EPA/600/A-96/077

ENVIRONMENTAL MODIFICATION AND SHOOT GROWTH IN A CLOSED ECOSYSTEM TO EVALUATE LONG-TERM RESPONSES OF TREE SEEDLINGS TO STRESS

D.M. Olszyk and D.T. Tingey
U.S. EPA, National Health and Ecological Effects Laboratory, Western Ecology Division, Corvallis, Oregon 97333

Abstract

Determination of ecosystem responses to stress requires careful control of the environment and measurement of biological effects. Closed chambers provide appropriate environmental control and measurement, but chamber impacts on the ecosystems must be documented so that treatment effects can be interpreted properly. A set of sun-lit chambers with control of atmospheric CO_2, air temperature, and dew point has been in operation for over 2 1/2 years. The chambers are being used to evaluate responses of Douglas-fir (*Pseudotsuga menziesii*) seedlings to elevated CO_2 and temperature. Comparison of trees grown in chambers at ambient CO_2 and temperature (ACAT) with corresponding outside "chamberless trees" (CL) indicates that ACAT trees have slightly less growth (e.g. smaller stem diameters, fewer branches, and shorter terminal buds) compared to CL trees. However, needle characteristics are essentially the same for ACAT and CL trees. The differences in growth for ACAT vs. CL trees likely can be attributed to less soil moisture and lower light intensities, and possibly slightly greater vapor pressure deficits in chambers *vs.* outside. Air temperature and CO_2 levels were very similar for ACAT and CL trees and likely do not affect tree growth. Thus, growth of the trees to CO_2 and temperature in the chambers are representative of the imposed climatic conditions, and can be used to estimate effects of climate stress.

Keywords: Controlled environment, vegetation, Douglas-fir, Pseudotsuga menziesii

1. Introduction

Careful control of environmental conditions is essential for precise description of factors affecting plant growth, and manipulation of climatic variables to test experimental hypotheses. Many types of facilities have been used in plant–environment studies (Allen et al., 1992) with closed environment chambers providing a high level of environmental control, especially in terms of atmospheric gases, air temperature, and dew point (Taylor et al., 1994). Sun-lit chambers (soil-plant-atmospheric-research or SPAR unit) has been intensively developed to provide for CO_2, air temperature, and dew point control (Jones et al., 1984), as well as a controlled soil system through use of an attached lysimeter. This type of chamber has been adapted at Corvallis, Oregon, for use with tree seedlings and their associated soil ecosystem (Tingey et al., 1996).

This paper describes shoot responses of Douglas-fir seedlings after >2 1/2 years of growth in the Oregon closed chambers with an ambient CO_2, temperature and dew point, *vs.* seedlings grown in outside air, to indicate if the performance of the chambers is providing conditions for acceptable plant growth. The trees are being grown as part of a study on the effects of global climatic change (elevated CO_2 and/or temperature) on Douglas–fir and the soil-rhizosphere ecosystem (Tingey et al., 1996).

2. Methods

2.1 Chamber Operation

The sun-lit, closed chambers ("terracosms") and associated equipment measure and control climatic and edaphic factors while maintaining natural environmental variability as described in detail by Tingey et al. (1996). Chambers are aligned on an east-west axis with front sides facing south. In brief, each chamber has a top enclosure 2 m wide, 1 m front-to-back, and 1.5 m tall in the back sloping to 1.3 m in the front, with an aluminum frame covered with 3 mil thick Teflon™ film (Du Pont Electronics, Wilmington, DE) on three sides and the top. The back (north) wall of the chambers is plexiglass. The back of each enclosure has "air handling" equipment to modify CO_2 concentration, temperature, and dew point, and to cycle air through the chamber. The above-ground chamber is attached to an insulated, water-tight, 6.4 mm thick aluminum soil lysimeter (1 m x 2 m x 1 m deep) which sits below ground level in a steel pit liner. The inside of the lysimeter is painted with white nontoxic epoxy paint covered with clear, 2.5 mil oriented-strand adhesive-backed Teflon™. Lysimeters are filled with a reconstituted (top, middle, and lower horizons) coarse textured sandy loam soil topped with forest floor litter. Soil temperature is measured with thermistors and soil moisture is measured with Time-Domain Reflectometry probes (TDR, Campbell Scientific, Logan, UT). Outside (chamberless "CL") plots have lysimeters but no enclosure.

Chamber environmental conditions, i.e., CO_2, air temperature, and dew point, are based on ambient data collected at a weather station near the chambers. Data are transferred each minute to processors at individual chambers and used to determine target levels inside the chambers. Targets can be either ambient or elevated above ambient, i.e. +200 $\mu l\ l^{-1}$ CO_2 and/or +4°C in the current experiment. After planting on 6 and 7 June, 1993, trees were exposed to ambient CO_2 and temperature until late August, 1993 when the CO_2 and temperature treatments were imposed. Climate data for this paper is for 1995, based on 1-min. averages. Data are reported here for ambient CO_2 and temperature chambers (ACAT). Weather station data are used for CL plots.

2.2 Tree Seedling Selection and Culture

Seedlings (1+1's, i.e. two years of growth) were obtained from the Weyerhaeuser Company in Aurora, OR, and grown from mixed seeds ("woods run") from five low elevation (<500 m) seed zones in the southern Willamette Valley and western Cascade Mountains. Fourteen trees were planted in each chamber or CL plot in three east-west rows of five (outer), four (middle) and five (outer) trees. Outer row trees are 18.5 cm from the north and south ends of the chamber and 19.5 cm from the east and west ends of the chambers. Middle row trees are 39.5 cm from the west and east ends of the chamber. Rows are 36.5 cm apart and trees within rows are 39.5 cm apart.

2.3 Tree Response Measurement and Statistical Analsysis

For ACAT chamber or CL plot stem diameter is measured on each tree with a Mitoya Digimatic digital caliper at a reference point just above the soil surface and first nodal swelling on the tree. Other parameters are measured on each tree with a ruler or meter stick: height from the diameter mark to the highest point on the terminal shoot, terminal shoot length from bud scar to longest point, length for one randomly selected needle on each tree midway along the terminal shoot, and terminal bud length from attachment to stem to tip. Leaf area index (LAI, or unit leaf area/unit ground surface area) is based on a function: leaf area = $1.65 + 1.682 * X - .086 * X^2$, where X=stem diameter^2 * height. Measurements are made weekly to monthly for spring-summer months; and bimonthly for fall-winter months. Branches numbers are determined from a subset of four trees. Needle areas and specific weights are based on pooled samples for all trees in a ACAT chamber or CL plot. Needles from this sample are measured for carbon and nitrogen concentrations [flame combustion analysis (Carlo–Erba)].

This analysis considers three replicate ACAT chambers and two CL plots (CL). Data from all available sampled trees were used to obtain ACAT or CL means. Statistical analysis on means is by an unpaired t–test (StatView®, Abacus Concepts).

3. Results

3.1 Chamber Environment

Climate data for 1995 indicate that CO_2, air temperature, and dew point are very similar for the ACAT *vs.* CL trees. Annual averages for ACAT and CL trees, respectively; were: CO_2 of 410 and 394 μmol mol^{-1}; air temperatures of 12.6 and 12.3°C; and dew points of 6.9 and 7.4°C. Annual growing degree days (5°C base) were 2816 and 2750, respectively, for ACAT and CL trees. Soil temperature for ACAT trees closely follows air temperature (data not discussed here). These results confirm the initial performance characteristics of the chambers for Nov. 1993 through Nov. 1994 (Tingey et al., 1996).

The difference in CO_2 concentrations is due primarily to higher levels (≈ 50 μmol mol^{-1}) at night for ACAT compared to CL trees due to the difficulty in fully venting the respiratory buildup in CO_2 in the chambers. Air temperature levels differ especially on cold winter nights when ACAT temperature cannot go much below 0°C due to the nature of the propylene glycol solutions used for temperature control. Furthermore, ice may form on chamber cooling coils when they approach 0°C, making them less efficient in removing heat and resulting in an increase in air temperature above ambient.

The major differences in environment between ACAT and CL trees are soil moisture, vapor pressure deficit (VPD), photosynthetically active radiation (PAR between 400–700 nm), and wind velocity. Soil moisture in chambers is dependent on irrigation which is quantitatively applied to mimic a normal seasonal (wet winter and dry summer) cycle for Pacific Northwest forests (Tingey et al., 1996). The ACAT trees receive only irrigation water, resulting in 821 mm of water applied in 1995 compared to a total of 2270 mm for CL trees which receive both irrigation and rainfall. The difference in water received results in lower soil moisture levels during the early and late growing season for ACAT compared to CL trees (Figure 1A). Based on the annual average air temperature and dew point data, annual average calculated VPD was slightly larger for ACAT *vs.* CL trees, at 0.47 and 0.40 kPa. The PAR varied diurnally and seasonally (Figure 2B, data for second half of 1995) across chambers due to shading and/or reflectance from chamber structural supports and absorption by the Teflon™ as well as condensation of moisture on the chamber walls during cool nights. Average PAR was 12% lower for ACAT compared to CL trees. Wind velocity for ACAT trees is at a constant rate and averaged 0.27 m s^{-1} within and 0.61 m s^{-1} above the tree canopy; resulting in ≈ 10 air changes per minute through the chambers (Tingey et al., 1996). In contrast, outside wind velocity varies diurnally and seasonally, with a average speed of ≈ 2.6 ± 2.7 m s^{-1} , based on daily aveages for approximately May--September 1994 from the for Corvallis area.

3.2 Plant Response

Stem diameters were first noticed to be significantly smaller ($p<0.05$) for ACAT compared to CL trees on 30 June 1994 (day 389, Figure 2A), becoming ≈ 3 mm less for ACAT than for CL trees by 18 October 1994 (day 489). This difference persisted through 11 December 1995 (day 918), when there was a significantly smaller stem diameter for ACAT compared to CL trees (Table 1). In contrast, heights were similar for ACAT and CL trees until 15 June 1995 (day 739, Figure 2B). Thereafter heights tended to be smaller for ACAT than for CL trees due to shorter main flush terminal shoots, but the differences in height and terminal shoot length were not statistically significant on day 918 (Table 1). The LAI was significantly lower for ACAT than for CL trees (Table 1). Branch numbers were significantly lower and terminal buds significantly shorter for ACAT than for CL trees (Table 1). However, needle characteristics (lenght, area, specific weight, % C and N) did not differ between ACAT and CL trees (Table 1).

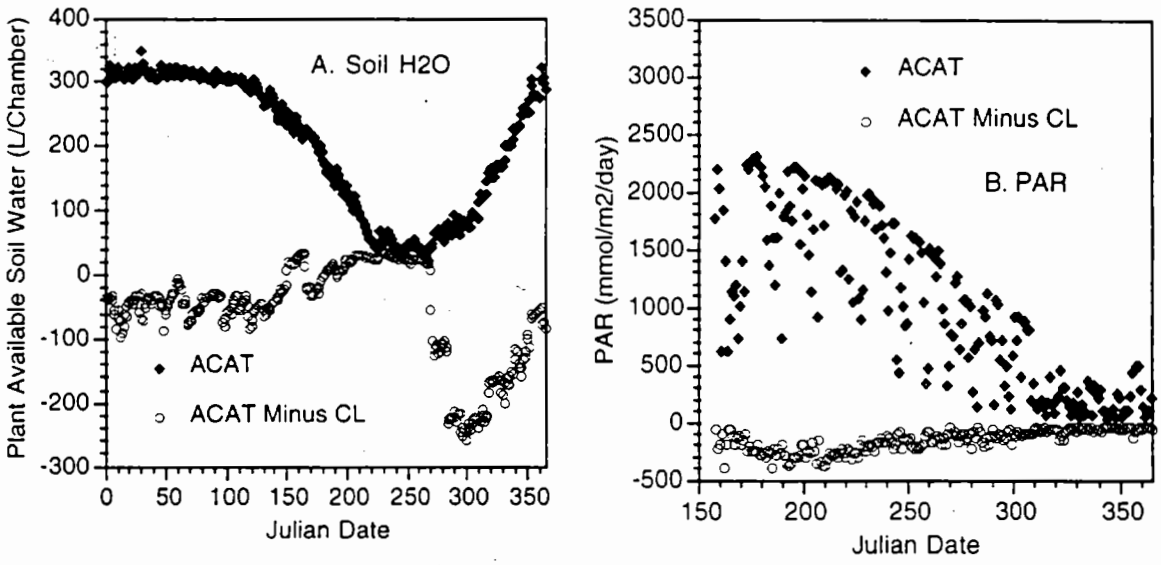

Figure 1. Plant available soil water (panel A), and photosynthetically active radiation (PAR) above the tree canopy (panel B), and in ambient chambers (ACAT) and difference between ACAT and outside air (CL) in 1995. Data are means for three ACAT chambers and two CL plots.

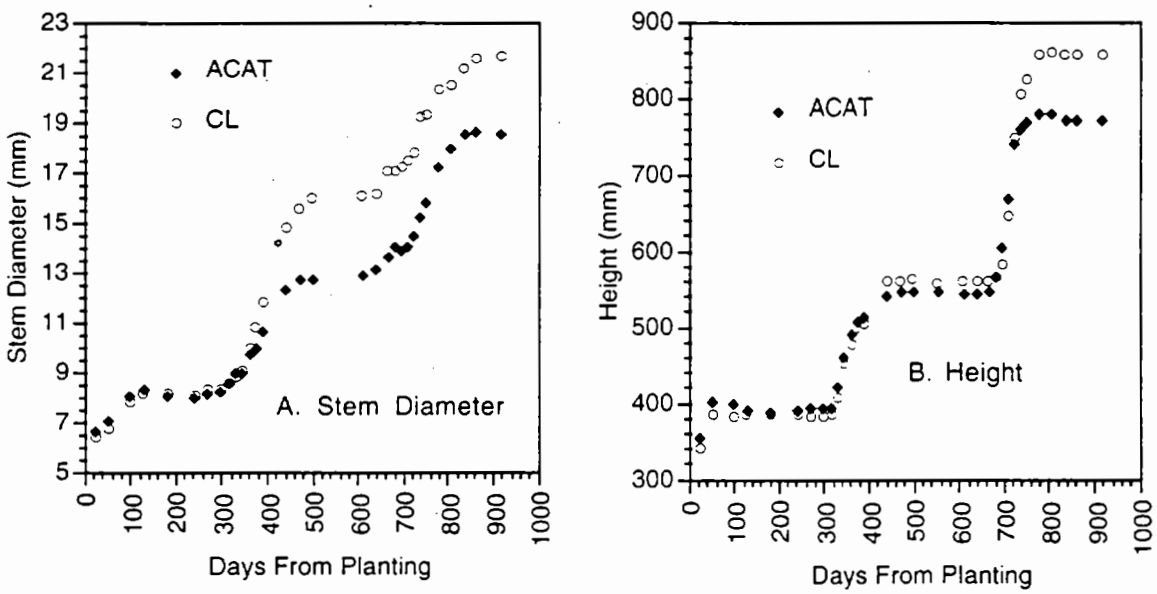

Figure 2. Stem diameter (panel A) and shoot height (panel B) for Douglas-fir seedlings growing in ambient chambers (ACAT) or outside air (CL) from 7 June 1993 (day 0) through 11 December 1995 (day 918). Data are means for three ACAT chambers and two CL plots. Average coefficients of variation for replicates across dates for ACAT and CL, respectively, are 4.9 and 4.3% for stem diameter and 3.6 and 3.3% for height.

4.0 Discussion

Over the first 2 1/2 years of the study, ACAT chamber trees grew well but were slightly smaller than CL trees. The differences in general growth may be attributed to

Table 1. Douglas-fir shoot responses in ambient CO_2 and temperature chambers (ACAT) and outside air (CL). Data from fall, 1995; dates between 862 to 918 days after planting depending on parameter. Values are means ± standard error for 3 ACAT and for 2 CL. Parameters averaged for 14 plants per chamber or plot; except for branch counts averaged for 4 plants per chamber or plot; and needle area, specific weight, nitrogen, and carbon based on needles pooled from all 14 plants. Terminal shoot and needle lengths are for main flush. The p values are from unpaired t–tests.

Parameter	ACAT	CL	p Value
Stem diameter (mm)	18.7 ± 0.4	21.7 ± 0.2	0.016
Height (mm)	774 ± 29	862 ± 32	0.143
LAI (mm^2/mm^2)	3.03 ± 0.06	4.00 ± 0.15	0.006
Terminal Shoot Length (mm)	248 ± 15	301 ± 3	0.075
Terminal Bud Length (mm)	10 ± 0.3	11 ± 0.2	0.032
Branch Count (#)	175 ± 14	264 ± 15	0.024
Needle Length (mm)	28 ± 1.3	26 ± 0.4	0.205
Needle Area (cm^2)	0.28 ± 0.01	0.25 ± 0.01	0.253
Needle Specific Wt. ($g\ cm^{-2}$)	0.012 ± 0.003	0.013 ± 0.004	0.181
Needle Nitrogen Conc. (% dry wt.)	1.46 ± 0.05	1.48 ± 0.04	0.816
Needle Carbon Conc.(% dry wt).	47.6 ± 0.9	47.6 ± 0.5	0.992

higher moisture stress (lower soil moisture coupled with higher VPD) and lower solar radiation for ACAT than for CL trees, both of which could reduce plant growth. The TREEGRO (Weinstein and Beloin, 1990) model is being used with the climate data for the chambers and outside plots to test this hypothesis. For woody plants, the small differences in early growth, as found for Douglas-fir, will likely become greater over time as shown for orange trees in open-top chambers (Olszyk et al., 1992). The slightly greater bud length at the end of 1995 suggested that the CL trees would start off with higher potential productivity for 1996, and this has been occurring (data not shown here).

There were no obvious differences in needle characteristics which would explain difference in growth for ACAT compared to CL trees. The same nitrogen concentrations in ACAT and CL trees suggest that atmospheric nitrogen deposition, which is an uncontrolled variable expected to occur only for CL and not for ACAT trees, is not a determining factor for nitrogen availability and subsequent tree growth in this study. Increased leaf nitrogen due to deposition could have affected overall tree growth due to the relatively low initial fertility of this soil and reliance on soil processes to provide nutrients in this study. Lack of differences in leaf carbon concentrations and photosynthetic rates (data not shown here) for ACAT and CL trees imply that carbon assimilation is not affected by the chambers. Essentially the same nitrogen concentrations for ACAT and CL needles also suggest similar carbon assimilation as leaf nitrogen is

closely correlated with photosynthetic rates (Field and Mooney, 1986). There is little information on dark respiration for needles and none for branches in this study, thus, effects of the chambers on overall carbon balance of the shoots could not be determined.

We recommend that the effect of the experimental system itself on plant response be an important consideration whenever any highly environmentally controlled facility is used, as discussed in detail for past studies using open-top chambers and ozone (Heagle et al., 1988). Our analysis to date shows that while some basic tree growth responses are different in the chambers *vs.* outside air; these difference are likely attributable to documented environmental conditions-- e.g. soil moisture, VPD, and light. Further analysis is necessary, but data to date show no evidence that basic tree responses to the imposed treatment variables (CO_2 and temperature) are different in the chambers *vs.* outside air, thus data obtained with the chambers can be used to determine effects on Douglas-fir (or any other plant) to environmental stress, especially global change.

5.0 Acknowledgement

The information in this document has been funded wholly by the U.S. Environmental Protection Agency. It has been subject to the agency's peer and administrative review. It has been approved for publication as an EPA document. Mention of trade names or commercial products does not constitute endorsement or recommendation for use. Soil moisture data from Dr. Mark Johnson, prepared under Dynamac Contract # 68-C6-0005 with the U.S. EPA, are greatly appreciated.

6.0 References

Allen, H.A., Jr., Drake, B.G., H.H. Rogers and J.H. Shinn. 1992. Field techniques for exposure of plants and ecosystems to elevated CO_2 and other trace gases. Critic. Rev. Plant Sci. 11:85-119.

Field, C., and H.A. Mooney. 1986. The photosynthesis-nitrogen relationship in wild plants. Pg. 25-56, In: Givnish T.J. (ed.) On the Economy of Plant Form and Function. NY: Cambridge University Press.

Heagle, A.S., L.W. Kress, P.J. Temple, R.J. Kohut, J.E. Miller and H.E. Heggestad. 1988. Factors influencing ozone dose-yield response relationships in open-top field chamber studies. pp. 141-179. In: Heck, W.W., O.C. Taylor and D.T.Tingey, Eds. Assessment of Crop Loss From Air Pollutants. Elsevier, New York.

Jones, P., J.W. Jones, L.H. Allen, Jr. and J.W. Mishoe. 1984. Dynamic computer control of closed environmental plant growth chambers: design and verification. Trans. ASAE 27:879–888.

Olszyk, D.M., B.K. Takemoto, G. Kats, P.J. Dawson, C.L. Morrison, J. Wolf Preston and C.R. Thompson. 1992. Effects of open-top chambers on 'Valencia' orange trees. J. Environ. Qual. 21:128-134.

Taylor, G.E. Jr. 1994. Controlled environment facilities in ecology and environmental sciences. Bull. Ecol. Soc. Am. 75:277–280.

Tingey, D.T., B. McVeety, R. Waschmann, M. Johnson, D. Phillips, P.T. Rygiewicz and D.Olszyk. 1996. A versatile sun-lit controlled-environment facility for studying plant and soil processes. J. Environ. Qual. 25:614-625.

Weinstein, D.A. and R. Beloin. 1990. Evaluating effects of pollutants on integrated tree responses: A model of carbon, water and nutrient balances. Pp. 313-323. In.: R.K. Dixon, R.S. Meldahl, G.A. Ruark and W.G. Warren (eds.), Process Modeling of Forest Growth Responses to Environmental Stress. Timber Press, Portland, OR.

TECHNICAL REPORT DATA
(Please read Instructions on the reverse before compl

1. REPORT NO.	2.	3.
EPA/600/A-96/077		

4. TITLE AND SUBTITLE	5. REPORT DATE
Environmental modification and shoot growth in a closed ecosystem to evaluate long-term responses of tree seedlings to stress	6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S)	8. PERFORMING ORGANIZATION REPORT NO.
D.M. Olszyk, D.T. Tingey	
9. PERFORMING ORGANIZATION NAME AND ADDRESS	10. PROGRAM ELEMENT NO.
USEPA, NHEERL, Corvallis, OR,	11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS US EPA ENVIRONMENTAL RESEARCH LABORATORY 200 SW 35th Street Corvallis, OR 97333	13. TYPE OF REPORT AND PERIOD COVERED Symposium Paper 14. SPONSORING AGENCY CODE EPA/600/02

15. SUPPLEMENTARY NOTES

1996. International Symposium on Plant Production in Closed Ecosystems, Aug 26-29, 1996, Narita, Japan

16. ABSTRACT

Determining ecosystem responses to stress requires careful control of the environment while measuring biological effects. Technologically advanced sun-lit closed chambers at WED were found to provide appropriate environmental control and measurement compared to chamberless controls. Chamber impacts on the ecosystems were documented so that treatment effects could be interpreted. Differences in growth can likely be attributed to less soil moisture and lower light intensities, and possibly slightly greater vapor pressure deficits in chambers vs. outside during parts of the year. Because of the advance control system, the effects of these sunlit chambers on plant growth appears to be negligible.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Controlled environment, vegetation, Douglas-fir, *Pseudotsuga menziesii*		

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
		6
	20. SECURITY CLASS *(This page)*	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE