**Tree Physiology** 21, 275–286


# Changes in physiological attributes of ponderosa pine from seedling to mature tree

NANCY E. GRULKE[1] and WILLIAM A. RETZLAFF[2]

[1] *USDA Forest Service, Pacific Southwest Research Station, 4955 Canyon Crest Drive, Riverside, CA 92507, USA*

[2] *Environmental Science Program, Southern Illinois University Edwardsville, Edwardsville, IL 62026, USA*

Received August 30, 1999

**Summary** Plant physiological models are generally parameterized from many different sources of data, including chamber experiments and plantations, from seedlings to mature trees. We obtained a comprehensive data set for a natural stand of ponderosa pine (*Pinus ponderosa* Laws.) and used these data to parameterize the physiologically based model, TREGRO. Representative trees of each of five tree age classes were selected based on population means of morphological, physiological, and nearest neighbor attributes. Differences in key physiological attributes (gas exchange, needle chemistry, elongation growth, needle retention) among the tree age classes were tested. Whole-tree biomass and allocation were determined for seedlings, saplings, and pole-sized trees. Seasonal maxima and minima of gas exchange were similar across all tree age classes. Seasonal minima and a shift to more efficient water use were reached one month earlier in seedlings than in older trees because of decreased soil water availability in the rooting zone of the seedlings. However, carbon isotopic discrimination of needle cellulose indicated increased water-use efficiency with increasing tree age. Seedlings had the lowest needle and branch elongation biomass growth. The amount of needle elongation growth was highest for mature trees and amount of branch elongation growth was highest for saplings. Seedlings had the highest biomass allocation to roots, saplings had the highest allocation to foliage, and pole-sized trees had the highest allocation to woody tissues. Seedlings differed significantly from pole-sized and older trees in most of the physiological traits tested. Predicted changes in biomass with tree age, simulated with the model TREGRO, closely matched those of trees in a natural stand to 30 years of age.

*Keywords: gas exchange, growth rates, mature trees, ontogenetic changes, TREGRO, whole tree biomass.*

## Introduction

We used site-specific data from a natural stand of ponderosa pine (*Pinus ponderosa* Laws.) to parameterize the physiologically based model, TREGRO (Weinstein et al. 1991), and to determine which physiological attributes change, and at which life stage, as seedlings grow to mature trees in a natural stand. We tested for age-related differences in needle gas exchange, tissue chemistry, and growth of needles or branches attached to different aged stems. Whole seedlings, saplings, and pole-sized trees were harvested to determine biomass and allocation to roots, stem and branches, and foliage.

Ponderosa pine is a widespread and common species with a distribution from Durango, Mexico in the south and southern British Columbia, Canada in the north, to the eastern slopes of the Rocky Mountains in Colorado and the western slope of the Sierra Nevada, Siskyous, and the Cascade Mountains (Oliver and Ryker 1990). It has a single bud flush each year. There are two well-described varieties: a west coast (var. *ponderosa*) and a continental variety (var. *scopulorum*). The two varieties differ with respect to water-use efficiency (WUE, Monson and Grant 1989), seasonal patterns of stomatal conductance and ability to respond to summer precipitation (Kolb and Stone 2000, Temple and Miller 1999), rooting distribution (e.g., Grulke et al. 1998 versus S.C. Hart, Northern Arizona Univ., AZ, unpublished data), as well as other attributes (Monson and Grant 1989).

The *P. ponderosa* var. *ponderosa*-dominated stand described here is located near Lassen Volcanic National Park, in the northern extent of the Sierran-mixed conifer forest type (*sensu* Barbour 1988). Although the site is not subject to atmospheric pollution (Grulke 1999), sites just 80 km south are exposed to moderate ozone pollution (> 50 ppb hourly means in summer, Quincy, CA; California Air Resources Board), which may alter physiological attributes as the trees mature (Grulke 1999). The site is dominated by a dry summer, Mediterranean-type climate. Effects of summer drought stress on several physiological parameters were tested by comparing seasonal patterns of gas exchange and growth of ponderosa pine during two summers differing significantly in total annual precipitation.

## Methods

### *Research site*

Research was conducted southeast of Lassen Volcanic National Park, CA in a mixed-age stand of ponderosa pine, with white fir (*Abies concolor* (Gord. & Glend.) Lindl. *ex* Hildebr.),

Jeffrey pine (*Pinus jeffreyi* Grev. & Balf.), sugar pine (*Pinus lambertiana* Dougl.), Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), lodgepole pine (*Pinus contorta* Dougl. *ex* Loud.), and incense cedar (*Libocedrus decurrens* Torr). Approximately 10% of the yellow pine at the site is Jeffrey pine. The site was lightly thinned about 40 years ago, leaving many older (100+ years old) trees as seed sources as well as saplings and pole-sized trees. The site is representative of ponderosa-pine-dominated, Sierran mixed-conifer stands (*sensu* Barbour 1988). The stand structure is open, which is typical of drought-prone sites, with 10–20-m spacings between patches of trees.

A meteorological station, maintained by research staff at Manzanita Lake, is located in the northwest corner of Lassen Volcanic National Park. A microenvironmental monitoring station was maintained from late-spring 1993 to late-autumn 1994 in Childs Meadow, 10 km west of the field site. Photosynthetic photon flux density (PPFD) was measured at a height of 2 m. Wind speed was measured at a height of 1.5 m. Needle and air temperatures were measured at a height of 1.5 m with copper-constantan thermocouples. Soil temperatures were measured in triplicate at 10- and 50-cm depths with 24-gauge wire. All microenvironmental data were measured every 5 min, averaged, and recorded hourly on a data logger (Model 21×, Campbell Scientific Inc., Logan, UT). Soil water was determined monthly with fiberglass moisture blocks, as well as gravimetrically in a soil pit at 10- and 45-cm depths in 1993. Soil water content of the upper 0–15-cm layer was spot checked with a time domain reflectometer (Soil Moisture Equipment Corp., Santa Barbara, CA) in 1994.

### *Tree and branch selection*

A 50 × 75 m plot was established in 1992. Representative trees of each of five tree age classes were chosen based on population means of morphological, physiological, and nearest neighbor attributes. All trees in the plot were sampled for the following data: age near the tree base, bole diameter at the base and at 1.4 m, height measured with a meter tape (cm) or estimated with a clinometer (m), number of needle age classes retained in mid-canopy, and distance (m) to the nearest conspecific and interspecific tree as a measure of inferred competition. Seedling age was estimated based on growth scars on the stem. Six trees with statistically average morphological characteristics in each of four tree age classes (3–9, 11–20, 21–40, and 41–60 years old) were selected for intensive monthly measurements. Trees less than 60 years old with their lowest branches greater than 3 m above the ground were not chosen because of the increased time required to collect gas exchange measurements. Because none of the mature trees in the plot had branches accessible by a 15-m scaffold, foliage of three mature trees (averaging 252 ± 43 years old) close to the plot was chosen for monthly measurements.

Measurements were made during one week each month from May to mid-October. The same branches and trees were measured in each age class on each occasion. Multiple tree age classes were sampled on each day during the week of intensive measurements. The third or fourth primary lateral branch from the terminus was sampled for both the 3–9- and 11–20-year-old tree age classes. For the older tree age classes, we consistently sampled from primary branches in the upper part of the lower third of the canopy. To quantify irradiance on the sampled primary branches, PPFD measurements were logged every 5 min diurnally with a Campbell 21× data logger, one day each month, throughout the growing season. Small PPFD sensors (Hamamatsu Corp., Bridgewater, NJ) were installed in each of the retained whorls on each of two branches, thus irradiances were integrated over the live foliage on each primary branch. The branches chosen for study received an irradiance of 1000 μmol $m^{-2}$ $s^{-1}$ for at least 3 h per day.

### *Gas exchange*

Gas exchange was measured between 0830 and 1330 h in May and June, and between 0830 and 1130 h during the remaining months at ambient $CO_2$ concentration (353 ppmv, overall mean for the data set), leaf temperature (30.5 °C), vapor pressure deficit (VPD, 3.6 kPa), and under saturating light conditions (> 1400 μmol $m^{-2}$ $s^{-1}$ for at least 20 min before and during measurement, and averaging 1722 μmol $m^{-2}$ $s^{-1}$ overall for the data set). Gas exchange rate of two fascicles per branch per tree was measured in 1992 through 1994 with a closed photosynthetic system (Model LI-6200, Li-Cor, Inc., Lincoln, NE) equipped with a 0.25-l cuvette. The instrument zero was checked every 2 h and gain for the $CO_2$ IRGA was checked daily with a tertiary calibration gas (± 1 ppmv; cross-checked with a secondary standard, National Institute of Standards and Technology). No drift in calibration gases was detectable over the growing season. Relative humidity calibration was checked on site several times each growing season with a dew point generator (Model 610, Li-Cor, Inc.).

Each measure of net assimilation rate (NAR) was matched with a measure of dark-adapted respiration at a similar leaf temperature (± 2 °C). After each NAR measurement, an insulated dark cloth with an outer surface of reflective material and inner surface of insulative material was wrapped around the cuvette. The time required to minimize photorespiration was determined to be 12 to 15 min for most trees.

Leaf area was calculated (for one fascicle, $(DL) + (3DL)$) from six measures of fascicle diameter ($D$), just above the sheath, and needle length ($L$). Needles on seedlings were not harvested to minimize disturbance. For the older tree age classes, the same foliage that was enclosed in the cuvette was flash frozen and stored in liquid nitrogen in the field, then prepared for measurements of both mass and nitrogen concentration. Needle area per unit weight (cm $g^{-1}$) was also calculated from these samples.

### *Needle xylem potential*

Predawn and midday needle xylem potentials ($\Psi$) were measured monthly on two to five fascicles from each of at least three trees in each tree age class (Pallardy et al. 1991). Water potential was measured on seedlings other than those used for gas exchange. The sheath was removed, a smooth surface was cut just distal to the petiole, and inserted into the pressure chamber (Pressure Measurement Systems, Corvallis, OR). Pressure was read to the nearest 0.05 MPa when apoplastic water first started bubbling at the cut fascicle surface, usually

through resin. Pressure potential was generally 0.2 MPa lower for measurements on non-resinous needles.

*Tissue chemistry*

Needle tissue for analysis of tissue chemistry was collected from three primary branches above those used to collect foliage for gas exchange, flash frozen in the field in liquid nitrogen, and transported and stored in liquid nitrogen, until lyophilized and ground. Total nitrogen and carbon were determined with an NA 1500 Series 2 analyzer (CE Instruments, Milan, Italy). Runs containing samples or a pine standard with > 2.5% error were reanalyzed.

Chlorophyll pigments were extracted with dimethyl sulfoxide (DMSO) at 65 °C for 7 h as described by Hiscox and Israelstam (1979). Extracts were filtered and analyzed spectrophotometrically (Model 80, Beckman Coulter, Inc., Fullerton, CA) at 645 nm (chlorophyll a), 652 nm (chlorophyll a + b), and 663 (chlorophyll b) against a DMSO blank. Values were checked for percent error between total chlorophyll concentration calculated from 645 + 663 nm with that from 652 nm. Duplicates differing by more than 5% from the mean were rerun with another subsample of tissue.

Cellulose for $\delta^{13}C$ determination was extracted from plant tissue by a series of solvents followed by delignification as described by Wise et al. (1945). Leaf cellulose was sent to the Waikato Stable Isotope Laboratory in Hamilton, New Zealand. The tissue sample was combusted with a Carlo Erba NA 1500 Analyzer, and the $CO_2$ produced was analyzed for $^{13}C$ abundance with a Finnigan/MAT 251 mass spectrometer (Finnigan Mat, San Jose, CA). The samples were analyzed against a laboratory standard of sucrose, pine standards, and compared with a certified standard (calibrated relative to Pee Dee belemnite) from CSIRO, Canberra, Australia. The largest error was between-run variability, which was corrected based on values from the known standards.

*Aboveground growth*

Lateral branch and needle elongation growth (mm), and needle retention were measured at the same canopy position, on the same trees, and at the same times as the gas exchange measurements. Three branchlets on the seedlings, five primary branches on six trees from the intermediate tree age classes, and three primary branches on the mature trees were measured and averaged to provide a single value per tree.

*Biomass*

Whole-tree harvests were made on three trees of the 3–9-, 11–20-, and 21–40-year-old tree age classes in early September 1993. All coarse and fine roots were excavated to bedrock, generally encountered at 1.5 to 2 m. Foliage was stripped from branches by needle age class, and packaged in paper sacks by canopy thirds based on the length of the live crown. Within 20 h of collection, foliage was dried at 105 °C for 2 days and weighed. Branches were bundled and the trunk was cut into five parts for transport. Roots were separated into fine (< 2 mm diameter), medium (2 mm diameter) and coarse (> 2 mm diameter) size classes, dried, and weighed. Branches, bole, and primary roots were dried at 105 °C and weighed at 2-day intervals until no additional weight loss was observed.

*Statistical analyses*

Multiple-year tests for differences in attributes between tree-age classes were performed with a three-way ANOVA (age class × sample date × year). This test was used rather than repeat measures because half of the intensively measured trees were harvested after the first year and replaced with new trees. When appropriate, a 1-way analysis of variance (ANOVA) was performed within a sampling date to demonstrate differences among tree age classes (Sokal and Rohlf 1969). Tests to determine whether regression lines were parallel or co-linear were performed according to Kleinbaum and Kupper (1978).

*Description of TREGRO*

TREGRO is a physiological simulation model of the carbon, water, and nutrient fluxes of an individual tree that was developed to analyze the responses of trees to multiple environmental stresses (Weinstein et al. 1991). In the model, the tree is divided into compartments: foliage, branches, stem, and coarse and fine roots. In each compartment, the model keeps track of three carbon pools: structure (living, respiring tissue); wood (the non-respiring tissue); and total nonstructural carbohydrate (TNC). Carbon assimilation of the tree is calculated hourly as a function of ambient environmental conditions and the availability of light in the canopy, water, and nutrients. Carbon is redistributed daily within the plant for respiration, growth, storage, and replacement of senescent tissues. Priority for carbon varies inversely with the distance between source and sink, and varies directly with relative sink strength.

In TREGRO, the interaction between tree growth and the environment is achieved through the linkage of separate data files. The parameter file includes: maximum photosynthetic rate, rates of maintenance and growth respiration, phenological patterns of growth, growth rates of individual tree compartments, initial patterns of carbon allocation among compartments, and partitioning within compartments among living structure, dead wood, and reserves (TNC). In addition to the data presented here, other sources include soils description (Grulke 1999), rooting distribution and biomass (Grulke et al. 1998), and whole plant carbohydrate content (Grulke et al. 2001). The meteorological file defines the site-specific hourly environmental conditions including air temperature (°C), relative humidity (%), rainfall (mm), and PPFD (μmol $m^{-2}$ $s^{-1}$) obtained from the archives of Lassen Volcanic National Park as well as this study. Biomass and allocation of 5-year-old trees harvested on site were used as the initial tree in these simulations.

## Results

*Representativeness of intensively measured trees and branches*

The distribution of tree age classes within the stand, and morphological and nearest neighbor attributes are given in Table 1. For all parameters except nearest neighbor proximity, the intensively measured trees were not statistically different

at $P = 0.05$ from the larger population of the same tree age class. Intensively measured trees in the 41–60-year-old tree age class were further away on average from both con- and interspecific neighbors than trees in the larger population of the same tree age class. Some of the average trees were rejected because of self-pruning of lower branches on the margins of the tree clusters, which significantly increased sampling time per tree for the gas exchange measurements.

Although a comprehensive analysis of canopy variability in physiological attributes was not conducted, gas exchange and leaf area per unit weight were compared in upper, middle and lower canopy thirds on five trees in the 41–60-year-old tree age class, based on midmorning measurements in June. Leaf area per unit weight of 1-year-old needles did not vary significantly across the canopy thirds ($P = 0.45$) and was 3.86 ± 1.39, 3.08 ± 0.28 and 4.54 ± 0.73 $cm^2\ g_{dw}^{-1}$ for the upper, mid and lower canopy, respectively (cf. Monserud and Marshall 1999). Stomatal conductance was similar across the canopy (68 ± 8, 70 ± 18 and 70 ± 10 mmol $H_2O\ m^{-2}\ s^{-1}$ for the upper, mid and lower canopy, respectively). Mencuccini and Grace (1996) reported that transpiration rates were similar throughout the canopy, and between 7- and 59-year-old tree age classes of Scots pine (*Pinus sylvestris* L.). Net assimilation rate (NAR) did not significantly differ within the canopy ($P = 0.58$), although it was 24% greater in the upper canopy (4.7 ± 0.1 μmol $CO_2\ m^{-2}\ s^{-1}$) relative to the mid (3.9 ± 0.9 μmol $CO_2\ m^{-2}\ s^{-1}$) and lower canopy (3.7 ± 0.7 μmol $CO_2\ m^{-2}\ s^{-1}$). Thus, measurements made in the lower third of the canopy may be representative of the 1-year-old foliage in the lower two thirds of the canopy. The 1-year-old needles had the greatest NAR until early August, when both current- and 1-year-old needles had similar NAR.

### *Climate*

The most important difference in climate between study years was in spring and fall precipitation (Table 2). Late spring pre-

Table 1. Representativeness of trees chosen for intensive study relative to the population of that tree age class (in years) within the stand. A one-way ANOVA was used to test for differences for each plant attribute. Mean values are given ± 1 SE.

| | Tree age class (year) | | | |
|---|---|---|---|---|
| | 0–10-year-old | 11–20-year-old | 21–40-year-old | 41–60-year-old |
| Proportion of trees in tree age class (%) | 15.9 | 12.1 | 40.7 | 20.6 |
| *Tree age* | | | | |
| Sampled | 5 ± 1 | 13 ± 2 | 27 ± 2 | 51 ± 4 |
| Population | 4 ± 1 | 15 ± 1 | 31 ± 1 | 50 ± 1 |
| *P*-Value | 0.42 | 0.26 | 0.05 | 0.61 |
| *Number of whorls retained* | | | | |
| Sampled | 2.6 ± 0.2 | 4.2 ± 0.3 | 5.6 ± 0.5 | 4.7 ± 0.4 |
| Population | 3.0 ± 0.2 | 4.3 ± 0.2 | 4.9 ± 0.1 | 4.8 ± 0.1 |
| *P*-Value | 0.33 | 0.76 | 0.08 | 0.74 |
| *Basal diameter (cm)* | | | | |
| Sampled | 0.25 ± 0.11 | 0.88 ± 0.12 | 3.77 ± 0.77 | 6.57 ± 0.69 |
| Population | 0.22 ± 0.07 | 0.89 ± 0.11 | 3.85 ± 0.32 | 4.90 ± 0.55 |
| *P*-Value | 0.82 | 0.99 | 0.94 | 0.26 |
| *Tree height (m)* | | | | |
| Sampled | 0.41 ± 0.14 | 0.92 ± 0.11 | 5.08 ± 0.96 | 6.83 ± 0.56 |
| Population | 0.48 ± 0.10 | 1.48 ± 0.23 | 4.89 ± 0.35 | 6.23 ± 0.49 |
| *P*-Value | 0.69 | 0.28 | 0.88 | 0.65 |
| *Number of needle age classes retained* | | | | |
| Sampled | 2.6 ± 0.2 | 4.2 ± 0.3 | 5.6 ± 0.5 | 4.7 ± 0.4 |
| Population | 3.0 ± 0.2 | 4.3 ± 0.2 | 4.9 ± 0.1 | 4.8 ± 0.1 |
| *P*-Value | 0.33 | 0.76 | 0.08 | 0.74 |
| *Nearest conspecific neighbor (m)* | | | | |
| Sampled | 5.2 ± 1.0 | 5.8 ± 1.1 | 2.3 ± 0.8 | 1.7 ± 0.4 |
| Population | 4.6 ± 0.5 | 2.1 ± 0.3 | 1.4 ± 0.1 | 1.1 ± 0.2 |
| *P*-Value | 0.61 | 0.0001 | 0.05 | 0.24 |
| *Nearest interspecific neighbor (m)* | | | | |
| Sampled | 5.9 ± 0.4 | 4.8 ± 1.0 | 3.7 ± 1.0 | 5.8 ± 2.1 |
| Population | 4.8 ± 0.4 | 3.2 ± 0.6 | 3.1 ± 0.3 | 1.7 ± 0.3 |
| *P*-Value | 0.22 | 0.24 | 0.62 | 0.0004 |

cipitation delayed the onset of summer drought. Soil water content was high in June in both growing seasons (> 15%), but dropped rapidly after mid-July. In 1994, soil water content dropped to 0% at 15-cm depth after mid-July, and remained at 0% until the end of September. In both years, soil pits dug to 100-cm depth in September yielded a soil water content of < 4%.

*Needle water potential*

In both years, predawn needle water potential (Ψ) was significantly lower for 3–9-year-old seedlings than for all older tree age classes. The lowest values obtained in 1993 and 1994 were –1.5 and –2.5 MPa, respectively (Figure 1). Twenty percent of tagged seedlings in the stand died by the end of the 1994 growing season. Based on whole-tree harvests, mean rooting depth of 3–9-year-old seedlings was 40 cm, and did not exceed 80 cm. Predawn needle Ψ did not correlate well with soil water content at depths of 10 and 45 cm ($P > 0.10$), except in 11–20-year-old trees where it was correlated with soil water content at 45-cm depth ($r = 0.82$, $P = 0.10$).

*Net assimilation rate*

Gas exchange measurements were representative of maximum values obtainable for NAR and $g_s$ for each tree on each sample date. The overall seasonal pattern of gas exchange (NAR, $g_s$) was marked by uniformly high early season and low late growing season values for all tree age classes. In a 3-way ANOVA on NAR, tree age class, Julian day, and year were significant as explanatory variables ($P < 0.001$ for each, Figure 1). The interactions between tree age class and Julian day ($P = 0.064$), and tree age class and year ($P = 0.49$), and tree age class, Julian day, and year ($P = 0.11$) were not significant, perhaps because the overall pattern of NAR was similar in the two years. Seedlings had lower NAR one month earlier than all other tree age classes in both years (early August 1993: $P = 0.05$; mid July 1994: $P = 0.01$). The interaction term between tree age class and Julian day was not significant in the mesic year ($P = 0.07$), but it was significant in the xeric year ($P = 0.05$).

Over the whole data set, irradiance (PPFD, $P = 0.56$), leaf temperature ($P = 0.85$), and VPD ($P = 0.85$) were not significantly different across the tree age classes during gas exchange measurements. Ambient $CO_2$ concentration ($C_a$; ppmv) at the time of NAR measurements was greater for the youngest two tree age classes than for the other tree age classes (0–9 years old, 357 ± 2; 11–20 years old, 354 ± 1; 21–40 years old, 350 ± 1; 41–60 years old, 350 ± 1; mature trees, 352 ± 2 ppm $CO_2$; $P = 0.02$). The relative difference in leaf to air temperature did not significantly differ among tree age classes (seedlings to mature trees: 1.8 ± 0.2, 1.3 ± 1.4, 1.4 ± 0.1, 1.4 ± 0.2, 1.1 ± 0.3 °C; $P = 0.13$).

Leaf nitrogen concentration and total chlorophyll concentration were higher for seedlings relative to all other tree age classes ($P < 0.001$ in both cases) (Table 3). Although not measured for seedlings, leaf area per unit weight of saplings was significantly greater than that of all older tree age classes ($P = 0.001$). However, seasonal values of net assimilation expressed on a leaf weight, leaf nitrogen concentration or leaf chlorophyll concentration did not change the seasonal pattern of net assimilation (data not shown).

*Needle respiration*

The relationship between daytime respiration and leaf temperature did not differ significantly across the five tree age classes for either slope (parallel: $P = 0.19$) or slope and $y$-intercept (coincident: $P = 0.08$). Respiration varied significantly with Julian day ($P < 0.001$), but not with tree age class ($P = 0.88$) or year ($P = 0.45$) (Figure 1). The interaction terms were not significant at $P = 0.05$. To determine whether seasonal changes in temperature, or subtle differences in needle temperature between tree age classes affected respiration, a regression was developed between dark-adapted respiration and temperature for each tree age class. When respiration was corrected to a constant temperature over the growing season, there were no significant differences between tree age classes ($P = 0.66$). Respiration varied significantly with Julian day ($P < 0.001$) and between years ($P = 0.05$). In both years, foliar respiration was highest during the period of greatest aboveground growth. Except during the growth period, foliar respiration was lower in the mesic year than in the xeric year.

Table 2. Summary of environmental conditions during the two intensive years of study, a mesic year (1993), and a xeric year (1994).

| | Apr 15–May 15 | May 16–Jun 15 | Jun 16–Jul 15 | Jul 16–Aug 15 | Aug 16–Sep 15 | Sep 16–Oct 15 |
|---|---|---|---|---|---|---|
| Julian day | 106–134 | 135–165 | 166–196 | 197–227 | 228–258 | 259–288 |
| *Mean air temperature at 1.5 m (°C)* | | | | | | |
| 1993 | 5.5 ± 3.7 | 10.8 ± 1.1 | 15.7 ± 0.4 | 15.4 ± 0.5 | 14.6 ± 0.4 | 10.3 ± 0.6 |
| 1994 | 6.7 ± 4.3 | 10.5 ± 4.3 | 14.6 ± 2.0 | 16.7 ± 1.5 | 12.5 ± 3.0 | 9.9 ± 5.5 |
| *Total monthly precipitation (cm)* | | | | | | |
| 1993 | 6.53 | 13.10 | 0.32 | 1.17 | 0.00 | 7.65 |
| 1994 | 8.10 | 2.62 | 0.03 | 0.33 | 0.46 | 2.29 |
| *Soil water (%; 1993 only)* | | | | | | |
| 10-cm depth | | 14.2 ± 3.2 | 2.8 ± 0.7 | 6.2 ± 0.6 | 0.4 ± 0.6 | 4.7 ± 0.8 |
| 45-cm depth | | 19.2 ± 12.2 | 7.2 ± 0.8 | 6.7 ± 0.9 | 3.1 ± 1.2 | 5.1 ± 0.2 |

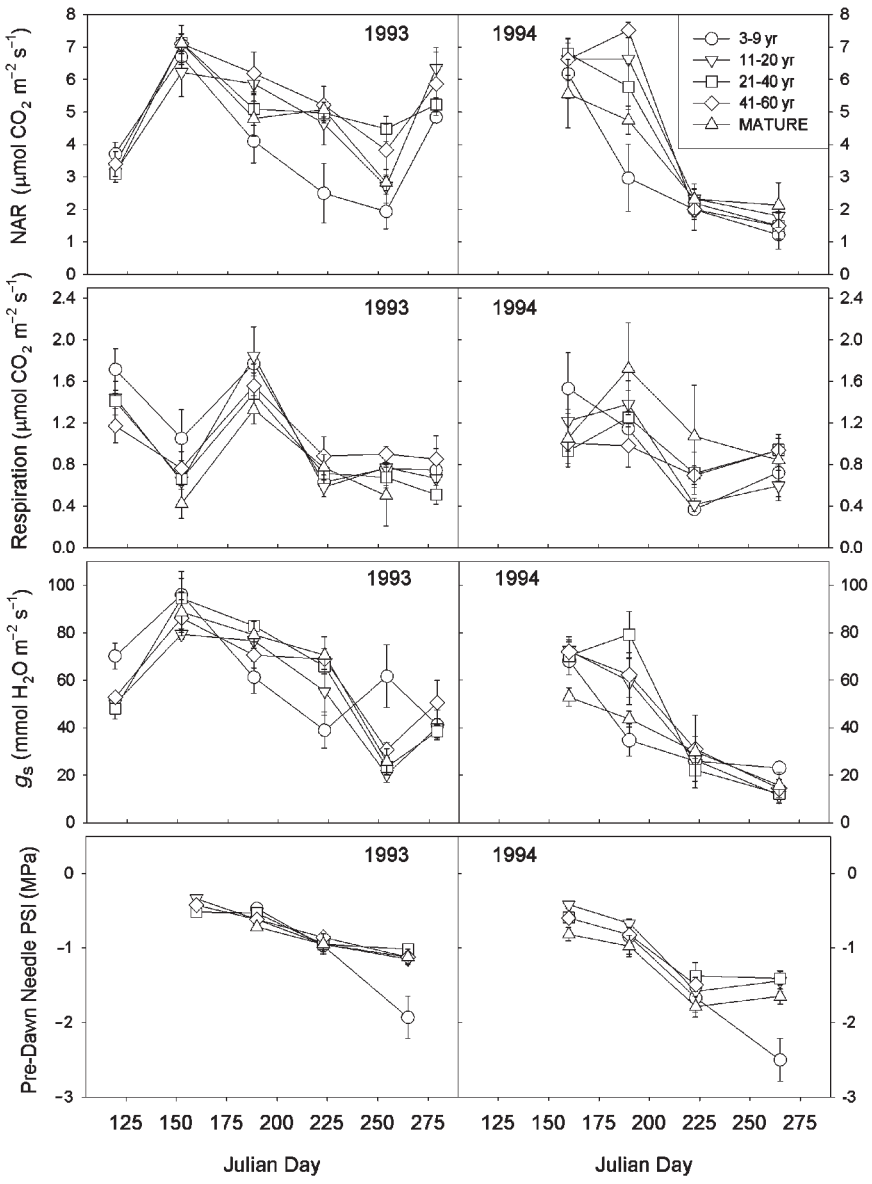


Figure 1. Seasonal course of net assimilation rate (NAR, µmol $CO_2$ $m^{-2}$ $s^{-1}$), daytime respiration rate (µmol $CO_2$ $m^{-2}$ $s^{-1}$), stomatal conductance ($g_s$, mmol $H_2O$ $m^{-2}$ $s^{-1}$), and predawn needle xylem potential (MPa) in a mesic (1993) and xeric year (1994). Each value represents the mean of six (± 1 SE) individuals in each tree-age class measured within 3 days of the Julian day indicated.

*Stomatal conductance*

In a 3-way ANOVA on $g_s$, tree age class ($P = 0.01$), Julian day ($P < 0.001$), and year ($P < 0.001$) were significant as explanatory variables (Figure 1). The interaction terms between tree age class and Julian day ($P = 0.30$), and tree age class and year ($P = 0.30$) were not significant, whereas the interaction term between tree age class, Julian day and year was significant ($P = 0.04$). Stomatal conductance was similar across all tree age classes except in April 1993 and July 1994. Among tree age classes, seedlings had the greatest $g_s$ in April 1993 ($P = 0.01$), and the lowest $g_s$ in July 1994 ($P = 0.03$).

A regression line was fit for the relationship between net assimilation and $g_s$ for each tree age class, for each month (Figure 2; graphed only for June through September). Changes in $g_s$ had little influence on NAR in late March, May, and June. The shift to more efficient water use occurred in all tree age classes by early August. All regressions were significant ($P < 0.001$) for August, September, and October for all tree age classes, but none differed significantly either in slope or *y*-intercept at $P = 0.05$. Seedlings shifted to a more efficient water use in July, a month earlier than all other tree age classes. The slope of the regression for seedlings was significantly greater than that for 11–20-year-old trees ($P = 0.04$), 21–40-year-old trees ($P = 0.06$), 41–60-year-old trees ($P = 0.06$) and mature trees ($P = 0.04$).

Figure 3 shows the relationship between maximum daily $g_s$ and VPD for the five tree age classes at non-limiting predawn needle xylem potentials, estimated at more than –0.8 MPa for seedlings, and more than –1.0 MPa for all older tree age classes. Although the slope of the regression between $g_s$ and predawn xylem potential was lower for seedlings than for other tree age classes ($P = 0.10$), the slopes of the regressions for seedlings through to 41–60-year-old trees were not significantly different at $P = 0.05$. At high VPDs, seedlings may have

Table 3. Summary of tissue chemistry reported at its phenological maximum across tree age classes. A one-way analysis of variance was used to test for differences among sites (mean ± 1 SE). Values with different letters or annotations indicate significant differences at $P < 0.05$. If not otherwise specified, foliage tissue is 1 year old. Chlorophyll, starch, and monosaccharide concentrations are reported in mg $g^{-1}$ dry weight Monosaccharide tissue concentration is summarized from Grulke et al. (2001).

| | Tree age class (year) | | | | | |
|---|---|---|---|---|---|---|
| | 3–9 | 11–20 | 21–40 | 41–60 | Mature | *P*-value |
| Total chlorophyll | 763 ± 51 a | 508 ± 91 b | 456 ± 72 b | 451 ± 41 b | 398 ± 37 b | 0.002 |
| Foliar N concentration, % | 1.24 ± 0.04 a | 0.98 ± 0.04 b | 0.99 ± 0.02 b | 1.03 ± 0.04 b | 1.04 ± 0.05 b | 0.001 |
| Foliar C concentration, % | 50.9 ± 0.2 a | 49.2 ± 0.3 b | 49.2 ± 0.4 bc | 49.2 ± 0.9 bd | 48.4 ± 0.99 bd | < 0.001 |
| Foliar soluble sugars | 415 ± 37 a | 208 ± 10 b | 186 ± 22 b | 184 ± 4 b | 220 ± 26 b | < 0.001 |
| *$\delta^{13}C$ of foliar cellulose* | | | | | | |
| 1993 | –25.4 ± 0.2 a | –24.9 ± 0.2 a | –24.9 ± 0.2 ac | –24.4 ± 0.4 bc | –24.0 ± 0.6 bc | 0.03 |
| 1994 | –24.9 ± 0.2 a | –24.1 ± 0.3 ac | –24.2 ± 0.2 ac | –23.8 ± 0.5 ac | –23.6 ± 0.7 bc | 0.07 |

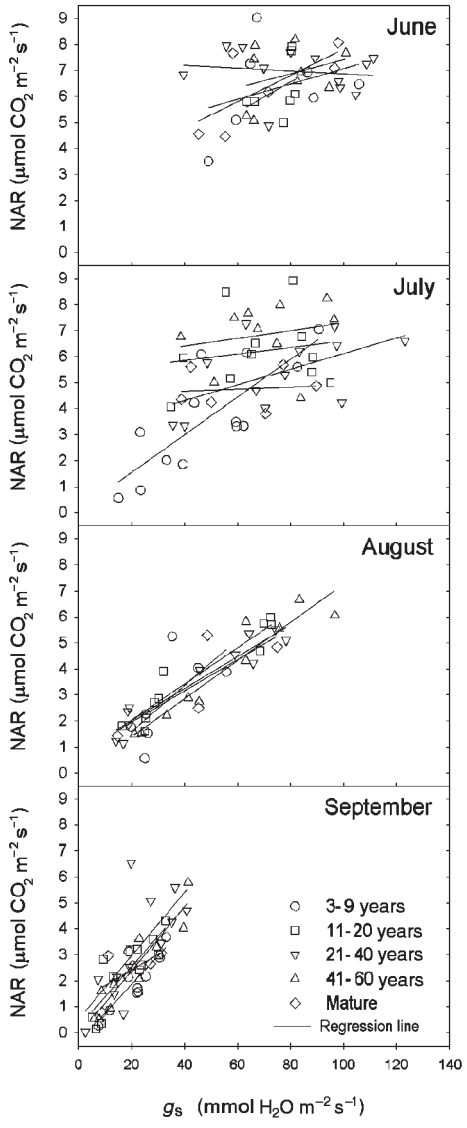


Figure 2. Seasonal change in water-use efficiency by tree age class. Late March and May responses were similar to those presented for June. October responses were similar to those presented for September.

lost stomatal control (Raschke 1975), or may have had incomplete stomatal closure (averaging 15 mmol $H_2O$ $m^{-2}$ $s^{-1}$). Mature trees appeared to be relatively unresponsive to changes in VPD during the midmorning measurements.

The relationship between $g_s$ and predawn needle xylem potential under non-limiting VPDs (< 4.5 kPa) was evaluated for the five tree age classes (Figure 3). To limit destructive sampling, predawn xylem potential was measured on seedlings other than those used in gas exchange measurements. Predawn needle xylem potential appeared to be limiting $g_s$ at values less than –0.8 MPa for seedlings, and less than –1.0 MPa for saplings and mature trees. Too few measurements of pole-sized trees were taken at low needle xylem potentials to evaluate their response. Slopes of the regression lines defining limits to $g_s$ did not differ significantly among the three tree age classes at $P = 0.05$.

### *Carbon isotope discrimination*

Needle elongation (and thus cellulose) was laid down between the end of May and was 90% complete before the onset of drought stress in mid July (Figure 4). Carbon isotope discrimination increased with increasing tree age (Table 3; Adjusted $r^2 = 0.33$, $P = 0.02$). Tree age, branch length, height of branch attachment, path length (branch attachment + branch length), needle WUE (averaged over the period of needle elongation), and chlorophyll a/b (a proxy for irradiance on sampled branches) all contributed to $\delta^{13}C$ in leaf cellulose (Rundel et al. 1988). Tree age was highly correlated with branch length (Adjusted $r^2 = 0.932$, $P < 0.001$), and moderately correlated with WUE (Adjusted $r^2 = 0.342$, $P = 0.02$) and height of foliar sample (Adjusted $r^2 = 0.649$, $P < 0.001$). When all variables were submitted to a multiple, stepwise regression, only path length (m) was a significant predictor of cellulose $\delta^{13}C$ (Adjusted $r^2 = 0.38$, $P = 0.02$).

### *Aboveground growth and foliar retention*

In both years, needle elongation growth was initiated at the end of May (Julian day 150) (Figure 4) and cessation of needle elongation occurred by mid-August (Julian day 225). Elongation growth was lowest in seedlings. Maximum needle length was greatest for mature trees in both the mesic and xeric years.

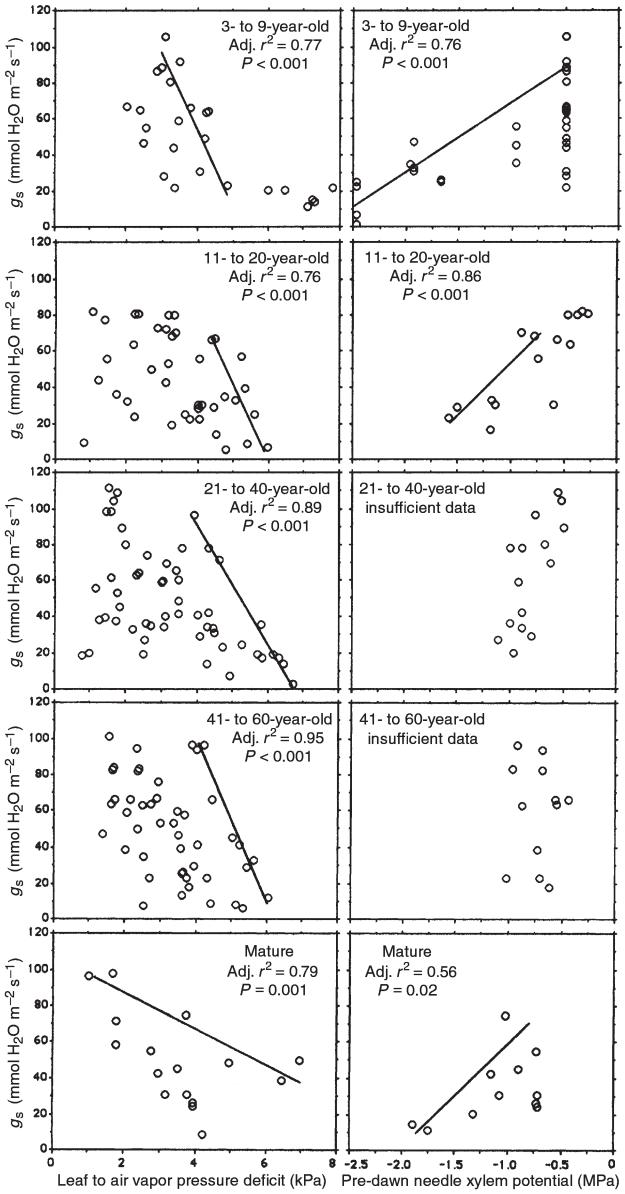


Figure 3. Relationships between stomatal conductance ($g_s$) and leaf to air vapor pressure deficit not limited by predawn xylem potential (more than –0.8 MPa for seedlings, and more than –1.0 MPa for all older tree age classes), and predawn needle xylem potential not limited by VPD (< 4.5 kPa) for the five tree age classes. A population mean for each sample date was used for seedling predawn xylem potential to reduce disturbance. Boundary line analysis (Webb 1972) was used to fit a regression line to the outer 10% of the observations in the range of physiological limitation.

Saplings 11–20-years-old had the greatest lateral branch elongation in both years. Measurements of lateral branch growth of the two younger tree age classes were made in the mid canopy, whereas lateral branch elongation was measured in the lower canopy of all older tree age classes. Maximum branch elongation was lower in 1994 than in 1993 for all tree age classes except for the 11–20-year-old class. Needle retention was lower for the youngest two tree age classes (< 20 years old) relative to the three older tree age classes (Figure 4). In the drought year, differences in needle retention between tree age classes were less distinct and not statistically significant.

### *Whole-tree biomass allocation*

Three seedlings, three saplings, and three 21–40-year-old trees were harvested in early September 1993. Estimates of seedling age based on terminal bud scars were accurate, but tree age determined at 8 cm varied significantly from tree age determined at the base of the tree (given in parentheses): 12 (versus 14 years), 14 (versus 16 years), 16 (versus 27 years), 25 (versus 36 years), 30 (versus 40 years), and 35 (versus 68 years old). Although the three trees in each tree age class were average, there was high within-age class variability in total tree biomass (Table 4). With increasing tree age, less biomass was allocated to belowground tissue, and more to aboveground support structures (bole + branch) (Table 5). Although the 27-year-old tree was placed in the 11–20-year-old tree age class based on its age at 8 cm, its allocation pattern was intermediate between the two tree age classes. It was a short tree with low total mass. For this tree, allocation to roots was more similar to the younger tree age class but higher allocation to needles was more similar to that of saplings. Trees were harvested at a time when fine root biomass was at a minimum and represented only 2% of whole-tree biomass (Grulke et al. 1998). Fine root biomass was sixfold higher before the onset of drought stress than in early September.

### *TREGRO simulations*

The biomass, carbon allocation, and other parameters of the initial ponderosa pine tree were set based on the data obtained from the field study. We first set parameters in the model to simulate the expected growth of a tree at the field site (Lassen, CA) under mesic conditions (Table 4). The simulation target was to grow a ponderosa pine tree with annual biomass increments that matched target values at 11–20, and 21–40 years old starting from a 3–9-year-old seedling. We assumed a constant rate of increase in all biomass components over the 10- and 25-year simulation periods. All TREGRO-simulated trees were calibrated by adjusting tissue growth rates and senescence rates in fine roots until two conditions were met: (1) when the simulated carbon gain of each of the trees compartments and the total tree carbon were within 10% of the field values, and (2) when the proportion of the biomass in each compartment closely matched that measured in the field. Not surprisingly, TREGRO simulations of total biomass, and proportion of biomass in needles, branch, stem, and coarse and fine roots matched well with total biomass and allocations determined in the field.

## Discussion

The Mediterranean climate, and adaptations of the local ecotype, dominated the physiological responses of ponderosa pine in this study. Determining the true site water availability in the Sierra Nevada and Transverse Ranges is one of the biggest challenges in modeling growth of ponderosa pine. Fine

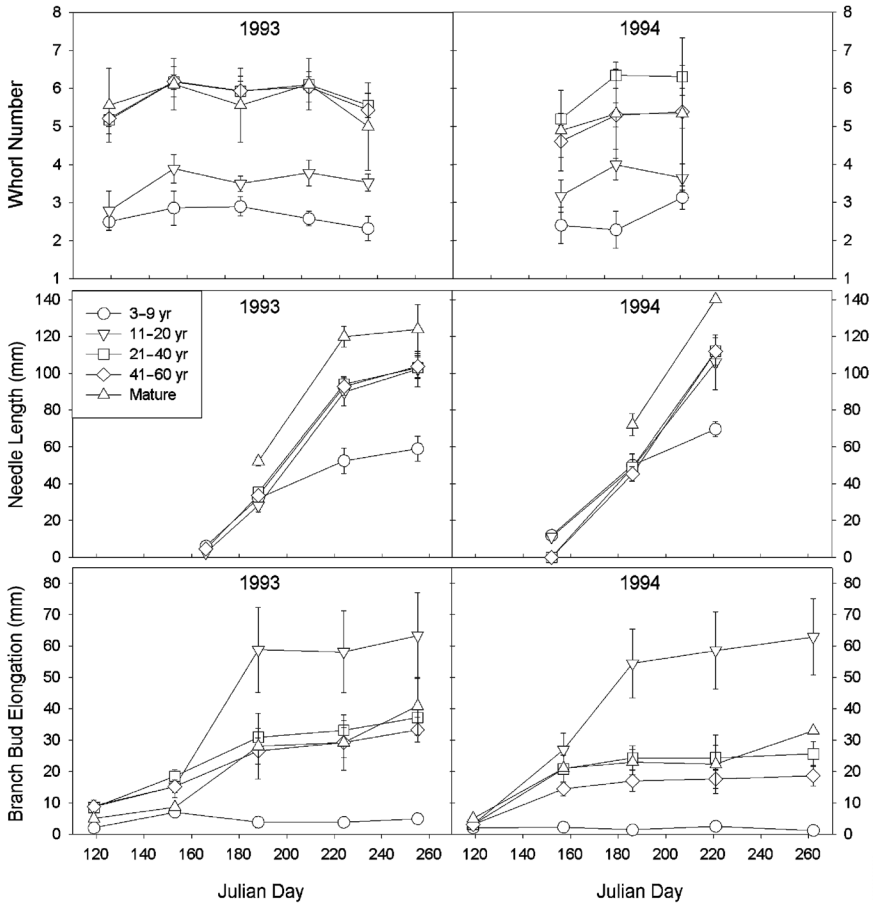


Figure 4. Seasonal course of the number of needle age classes retained, and elongation growth of needles and lateral branches in a mesic (1993) and xeric (1994) year.

Table 4. Total biomass (g dry weight), and biomass by compartment for ponderosa pine in the 3–9, 11–20, and 21–40-year-old tree age classes corresponding to allocation presented in Table 5. The % error in estimate for total biomass, and biomass by compartment is given for TREGRO simulation, targeting the mean field biomass of 15- and 40-year-old trees. The initial tree size was 5-year-old ponderosa pine on site.

| | Tree age class (years) | | |
|---|---|---|---|
| | 3–9 | 11–20 | 21–40 |
| Whole tree biomass (g) | 1.58 ± 0.39 | 370 ± 180 | 31,160 ± 19,220 |
| Simulation estimate (g) | | 340 | 30,951 |
| % Foliage | 23 | 37 | 12 |
| Simulation estimate | | 32 | 11 |
| % Branch | 7 | 21 | 17 |
| Simulation estimate | | 23 | 17 |
| % Stem | 12 | 4 | 59 |
| Simulation estimate | | 4 | 60 |
| % Coarse root | 5 | 34 | 11 |
| Simulation estimate | | 36 | 11 |
| % Fine root | 53 | 0.4 | 0.4 |
| Simulation estimate | | 0.4 | 0.4 |

root mortality in response to drying in the upper soil horizons and associated hormone signals (Loewenstein and Pallardy 1998) induced fundamental changes in leaf-level WUE, and dominated elongation growth patterns.

The correlations between $g_s$ and soil water availability in the upper soil horizons were poor, indicating that soil water availability in the upper 50 cm of soil does not adequately reflect deep water sources available to the trees (DeLucia et al. 1988, Hubbert 1999). Only the saplings had a marginally significant correlation coefficient between $g_s$ and soil water at 45-cm

Table 5. Proportion of tree biomass allocated to fine and coarse root, bole, branch, and foliage for ponderosa pine in three tree age classes in a natural stand. For the seedlings, there were no coarse roots and stem (bole) was reported as branch tissue. Mean ± 1 SE is given.

| | Tree age class (years) | | |
|---|---|---|---|
| | 3–9 | 11–20 | 21–40 |
| Age, years | 4 ± 1 | 16 ± 6 | 48 ± 10 |
| % foliage | 25 ± 6 | 32 ± 8 | 14 ± 2 |
| % branch | 20 ± 2 | 24 ± 5 | 17 ± 2 |
| % stem | – | 4 ± 1 | 60 ± 3 |
| % coarse root | – | 34 ± 6 | 9 ± 2 |
| % fine root | 55 ± 4 | 6 ± 3 | 1 ± 1 |

depth. Because trees at Lassen, CA were physiologically active during at least some part of the day in August, September, and October despite the lack of precipitation, trees older than 20 years must have been relying on water obtained from roots deep (> 100-cm deep) within interstices of cracked bedrock (Jones and Graham 1993, Hubbert 1999). Unfortunately, in ponderosa pine ecosystems with deeply weathered bedrock, the only proxy for available soil water is predawn needle Ψ. Maximum daily $g_s$ of seedlings, saplings, and mature trees was significantly correlated to predawn needle Ψ at non-limiting VPDs (< 4.5 kPa). Among tree age classes, $g_s$ of mature trees was least responsive to changes in VPD at non-limiting predawn needle Ψ.

Gas exchange measurements in the field vary with ecotype, season and phenology, canopy position, foliar age class, and microenvironment of the tree. However, when trees were at a daily maximum of hydration in the morning, leaf specific gas exchange was similar across tree age classes. Similarly, under non-limiting conditions in the morning, maximum NAR measured in the upper canopy of ponderosa pine trees in central Oregon was similar between pole-sized and mature trees (54- and 230-year-old trees, Yoder et al. 1994), and comparable with the values reported for the lower one third of the mature tree canopy in this study. Our leaf-specific maximum NAR and $g_s$ values were also similar to those reported for *P. ponderosa* var. *scopulorum* in Arizona (Kolb and Stone 2000).

Although maximum gas exchange rates were similar across tree age classes, physiological limitations to water movement in mature trees over the course of the day (Yoder et al. 1994, Hubbard et al. 1999) and growing season were reflected in an integrated measure of WUE, provided by carbon isotopic discrimination. In general, NAR and $g_s$ in conifers decline with increasing tree age (e.g., ponderosa pine and lodgepole pine, Yoder et al. 1994, Ryan and Waring 1992, bristlecone pine (*Pinus aristata* Engelm.), Schoettle 1994, giant sequoia (*Sequoiadendron giganteum* (Lindl.) Buchholz), Grulke and Miller 1994, Norway spruce (*Picea abies* L. Karst), Kull and Koppel 1987). Greater resistance to water flow as a result of longer stems (Mencuccini and Grace 1996) and branch length (Walcroft et al. 1996) contributed to lower $g_s$ (higher WUE) in older trees compared with younger trees. The trend to less negative $\delta^{13}C$ values with increasing tree age indicates that stomatal conductance decreases with tree age. Qualitatively similar trends have been reported for pole-sized versus mature ponderosa pine and lodgepole pine (Yoder et al. 1994). Our values of $\delta^{13}C$ for ponderosa pine in the dry year were similar to those reported by Carey et al. (1998) for montane ponderosa pine in Nevada. Generally, $\delta^{13}C$ varied little across a broad range in tree age (from 5 to 50 years old). We found that path length (bole + branch length) was the best explanatory variable for carbon isotopic discrimination.

It is not necessarily the maximum gas exchange, but the seasonal phenological patterns and site-specific environmental limitations to gas exchange that are important for modeling efforts. In Arizona, there are two transitions in WUE corresponding to two soil drying cycles: one by mid-June, and one by mid- to late August when thunderstorms rewet the upper soil surfaces (Kolb and Stone 2000). In our study, there was only one soil drying cycle and one shift to increased water-use efficiency. Water-use efficiency was similar across all tree age classes early and late in the growing season. Seedlings shifted to more efficient water use one month earlier than all older tree age classes. The integrated measure of seedling WUE indicated that seedlings were less efficient overall than older tree age classes. The $CO_2$ concentration during the instantaneous gas exchange measurements was significantly higher for seedlings and saplings than for all older tree age classes. Respired $CO_2$ from the soil is much lighter than that of the atmosphere (Andreux et al. 1990), and could have contributed to a more negative $\delta^{13}C$ in seedlings.

In Arizona populations of ponderosa pine, the two transitions in WUE within the growing season corresponded to two phases of needle elongation growth (Kolb and Stone 2000). In our study, the phenological pattern of needle growth was similar for all tree age classes: elongation ceased within two to three weeks of the drying of the upper soil horizon, and the pattern was consistent in both mesic and xeric years. Needle elongation and integrated WUE were lowest in seedlings, greatest in mature trees, and intermediate over a broad tree age range (11–60-year-old trees). Lateral branch elongation was lowest for seedlings and greatest for saplings, despite sampling in mid-canopy for both tree age classes. We conclude that site-specific phenology confounds more general modeling efforts in this species.

Branch elongation slowed during the period of needle growth, providing support for the idea that growth is limited by internal competition among sinks for carbon resources (Waring and Schlesinger 1985, Luxmoore et al. 1995). Branch elongation growth of mature trees appeared to increase slowly until the end of October. Although water was available to mature trees throughout the growing season (Hubbert 1999), rate of delivery was probably limited by low hydraulic conductance in mature trees (Yoder et al. 1994, Hubbard et al. 1999, Kolb and Stone 2000). Within the canopy, the greatest current-year shoot growth occurred in the mid-canopy (Solling 1972, as seen in Schulze et al. 1977). Lateral branch growth is suppressed in the upper canopy by high auxin concentrations (Denne 1979), and by light-limited carbon acquisition in the lower canopy (Luxmoore et al. 1995). Although irradiance at the primary branches did not appear to be limiting, a better understanding of within-canopy variability of elongation growth and its response to environmental stressors may be needed to adequately model mature trees in natural stands. Tools such as multiple elemental isotopic discrimination may aid in understanding within-canopy variability of gas exchange, although ambient $CO_2$ concentrations and possible differences in the timing of cell wall growth may confound efforts.

Foliar retention was significantly lower for seedlings and saplings than for all older tree age classes. Tissue chemistry of saplings was similar to that of all older tree age classes, whereas seedlings had higher nitrogen and carbon contents, and higher carbohydrate (Grulke et al. 2001) and chlorophyll concentrations in foliage. Thus, needle loss in seedlings represented a much greater proportion of whole-plant resources

than for any other tree age class. Ponderosa pine seedlings and saplings exhibited strong conservation of resources: despite severe drought stress, surviving seedlings and saplings had no fewer needle age classes in the xeric year than in the mesic year. In contrast, mature and 41–60-year-old pole-sized trees lost multiple needle age classes in the xeric year (this study, and Grulke and Balduman 2000). Changes in needle retention with tree age class will have a significant effect on modeled ponderosa pine responses, especially to stressors that reduce foliar retention (drought, ozone exposure, high nitrogen deposition).

Whole-tree biomass was lower than that reported for greenhouse-, chamber-, or plantation-grown ponderosa pine. Sapling biomass was comparable with that of saplings growing in small gaps (9 m in diameter) on a nearby site (McDonald and Reynolds 1999), although distances between tree clusters was much greater in this study (estimated as 5–20 m). Pole-sized trees in our natural stand (21–40-year-old trees) had similar diameter growth to that of 10-year-old plantation-grown trees at a nearby site. There was little competition from herbs or shrubs at the Lassen site.

The TREGRO simulations of total biomass, and proportion of biomass in needles, branch, stem, and coarse and fine roots matched well with total biomass and allocations determined in the field (Table 4). Ponderosa pine seedlings had high biomass allocation to roots, saplings had high foliar and branch biomass allocation, and pole-sized trees had the highest biomass allocation to support structure tissues (branches and bole). Both tree age and size influenced biomass allocation patterns. Lodgepole pine also showed increases in allocation to stems as trees aged from 15 to 30 years (Smith and Resh 1999). However, reductions in belowground carbon allocation only occurred in a very old lodgepole stand, whereas reductions in belowground carbon allocation occurred in a 30-year-old ponderosa pine tree in this study. In Scots pine, allocation to foliage, woody tissue, and roots was 20, 49 and 30%, respectively, in 14-year-old trees and 15, 77 and 30%, respectively, in 23-year-old trees (Ovington 1957, as seen in Miller et al. 1990). We observed a much greater increase in woody tissue between comparable tree age classes at the expense of both foliage and root biomass. In western juniper, foliar mass was constant between comparable tree age classes, but woody tissue increased at the expense of root biomass. Foliar mass did not increase appreciably with increasing tree age, but bole mass continued to increase in monospecific stands of *Pinus* species (Gower et al. 1995). In slash pine (*Pinus elliotii* Engelm.) plantations, the shift to increased woody tissue biomass occurred in 5-year-old trees, whereas in our natural ponderosa pine stand it did not occur until the trees were 35 years old.

This study marks one of the first attempts to parameterize TREGRO over a series of life stages of a single tree species. Red oak (*Quercus rubra* L., Weinstein et al. 1993) is the only other species for which both seedling and mature tree simulations have been made. The most common application of TREGRO has been to simulate a short-term (3 year) response of a seedling (Laurence et al. 1993, Weinstein et al. 1998) or mature tree (Constable et al. 1996, Constable and Taylor 1997, Retzlaff et al. 2000) to an environmental stressor, consequently, the simulations have not been long enough to develop the decadal changes in biomass presented here. When extrapolating seedling, sapling, or plantation-grown tree responses to mature trees in natural stands, special consideration for the amount of time the seedling remains small, and the timing of the changes in resource allocation as the individual matures will improve modeling success. Providing for deep water access in weathered bedrock will be a critical next step in the development of physiologically based models and their application to ecosystems characterized by summer droughts.

## Acknowledgments

This research was funded by US Environmental Protection Agency agreement, DW-12934530, but has not been subjected to the Agency's review and therefore does not necessarily reflect the views of the Agency. No official endorsement should be inferred. Special thanks to Bill Hogsett, who supported the field research. Janet Kirsch, Lynn Morrison, Patti Haggerty, and Leanne Mitchell all significantly contributed to the field data collection. Diane Fisher processed all tissue samples for analysis and determined chlorophyll concentrations. Lisa Balduman is credited for data entry, analysis, and quality assurance.

## References

Andreux, F., C. Cerri, P.B. Vose and V.A. Vitorello. 1990. Potential of stable isotope, $^{15}N$ and $^{13}C$, methods for determining input and turnover in soils. *In* Nutrient Cycling in Terrestrial Ecosystems. Eds. A.F. Harrison, P. Ineson and O.W. Heal. Elsevier Applied Science, New York, pp 260–275.

Barbour, M.G. 1988. Californian upland forests and woodlands. *In* North American Terrestrial Vegetation. Eds. M.G. Barbour and W.D. Billings. Cambridge Univ. Press, New York, pp 131–164.

Carey, E.V., R.M. Callaway and E.H. DeLucia. 1998. Increased photosynthesis offsets costs of allocation to sapwood in an arid environment. Ecology 79:2281–2291.

Constable, J.V.H., G.E. Taylor, Jr., J.A. Laurence and J.A. Weber. 1996. Climatic change effects on the physiology and growth of *Pinus ponderosa*: expectations from simulation modeling. Can. J. For. Res. 26:1315–1325.

Constable, J.V.H. and G.E. Taylor, Jr. 1997. Modeling the effects of elevated tropospheric $O_3$ on two varieties of *Pinus ponderosa*. Can. J. For. Res. 27:527–537.

DeLucia, E.H., W.H. Schlesinger and W.D. Billings. 1988. Water relations and the maintenance of sierran conifers on hydrothermally altered rock. Ecology 69:303–311.

Denne, M.P. 1979. Wood structure and production within the trunk and branches of *Picea sitchensis* in relation to canopy formation. Can J. For. Res. 9:406–427.

Gower, S.T., J.G. Isebrands and D.S. Sheriff. 1995. Carbon allocation and accumulation in conifers. *In* Resource Physiology of Conifers. Eds. W.K. Smith and T.M. Hinckley. Academic Press, San Diego, CA, pp 217–254.

Grulke, N.E. 1999. Physiological responses of ponderosa pine to gradients of environmental stressors. *In* Oxidant Air Pollution Impacts in the Montane Forests of Southern California. Eds. P.R. Miller and J.R. McBride. Springer-Verlag, New York, pp 126–163.

Grulke, N.E. and L. Balduman. 2000. Deciduous conifers: high N deposition and $O_3$ exposure effects on growth and biomass allocation in ponderosa pine. Water Air Soil Pollut. 116:235–248.

Grulke, N.E. and P.R. Miller. 1994. Changes in gas exchange characteristics during the life span of giant sequoia: implications for response to current and future concentrations of atmospheric ozone. Tree Physiol. 14:659–668.

Grulke, N.E., C.P. Andersen, M.E. Fenn and P.R. Miller. 1998. Ozone exposure and nitrogen deposition lowers root biomass of ponderosa pine in the San Bernardino Mountains, California. Environ. Pollut. 108:63–73.

Grulke, N.E., C.P. Andersen and W.E. Hogsett. 2001. Seasonal changes in above- and belowground carbohydrate concentrations of ponderosa pine along a pollution gradient. Tree Physiol. 21: 173–181.

Hiscox, J.D. and G.F. Israelstam. 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot. 57:1332–1334.

Hubbard, R.M., B.J. Bond and M.G. Ryan. 1999. Evidence that hydraulic conductance limits photosynthesis in old *Pinus ponderosa* trees. Tree Physiol. 19:165–172.

Hubbert, K.R. 1999. The edaphic role of weathered bedrock in a seasonally-dry Jeffrey pine plantation. Ph.D. Diss., Dept. Soil Water Sciences, Univ. California, Riverside, CA, 139 p.

Jones, D.P. and R.C. Graham. 1993. Water-holding characteristics of weathered granitic rock in chaparral and forest ecosystems. Soil Sci. Soc. Am. J. 57: 256–261.

Kleinbaum, D.G. and L.L. Kupper. 1978. Applied regression analysis and other multivariable methods. Duxbury Press, North Scituate, MS, pp 188–208.

Kolb, T.E. and J.E. Stone. 2000. Differences in leaf gas exchange and water relations among species and tree sizes in an Arizona pine–oak forest. Tree Physiol. 20:1–12.

Kull, O. and A. Koppel. 1987. Net photosynthetic response to light intensity of shoots from different crown positions and age in *Picea abies* (L.) Karst. Scand. J. For. Res. 2:157–166.

Laurence, J.A., R.J. Kohut and R.G. Amundson. 1993. Use of TREGRO to simulate the effects of ozone on the growth of red spruce seedlings. For. Sci. 39:453–464.

Loewenstein, N.J. and S.G. Pallardy. 1998. Drought tolerance, xylem sap abscisic acid and stomatal conductance during soil drying: a comparison of canopy trees of three temperature deciduous angiosperms. Tree Physiol. 18:431–439.

Luxmoore, R.J., R. Oren, D.W. Sheriff and R.B. Thomas. 1995. Source–sink-storage relationships of conifers. *In* Resource Physiology of Conifers. Eds. W.K. Smith and T.M. Hinckley. Academic Press, San Diego, CA, pp 179–216.

McDonald, P.M. and P.E. Reynolds. 1999. Plant community development after 28 years in small group-selection openings. USDA Forest Service Research Paper PSW-241, 17 p.

Mencuccini, M. and J. Grace. 1996. Developmental patterns of above-ground hydraulic conductance in a Scots pine (*Pinus sylvestrus* L.) age sequence. Plant Cell Environ. 19:939–948.

Miller, P.M., L.E. Eddleman and S. Kramer. 1990. Allocation patterns of carbon and minerals in juvenile and small-adult *Juniperus occidentalis*. For. Sci. 36:734–747.

Monserud, R.A. and J.D. Marshall. 1999. Allometric crown relations in three northern Idaho conifer species. Can. J. For. Res. 29: 521–535.

Monson, R.K. and M.C. Grant. 1989. Experimental studies of ponderosa pine. III. Differences in photosynthesis, stomatal conductance, and water-use efficiency between two genetic lines. Am. J. Bot. 76:1041–1047.

Oliver, W.W. and R.A. Ryker. 1990. *Pinus ponderosa* Dougl. *ex* Laws. *In* Silvics of North America. Vol. 1. Conifers. Tech. Coor. R.M. Burns and B.H. Honkala. USDA Forest Service Agricultural Handbook 654, Washington, DC., pp 413–424.

Pallardy, S.G., J.S. Pereira, and W.C. Parker. 1991. Measuring the state of water in tree systems. *In* Techniques and Approaches in Forest Tree Ecophysiology. Eds. J.P. Lassoie and T.M. Hinckley. CRC Press, Inc., Boca Raton, FL, pp 27–76.

Raschke, K. 1975. Stomatal action. Ann. Rev. Plant Phys. 26: 309–340.

Retzlaff, W.A., M.A. Arthur, N.E. Grulke, D.A. Weinstein and B. Gollands. 2000. Use of a single-tree simulation model to predict effects of ozone an drought on growth of a white fir tree. Tree Physiol. 20:195–202.

Rundel, P.W., J.R. Ehleringer and K.A. Nagy. 1988. Stable Isotopes in Ecological Research. Springer-Verlag, New York, 409 p.

Ryan, M.G. and R.H. Waring. 1992. Maintenance respiration and stand development in a subalpine lodgepole pine forest. Ecology 73:2100–2108.

Schoettle, A.W. 1994. Influence of tree size on shoot structure and physiology of *Pinus contorta* and *Pinus aristata*. Tree Physiol. 14:1055–1068.

Schulze, E.-D., M. Fuchs and M.I. Fuchs. 1977. Spatial distribution of photosynthetic capacity and performance in a mountain spruce forest of northern Germany. Oecologia 30:239–248.

Smith, F.W. and S.C. Resh. 1999. Age-related changes in production and below-ground carbon allocation in *Pinus contorta* forests. For. Sci 45:333–341.

Sokal, R.R. and F.J. Rohlf. 1969. Biometry: the principles and practice of statistics in biological research. W.H. Freeman and Co., San Francisco, CA. pp 204–287.

Temple, P.J. and P.R. Miller. 1999. Seasonal influences on ozone uptake and foliar injury to ponderosa and Jeffrey pines at a southern California site. *In* Proc. Int. Symp. Air Pollution and Climate Change Effects on Forest Ecosystems. Eds. A. Bytnerowicz, M.J. Arbaugh and S.L. Schilling. USDA Forest Service General Technical Report PSW-166, pp 221–228.

Walcroft, A.S., W.B. Silvester, J.C. Grace, S.D. Carson and R.H. Waring. 1996. Effects of branch length on carbon isotope discrimination in *Pinus radiata*. Tree Physiol. 16:281–286.

Waring, R.H. and W.H. Schlesinger. 1985. Forest ecosystems. Academic Press, New York, 325 p.

Webb, R.A. 1972. Use of the boundary line in the analysis of biological data. J. Hort. Sci. 42:309–319.

Weinstein, D.A., R.M. Beloin and R.D. Yanai. 1991. Modeling changes in red spruce carbon balance and allocation in response to interacting ozone and nutrient stresses. Tree Physiol. 9:127–146.

Weinstein, D.A., M.A. Arthur and L. Samuelson. 1998. Simulating the differences in $O_3$ response between seedlings and mature red oak trees. Environ. Pollut. 102:307–320.

Wise, L.F., M. Murphy and A.A. D'Addieco. 1945. Chlorite holocellulose, its fractionation and bearing on summative wood analysis and on studies on the hemicelluloses. Section Tech. Assoc. Pulp Paper Indust. 122:11–19.

Yoder, B.J., M.G. Ryan, R.H. Waring, A.W. Schoettle and M.R. Kaufmann. 1994. Evidence of reduced photosynthetic rates in old trees. For. Sci. 40:513–527.