seedlings of the Rocky Mountain variety; and (3)

Figure 4. The results of the three factor ANOVA with carotenoid concentration as the dependent variable. The three main factors are CO₂ level, variety, and needle age. Within each of the three main factors, values followed by the same letter are not statistically different. (Student-Newman-Keuls Multiple Range Test. See also, "Materials and Methods").

greater total chlorophyll concentration in the 0-year-old age class of needles than in the 1-year-old age class of needles. However, the statistical analysis showed that total chlorophyll and chlorophyll b concentrations had similar interactions. Total chlorophyll had significant CO₂ level x variety and CO₂ level x age interactions (Tables 2 and 3).

Carotenoids. The 1-year-old age class of needles from seedlings of the Rocky Mountain variety showed a decline in carotenoid concentration with increasing CO₂ concentration (Table 1). For the 0-year-old age class of needles from seedlings of the Rocky Mountain variety, the highest carotenoid concentration occurred at +0 µLL⁻¹ CO₂, with an equal reduction in carotenoid concentration at all elevated CO₂ concentrations. The seedlings of the Sierran variety showed a lower carotenoid concentration at +150 µLL⁻¹ and +300 µLL⁻¹

Table 3. Resultant mean value pigment concentrations ($\mu\text{g cm}^{-2}$) for CO_2 level x needle age class interaction (data was pooled across both varieties). Within each pigment class, values followed by the same superscript letter are not statistically different (Student-Newman-Keuls Multiple Range Test. See also "Materials and Methods"). Multiple range results only apply to those pigment classes that showed a significant ANOVA interaction. 0 = current age class of needles; 1 = one-year old age class of needles.

Pigment Class	Pigment Concentrations ($\mu\text{g cm}^{-2}$)							
	CO ₂ Concentration (μLL^{-1} above ambient)							
	+0		+75		+150		+300	
	Needle Age Class							
	0	1	0	1	0	1	0	1
chlorophyll a	30.6	27.0	27.6	27.9	24.2	17.5	24.0	17.9
chlorophyll b	15.1 ^{ab}	20.2 ^a	16.4 ^{ab}	19.7 ^a	15.1 ^{ab}	6.3 ^c	13.5 ^{ab}	11.7 ^b
total chlorophyll	45.6 ^a	47.1 ^a	44.0 ^a	47.7 ^a	39.3 ^{ab}	23.8 ^c	37.4 ^{ab}	29.6 ^{bc}
carotenoids	10.0	7.1	8.1	6.9	6.7	6.8	7.4	5.3

for 0-year-old age class of needles, and at +300 μLL^{-1} for 1-year-old age class of needles.

In Figure 4 is shown the results of the main factors (needle age, variety, CO_2 level) of the ANOVA for the carotenoids. All main factors were significant; however, none of the interactions were significant. Carotenoid concentration did not demonstrate the same patterns seen in the chlorophylls with respect to CO_2 level (Figures 1,2, and 4; Tables 2 and 3). The multiple range test showed that leaf tissue from seedlings growing under +0 μLL^{-1} CO_2 had the greatest carotenoid concentration. There tended to be a decrease in carotenoid concentration as CO_2 concentration increased. The effect of variety and needle age was the same as that of the chlorophylls. The ANOVA indicated that the carotenoid concentration of leaf tissue from seedlings of the Sierran variety was statistically greater than that of leaf tissue from seedlings of the Rocky Mountain variety and that the carotenoid concentration of leaf tissue from the 0-year-old

Table 4. Results from the MANOVA. Resultant p values > F for the test variables derived from the MANOVA, where the dependent variable is total leaf pigmentation.

Effect	Wilkes' Criterion	Pillai's Trace	Hotelling-Lawley Trace
CO ₂	0.0011	0.0023	0.0005
Variety	0.0001	0.0001	0.0001
Age	0.0104	0.0104	0.0104
CO ₂ x Variety	0.0382	0.0512	0.0282
CO ₂ x Age	0.0162	0.0226	0.0115
Variety x Age	0.5126	0.5126	0.5126

needles was statistically greater than that of leaf tissue from the one-year-old needles.

Total Pigmentation Analysis. The results of the MANOVA are given in Table 4. Leaf pigmentation is defined as the collective concentrations of chlorophyll a, chlorophyll b, and carotenoids. All of the MANOVA test statistics indicated that the effects of CO₂ level on leaf pigmentation were highly significant ($p < 0.01$). Though all main factors showed significance in the MANOVA, only the CO₂ level x needle age and CO₂ level x variety showed significant interactions. The variety x needle age interaction showed no significance. The decreases in total pigmentation at the elevated CO₂ concentrations was consistent within varieties and within age classes. In general, total leaf tissue pigment concentrations in *P. ponderosa* needles decreased after extended elevated CO₂ exposure.

Discussion

There are at least two possible explanations for the observed reduction in pigmentation due to elevated CO₂ concentrations. The first possibility is that the seedlings were not stressed by the elevated CO₂, but rather an adaptive alteration in their physiology had occurred. In conjunction with an increase in leaf senescence and mid-needle abscission (Houpis et al., 1986), the reduction

in pigmentation at the higher CO₂ levels may be correlated to the plant not requiring as much photosynthetic apparatus. This may possibly be due to higher photosynthetic rates that are inherent in C3 species due to moderate increases in ambient CO₂ (Ludwig, 1974). A reduction in chlorophyll concentration with a corresponding increase in growth under elevated CO₂ has been previously reported (Downton et al., 1980; Patterson and Flint, 1982; Delucia et al., 1985; Oberbauer et al., 1985). Complicating the issue, however, is that others have found a reduction in chlorophyll does not necessarily occur with increases in growth (Hesketh, 1963; Havelka et al., 1984; St.Omer and Horvath, 1984). Likewise, Patterson and Flint (1982) found delayed leaf senescence due to elevated CO₂, whereas we found the opposite. If we restrict our discussion to tree species, there was an increase in reported growth and chlorophyll by Koch et al. (1983), but an increase in growth with a concomitant decrease in chlorophyll was reported by Oberbauer et al. (1985). Therefore, because of the varied results found in the literature, generalizations concerning chlorophyll content and growth cannot be made with information currently available. Furthermore, our study indicated that the magnitude of the reduction in pigmentation may be mediated by intraspecific variation in their response to elevated CO₂.

A second possible explanation is that the seedlings were under stress due to increasing CO₂ concentrations. This effect would occur at CO₂ concentrations above +75 μLL^{-1} . This may be a more plausible explanation because all of the above mentioned studies reported an increase in growth, where we found a decrease in growth at the highest CO₂ concentrations (Houpis et al., 1986). Furthermore, this decrease in growth was more pronounced in the Sierran variety, which also had the greatest reduction in chlorophyll. Also, the CO₂ level x needle age interaction on pigmentation corresponds to the increase in senescence and mid-needle abscission that was observed to a much greater extent in the older age class of needles. Both of these effects, along with a reported increases in water stress symptoms, needle temperatures, and ethylene production, increased with CO₂ concentration (Surano et al., 1986; Houpis et al., 1987).

References

- Bacastow, R. and C.D. Keeling. 1973. Atmospheric carbon dioxide and radiocarbon in the natural carbon cycle. II. Changes from AD 1700 to 2070 as deduced from a geochemical model. In Carbon and the Biosphere. Eds. G. Woodwell and E. Pecan. USAEC, Washington, D.C. pp.86-134.
- Beckerson, D.W. and G. Hofstra. 1979. Effect of sulphur dioxide and ozone singly or in combination on leaf chlorophyll, RNA, and protein in white bean. Can. J. Bot. 57:1940-1945.
- Carter, D.R. and K.M. Peterson. 1983. Effects of a CO₂-enriched atmosphere on the growth and competitive interaction of a C3 and C4 grass. Oecologia 58:188-193.
- Cave, G., L.C. Tolley and B.R. Strain. 1981. Effect of carbon dioxide enrichment on chlorophyll content, starch content and starch grain structure in *Trifolium subterraneum* leaves. Physiol. Plant. 51:171-174.
- Clark, W.C., K.H. Cook, G. Marland, A.M. Weinburg, R.M. Rotty, P.R. Bell, L.J. Allison and C.L. Cooper. 1982. The carbon dioxide question: perspectives for 1982. In Carbon Dioxide Review: 1982. Ed. W.C. Clark. Oxford University Press, New York. pp.3-44.
- DeLucia, E.H., T.W. Sasek and B.R. Strain. 1985. Photosynthetic inhibition after long-term exposure to elevated levels of atmospheric carbon dioxide. Photosyn. Res. 7:175-184.
- Downton, W.J.S., O. Bjorkman and C.S. Pike. 1980. Consequences of increased atmospheric concentrations of carbon dioxide for growth and photosynthesis of higher plants. In Carbon Dioxide and Climate. Ed. G.I. Pearman. Australian Research, Australian Academy of Science, Canberra, Australia. pp.143-151.
- Gilbert, O.L. 1968. Biological Indicators of Air Pollution. Ph.D. Thesis, University of Newcastle-on-Tyne, Great Britain.
- Havelka, U.D., V.A. Wittenbach and M.G. Boyle. 1984. CO₂-enrichment effects on wheat yield and physiology. Crop Sci. 24:1163-1168.
- Heagle, A.S., D.E. Body and W.W. Heck. 1973. An open-top field chamber to assess the impact of air pollution on plants. J. Environ. Qual. 2:365-368.
- Hesketh, J.D. 1963. Limitations to photosynthesis responsible for differences among species. Crop Sci. 3:493-496.
- Houpis, J.L.J., K.A. Surano, P.F. Daley and J.H. Shinn. 1986. Growth and morphology of *Pinus ponderosa* seedlings fumigated for two years with elevated CO₂. Proc. Physiological and Genetic Basis of Forest Decline.

Ninth North American Forest Biology Workshop, Stillwater, Oklahoma.
pp.19-26.

Houpis, J.L.J., K.A. Surano and J.H. Shinn. 1987. Seasonal and diurnal water use patterns of *Pinus ponderosa* seedlings exposed for two years with elevated carbon dioxide. Supplement to: Plant Physiol. 83:45.

Knudson, L., W. Tibbitts and G. Edwards. 1977. Measurements of ozone injury by determination of leaf chlorophyll concentration. Plant Physiol. 60:606-608.

Koch, K.E., D.W. White, P.H. Jones and L.H. Allen. 1983. CO₂ enrichment of carrizo citrange and swingle citrumelo rootstocks. Proc. Fla. State Hort. Soc. 96:37-40.

Lauenroth, W.K. and J.L. Dodd. 1981. Chlorophyll reduction in western wheatgrass (*Agropyron smithii* Rydb.) exposed to sulfur dioxide. Water, Air, and Soil Pollution 15:309-315.

Lugwig, L.J. 1974. Effects of light flux density, CO₂ enrichment and temperature on leaf photosynthesis. Rep. Glasshouse Crops. Res. Inst. 1973:47-49.

Moran, R. and D. Porath. 1980. Chlorophyll determination in intact tissues using N,N-dimethylformamide. Plant Physiol. 65:478-479.

Oberbauer, S.F., B.R. Strain and N. Fetcher. 1985. Effect of CO₂ enrichment on physiology and growth of seedlings of two tropical tree species. Physiologia plantarum 65:352-356.

Patterson, D.T. and E.P. Flint. 1982. Interacting effects of CO₂ and nutrient concentration. Weed Sci. 30:389-394.

Rogers, H.H., W.W. Heck and A.S. Heagle. 1983. A field technique for the study of plant responses to elevated carbon dioxide concentrations. J. Air Pollution Control Assoc. 33:42-44.

St.Omer, L. and S.M. Horvath. 1984. Potential effects of elevated carbon dioxide levels on seed germination of three native plants species. Bot. Gaz. 144:477-480.

Surano, K.A., P.F. Daley, J.L.J. Houpis, J.H. Shinn, J.A. Helms, R.J. Palassou and M.P. Costella. 1986. Growth and physiological responses of *Pinus ponderosa* Dougl ex P. Laws. to long-term elevated CO₂ concentrations. Tree Physiol. 2:243-259.

Wray, S.M. and B.R. Strain. 1987. Competition in old-field perennials under CO₂ enrichment. Ecology 68:1116-1120.

**EXAMINING THE USE OF AN EXISTING
WESTERN CONIFEROUS FOREST GROWTH AND SUCCESSION
MODEL IN ELEVATED CO₂ SCENARIOS**

K.A. Surano¹, P.J. Tate¹, J.R. Kercher², J.H. Shinn² and J.L.J. Houpis²

¹Environmental Protection Department, L-621

²Health and Ecological Assessment Division, L-524

Lawrence Livermore National Laboratory

P.O. Box 808

Livermore, CA 94550

**LAWRENCE LIVERMORE NATIONAL LABORATORY
University of California • Livermore, CA • 94550 • USA**

Abstract

Atmospheric carbon dioxide (CO₂) concentrations are continuing to rise and will double during the next century. All species of plants will be affected by these increases, but differential species responses are likely. Changes in plant community and ecosystems may therefore result.

Very little information is available regarding the responses of plant communities and ecosystems to elevated CO₂. Simulation modelling may give insight into critical data needs so future research can be directed. Existing models may provide a starting point for simulation research. While accurate predictions using these models are impossible because of the assumptions that must be made in the simulations, the modelling exercises may serve useful purposes.

An existing forest simulation model, SILVA was adapted to run under elevated atmospheric CO₂ scenarios using differential species responses. A sensitivity analysis performed on the CO₂ response variable indicated that SILVA was moderately sensitive to CO₂. Two types of scenarios were run, one with CO₂ remaining constant, and the other with CO₂ increasing at 1 μLL^{-1} per year. Both positive and negative growth responses were incorporated into the scenarios.

Elevated CO₂ concentrations, depending on the scenario, had various impacts on the composition and structure of the forest. Total production either increased, decreased, or remained constant. Differential species responses were demonstrated, causing changes in dominance and community structure. One species exhibited increased production even when negatively impacted at CO₂ concentrations above 500 μLL^{-1} . Another species remained a minor component of the community, but played a significant role in the relative success of another species.

Simulation may be the only method to predict the responses of long-lived species to long-term elevated CO₂ concentrations. However, more experimental and modelling research is required before accurate predictions can be made.

Introduction

Global atmospheric carbon dioxide (CO₂) concentrations have increased significantly during this century, due mostly to fossil fuel emissions (Strain and Cure 1985; Goudriaan and Ketner, 1984). Because fossil fuel use is projected

to increase in the future, there is no longer a question of whether CO₂ concentrations will increase, but rather a question of how rapidly and high they will rise (Hoffman, 1984). Most scenarios predict CO₂ concentrations will continue to increase at a rate of 1-1.5 μLL^{-1} per year until they double sometime during the next century (Keeling and Bacastow, 1977).

Predicting the future fate of the biosphere is difficult because future conditions, including future atmospheric CO₂ concentrations, must be estimated. Most models and predictions of the future biosphere have generally been based on the simplifying assumption that atmospheric CO₂ concentrations remain essentially constant (Strain, 1985). Because that fundamental assumption appears invalid, forecasts of future conditions and their effects on the biosphere must be adjusted.

All species of plants will be affected by elevated CO₂ concentrations (see reviews by Lemon, 1983; Strain and Cure, 1985). Gates et al. (1983) stated that because carbon is the basic building block of life and CO₂ is the primary source of inorganic carbon, CO₂-induced effects on primary producers (plants) would also affect consumers and decomposers. If, as stated by Peterson (1969), the carbon cycle is the most sensitive of all systems controlling life, the forecasted changes in atmospheric CO₂ concentrations could have serious implications for the global biosphere.

There has been some evidence, through tree-ring examination, that effects of elevated atmospheric CO₂ are already discernable (LaMarche et al., 1985). The expression of plant effects from elevated CO₂ will likely become more dramatic with further increases in CO₂ concentrations. However, all species of plants will not be affected by elevated CO₂ concentrations in the same manner or magnitude. Therefore, changes in plant community structure and dynamics may occur.

A stated goal of CO₂ research is the estimation of future responses for both crops and ecosystems (Dahlman, 1984). Dahlman (1985b) expressed a need for scientifically-based ecosystem-level predictions to aid analyses of energy policy issues related to future fossil fuel emissions. Dahlman (1984) indicated researchers should study the effects of CO₂ on species relationships and the structure and functioning of communities and ecosystems. He also called for evaluations of the effects of CO₂ on ecosystem productivity and the potential for carbon storage, and for the development of models for predicting the effects of CO₂ alone and in combination with other principal factors

governing the structure and functioning of ecosystems. However, little data is currently available towards these ends.

Nearly all previous studies to date have involved relatively short-term exposures of crop species grown in typical monocultural conditions (Strain and Cure, 1985). While much knowledge has been gained about crop responses, results from crop studies are of little use for prediction of community and ecosystem responses to long-term increasing CO₂ concentrations (Strain and Cure, 1985; Dahlman 1984). Most crop studies have addressed the CO₂ effects on productivity and yield because of the importance of food. The questions asked about ecosystem and community responses are likely to be very different (Gates, 1983). The dearth of information regarding the responses of plant communities, and of perennial plants in particular, to elevated CO₂ has been mentioned often (Strain and Cure, 1985; Strain and Bazzaz, 1983; Dahlman, 1984; Gates, 1985; Lemon 1983).

Plant community responses to increased CO₂ may include changes in productivity (including changes in amounts of carbon sequestered in plant tissues), altered species composition, changes in dominance and altered plant distributions in space and time (Strain and Cure, 1985; Strain and Bazzaz, 1983; Bazzaz, 1985a; Reynolds and Acock, 1985a,b; Kimball, 1985; Idso, 1984; Dahlman 1985b; Wittwer, 1983; Lemon, 1983). Wittwer (1983) mentioned that the stability and magnitude of CO₂-induced production changes would be of importance. If differential species responses result in changed competitive interactions, succession will be affected. The stability of the community would then be altered. The actual effects on individual plant communities may be difficult to ascertain because interactions at that level are extremely complex.

Some limited experimental evidence does exist supporting the hypothesis that elevated CO₂ concentrations will affect plant communities and ecosystems. Direct competition experiments with annual plants have shown differential species responses to elevated CO₂ can cause changes in community composition, dynamics and productivity (Billings et al., 1984; Overdieck et al., 1984; Bazzaz and Carlson, 1984; Bazzaz et al., 1985b; Carter and Peterson, 1983; Patterson et al., 1984; Patterson and Flint, 1980; Carlson and Bazzaz, 1980; Zangerl and Bazzaz, 1984). Indirect evidence, based on photosynthesis measurements, of differential responses to elevated CO₂ in naturally competing perennial species has been reported (Wright, 1974; Teskey and Shrestha, 1985). One study reported a broad-leaved tree species

maintained height dominance over its conifer competitor at all CO₂ concentrations, but future interactions could cause changes in community composition (Sionit et al., 1985). Surano et al. (1986) showed elevated CO₂ exposures over two years caused detrimental effects to trees at the highest concentrations. Only a single study has reported the effects of elevated CO₂ on perennial species in direct competition (Williams et al., 1986). That study took place over a single growing season and indicated the total growth in the communities studied was not significantly affected, but the relative biomass of each species changed.

Forests occupy 1/3 of global land area and carry on 2/3 of global photosynthesis (Kramer, 1981), so effects on them may have global impacts. Primary production in forests could potentially increase under elevated CO₂, resulting in increased standing biomass and therefore increased sequestering of carbon (Botkin, 1977). However, forests have long generation times, and will be unable to adapt to increasing CO₂ concentrations as quickly as annual species (Kimball, 1985); trees currently beginning growth will reach their periods of greatest growth under much higher CO₂ concentrations. Instead, the response of long-lived tree species may be determined by their ability to acclimate to elevated CO₂ conditions (Kimball, 1985.)

Temperate forests may be a very important community type with respect to the CO₂ question. These forests have been estimated to be a sink for 20-60 percent of CO₂ released through fossil fuel combustion in the last 30 years (Armentano and Ralston, 1980). Additionally, temperate forests have great economic, ecological, recreational, and aesthetic value, so any future effects on them are of concern (Surano et al., 1986). However, very little previous work has been conducted regarding CO₂ effects on temperate forest species. Short-term studies on temperate forest species have shown photosynthesis increases and transpiration decreases under elevated CO₂ (Wright, 1974; Green and Wright, 1977; Bryan and Wright, 1976). Experiments with seedlings subjected to very high CO₂ concentrations in greenhouses have shown that growth can be increased (Funch et al., 1970; Tinus, 1972). Only two studies have looked at sapling-sized trees (Williams et al., 1986; Surano et al., 1986). There is almost no data on the effects of elevated CO₂ on whole mature trees (Baker and Enoch, 1983; Pollard, 1985; Strain and Bazzaz, 1983).

It is easy to lament over the lack of studies on the effects of elevated CO₂ on mature forest species, but it is difficult to suggest how such studies might be

readily accomplished. The technical difficulties and costs of scaling for such studies are staggering (Dahlman, 1984; Shugart and Emanuel, 1985). Experiments would need to be run over many years, because of the longevity of the species. The complexities of forest ecosystems would also hinder large-scale, long-term studies. Carbon dioxide does not act alone on plants; it is only one of many factors affecting ecosystems. For example, production in unmanaged ecosystems is more often limited by water and nutrients than by CO₂ (Kramer, 1981). Experiments designed to test all the interactions of CO₂ and other plant growth controlling factors would be impossible. One approach that may help understand and predict the responses of complex communities and ecosystems to elevated CO₂ is the use of simulation modelling techniques (Reynolds and Acock, 1985a,b; Dahlman, 1985a).

Eventually, models covering many life cycles and capable of extrapolation under elevated CO₂ will be developed. These models will begin on the process level and will scale up in complexity to the ecosystem level (Strain and Bazzaz, 1983; Strain and Cure, 1985; Reynolds and Acock, 1985a; Dahlman, 1985a). However, no such models currently exist (Dahlman, 1985a; Reynolds and Acock, 1985a). A strategy for developing suitable models of CO₂ effects on communities and ecosystems may involve adaptation of existing models (Dahlman, 1985b; Botkin, 1977).

Many process level photosynthesis models exist, but cannot be used to predict whole-plant production, let alone forest or ecosystem effects (Reynolds and Acock, 1985b; Allen et al., 1971; Lemon, 1973; Acock et al., 1976). Existing ecosystem models have limitations for prediction because of the paucity of suitable parameterization data (Miller et al., 1983; Reynolds and Acock, 1985a; Oechel and Strain, 1985). However, existing models can aid evaluations of existing data and can point out critical data needs so future research can be suitably directed (Strain and Bazzaz, 1983; Strain and Cure, 1985; Dahlman, 1984).

Existing forest succession models may be currently useful in evaluating the effects of elevated CO₂ on forest ecosystems before field-scale experiments are attempted (Dahlman, 1984). In the future, these models may be extremely useful for predictions if they can be adequately interfaced with mechanistic models of short-term growth dynamics (Reynolds and Acock, 1985a).

Previous attempts have been made at adapting successional models for use in elevated CO₂ scenarios. Botkin et al. (1973) used the JABOWA model

(Botkin et al., 1972) to estimate the effects of elevated CO_2 on a northeastern-U.S. forest by assuming the annual diameter increment for all species increased by equal amounts due to CO_2 . They used 4 scenarios, where the responses were assumed to be 10, 20, 50, and 100% increases in annual individual tree growth. They concluded elevated CO_2 concentrations would favor shade-tolerant, long-lived species and that succession would be accelerated. The stochastic properties of birth and death masked CO_2 effects and interactions among the species buffered the total ecosystem response. The final relative importance of each species was not greatly affected. Shugart and Emanuel (1985) made similar assumptions in their use of existing successional models in elevated CO_2 scenarios. They indicated the biomass response was less than the growth rate response. They admitted their scenarios were unrealistic and their model runs should not be used for predictive purposes. Additionally, they hypothesized that if changes in growth rate are not uniform (as they had assumed) the potential expression of CO_2 fertilization may involve changes in the structure of the community due to changes in competitiveness.

Another forest ecosystem model, SILVA, originally developed to explore the effects of sulfur dioxide pollution and fire on western-U.S. forest resources, may prove useful in the early stages of the modelling contexts mentioned above. The population dynamics of SILVA are similar to that of JABOWA, the northeastern-U.S. forest simulator of Botkin et al. (1972, 1973). Species composition and the age and size structures of each species are followed over time. The model consists of parameters which define silvicultural characteristics such as maximum height, diameter at breast height, and age, and a range of shade and moisture tolerances in which species can grow. SILVA calculates birth, growth, and death of trees each year and simulates competition between individuals.

The research reported here was an attempt to adapt SILVA for use in elevated CO_2 scenarios using differential species responses, and the assessment the usefulness of the model in that regard. It is stressed that the results from these primitive simulations are not predictive, and should be used with caution. However, the modelling exercises may help to determine if the model can be adapted for future projections, once appropriate data become available and the model is suitably parameterized.

Materials and methods

Description of the model. Complete descriptions of SILVA are found in Kercher and Axelrod (1981, 1982, 1984a). SILVA was written in ANSI FORTRAN and does not require any special libraries. It was developed on Control Data Corporation 7600 computers with the CDC RUN compiler at Lawrence Livermore National Laboratory, Livermore, CA. The model has recently been adapted to run on IBM personal computers using the Microsoft Fortran compiler version 3.31. The personal computer version of SILVA requires a minimum of 256K memory and is most efficiently run on machines that have the 8087 math co-processor.

SILVA simulates forests typical of the lower and mid-elevations of the Sierra Nevada. The mixed conifer forest is of great interest because it is the largest single vegetation type in California and has significant economic, ecologic, recreational and aesthetic value. The major tree species are ponderosa pine (*Pinus ponderosa* Laws.), white fir (*Abies concolor* (Gord. and Glend.) Lindl.), incense cedar (*Libocedrus decurrens* Torr.), Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco), sugar pine (*Pinus lambertiana* Dougl.) and black oak (*Quercus kelloggii* Newb.).

In the model, birth and death are stochastic processes where the probability of an event occurring is determined by comparison of a random number with the probability of occurrence. The probability of occurrence is a function of the state of the system at the time the event is possible. SILVA has several major subroutines which are called each year of the simulation. These include BIRTH, GROW, FIRE, and KILL. The BIRTH subroutine generates and establishes new seedlings in the stand. Subroutine GROW deterministically increments the biomass of each of the trees in the stand as a function of available light, leaf area, climate, soil conditions, water stress, respiratory losses and pollution insult. FIRE is the controlling subroutine for fire ecology. It calls routines that calculate fuel loading and determine if a fire will occur in a given year. If a fire does occur, fire intensity, the probability of death for each tree in the stand, and a reduction of fuel load are calculated. The KILL subroutine stochastically decides which trees are killed based on each tree's probability of death, which is determined by ecological risk, lack of growth and fire damage.

Modifications for CO₂ Simulations. Two subroutines, CO2CON and NHANCE, were added to SILVA for these modelling exercises. CO2CON is called at the beginning of each simulation and determines the CO₂

concentration scenario to be used in the experimental run. In one scenario, labeled "constant CO₂," it was assumed CO₂ concentration remains unchanged over time from current ambient levels (350 µLL⁻¹). All other scenarios used CO₂ concentrations that increased at the rate of 1 µLL⁻¹ per year.

Subroutine NHANCE is called for each year of the simulation. The subroutine calculates a growth multiplier (GRCO2) for the diameter increment equation in subroutine GROW. The growth multiplier is a function of the individual species responses and the CO₂ concentration during the particular year of the simulation. The equation for GRCO2 is:

$$\text{GRCO2} = \text{RESPO} * (\text{CONC} - \text{AMCONC}) + 1$$

where

RESPO = individual species response to elevated CO₂
CONC = the CO₂ concentration calculated in CO2CON
AMCONC = current ambient CO₂ concentration (350 µLL⁻¹).

Positive CO₂ Responses. Much of the existing literature regarding the effects of elevated CO₂ on plants is related to changes in net photosynthesis. However, the relationship between growth and net photosynthesis is uncertain. Since data was unavailable for differential species growth responses to elevated CO₂, a 1 to 1 correlation between increased net photosynthesis and increased growth was used. Although this was clearly an unrealistic assumption, it was a reasonable approach in light of the absence of appropriate data. The assumption allowed the model to run with differential species responses, unlike the simplifying assumptions of equal species responses made in previous modelling attempts (Botkin et al, 1973; Shugart and Emanuel, 1985).

Wright (1974) examined the effects of elevated CO₂ concentrations on photosynthesis of several competing San Bernardino mountain species. Doehlert and Walker (1981) examined the CO₂ effects on Douglas fir photosynthesis. Data from these studies was used to derive the CO₂ "growth" responses (RESPO variable) used in these simulations (Table 1).

Negative CO₂ Responses. Recent evidence from a pilot study suggested that long-term exposures to elevated CO₂ may cause detrimental effects on ponderosa pine (Surano et al., 1986; Houpis et al., 1986, 1988). In that study, the growth of ponderosa pine saplings and seedlings responded positively to

Table 1. Percent increase in net photosynthesis as a function of a 1 $\mu\text{L L}^{-1}$ increase in CO_2 concentration. Values are based on data from Wright (1974) and Doehlert and Walker (1981)

Species	Percent Increase
Douglas fir	0.0014
White fir	0.0018
Sugar pine	0.0020
Ponderosa pine	0.0031
Incense-cedar	0.0034
Black oak	0.0035

increased CO_2 concentrations up to 150 μLL^{-1} above present ambient concentrations, but serious reductions in growth were observed at higher concentrations. If some species show negative growth responses and others show positive responses, the effects on communities and ecosystems may be even larger than previously hypothesized. Therefore, some model runs were made with species having negative growth responses at CO_2 concentrations above 500 μLL^{-1} . Because ponderosa pine is the dominant species in the modelled community and data exist showing that it may respond in a negative fashion, its response was modelled as negative in all scenarios where negative responses occurred. The responses of other species alternated between positive and negative.

Negative responses of all species, when applicable, were assumed to be identical to the negative responses of ponderosa pine, based on data from the previously mentioned experiment. The negative growth response used was -0.0017 percent for each μLL^{-1} increase in CO_2 concentration. In the simulations, all species increased positively according to their previously mentioned differential positive rates until the simulation CO_2 concentration exceeded 500 μLL^{-1} (150 years). At that point, depending on the chosen scenario, species that were deemed to have negative responses began to respond negatively. In the data tables and figures, species names are followed by either + or -. These signs refer to the mode of response for each species in that particular scenario. A + sign means that species responded positively to

CO₂ over the entire model run. A - sign means that species responded positively for 150 years, and then responded negatively for 150 years.

Simulations. The simulations were run on a plot size of 400 m² at an elevation of 1525 m in the central Sierra Nevada. This xeric area of the western conifer forest is dominated by ponderosa pine, with the growth of white fir restricted due to lack of precipitation and available water. The simulations were begun at clearcut (year 0) and proceeded for 300 years. Results from the simulations are presented as yearly basal area per ha (m² ha⁻¹) for each species. The reported values are the calculated averages of 25 model runs due to the stochastic elements in the model. Similar averages were reported in previous modelling exercises. (Shugart and Emanuel, 1985).

Sensitivity Analysis. A sensitivity analysis was performed on the CO₂ response variable, RESPO, identical to that reported by Kercher and Axelrod (1984b). The sensitivity analysis was based on ponderosa pine basal area evaluated at year 50. The RESPO variable was changed by 10% of its original value and a sensitivity value was computed and compared to the sensitivity scale reported by Kercher and Axelrod (1984b).

Results

All six of the tree species found in the western coniferous forest were included in these simulations. However, Shinn and Daley (1984) showed that Douglas fir and black oak, the early successional species, were virtually unaffected by CO₂ concentrations and disappeared from the community by year 100. They also indicated that sugar pine was a minor constituent of the forest at all times and at all CO₂ concentrations. Results from the current model runs mirrored these previously reported responses. In each of the present model runs, the only significant basal area responses to CO₂ occurred in the three main species (ponderosa pine, white fir, and incense cedar). Therefore, for the sake of simplicity, only data from the three main species are displayed in the data tables and figures. For the remainder of the results section, as well as in the discussion, Table 2 and Figures 1-2, pine refers to ponderosa pine, fir refers to white fir and cedar refers to incense cedar.

Plots of basal area for each of the three main species over the 300-year model runs are shown in Figures 1 and 2. Results at 100-year intervals from each of the scenarios are found in Table 2. Comparisons between SILVA

Table 2. Specific basal area and total basal area (m²/ha) every 100 years for each of the model scenarios. Comparisons (% difference) between each elevated-CO₂ scenario and the constant-CO₂ scenario are made for each species and for total basal area in each year shown.

Scenario: Constant CO ₂ (350 μ L/L)			
	year 100	year 200	year 300
pine	31.6	40.3	46.9
fir	2.2	3.9	4.2
cedar	3.1	7.6	4.0
TOTAL	36.9	51.8	55.1

Scenario: pine+ fir+ cedar+						
	year 100	% Diff	year 200	% Diff	year 300	% Diff
pine	29.9	-5.4	41.7	3.5	48.8	4.1
fir	1.3	-40.9	3.7	-5.1	4.7	11.9
cedar	5.2	67.7	15.5	103.9	19.3	382.5
TOTAL	36.4		60.9		72.8	
% Diff	-1.4		17.6		32.1	

Scenario: pine- fir+ cedar+						
	year 100	% Diff	year 200	% Diff	year 300	% Diff
pine	36.0	13.8	29.2	-27.5	17.2	-63.4
fir	1.9	-15.5	4.5	14.1	4.2	0.0
cedar	4.1	32.9	15.7	106.6	35.2	780.5
TOTAL	41.9		49.4		56.6	
% Diff	13.6		-4.7		2.7	

Scenario: pine- fir- cedar+						
	year 100	% Diff	year 200	% Diff	year 300	% Diff
pine	33.0	4.5	33.2	-17.6	19.4	-58.7
fir	1.3	-42.7	2.6	-33.3	1.6	-61.9
cedar	4.4	41.9	8.5	12.4	23.5	487.3
TOTAL	38.7		44.4		44.5	
% Diff	4.9		-14.4		-19.3	

Scenario: pine- fir+ cedar-						
	year 100	% Diff	year 200	% Diff	year 300	% Diff
pine	32.2	1.9	36.0	-10.7	27.1	-42.2
fir	1.9	-15.5	2.9	-24.6	12.3	192.4
cedar	4.8	53.9	13.5	77.6	9.0	125.5
TOTAL	38.8		52.4		48.4	
% Diff	5.3		1.2		-12.1	

Scenario: pine- fir- cedar-						
	year 100	% Diff	year 200	% Diff	year 300	% Diff
pine	31.0	-1.9	37.2	-7.6	27.1	-42.1
fir	1.3	-42.7	2.6	-34.6	2.6	-39.3
cedar	4.8	53.9	10.2	34.5	13.3	233.5
TOTAL	37.0		50.0		43.0	
% Diff	0.4		-3.5		-21.9

Figure 1. Model output from SILVA of basal area of main species in the western coniferous forest over 300 years following clearcut: (a) CO_2 concentration remained constant at $350 \mu\text{L/L}$ for entire simulation; (b) CO_2 concentration increased at $1 \mu\text{L/L}$ per year and all species exhibited positive growth responses throughout the simulation.

Figure 2. Model output from SILVA of basal area of main species in the western coniferous forest over 300 years following clearcut. All scenarios assumed CO_2 increased at $1 \mu\text{LL}^{-1}$ per year. Species names followed by + had positive responses to elevated CO_2 throughout the course of the simulation. Species names followed by - had positive growth responses for the initial 150 years and negative responses for the last 150 years.

output prior to adaptation for CO_2 scenarios (constant CO_2 scenario) and all other scenarios are also found in Table 2.

Under conditions of constant CO_2 , ponderosa pine, with more than 10 times the basal area of the other two species, was clearly the dominant species (Figure 1a, Table 2). The amounts of fir and cedar basal areas were roughly equivalent, but were minor components of total community basal area. However, under conditions of elevated CO_2 concentrations, changes became apparent in community structure and productivity.

With all species exhibiting positive responses to increasing CO_2 , pine remained dominant (Figure 1b). However, pine exhibited only a 4% increase in basal area by year 300, and only showed 2.5 times as much basal area as cedar at that time (Table 2). Fir basal area essentially remained the same as in the constant CO_2 scenario. However, cedar basal area increased very

significantly. Total community productivity increased 32% over that produced in the constant CO₂ scenario, mostly due to increased cedar basal area production (Table 2). This was the only scenario that exhibited significantly increased community productivity.

When the model was run with pine alone responding negatively at CO₂ concentrations greater than 500 μLL^{-1} , cedar basal area increased most dramatically (Figure 2a). Cedar became the dominant species, with nearly twice the amount of basal area as ponderosa pine (Table 2). Fir basal area remained unchanged from that produced in the constant CO₂ scenario. At the end of 300 years, total community basal area productivity was nearly equal to that produced under constant CO₂, but the contribution of pine had declined while that of cedar had increased.

When both pine and fir exhibited negative responses to CO₂ concentrations greater than 500 μLL^{-1} , cedar became co-dominant with pine (Figure 2b). Pine was impacted less severely in this scenario than when it was the only species with a negative response (Table 2). Fir was nearly eliminated (Figure 2b). Total community basal area production decreased nearly 20%, although cedar basal area increased nearly 500% (Table 2).

When fir was the only species exhibiting positive responses throughout the model runs, it still was not a major component of community basal area (Figure 2c). Although cedar exhibited a negative growth response, its basal area increased 125% at 300 years over that produced under constant CO₂ conditions (Table 1). Total community basal area production was moderately reduced, mainly due to decline in ponderosa pine basal area (Figure 2c).

When the model was run with all species exhibiting negative responses to elevated CO₂ concentrations greater than 500 μLL^{-1} , total community basal area production decreased nearly 22% (Table 2). Pine remained the dominant species, although its contribution to the total community area was reduced (Figure 2d). Cedar basal area increased 233%, making its contribution to total community basal area more significant (Table 2). Fir basal area was reduced (Figure 2d).

The sensitivity value of the CO₂ response variable, RESPO, determined from the sensitivity analysis was 0.58. This corresponds to a moderately sensitive ranking according to the scale developed by Kercher and Axelrod (1984b).

Discussion

Elevated CO₂ concentrations had definite impacts on the composition and structure of the modelled western coniferous forest in these exercises. Although only major tree species were used in the simulations, it can be assumed that effects would be imposed at all levels in the ecosystem.

SILVA, using the crude assumptions discussed earlier, predicted increases in production similar to those reported by other investigators. All other investigators assumed that all species respond positively to elevated CO₂ concentrations. Under similar assumptions in these exercises, total community basal area increased by 32% by the end of the simulation. Shugart and Emanuel (1985) reported an estimated 35% increase in production under elevated CO₂ with a doubling of growth rates. Additionally, estimates of total agricultural production increases due to a doubling of CO₂ concentrations are usually in the 30-35% range (Strain and Cure, 1985).

However, when negative responses were considered, very different results were attained in the simulations. Total community basal area production either remained the same or decreased. The response of individual species in each scenario was also affected.

Because fir is not a major component of the western coniferous forest at this elevation, it was expected that CO₂ effects on its production would not have significant impacts in the community. In general, the role of fir was minor in all scenarios. For example, when fir was the only species exhibiting positive responses throughout the model run, it was unable to impact the other species greatly (Figure 2c). However, the response of fir was important in mediating the responses of cedar and pine in other scenarios.

When pine was the only species expressing negative responses, cedar was able to increase its total basal area at year 300 by over 700% when compared to the constant CO₂ scenario results (Figure 2a, Table 2). Cedar became the dominant species in the forest and total community production increased. When both fir and pine expressed negative effects, it was expected that cedar would become even more dominant. However, this was not the case. Cedar basal area in that scenario increased by approximately 500% (Figure 2b, Table 2). Cedar became co-dominant with pine and total community production was not increased. In the absence of a positive response from fir, cedar was unable to compete as strongly with pine.

The simulation results can be interpreted in the context of competition for light. Fir is a shade-tolerant species, pine is shade-intolerant and cedar is intermediate in shade tolerance. In this community, fir appears to be the strongest competitor for pine, and pine appears to be the most important competitor for cedar. Pine is clearly dominant under most conditions, probably because it is fast growing and is less likely to be fatally scorched by minor fires (Shinn and Daley, 1984). However, when both cedar and fir had positive responses, pine was replaced as the dominant species by cedar. If the competitiveness of fir did not increase, the positive responses of cedar were reduced.

The basal area of cedar, in comparison with that of other species, was most likely to increase under elevated CO₂ concentrations. The basal area of cedar increased in all scenarios, even when cedar exhibited negative responses to elevated CO₂. Pine production, on the other hand, was reduced in all scenarios except when all species responded positively.

When all species responded negatively, the results were similar to those produced when all responded positively (Figures 1b, 2d). Although cedar production increased, pine remained dominant, and fir remained a minor component of total community basal area. Total community basal area was, of course, much reduced when all species responded negatively especially when compared to that from the scenario when all species responded positively.

The moderately sensitive ranking for the CO₂ response variable from the sensitivity analysis is indicative that CO₂ was not an overwhelming factor in the community. Carbon dioxide was only one of many factors influencing community structure and dynamics. However, elevated CO₂ concentrations were clearly shown to have potentially significant effects on this community.

The modelling exercises reported here showed many different community responses. Total production either increased, decreased, or remained constant (Table 2). Differential species responses were demonstrated. Expected positive results were observed. However, one species exhibited increased basal area even when negatively impacted at concentrations above 500 μLL^{-1} . Still another species remained a minor component of the community, but played a significant role in the relative success of another species.

Evidence of a high potential for large CO₂-induced community effects in the western coniferous forest has been shown both experimentally and through

simulation modelling. Future experiments should be conducted so that models can be adapted and properly parameterized. Future experimental work should be focused to explicitly determine appropriate response terms for modelling. Future modelling should address other parameters of community ecology that may interact with elevated CO₂ concentrations, including nutrient and water availability.

If we are resigned to a future environment where atmospheric CO₂ concentrations are greatly increased, there will be effects on plant communities and ecosystems. Dahlman (1984) indicated the ecosystems of greatest interest for study were those that had 1-large geographic expanse, 2-high economic value, 3-potentially significant effects on global carbon balance, and 4-had investigators with scientific expertise and facilities available to work in them. The western coniferous forest meets all of these criteria. It is the largest single vegetation type in California, it has high economic value for logging and recreation, and it is a major sink of CO₂ (Armentano and Ralston, 1980) with much carbon sequestered in standing biomass. Additionally, experienced investigators and existing, validated models are available. Simulation may be the only method to predict the responses of such long-lived species to long-term elevated CO₂ concentrations. However, more experimental and modelling research is required before accurate predictions can be made. SILVA may be a good candidate for future adaptations.

References

- Acock B, Hand DW, Thornley JHM, Wilson JW (1976) Photosynthesis in stands of green peppers: An application of empirical and mechanistic models to controlled-environment data. *Ann Bot* 40:1293-1307
- Allen LH, Jensen SE, Lemon ER (1971) Plant response to carbon dioxide enrichment under field conditions: A simulation. *Science* 173:256-258
- Armentano TV, Ralston CW (1980) The role of temperate forests in the global carbon cycle. *Can J For Res* 10:53-60
- Baker DN, Enoch HZ (1983) Plant growth and development. In: Lemon, ER (ed) CO₂ and plants. Westview Press, Boulder, CO
- Bazzaz FA, Carlson RW (1984) The response of plants to elevated CO₂ I. Competition among an assemblage of annuals at different levels of soil moisture. *Oecologia* (Berlin) 62:196-198

- Bazzaz FA, Garbutt K, Williams WE (1985a) Effect of increased atmospheric carbon dioxide concentration on plant communities. In: Strain BR, Cure JD (eds) Direct effects of increasing carbon dioxide on vegetation. DOE/ER-0238, U.S. Department of Energy, Office of Energy Research, Carbon Dioxide Research Division, Washington, DC
- Bazzaz FA, Garbutt K, Williams WE (1985b) The effect of elevated atmospheric CO₂ on plant communities. DOE-EV-04329-5, U.S. Department of Energy, Office of Energy Research, Carbon Dioxide Research Division, Washington, DC
- Billings WD, Peterson KM, Luken JO, Mortensen DA (1984) Interaction of increasing atmospheric carbon dioxide and soil nitrogen on the carbon balance of tundra microcosms. *Oecologia* (Berlin) 65:26-29
- Botkin DB (1977) Forests, lakes, and the anthropogenic production of carbon dioxide. *BioScience* 27:325-331
- Botkin DB, Janak JF, Wallis JR (1972) Some ecological consequences of a computer model of forest growth. *J Ecol* 60:849-872
- Botkin DB, Janak JF, Wallis JR (1973) Estimating the effects of carbon fertilization on forest composition by ecosystem simulation. In: Woodwell GM, Pecan EV (eds) Carbon and the biosphere. CONF-720510, U.S. Atomic Energy Commission, Washington, DC
- Bryan W, Wright RD (1976) The effect of enhanced CO₂ levels and variable light intensities on net photosynthesis in competing mountain trees. *Am Midl Nat* 95:446-450
- Carlson RW, Bazzaz FA (1980) The effects of elevated CO₂ concentrations on growth, photosynthesis, transpiration, and water use efficiency of plants. In: Singh JJ, Deepak A (eds), Environmental and climatic impact of coal utilization. Academic Press, New York, NY
- Carter DR, Peterson KM (1983) Effects of a CO₂-enriched atmosphere on the growth and competitive interaction of a C3 and C4 grass. *Oecologia* (Berlin) 58:188-193
- Dahlman RC (1984) Vegetation response to carbon dioxide research plan. DOE-ER-0187, U.S. Department of Energy, Office of Energy Research, Carbon Dioxide Research Division, Washington, DC
- Dahlman RC (1985a) Preface. In: Strain BR, Cure JD (eds) Direct effects of increasing carbon dioxide on vegetation. DOE/ER-0238, U.S. Department of Energy, Office of Energy Research, Carbon Dioxide Research Division, Washington, DC

- Dahlman RC (1985b) Modeling needs for predicting responses to CO₂ enrichment: Plants, communities, and ecosystems. *Ecol Modelling* 29:77-106
- Doehlert DC, Walker RB (1981) Photosynthesis and photorespiration in Douglas-fir as influenced by irradiance, CO₂ concentration, and temperature. *Forest Sci* 27:641-650
- Funsch RW, Mattson RH, Mowry GR (1970) Carbon dioxide-supplemented atmosphere increases growth of *Pinus strobus* seedlings. *Forest Sci* 16:459-460
- Gates DM (1983) An overview. In: Lemon ER (ed) CO₂ and plants. Westview Press, Boulder, CO
- Gates DM (1985) Global biospheric response to increasing atmospheric carbon dioxide concentration. In: Strain BR, Cure JD (eds) Direct effects of increasing carbon dioxide on vegetation. DOE/ER-0238, U.S. Department of Energy, Office of Energy Research, Carbon Dioxide Research Division, Washington, DC
- Gates DM, Strain BR, Weber JA (1983) Ecophysiological effects of changing atmospheric CO₂ concentration. In: Lange OL, Nobel PS, Osmond CB, Ziegler H (eds) Physiological plant ecology IV. Springer-Verlag, New York, NY
- Goudriaan J, Ketner P (1984) A simulation study for the global carbon cycle, including man's impact on the biosphere. *Climatic Change* 6:167-192
- Green K, Wright RD (1977) Field response of photosynthesis to CO₂ enhancement in ponderosa pine. *Ecology* 58:687-692
- Hoffman JS (1984) Carbon dioxide and future forests. *J Forestry* 82:164-167
- Houpis JLJ, Surano KA, Cowles S, Shinn JH (1988) Chlorophyll and carotenoid concentrations in two varieties of *Pinus ponderosa* seedlings subjected to long-term elevated carbon dioxide. *Tree Physiol.* 4:187-193.
- Houpis JLJ, Surano KA, Daley PF, Shinn JH (1986) Growth and morphology of *Pinus ponderosa* seedlings exposed to long-term elevated atmospheric carbon dioxide concentrations. In: Tauer CG, Hennessey TC (eds) Proceedings of the ninth North American forest biology workshop. Department of Forestry, Division of Agriculture, Oklahoma State University, Stillwater, OK
- Idso SB (1984) Atmospheric CO₂ variability: A cause for concern in the reconstruction of past climates. *Speculations in Sci and Technol* 7:37-40

- Keeling DC, Bacastow RB (1977) Impact of industrial gases on climate. In: National Academy of Sciences (eds) Energy and climate. Geophysics Research Board, Geophysics Study Committee, Washington, DC
- Kercher JR, Axelrod MC (1981) SILVA: A model for forecasting the effects of SO₂ pollution on growth and succession in a western coniferous forest. UCRL-53109, Lawrence Livermore National Laboratory, Livermore, CA
- Kercher JR, Axelrod MC (1982) Modeling the individual and community effects of air pollution stress in a western coniferous forest. UCRL-87747, Lawrence Livermore National Laboratory, Livermore, CA
- Kercher JR, Axelrod MC (1984a) A process model of fire ecology and succession in a mixed conifer forest. Ecology 65:1725-1742
- Kercher JR, Axelrod MC (1984b) Analysis of SILVA: a model for forecasting the effects of SO₂ pollution and fire on western coniferous forest. Ecol Modelling 23:165-184
- Kimball BA (1985) Adaptation of vegetation and management practices to a higher carbon dioxide world. In: Strain BR, Cure JD (eds) Direct effects of increasing carbon dioxide on vegetation. DOE/ER-0238, U.S. Department of Energy, Office of Energy Research, Carbon Dioxide Research Division, Washington, DC
- Kramer PJ (1981) Carbon dioxide concentration, photosynthesis and dry matter production. BioScience 31:29-33
- LaMarche VC Jr, Graybill DA, Fritts HC, Rose MR (1985) Increasing atmospheric carbon dioxide: Tree ring evidence for growth enhancement in natural vegetation. Science 225:1019-1021
- Lemon ER (1973) Predicting crop climate and net carbon dioxide exchange. Photosynthetica 7:408-413
- Lemon ER (ed) (1983) CO₂ and plants. Westview Press, Boulder, CO
- Miller PC, Kendall R, Oechel WC (1983) Simulating carbon accumulation in northern ecosystems. Simulation 40:119-131
- Oechel WC, Strain BR (1985) Native species responses to increased carbon dioxide concentration. In: Strain BR, Cure JD (eds) Direct effects of increasing carbon dioxide on vegetation. DOE/ER-0238, U.S. Department of Energy, Office of Energy Research, Carbon Dioxide Research Division, Washington, DC
- Overdieck D, Bossemeyer D, Lieth H (1984) Long-term effects of an increased CO₂ concentration level on terrestrial plants in model-ecosystems I.

Phytomass production and competition of *Trifolium repens* L. and *Lolium perenne* L. *Progress in Biometeorol* 3:344-352

Patterson DT, Flint EP (1980) Potential effects of global atmospheric carbon dioxide enrichment on the growth and competitiveness of Carbon 3 and Carbon 4 weed and crop plants. *Weed Sci* 28:71-75

Patterson DT, Flint EP, Beyers JL (1984) Effects of CO₂ enrichment on competition between a C4 weed and C3 crop. *Weed Sci* 32:101-105

Peterson EK (1969) Carbon dioxide affects global ecology. *Environ Sci and Technol* 3:1162-1169

Pollard DFW (1985) A forestry perspective on the carbon dioxide issue. *Forestry Chron* (August): 312-318

Reynolds JF, Acock B (1985a) Modeling approaches to evaluating vegetation responses to carbon dioxide concentration. In: Strain BR, Cure JD (eds) *Direct effects of increasing carbon dioxide on vegetation*. DOE/ER-0238, U.S. Department of Energy, Office of Energy Research, Carbon Dioxide Research Division, Washington, DC

Reynolds JF, Acock B (1985b) Predicting the response of plants to increasing carbon dioxide: A critique of plant growth models. *Ecol Modelling* 29: 107-129

Shinn JH, Daley PF (1984) Response of plants to increased carbon dioxide. *Energy and Technol Review*, UCRL-52000-84-9. Lawrence Livermore National Laboratory, Livermore, CA

Shugart HH, Emanuel WR (1985) Carbon dioxide increase: The implications at the ecosystem level. *Plant, Cell and Environ* 8:381-386

Sionit N, Strain BR, Hellmers H, Riechers GH, Jaeger CH (1985) Long-term atmospheric CO₂ enrichment affects the growth and development of *Liquidambar styraciflua* and *Pinus taeda* seedlings. *Can J For Res* 15:468-471

Strain, BR (1985) Physiological and ecological controls on carbon sequestering in terrestrial ecosystems. *Biogeochem* 1:219-232

Strain BR, Bazzaz FA (1983) Terrestrial plant communities. In: Lemon ER (ed) *CO₂ and plants*. Westview Press, Boulder, CO

Strain BR, Cure JD (eds) (1985) *Direct effects of increasing carbon dioxide on vegetation*. DOE/ER-0238, U.S. Department of Energy, Office of Energy Research, Carbon Dioxide Research Division, Washington, DC

- Surano KA, Daley PF, Houpis JLJ, Shinn JH, Helms JA, Palassou RJ, Costella MP (1986) Growth and physiological responses of *Pinus ponderosa* Dougl ex P. Laws. to long-term elevated CO₂ concentrations. *Tree Physiol* 2: 243-259
- Teskey RO, Shrestha RB (1985) A relationship between carbon dioxide, photosynthetic efficiency and shade tolerance. *Physiol Plant* 63:126-132
- Tinus RW (1972) Carbon dioxide enriched atmosphere speeds growth of ponderosa pine and blue spruce seedlings. *Tree Planter's Notes* 23: 12-17
- Williams WE, Garbutt K, Bazzaz FA, Vitousek PM (1986) The response of plants to elevated CO₂ IV. Two deciduous-forest tree communities. *Oecologia* (Berlin) 64: 454-459
- Wittwer SH (1983) Conference background. In: Lemon ER (ed) CO₂ and plants. Westview Press, Boulder, CO
- Wright RD (1974) Rising atmospheric CO₂ and photosynthesis of San Bernardino Mountain plants. *Am Midl Nat* 91:360-370
- Zangerl AR, Bazzaz FA (1984) The response of plants to elevated CO₂ II. Competitive interactions among annual plants under varying light and nutrients. *Oecologia* 62: 412-417

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