

FINAL TECHNICAL REPORT FOR ENERGY RD&D PROJECT:

“What causes the density effect in young forest plantations?”

Project DE-FC07-97ID13530

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Executive Summary

In young forest plantations, trees planted at high densities frequently show more rapid height and diameter growth than those plants at lower densities. This positive growth response to density (the "density effect") often manifests long before seedlings are tall enough to shade one another, so it is not a simple response to shade. The mechanism(s) which trigger and sustain this growth enhancement are unknown. Our objectives were to document the temporal dynamics of positive growth response to increasing density in Douglas-fir plantations and to test two hypotheses as potential mechanisms for this response. The hypotheses are 1) a canopy boundary layer effect, and 2) alterations in the quality of light reflected from neighboring trees.

The "boundary layer" hypotheses proposes that changes in atmospheric mixing occur in high-density plantations, promoting increased concentrations of CO₂ and H₂O vapor during early morning hours, which in turn would enhance carbon assimilation. The "light quality" hypothesis proposes that the presence of neighbors alters the ratio of red to far red light in the canopy environment. Plant sensors detect this change in light quality, and growth and development is altered in response.

We found that boundary layer conductance was higher, as we predicted, in low-density Douglas-fir stands than in high-density stands five years after planting. The changes in boundary conductance were accompanied by higher CO₂ and H₂O vapor during early morning hours. However, we also found that the primary manifestation of the density effect in Douglas-fir occurs two to four years after planting, and we were not able to measure differences in boundary conductance in different densities at that time. Also, we found no difference in carbon isotope composition of wood cellulose formed in high- vs. low-density stands two to three years after planting. We conclude that although stand density may have a significant impact on boundary layer conductance in young stands, it does not account for the "density effect".

Our tests of the light quality hypothesis were slowed due to poor plantation establishment in the early phase of this study. In a variable density experiment we detected significant changes in R:FR related to density. Also in that study we measured a significant enhancement of tree height at high density. However, after three years of growth, the study trees did not show significant differences in stem diameter related to density. Experimenters at Weyerhaeuser therefore decided not to harvest the trees at the end of the 3rd growth year, as originally planned. In a 1-year study of seedlings planted in raised beds subjected to different light quality treatments using transparent plastic film, we found that tree height but not diameter increased in response to decreased R:FR. At this point, we conclude that R:FR remains a viable hypothesis for the "density effect", but evidence is not conclusive. We expect that continued measurements in the variable density test plot at Weyerhaeuser will add more evidence in the future.

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Project Goals, Objectives and Tasks

In young forest plantations, trees planted at high densities frequently show more rapid height and diameter growth than those plants at lower densities. This positive growth response to density (the "density effect") often manifests long before seedlings are tall enough to shade one another, so it is not a simple response to shade. It occurs even when competition from other species is well controlled. The effect is ephemeral, typically lasting for a few to several years, and has been observed in several hardwood and coniferous species.

The mechanism(s) which trigger and sustain this growth enhancement are unknown. Possibilities, include: enhanced nutrient availability at higher densities (due to greater mycorrhizal development, root exudations, more litter, or other causes); improved soil water availability at higher densities (deemed not likely but possible due to condensation of atmospheric moisture on needles); phytochrome response to changes in light quality due to reflectance from foliage of neighboring trees; reduced weed competition; reduced animal browsing; and increased resistance in the atmospheric boundary layer in the high density plots. In an experiment at Weyerhaeuser that preceded this project, potted Douglas-fir seedlings were placed at varying density (265 cm² to 2555 cm² per plant) in a greenhouse. After 30 weeks, mean plant height, crown biomass and branch number increased significantly with decreasing growing space (Ritchie, manuscript in review). This indicates that root or soil phenomena are not likely to be important sources of the density effect, and provides support for the above-ground mechanisms. This study was designed to test two of these hypotheses:

The R:FR hypothesis. This hypothesis proposes that plants are able to perceive the presence of neighboring plants by the R:FR ratio of the light reflected from their foliage. This ratio is depleted when a plant is growing with neighbors because chlorophyll absorbs preferentially in the red region. The perception is made by the phytochrome system and is transduced into greater height and diameter growth to avoid being overtopped by these future competitors. Whether this growth adjustment involves an increase in photosynthetic efficiency or preferential allocation of carbon to above-ground biomass is not known. Such behavior has been noted in several annual plant species and fits well with competition hypotheses.

The canopy boundary layer hypothesis: This hypothesis proposes that the microclimate around individual plants is altered by neighbors, and this alteration may improve plant growth when the density is not so high that neighbors are competing for resources. As plants absorb or release gases and energy, concentration gradients develop in the atmosphere. Canopy boundary layers reduce atmospheric mixing, and can lead to very different gas concentrations and temperatures within the canopy than above it. Low wind speed, an even canopy surface, small plant stature, and high plant density all increase the boundary layer and inhibit mixing. Atmospheric mixing is usually lowest at night, when wind speed is low and CO₂ is released due to respiration by soil and

plants. A substantial increase in the CO₂ concentration around plant foliage may result, producing higher morning photosynthetic rates. A high canopy boundary layer will also increase the local humidity around leaves because water vapor from soil evaporation and transpiration will be trapped. Stomata are likely to respond to increased humidity by opening more or remaining open longer in the day, increasing the daily net photosynthesis as well as photosynthetic water-use efficiency (CO₂ gained/H₂O transpired).

The goal of our project was to test these two hypotheses using Douglas-fir as a model species. Five tasks were outlined for these tests: 1) Measurements of Red:Far Red light in a newly-planted variable density test site, 2) Growth and carbon allocation studies in the variable-density test site, 3) Photosynthetic efficiency studies (through measurements of chlorophyll fluorescence) in the variable-density test site, 4) Analyses of CO₂ and H₂O vapor profiles, growth dynamics and carbon isotope ratios of annual rings in 7- to 12-year-old density test plots established by Weyerhaeuser, and 5) Measure gas exchange in the 7- to 12-year-old density test plots to evaluate impact of density-altered microclimate on net photosynthesis. The first three tasks are relevant to the R:FR hypothesis, and were the primary responsibility of scientists at Weyerhaeuser as the cost-share component of this project. The final two tasks are relevant to the boundary layer hypothesis and were the primary responsibility of OSU scientists.

Actual Accomplishments

We encountered difficulties in establishing the new variable-density test site for Tasks 1-3. The test site at Weyerhaeuser was planted in the first year after we learned that the project would be supported, but unfortunately by then the growing season was well under way, and most of the seedlings died. The site was replanted in the second year of the study, but weed control was inadequate and again, mortality was high. The site was replanted successfully near the end of the 2nd year of the project (details provided below) and the experiment progressed well from there, but unfortunately this delayed this part of the study by two years. Nevertheless, task 1 has been accomplished very well and significant progress has been made with tasks 2 and 3.

Because of the delays in establishing the new variable density plot, and because additional funding became available, we added a new, complementary task to test the R:FR hypothesis. Douglas-fir seedlings were planted at a low density in raised beds at Oregon State University. After establishment, the seedlings were surrounded by transparent, colored film to alter the quality of light reaching the seedling. Details are provided below. With this study we were able to examine how the growth and morphology of Douglas-fir is altered in response to R:FR without manipulating planting density.

As we initiated the study, it became apparent that task #4 was very large, so it was divided into “sub-tasks”. These were, 4a: through retrospective growth analyses, determine the dynamics of the positive growth enhancement in different densities, 4b: evaluate carbon isotope ratios in wood cellulose of trees growing at different densities during years in which the “density effect” is apparent to evaluate possible physiological mechanisms. For example, if growth enhancement is caused by CO₂ enrichment due to a boundary layer effect, one expects depletion of ¹³C relative to ¹²C in wood cellulose, 4c: measure CO₂ and H₂O vapor in vertical profiles in different densities to test the hypothesis that concentrations are higher in high-density plots in the morning, and 4d: through direct measurements of microclimate and stomatal conductance, calculate the boundary layer conductance in different planting densities at different times of day. As explained in detail in the following sections, the results of tasks 4a and 4b effectively disproved the possibility that boundary layer dynamics could be responsible for the “density effect”. We learned that the “density effect” occurs only in the first few years after planting; size differences persist for many years simply because of the early growth boost. We also found no significant differences in microclimate, or boundary layer, among different densities during the early years when the growth enhancement occurred. Nevertheless, we continued with the initial plans for measuring boundary layer differences (tasks 4c and 4d), only we conducted the measurements in stands that were slightly older than the age when we knew that the density effect occurred. On the other hand, we dropped task 5 (evaluating the impact of boundary layer differences on photosynthesis) once we knew that differences in boundary layer were not responsible for the “density effect”.

Project Activities

Testing the R:FR Hypothesis

The variable density plot at Weyerhaeuser

This part of the study plan involves measurements in a “Nelder”-style variable density plot. The “Nelder” configuration is a circular plot, with seedlings planted in concentric circles, and the distance between subsequent circles increases from the inside to the outer ring. Thus, the planting density decreases from the inside out, establishing a large variation in density in a relatively small space.

The start-date of the study was initially planned for early spring, 1997. By that time we had prepared a test site on Weyerhaeuser’s Mima Nursery grounds to contain the Nelder, but we delayed planting the site until official notification of grant acceptance. When we were notified that the grant had been approved on about June 15, 1997, we immediately planted containerized Douglas-fir seedlings on the site in the Nelder configuration. We were concerned about the lateness of the planting date but were optimistic that the container stock would perform adequately. Unfortunately it did not. At the beginning of the second growing

season we replaced dead and low vigor seedlings. By the end of the second growing season it was clear that the seedlings lacked uniformity, both in size and vigor, which would confound and compromise the experimental design, so we decided to replant the entire test. Because of the two-year delay in establishment of this experiment, we ended up requesting two years of no-cost extension for the project (all other components were completed on time). Indeed, the final stages of the Nelder experiment are still in progress as we write this final report.

At the beginning of the third season (March 1999) we replanted with large Douglas-fir container stock, and this stock has performed extremely well (Fig. 1). We employed a local forestry contractor to hand remove all weeds (mostly thistle) from the plot and to hoe out any newly emerging weeds on a weekly basis. As a result, the plot remained very clean and weed-free throughout the growing season (Figure 2).



Figure 1. Photograph of trees in the Nelder plot taken on May 24, 2000. Note vigorous growth and shoot elongation. Taken from the plot center looking west.



Figure 2. Photograph taken on July 26 showing nearly total weed control on Nelder plot. View is to the south looking into the center of the plot.

R:FR measurements in the Nelder Plot (Task #1)

We conducted R:FR measurements during the 1999, 2000 and 2001 growing seasons. In 1999, we were not able to detect any consistent pattern of horizontally reflected R:FR ratios, nor did we observe any differences in height growth with distance from the plot center (decreasing planting density). However, we did find significantly different R:FR ratios related to density in subsequent years. On May 24, 2000, measurements taken within the plot with two different sensors showed the expected trend - both along north-south and east-west transects (Figure 3).

R:FR values were lowest at the plot center where they ranged around 0.80, then followed a logarithmic increase out to about 12 m from the plot center, where they appeared to reach a plateau. To follow up on this encouraging result we employed a summer intern, Tim Koontz, an Olympia-area high school science teacher. Tim spent about five weeks during July and early August conducting intensive light measurements in the Nelder plot to confirm and broaden this early observation.

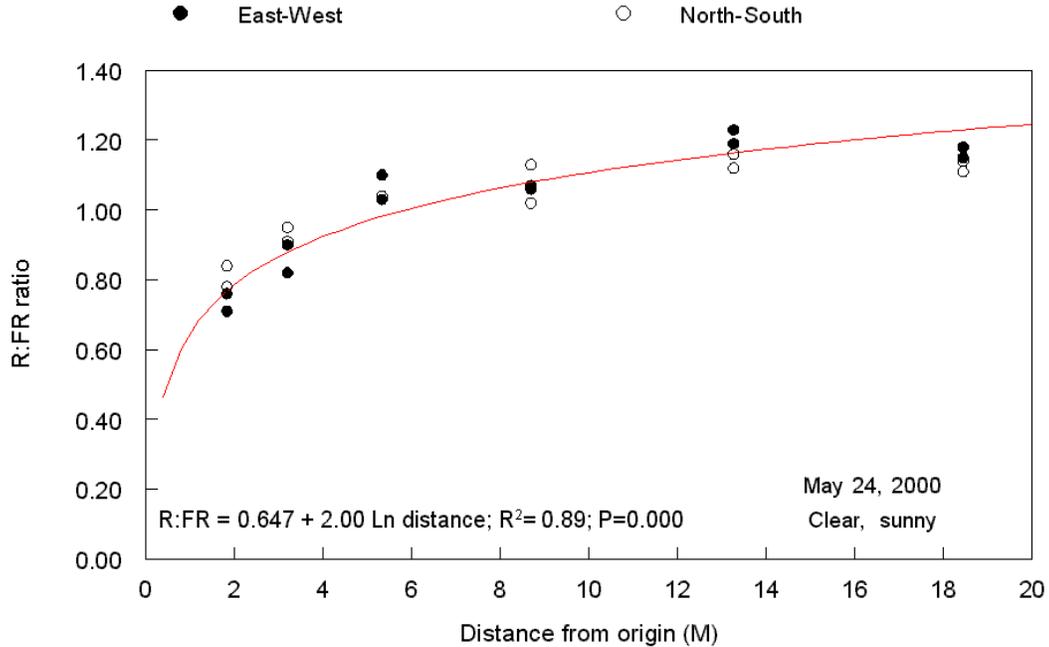
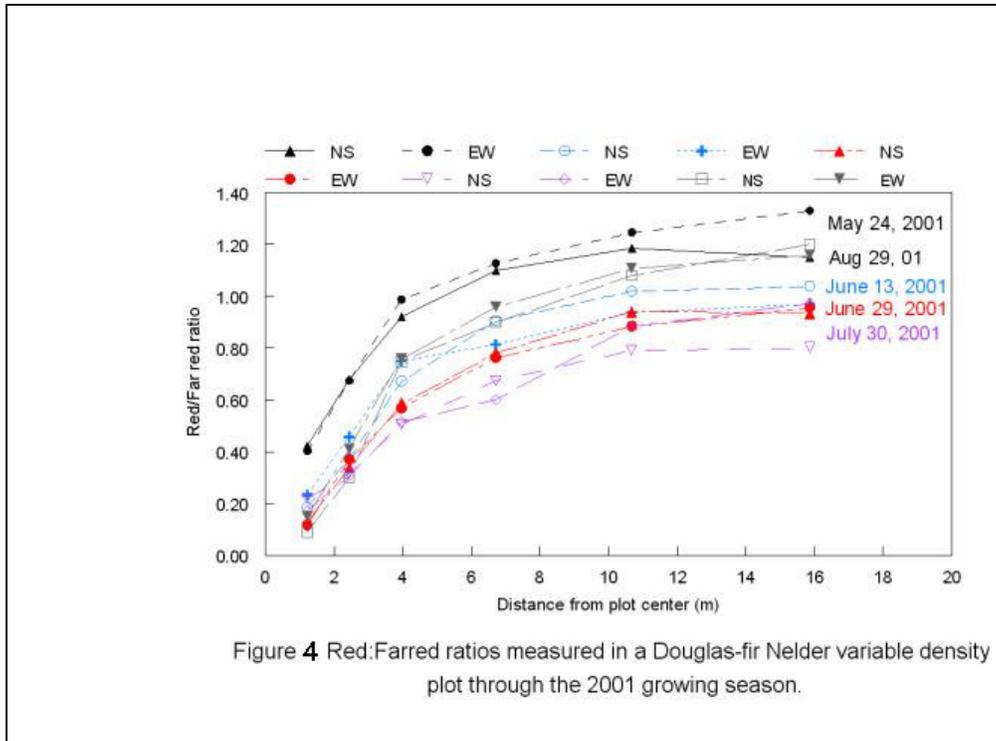


Figure 3. Horizontally measured R:FR light as a function of distance from the center of the Nelder plot on May 24, 2000. Data pooled from two sensors and two transects.

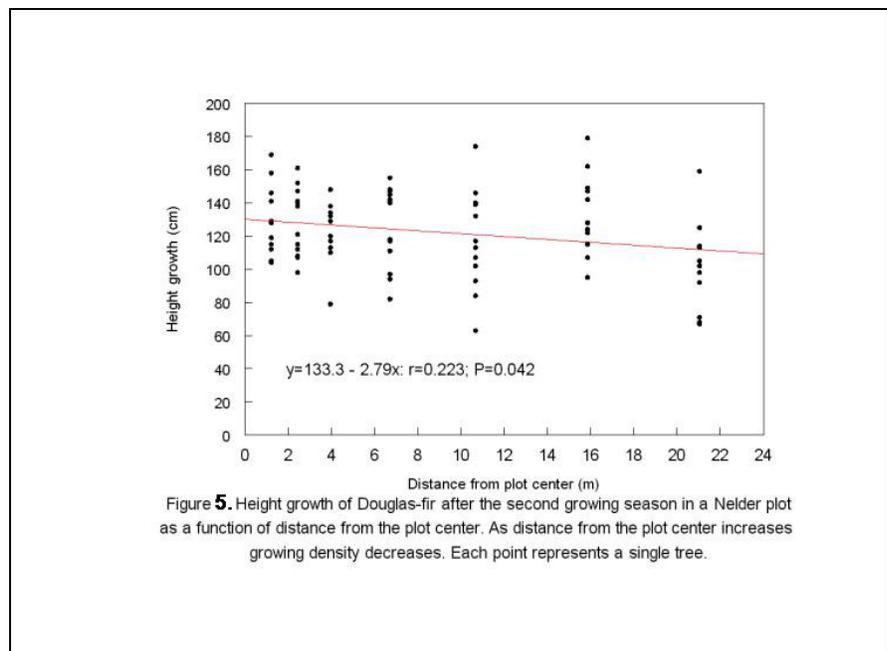
Throughout the measurement period (May 24 through July 20, 2000) observations were made using two sensor heads, and along both the north-south and east-west transects. Both sensors gave nearly identical values and the north-south profiles were similar to the east-west profiles on all measurement days. During this period, all days were clear and sunny with the exception of July 6 and 13, which were overcast. All measurements were made between 11:00am and 2:00pm.

The density-related variations in R:FR became more pronounced in 2001. The ratio of red to far red (R/FR) light increased sharply with distance from the center (higher to lower planting density) (Figure 4). This result supports the hypothesis under test, i.e., that the presence of trees depletes the R/FR ratio in proportion to tree density and that this can be used by trees to signal the presence of competitors.

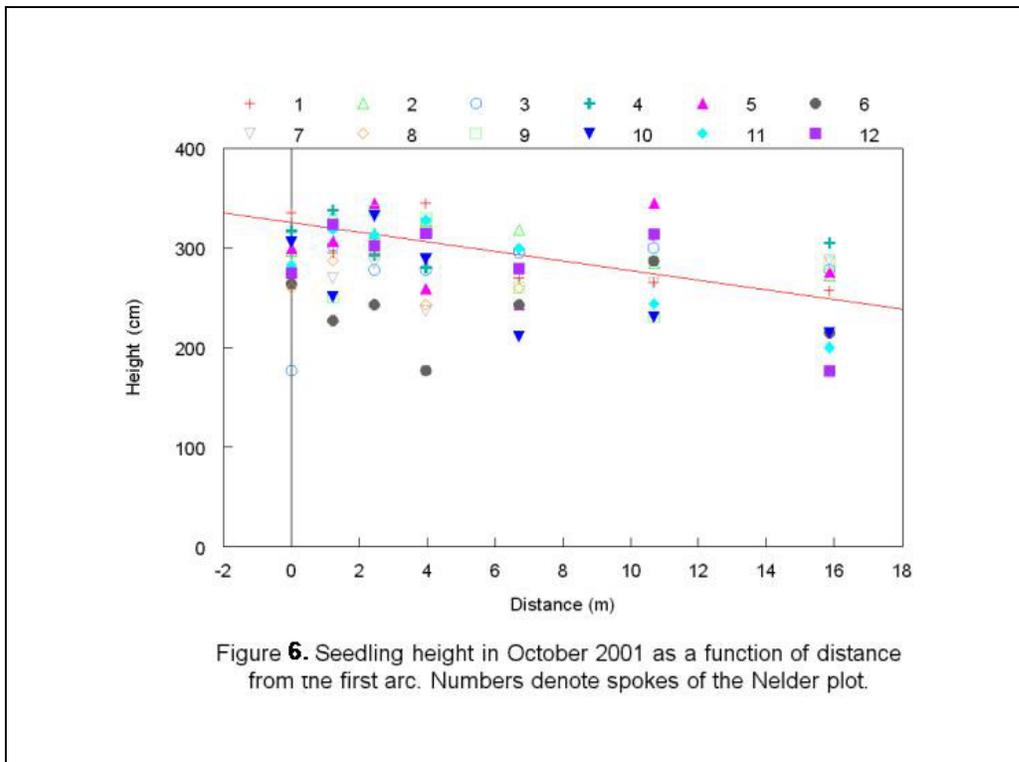


Growth and Biomass Allocation in the Nelder Plot (Task #2)

We measured height and diameter of all seedlings each year of the experiment. Height growth measurements made at the end of the first growing season did not show any differences with growing density. Heights were measured again (along with stem diameters) at the end of the 2000 growing season (October 10). These results showed a slight, but statistically significant ($P=0.042$) decline in height growth from the highest to the lowest growing densities (Fig. 5). We did not find any relationship between density and stem diameter in the second year.



Height differences among the different densities became pronounced in the third year (Fig. 6).



When height data were analyzed separately by “spoke” (i.e., the radii of the Nelder circle) an interesting and unexpected pattern emerged. Slopes were statistically significant and R^2 values were high for Spokes 1, 2, 10, 11 and 12, only (Table 1). This represents the northwestern section of the Nelder plot. This is shown schematically in Figure 7, which is a plan view of the Nelder. Dark lines represent spokes in which the hypothesized density relationship was observed. The reason for this pattern is not clear. Mean heights of trees in each spoke, although significantly different among spokes, did not provide any clues.

We currently have two hypotheses to explain this pattern. First is that the pattern may have something to do with sun angle. At this latitude ($\sim 46^\circ\text{N}$) the sun tracks across the southern sky during the growing season and may somehow influence reflective patterns in such a way that the R/FR effect is skewed to the north. The other hypothesis is that a row of fir trees to the south side of the plot at a distance of about 20 meters (approximate) is producing R/FR signals that are swamping any signals produced by the trees in the plot itself. Alternatively, the observed pattern might be a sampling anomaly or could be related to some unmeasured edaphic factors.

Table 1. Summary of regression results for the entire test and for each spoke of the Nelder plot. Bold numbers denote statistical significance at Alpha = 0.10.

Spoke	Intercept	Slope	R ²	P value
<i>Combin ed</i>	294	-2.24	0.09	0.007
1	325	-4.84	0.614	0.037
2	324	-2.94	.0550	0.056
3	255	3.08	0.163	0.369
4	309	-0.52	0.025	0.734
5	302	-0.93	0.018	0.777
6	236	0.04	0.000	0.999
7	277	-0.24	0.004	0.892
8	266	2.55	0.146	0.455
9	291	-1.68	0.060	0.605
10	297	-5.95	0.513	0.070
11	324	-6.94	0.731	0.000
12	319	-6.07	0.468	0.090

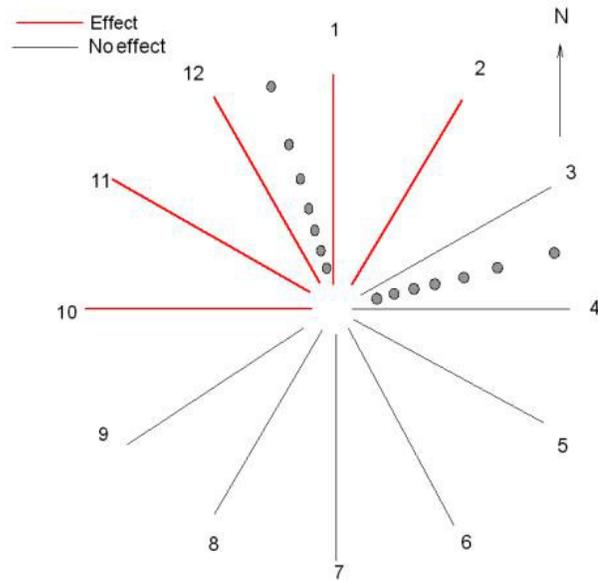


Figure 7. Plan view of Nelder plot showing rows with and without the height effect. Dots represent light measurement points.

At the end of 2001 there were still no statistically significant differences in stem diameter as a function of density, although mean diameters were greatest in higher densities. On the other hand, our retrospective analyses of diameter growth in older density trials showed that high density plots had significantly greater diameter growth in years 3 and 4. Therefore, we decided not to harvest the study after the 3rd year (as originally planned). We now expect to harvest this study (and compare above/below-ground biomass) at the end of the current growing season (the fall of 2002).

Chlorophyll fluorescence emissions in the Nelder Plot (Task #3)

One hypothesis that has been suggested to partially explain the increase in growth at high density is that following R:FR signal perception, it is transduced into an increase in photosynthesis, resulting in faster growth. In order to obtain preliminary insight into this hypothesis, we measured chlorophyll fluorescence of each tree in one spoke at about 10:00 am on July 25. Three small twigs (approx. 2 cm in length) were sampled from each tree and used to perform a “Quenching analysis” using a PAM-2000 modulated chlorophyll fluorometer (Mohammed et al. 1995 and references listed therein). Twigs were collected in the field, then transported back to the laboratory in sealed plastic bags on moist filter paper.

There was no systematic change in either Fv/Fm or Y (indicators of photosynthetic efficiency) or the two energy quenching terms qP (photochemical quenching) or qN (nonphotochemical quenching) along the density gradient (Table 1). These results do not support the above hypothesis. However, this very limited set of observations is far from conclusive.

Table 1. Chlorophyll fluorescence emissions parameters from three-year old Douglas-fir growing in a Nelder plot.				
Tree distance (m) from plot center	Fv/Fm	Y	qP	qN
1.22	0.773	0.488	0.823	0.684
2.44	0.797	0.483	0.817	0.729
3.96	0.797	0.545	0.856	0.674
6.71	0.789	0.505	0.833	0.696
10.67	0.754	0.422	0.757	0.730
15.85	0.780	0.490	0.815	0.700

Altering light quality for seedlings in raised beds using colored-transparent films
(THIS STUDY WAS NOT INCLUDED IN OUR INITIAL PROPOSAL. IT WAS ADDED AS AN ADDITIONAL TEST OF THE R:FR HYPOTHESIS WHEN THE NELDER EXPERIMENT WAS DELAYED.)

The primary objective of this study was to determine of effects of varying light quality on growth of Douglas-fir seedlings. We developed an experimental system that directly manipulates light quality by using colored transparent films.

We examined the effects of placing plastic films around the seedlings by controlling for both reduced photosynthetically active radiation (PAR) and altered seedling microclimate. We also sought to determine whether responses could be detected in established seedlings treated with altered light for only one growing season and to quantify the variability in growth response among seedlings in similar treatments. Finally, we wanted to compare commonly used statistical methods with other suggested methods in the analysis of the response variables.

Two-year-old, bare-root Douglas-fir seedlings were planted in outdoor raised planting beds on the campus of Oregon State University, OR in March 1999. We planted 6 seedlings in each of 13, 2.13 m x 1.47 m raised beds. Seedlings were planted 0.3 m from the edges of the bed and the minimum distance between seedlings was 0.86 m. The density of seedlings was kept constant. Seedlings were given a complete fertilizer at the time of planting and again in June 1999. A drip irrigation system provided ample water to all seedlings throughout the growing season. Wire mesh cages (diameter, 40 cm; height, 60 cm) were erected around each seedling, leaving 20 cm between the soil surface and the base of the cage (Figure 1). These cages were used to support the plastic film used in the experimental treatments that are described below.

Treatments included reduced red light (-R), reduced blue light (-B), neutral shade (-N), clear-plastic procedural control (C), and a no-plastic control (O). Each of these treatments (except O) included no-cap (^{NC}) and cap (^C) treatments to manipulate side light and total light, respectively (Figure 8). This dichotomy was meant to mimic natural systems, where side light quality is often altered when direct sky radiation is not. A 5-cm gap between the cap and the peripheral plastic was used along with a 20-cm gap between the soil surface and the bottom of the enclosure for convective heat transfer (Figure 8). The -R plastic produced a 70% reduction in R without appreciable FR reduction (Azure Blue Roscolux #72). The -B plastic produced a 95% reduction in B (Golden Amber Roscolux #21). The -N treatment included a combination of the C treatment plastic (Warp Brothers, Chicago) and a shade cloth that produced a 40% reduction in total PAR, approximately the same total PAR reduction as the filters that altered light quality. We used a completely randomized experimental design with 8 seedlings as replications for each

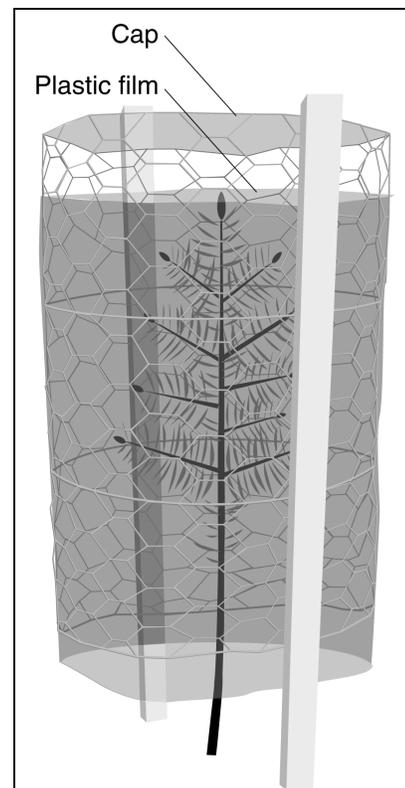


Figure 8. Enclosure design for treated seedlings. The cap is composed of identical plastic film to that which surrounds the seedling. Capped treatments (^C) have the cap as shown here and no-cap treatments (^{NC}), do not. Note the two gaps present at the top and bottom of the enclosure for convective heat transfer.

treatment and 14 seedlings in the O treatment. All treatments were applied a few days after planting and remained present until seedling harvest in October 1999. Weeds were manually removed from the planting beds biweekly so as to maintain bare soil around the seedlings.

Environmental conditions

We assessed light quantity and quality with a spectroradiometer (Model 1800, Li-Cor, Inc., Lincoln, NE) fitted with a remote cosine receptor measured photon flux density ($\mu\text{moles m}^{-2} \text{s}^{-1}$) within each seedling enclosure on cloudless days. Hobo dataloggers (Onset Computer Corporation, Bourne, MA) were installed in 20 seedling enclosures (2 per treatment) and programmed to record air temperature every 10 minutes for the 6-month period of treatment. Air temperature differences were assessed on three levels, ΔT_{O} , ΔT_{C} , $\Delta T_{\text{-N}}$; where ΔT_{O} was the difference between all plastic containing treatments (-R, -B, -N, C) and the no plastic control (O); ΔT_{C} was the difference between shading plastic treatments (-R, -B, -N) and the clear plastic control (C); and $\Delta T_{\text{-N}}$ was the difference between light filtering treatments (-R, -B) and the neutral shade treatments (-N).

Seedling measurements

Before planting, we destructively analyzed 20 seedlings to develop allometric equations by stepwise linear regression that predicted initial dry mass of roots, stems, and foliage of the experimental trees using seedling height, stem diameter 2 cm above soil level, and crown area. Crown area was calculated using mean crown diameter (maximum and orthogonal to maximum diameters). At the time of planting experimental seedlings, we measured each seedling's height, stem diameter 2 cm above soil level, and crown area for use as biomass predictors. Experimental seedlings were harvested in October 1999 after we measured final stem height and diameter at 2 cm above the soil. Approximately 50 needles per seedling were selected randomly and removed at the time of harvest for specific leaf area (SLA) calculation (leaf area per unit leaf mass). Roots, stems, and leaves were dried separately to a constant weight in a 65°C oven. Response variables included root dry mass, stem dry mass, total plant dry mass, leaf area, SLA, mean relative growth rate (RGR), root-dry-mass:shoot-dry-mass ratio (root:shoot), leaf-dry-mass:stem-dry-mass ratio (leaf:stem), stem-height:stem-dry-mass ratio (height:stem), root-dry-mass:total-plant-dry-mass ratio (root:total), stem-dry-mass:total-plant-dry-mass ratio (stem:total), and leaf-dry-mass:total-plant-dry-mass ratio (leaf:total).

Results

Effects of treatments on environmental conditions. Treating O as 100%, the treatments reduced total PAR by 32% (-R^{NC}), 43% (-R^C), 30% (-B^{NC}), 56% (-B^C), 27% (-N^{NC}), 53% (-N^C), 12% (C^{NC}), and 17% (C^C). The R:FR ratio of side light was reduced in both -R treatments compared with side light of all other

treatments. Daytime air temperatures averaged about 4° greater in enclosures with plastic on both sunny and cloudy days in comparison with no plastic controls, but there was little or no difference in temperature among plastic treatments.

Seedling responses.

Treatments with a reduced R:FR ($-R^{NC}$ and $-R^C$) exhibited the greatest mean height among low PAR treatments (Figure 9); however, this trend was not statistically significant ($df=5$; $F=2.13$; $p=0.087$). The $-B^C$ and $-N^C$ treatments had the lowest mean seedling height (Figure 9). The root:shoot, height:stem, and leaf:stem ratios did not differ among low PAR treatments ($df=5$, $F=0.42$, $P=0.830$; $df=5$, $F=0.80$, $P=0.556$; $df=5$, $F=0.59$, $P=0.705$; respectively) (Figure 9). Among low PAR treatments, mean height, diameter, root dry mass, stem dry mass, total plant dry mass, and leaf area were greatest in $-R^C$ treatment. Among all treatments, allocation of mass to the stems appears to be less in high PAR treatments as inferred from the leaf:stem and stem:total ratios. Specific leaf area was lowest in high PAR treatments and RGR was lowest in the $-N^C$ treatment. High variability within treatments was apparent among all response variables. Individual contrasts for enclosure effects, cap effects, and $-B$ effects had high P values compared with R:FR effects and particularly with PAR effects.

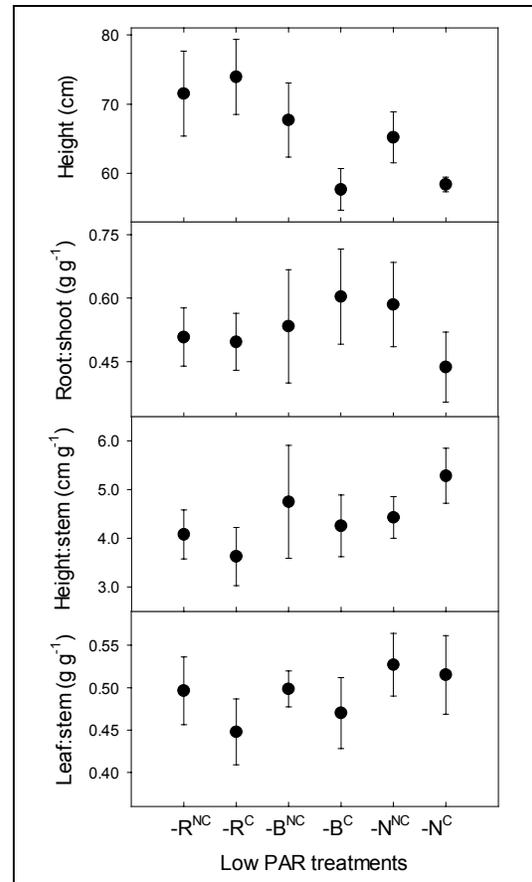


Figure 9. Mean height, root-dry-mass:shoot-dry-mass ratio (Root:shoot), stem-height:stem-dry-mass ratio (Height:stem), and leaf-dry-mass:stem-dry-mass ratio (Leaf:stem) for low-PAR treatments. Bars indicate standard error.

Conclusions

The plastic film used in light filtering treatments altered light quality by removing R or B, but it also altered microclimate and total PAR, and not surprisingly, total PAR did have a significant effect on overall seedling growth. Anticipating these effects, we included neutral-filter controls in the experimental design. Interestingly, the seedlings with reduced R:FR only from side-light (i.e, with the colored plastic wrapped around the seedling but without a “cap” over it) showed nearly the same responses as seedlings with reduced R:FR from above as well as from the sides. The seedlings in reduced R:FR treatments were taller than the neutral-filter controls, and their overall biomass was slightly greater. These

results suggest that R:FR is an important environmental factor to the growth of Douglas-fir seedlings. However, the statistical power of the experiment was weak, so the results are not as conclusive as we would have liked. To obtain statistically significant results might require a larger sample size, a greater reduction in R, or a longer treatment period.

Testing the Boundary Layer Hypothesis

Determining the temporal dynamics of “The Density Effect” (Task #4a)

Where it has been noted, positive growth response to density is a short-lived phenomenon. Knowledge of the timing involved could allow foresters to develop thinning and silvicultural prescriptions to maintain ideal stocking densities for maximum tree growth. In addition, increased knowledge of this phenomenon could lead to new ways of thinking about the biology and physiology of plant interaction and competition. The first objective of this part of our study was to provide further evidence that high initial stocking density resulting in a short-lived increase in height and diameter growth of Douglas-fir seedlings. The second objective was to determine the timing and duration of the positive response to density.

The measurements for this study were conducted in 1997, using density trials previously established by Weyerhaeuser Co. at three sites in Washington state. Sites 1 and 2 were planted in 1989 and site 3 was planted in 1985. Douglas-fir seedlings were planted in a randomized block design with a single replicate of density treatments at each site and plot sizes of approximately 2 ha. Weyerhaeuser Co. randomly assigned treatment densities to plots; for this analysis we used three stocking densities, 300, 1360 and 2960 trees ha⁻¹ (120, 550, and 1200 trees acre⁻¹), referred to hereafter as low-, mid- and high-density treatments. Within each plot we randomly selected 50 measurement trees. An increment borer was used to extract a core from the bole of each tree 30 cm above ground on the north side; additional cores were extracted if necessary until core included the pith. From the cores, annual diameter growth was measured using a Metrics Quick Check QC-1000-M-AR digital readout unit, a 0.001 mm resolution Accu-Rite linear encoder and a Velmex Unislide measuring stage. Tree heights were measured with a height pole to a 0.1 m resolution. Annual height growth was determined retrospectively by measuring the distance between branch whorls for each year of growth. Trees in site 3 were 4 years older than those in the other two sites and too large for measurement with a height pole. Weyerhaeuser Co. measured tree heights annually at this site commencing three years after planting, and we used these measurements to calculate height growth commencing at year four. However, these measurements were not included in statistical analyses because the sampling design was different from the rest of the experiment.

For sites 1 and 2, we calculated a tree biomass index as d^2h , where d is stem diameter at 30 cm above ground and h is height. An analysis of d^2h could not be conducted for site 3 because trees sampled for height growth were different from the trees sampled for diameter growth.

In determining diameter growth of annual rings, we began measuring from the current year and counted inward. For some trees, the number of growth rings was less than the total number of years since planting because the end of the first or second year of growth occurred below the sampling height of 30 cm. Similarly, we measured height growth from the top whorl (current year) down, and for some trees the number of internodes was less than the number of years since planting. Therefore, the sample size during early growth years was less than the 50 trees per plot that we selected for measurement.

Analyses of growth

Mean annual height increment was greater in the high-density treatment than in the low-density treatment, and height growth of the mid-density treatment was typically between the other treatments (Figure 10). Averaged across sites 1 and 2, the greatest absolute difference among treatments in mean annual height increment occurred in year 5. Significant differences in height growth occurred in years 2, 5 and 6. Proportionally, the greatest differences in height increment occurred during years 2 and 3. Mean second and third-year height increment averaged across sites 1 and 2 in the high-density treatment was 133% and 136% of that in the low-density treatment, respectively. The positive growth response to density declined over time such that by the eighth year after planting, height growth in the high-density treatment was only 3% greater than that in the low-density treatment. Differences in mean annual height increment between mid- and high-density treatments were typically greater than those between low- and mid-density treatments. There were no significant differences in mean annual height increment between the low- and mid-density treatments in any single year, although the mean value of height growth was greater for the mid-density treatment at all sites in almost every year (Figure 10).

In years 2-5 mean annual diameter increment was consistently greater in the high-density treatment than the low-density treatment (Figure 11). Mean annual diameter increment of the mid-density treatment was between that of the

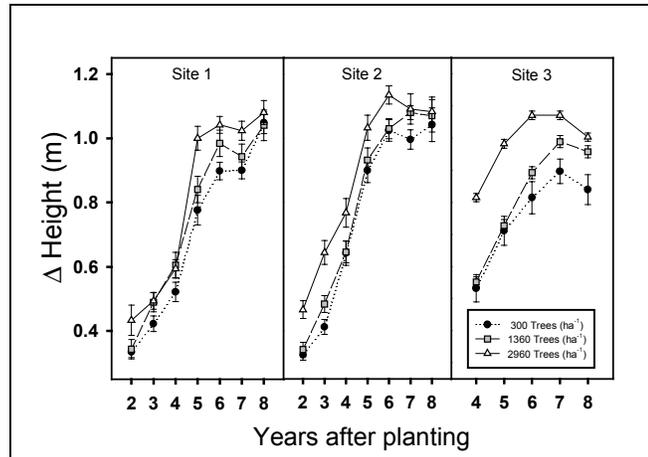


Figure 10. Annual height growth for trees in the low-, mid-, and high-density treatments. Error bars are standard errors of the mean of up to 50 trees for sites 1 and 2, and between 28 and 323 trees for site 3.

low- and high-density treatments. During this period the greatest absolute difference in mean annual diameter increment occurred between the low- and high-density treatments in year 4. Diameter growth in the high-density treatment was significantly greater than that of the low-density treatments in years 2, 3, 4 and 5. Proportionally, the greatest differences in diameter increment occurred during years 2 and 3. Averaged across all

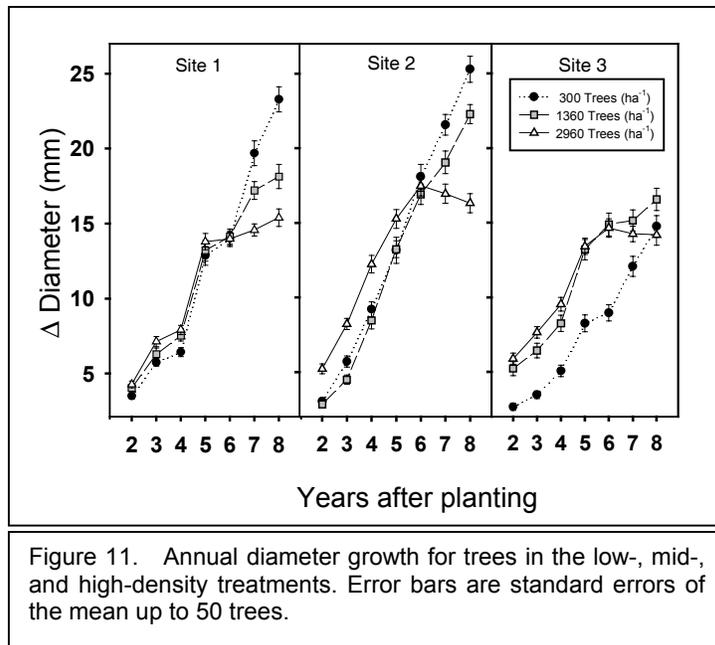


Figure 11. Annual diameter growth for trees in the low-, mid-, and high-density treatments. Error bars are standard errors of the mean up to 50 trees.

sites, mean diameter increment in years 2 and 3 in the high-density treatment were 165% and 154% of that in the low-density treatment, respectively. This positive growth response to density declined over time such that by the seventh year after planting diameter increment was greatest in the low-density treatment. By the eighth year after planting diameter increment was significantly greater in the low-density treatment than in the high-density treatment. Averaged across all three sites, mean diameter increment for the eighth year after planting in the high-density treatment was 72% of that in the low-density treatment.

This study confirmed that both height and diameter growth of Douglas-fir trees were positively correlated with initial stocking density for the first few years after planting. The trend reversed in later years, resulting in decreased growth (at the individual tree level) in stands of higher initial stocking density. The greatest positive correlation between growth and density occurred in the second and third years after planting.

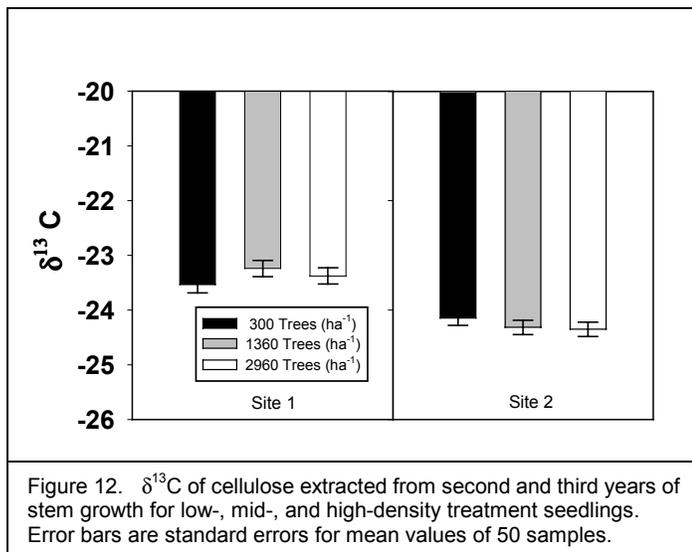
Carbon isotopes in annual rings of trees grown at different densities (Task #4b)

Analysis of stable carbon isotopes (¹²C and ¹³C) can provide a means to investigate a variety of issues in plant physiology. During photosynthesis, C₃ plants discriminate against the heavier stable isotope of carbon, ¹³C. Differences in moisture availability (in soil or atmosphere) and nutrient availability can affect the degree of discrimination. Greater moisture availability tends to increase stomatal conductance, leading to increased discrimination against ¹³C, and greater nutrient availability tends to decrease discrimination. Also, if respired CO₂ is "trapped" in the canopy atmosphere because of low mixing with the bulk atmosphere, the relative abundance of ¹³C in the CO₂ used in photosynthesis is decreased. As the isotopic signal for any given year of tree growth is contained

within the annual growth rings of stems, stable isotope analysis of wood cellulose can be used to test for variations in water availability, nutrient availability, or CO₂ concentration of source air between years or between treatments or sites within years. However, the interpretations must be made cautiously because many environmental variations may effect similar changes in C isotope discrimination, and it is also possible that changes in more than one of these factors can cancel each other out. The objective of this part of our study was to analyze carbon isotope ratios of annual rings in stemwood of Douglas-fir seedlings during years of positive growth response to density to differentiate among potential causes of the increased growth.

Methods. For this study we the increment cores extracted from the trees in the density trials that were measured for the study to examine the dynamics of the density effect (above). The growth rings for the second and third years after planting were excised from the core samples with a razor blade. These years were chosen for stable isotope analysis because height and diameter growth was greater in high-density than in low-density treatments in both of these years. The excised xylem was dried at 70° C for at least 72 h. It was then ground with a coffee grinder and pulverized in liquid nitrogen with a mortar and pestle. Cellulose was extracted from pulverized xylem samples according to the procedures described by Wise et al. (1945). Stable carbon isotope ratios of the extracted cellulose were determined at the SIRFER laboratory (Stable Isotope Ratio Facility for Environmental Research) at the Biology Department of the University of Utah. All isotope data are expressed in delta notation (δ) and are presented relative to the Pee Dee belemnite standard for $\delta^{13}\text{C}$.

We found no significant differences among the three planting densities in carbon isotopic composition of wood cellulose in the second and third years after planting for sites 1 and 2 (Figure 12). In



fact, the difference in isotope composition between sites 1 and 2 was much greater than the difference among treatments within sites, indicating that the lack of significance due to density was meaningful biologically in addition to statistically. Carbon isotopes were not analyzed for samples collected from site 3; we decided that the analysis cost was not warranted since we found no significant differences at the other two sites.

This study suggests that the increased growth at higher planting densities did not result from a CO₂ “fertilization” effect caused by higher boundary layer resistance at the higher planting densities. If high CO₂ concentrations contributed to increased photosynthesis at high densities, we would have expected a lower (i.e., more negative) δ¹³C value for high-density trees. It is possible that other environmental differences in the high-density plots affected isotope discrimination in a way that counter-balanced CO₂-enrichment effects. Soil moisture stress in high-density plots, for example, could reduce isotopic discrimination – causing an enrichment in ¹³C that might mask the depletion in an enriched-CO₂ atmosphere. But it is unlikely that soil moisture stress was significant in high-density plots during the time when height and diameter growth in these plots was greater than that in low-density plots.

The combined results from the studies of growth dynamics in different densities (task #4a) and the isotope measurements (task #4b) cast serious doubt on the boundary layer hypothesis. The positive growth response to density occurs while trees are still very small, and even in the high-density plots, individual trees are not close. Our preliminary measurements showed no difference in atmospheric dynamics in the different densities two to four years after planting.

Measurements of CO₂ and H₂O vapor in vertical profiles and calculations of boundary layer conductance in different planting densities. (Tasks 4c and 4d)

As mentioned previously, our preliminary measurements indicated no difference in atmospheric conditions in different densities during the years that we knew the “density effect” occurred, and our isotope measurements suggested that the increased growth did not result from CO₂ enrichment. Having discarded the “boundary layer hypothesis”, is there any point at all to completing tasks 4C and 4D? We decided there was, not as part of the quest to understand the “density effect”, but in as part of a larger effort to understand the multiple impacts of different planting densities on tree growth and development. Even if differences in boundary layer conductance might not explain the early manifestations of the “density effect”, they could have important impacts on growth slightly later in stand development. The purpose of the study was to investigate the effects of stand density on the micro-climate and boundary layer of young conifer forests. The hypothesis was that stands of higher density generally have lower boundary layer conductance, especially during the night and morning. This lower canopy boundary layer conductance functions to “trap” morning high levels of respired CO₂ and transpired water vapor within the stand. The higher concentrations of CO₂ and H₂O may enhance photosynthesis within the higher density stands.

Methods. For this part of the study we used 5-year-old stands of Douglas-fir planted at densities of 300 and 1360 trees ha⁻¹ on Weyerhaeuser Co. land at Marcusson Creek, near Dryad, Washington. Measurements were conducted in one plot at a time due to equipment limitations. Different-density treatments were initially measured on separate half-days, and then on separate whole days.

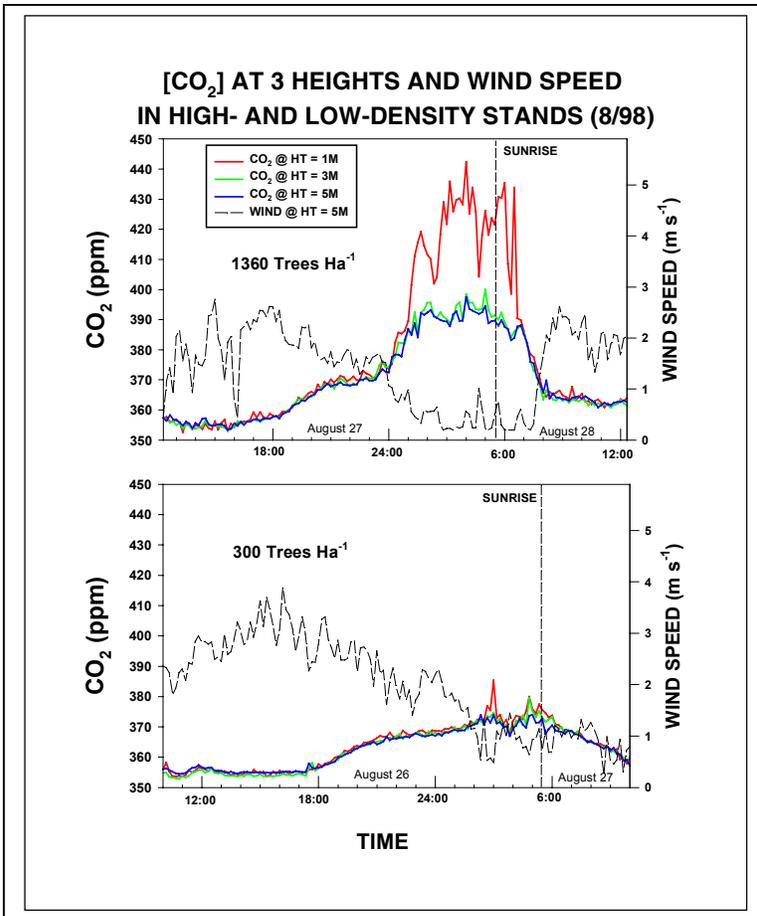
Variations in weather conditions between measuring periods of different densities were accounted for by analyzing the differences in climatic variables relative to local weather station values.

Measurements were conducted during the summer of 1998. Low-density plots were measured on July 28th (morning), 29th (evening), and 30th (evening); August 25th, and 28th; and September 9th. High-density plots were measured on July 28th (evening), 29th (morning); August 26th, and 27th; and September 10th. Evaporation was measured directly using the mass exchange method designed by Teklehaimanot et al. (1991). In this method, rate of evaporation from foliage is measured directly by weighing a saturated tree over the course of its drying.

A complete tree, similar in size to the trees in the study stands, was cut from a nearby stand and suspended with a 5-m tripod from a load cell, and water was sprayed onto the tree from a fine spray nozzle. Spraying was continued until the weight of the tree reached a maximum and remained constant for at least 60 seconds. Saturation typically required approximately ten minutes of continual spraying of all sides of the tree. The wetting process was then stopped and the tree was allowed to dry. The weight of the tree was recorded at 1-min intervals on a datalogger. The rate of evaporation was calculated over a time period that commenced ten minutes after spraying stopped, continuing until 75% of the total amount of water on the foliage had evaporated. Evaporation from foliage was normalized by leaf area of the cut tree.

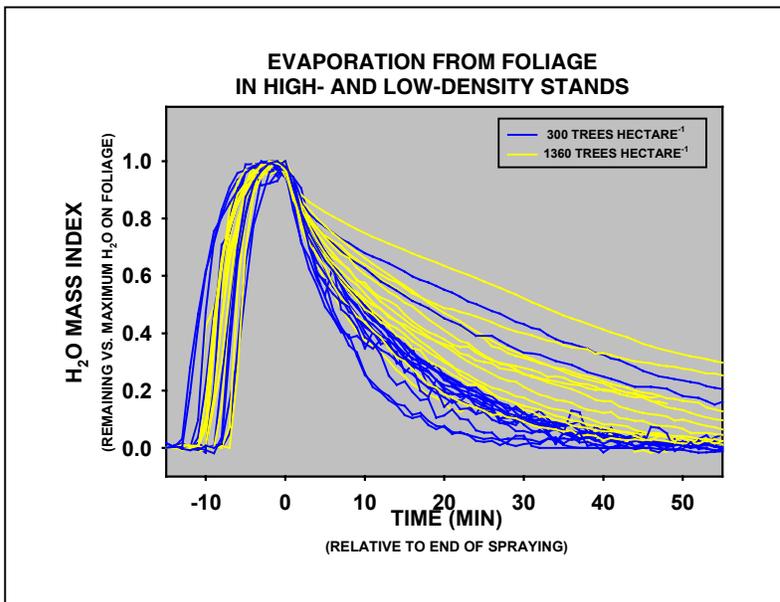
Stomatal conductance on five trees was measured concurrently with evaporation measurements with a Li-Cor 1600 porometer. Air temperature, relative humidity, wind speed, and [CO₂] were recorded at three elevations within the canopy: one meter above soil surface, 1/2 of the distance from the ground to the tree tops (three meters), and one above tree tops (five meters). The sampling equipment was secured to a mast placed at the center of each plot. These micro-meteorological measurements were recorded continually for the 24-hour periods during which evaporation measurements were conducted with the suspended tree. However, analyses were restricted to a one-hour average commencing two hours after sunrise. This time was selected because it occurred both during daylight when photosynthesis could take place and during a period when atmospheric mixing was still low.

Results. Between one and two hours after sunrise, mean [CO₂] in the high-density stand averaged 32.8, 23.74, and 23.2 ppm greater than that in the low-density stand at one, three and five meters respectively; between two and three hours after sunrise, mean [CO₂] in the high-density stand was 8.89, 7.84, and 10.22 ppm greater than that in the low-density stand at these same heights (data in Fig. 13 shows only one sampling date). Two to five hours after sunrise, relative humidity averaged about 10% greater in high density plots – the results for high vs. low density plots were significant at heights of 1 and 3 m above the ground. Mean air temperature was greater in the low-density stand compared with the



high-density stand between two and three hours after sunrise. The greatest difference in temperature occurred at the one-m height. Mean temperature at the one-m height in the high-density treatment was 67% of that in the low-density treatment.

Fig. 13. [CO₂] for high- and low-density stands at heights of one, three and five meters and wind speed at five meters. Vertical line indicates time of sunrise.



Significant differences in mean evaporation rates were observed between the two densities. Mean evaporation rate in the low-density treatment was 164% of that in the high-density treatment. (Fig. 14).

Fig. 14. Evaporation of water from foliage in high- and low-density stands.

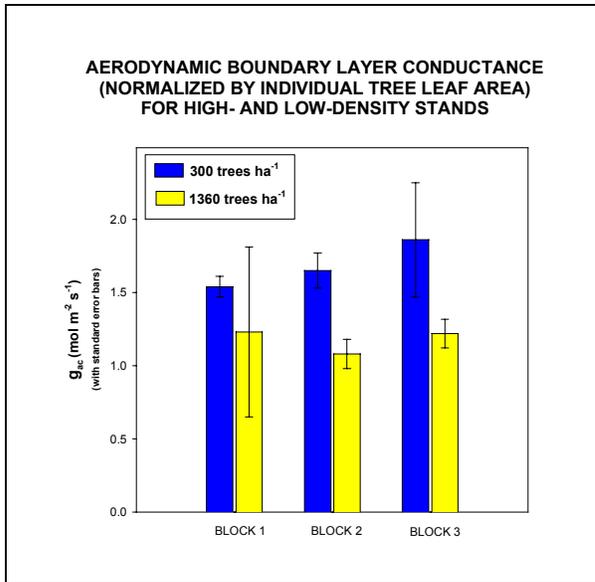


Figure 2.9 Mean g_{ac} for blocks one, two and three. Bars are standard errors for three, eight, and four sampling sessions per density for blocks one, two and three respectively.

We calculated canopy boundary layer conductance (is g_{ac}) using a derivation of the Penman equation for evaporation. Details are described in David Woodruff's thesis (submitted separately), but briefly, the equation is $g_{ac} = (\epsilon + 1)PaE_i/VPD$, where E_i is evaporation of intercepted water (from data shown in Fig. 14), ϵ is a coefficient for change in the ratio of sensible and latent heat contents of air with respect to temperature, VPD is water-vapor pressure deficit of the air, and Pa is atmospheric pressure. The calculations indicated that boundary layer conductance is, in general, significantly higher in low density plots (Fig. 15).

Conclusions. The 'density effect' occurs in stands much younger and smaller than those used for the atmospheric measurements in our study. We do not believe (as explained earlier) that the boundary layer effect plays a significant role in the enhanced growth that is often observed in younger stands at high density. However, our measurements do show that the microclimate within stands is impacted by stand density. These differences in microclimate are likely to affect tree growth. Although environmental effects due to competition (changes in soil moisture, for example) probably cause a net reduction in growth of trees in high-density plots at this time, it is likely differences in the canopy atmosphere create "facilitation" effects that partially compensate for the competition effects. Future studies may be needed to quantify the facilitation in greater detail.

Products

Presentations at meetings

Woodruff, D.R., B.J. Bond, G.A. Ritchie, E. Watrud, J. Coate and N. Phillips. The effects of stand density on the canopy boundary layer conductance and microclimate of young conifer forests. 85th annual meeting of the Ecological Society of America, Snowbird, Utah, Aug. 6-10, 2000.

Schauer, A.J., B.J. Bond, S. Radosevich, G.A. Ritchie and D. Woodruff. Douglas-fir seedling growth response to different light environments. 85th annual meeting of the Ecological Society of America, Snowbird, Utah, Aug. 6-10, 2000.

Thesis

Woodruff, D. 2000. The effects of stand density on the growth and microclimate of young Douglas-fir stands. M.S. Thesis, Oregon State University.

Papers

Woodruff, D., B.J. Bond, G.A. Ritchie and W. Scott. 2002. Effects of stand density on the growth of young Douglas-fir trees. *C.J.F.R.* In Press.

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Mohammed, G. H., W. D. Binder and S. L. Gillies. 1995. Chlorophyll fluorescence: A review of its practical forestry applications and instrumentation. *Scand. Jour. For., Res.* 10:383-410.