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The Density Effect: Red/Far Red Signaling and Douglas-fir Seedling Growth in a Variable Density Field Test

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Abstract: Coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings, when planted in a reforestation setting, exhibit early height and diameter growth that is inversely proportional to planting density. One hypothesis to explain this observation is that they are able to detect the presence of nearby trees using phytochrome by sensing the ratio of red to far red light (R/FR) reflected from their crowns. This provides an "early warning signal" of future competition. This signal then triggers a change in growth rate or allometry, which leads to greater height and diameter growth as trees attempt to avoid being overtopped. This is called the "Density Effect." As a partial test of this hypothesis, we established a variable density field test to determine whether changes in R/FR could be detected at different growing densities, and if this was associated with variations in height and diameter growth. The predicted reduction in R/FR, and concomitant increase in height growth at higher plant densities, were observed beginning early in the second growing season. These observations lend support to the R/FR signaling hypothesis.

Keywords: phytochrome, allometry, chlorophyll fluorescence, plant competition, stand development

Introduction

Coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) seedlings, when planted in a reforestation setting, exhibit early height and diameter growth that is inversely proportional to planting density. Several test plantations that were established across approximately 50 sites in western Washington and Oregon at six initial planting densities (120 to 1,200 trees/ac [300 to 3,000 trees/ha]) were assessed 11 years after planting. Tree height and DBH (diameter at breast height) became progressively greater as planting density increased (Scott and others 1998). This has become known as the "Density Effect."

Several hypotheses have been proposed to account for this surprising observation. One hypothesis, based on the pioneering research of Carlos Ballaré and his co-workers (Ballaré and others 1987, 1990, 1995), proposes that many plants are able to perceive the presence of nearby future competitors very early in life, and to respond by increasing height and diameter growth to avoid being overtopped by these competitors. This response follows detection, via the phytochrome system, of alterations in the natural ratio of red to far red light (R/FR), which is reflected horizontally from the canopies of the adjacent trees. This signal is propagated long before any overtopping has occurred, providing the seedlings with an "early warning signal" of future competition.

This hypothesis was tested in a greenhouse experiment where Douglas-fir seedlings were sown at variable densities providing a range of individual plant growing areas from 265 to 2,555 cm^2 (41 to 396 in^2) (Ritchie 1997). The seedlings were arrayed in a "Nelder" design, that is, planted in concentric arcs of gradually increasing diameter providing an array of planting densities within each test plot (Nelder 1962). Their growth, and R/FR light reflectance, were monitored along this density gradient throughout one growing season.

After 30 weeks, seedling height, crown biomass, and branch number increased with planting density. Further, R/FR was lowest at the highest density and decreased as density increased. The growth response first became apparent after only 55 days from sowing, or 15 days from seedling emergence. While these observations lent strong support to the R/FR hypothesis, the experiment was conducted in an artificial enclosure with very young seedlings growing at very close spacing. Extrapolation of these results to full size trees growing in a natural outdoor setting at much wider spacing would not be justified without experimental confirmation.

Therefore, the main objective of the present study was to confirm the repeatability of the above greenhouse trial results in a natural field setting using Douglas-fir reforestation stock. We were also interested to determine at what time any growth differences and alterations in R/FR ratio might become apparent, and whether we could detect differences in photosynthetic efficiency among trees growing at different rates.

Methods

Study Site

The study was conducted on a non-cropped portion of Weyerhaeuser Company Mima Forest Nursery, near Olympia, WA. The site slopes very gently toward the south. The soil is a sandy loam and, up until study establishment, supported a dense sod cover. Before planting, it was sprayed with Glyphosate®, and rototilled and fenced to exclude deer and rabbits. Douglas-fir container nursery stock was planted in spring 1999. The site was treated each spring with Goal®, a pre-emergent herbicide registered for use around conifer seedlings. The plot was hand-weeded weekly by a contractor throughout the study.

Nelder Design

Nelder tests are laid out as circular plots resembling wheels, with spokes and concentric circles. These are highly efficient designs for testing effects of growing density on plants, and have been previously used in forest research (for example, Namkoong 1966; Galinski 1994). The Nelder plot was approximately 140 ft (43 m) in diameter and contained 12 spokes and 7 arcs, with the spokes spaced 30° apart (fig. 1). The first spoke (#1) was aligned north-south. The first arc was located ata distance of 4 ft(1.22 m)from the plot center. Light measurement points were established between spokes #12 and #1 (north-south) and spokes #3 and #4 (east-west).

Distances of each arc from the plot center and from the previous arc are listed in table 1. Also listed are estimates of the mean plant distance (MPD) at each arc-spoke intersect. This is calculated simply as the mean distance of the four closest trees to the subject tree.

A container Douglas-fir rooted cutting (Ritchie 1994) was planted at the intersections of each spoke and arc (total = 84). These trees were produced in a greenhouse using D16 Deepot™ cells (2 in [5 cm] diam by 7 in [18 cm] deep) and were uniform in height and diameter at time of planting.

Tree heights were analyzed using linear regression, with each spoke considered one replicate. Therefore, there were 12 replicates, each containing seven trees. R/FR measurements were also analyzed with linear regression.

Site Visits

The site was visited several times during the 1999, 2000, and 2001 growing seasons. During each of these visits, R/FR was measured at each scan point shown in figure 1. Tree heights and root collar diameters were determined at the end of each growing season.

R/FR Measurements

Measurements of horizontally reflected radiation at each scan point were made with a Li-Cor® 1800 spectroradiometer (Li-Cor® Biosciences, Lincoln, NE) fitted with an integrating cylinder (Ballaré and others 1987) with a ±10° acceptance angle. Horizontally mounted, this cylinder "sees" only that light that is reflected inward from the sides, while blocking sky light and ground reflections. Ballaré and others (1990) argue that the integrating cylinder is necessary for these measurements because the normally used cosine corrected planar sensors do not adequately characterize the radiation conditions at the photoreceptive sites in even-aged plant communities. The tripod-mounted sensor head was placed directly over a permanent marker that was installed at each scan point. The instrument was programmed to perform triple scans at 1-nm band intervals from 650 to 740 nm, which were then averaged. One scan was performed at each position. R/FR was calculated as the ratio of spectral irradiance in the bands at 655 to 665 nm (red) over irradiance in the bands 725 to 735 nm (far red) (Smith 1994).

Chlorophyll Fluorescence Measurements

One hypothesis that has been suggested to partially explain the increase in growth at high density is that the R/FR signal perception is transduced into an increase in photosynthetic rate or efficiency, 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 25 July 2001 using a PAM-2000 pulse amplitude modulated chlorophyll fluorometer (Heinz Walz, Eichenrig, Germany). For an explanation of this technique and a guide to interpreting results, see Ritchie (2006).

Results

R/FR Light Measurements

We conducted measurements ofhorizontally reflected R/FR ratios during the 1999, 2000, and 2001 growing seasons. In 1999, we were not able to detect any consistent R/FR patterns with respect to planting density (mean plant distance). In 2000, however, we found a strongly significant trend of increasing R/FR with mean plant distance (fig. 2). This trend was consistent along both the N-S and E-W transects. R/FR values at the plot center averaged between 0.60 and 0.90, then followed a logarithmic increase out to about 7 m (23 ft)

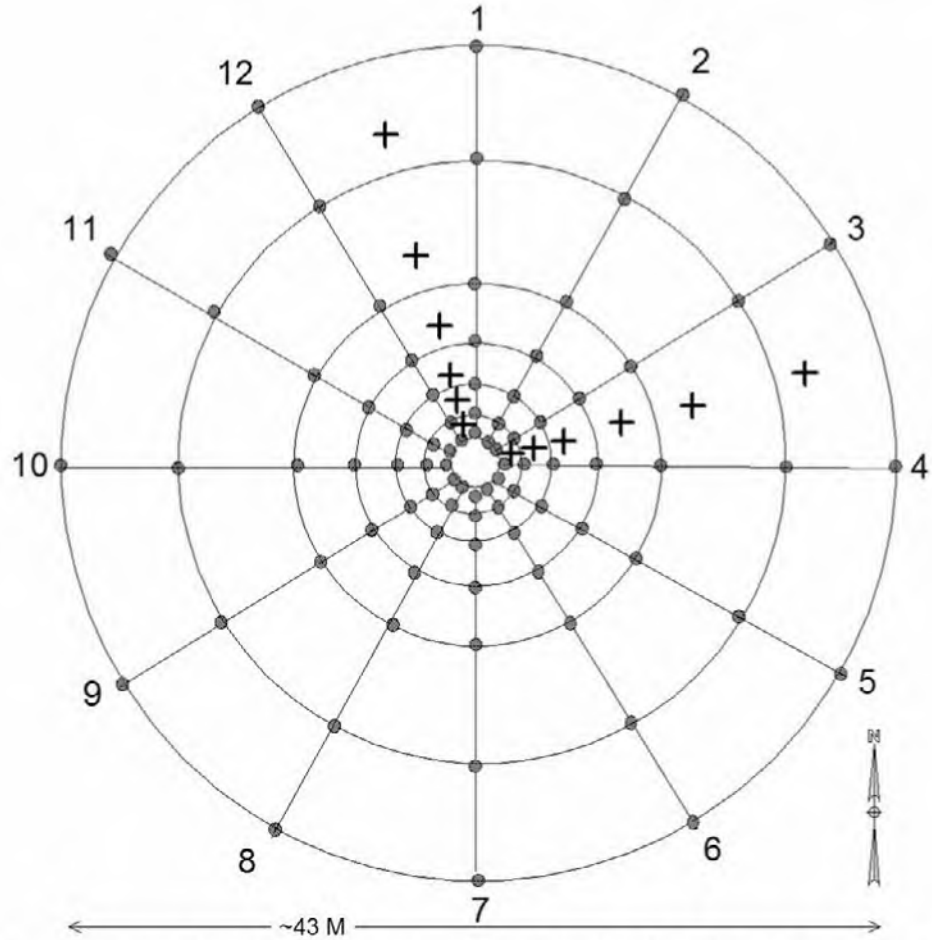

Figure 1—Diagram of the Nelder (1962) plot established to test the effect of planting density on R/FR light and tree growth. Each point indicates the location of one Douglas-fir plant. Each **+** is a light measurement point. "Spokes" are numbered 1 to 12.

Table 1—Arc distances from plot center and previous arc, and calculated Mean Plant Distance (MPD) for trees growing in each arc. MPD = mean distance to four nearest trees.

Arc	Distance from plot center	Distance from previous arc	Mean plant distance
	ft (m)	*ft (m)*	*ft (m)*
1	4 (1.22)	–	4.0 (1.22)
2	8 (2.44)	4 (1.22)	4.3 (1.31)
3	13 (3.96)	5 (1.52)	7.0 (2.13)
4	22 (6.71)	9 (2.74)	11.5 (3.51)
5	35 (10.67)	13 (3.96)	16.5 (5.03)
6	52 (15.85)	17 (5.18)	22.5 (6.86)
7	69 (21.03)	17 (5.18)	30.8 (9.39)

from the plot center, where they appeared to reach a plateau between roughly 0.90 and 1.30. R/FR tended to be higher on cloudy days than on clear days.

These density-related variations in R/FR became stronger in 2001 (fig. 3). R/FR was appreciably lower near the plot center (average about 0.30) than in 2000. R/FR then increased sharply with mean plant distance out to about 11 m (36 m) (MPD = 2.1 m [6.9 ft]). This was observed in both N-S and E-W transects. There was a tendency for R/FR to decrease at all densities later in the growing season.

Linear regressions conducted on the individual sample days, as well as pooled sample days for both 2000 and 2001, had highly significant ($P \leq 0.0001$) positive slopes (that is, R/FR increased as MPD increased).

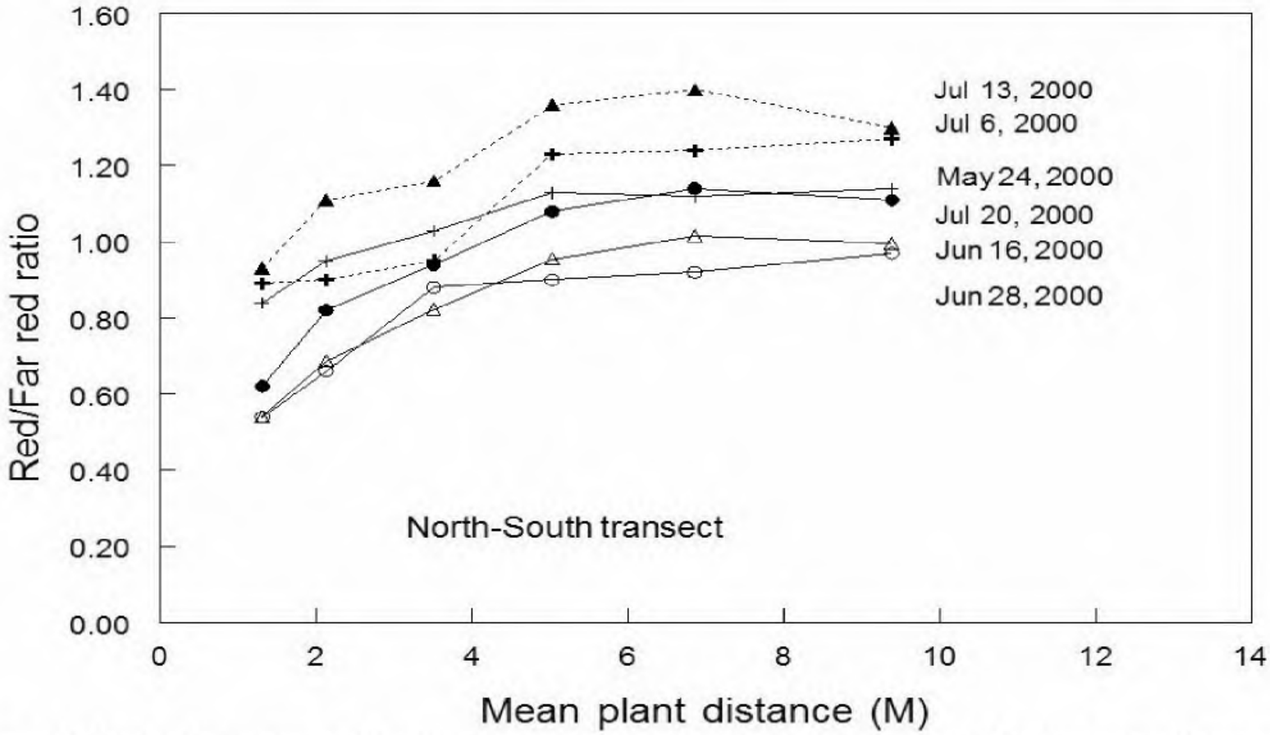

Figure 2—Red/far red ratios measured at varying mean plant distances during the 2000 growing season. Dotted lines represent measurements made on overcast days; solid lines represent measurements made on clear days.

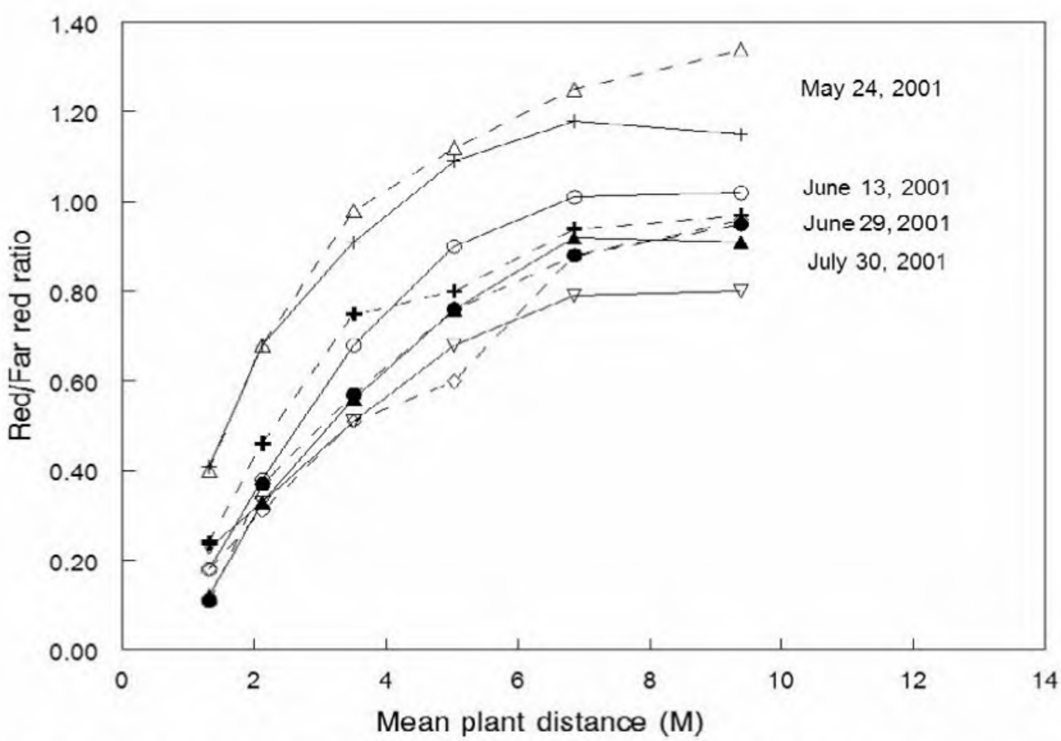

Figure 3—Red/far red ratios measured at varying mean plant distances during the 2001 growing season. Solid lines represent measurements made along a north-south transect; dashed lines represent measurements made along an east-west transect.

Tree Growth

At time of planting, mean tree height was 20.6 cm (8.1 in) (range = 18.5 to 24.0 cm [7.3 to 9.4 in]). First year height growth averaged about 4.3 cm across arcs and was greatest in the 2 outermost arcs. Height growth during the 2000 year showed a statistically significant negative trend with MPD (fig. 4). At the end of the third growing season (2001), total plant height showed a steeper statistically significant negative slope with increasing MPD (fig. 5). No differences in root collar diameter with density were observed.

Chlorophyll Fluorescence Emissions

Chlorophyll fluorescence emissions were measured on each tree in one spoke at about 10:00 AM on 25 July 2001. Tree heights in this spoke showed a significant negative slope with MPD from the center of the plot, indicating that they had been growing more rapidly at higher density. A "quenching analysis" was performed on 3 small current-season lateral twigs approximately 2 cm (0.8 in) in length from each tree. 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 (non-photochemical quenching) along the gradient of mean plant distance (table 2).

Discussion

The main reason for conducting this study was to test the repeatability of the results of the earlier greenhouse study (Ritchie 1997). If we were not able to achieve repeatability, the R/FR signaling hypothesis would be difficult to support for trees growing in the field. The main results to be confirmed were: (1) an increase in the R/FR ratio with increasing mean plant distance; and (2) a decrease in height growth with increasing mean plant distance. Both of these results were confirmed in this field study.

We were also interested to learn how soon after plantingthe R/FR signal and growth response could be detected. Results suggest that the first alterations in R/FR were detectable as early as May of the second growing season (fig. 2), and that the height growth response was also clearly apparent and statistically significant at the end of the second growing season (fig. 4). These results suggest that if the height response is triggered by R/FR differences, the light signal was perceived early in the second year after planting, with the growth response following during the same growing season.

Both Scott and others (1998) and Woodruff and others (2002) reported that a visual density-related height effect could first be "seen" 3 or 4 years after planting. Woodruff and others (2002)found that statically significant differences in height growth persisted from the third through the sixth year after planting. We were able to detect this response

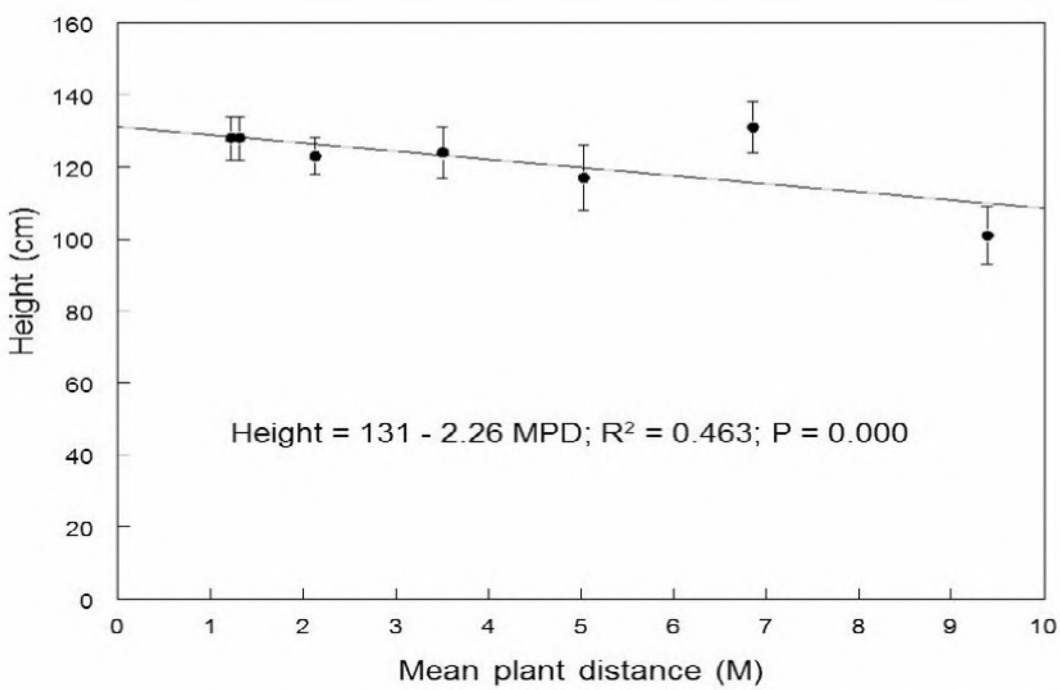

Figure 4—Total height of trees growing at different mean plant distances at the end of the second growing season in a Nelder plot. Each point is a mean (± 1 SE) of 12 trees.

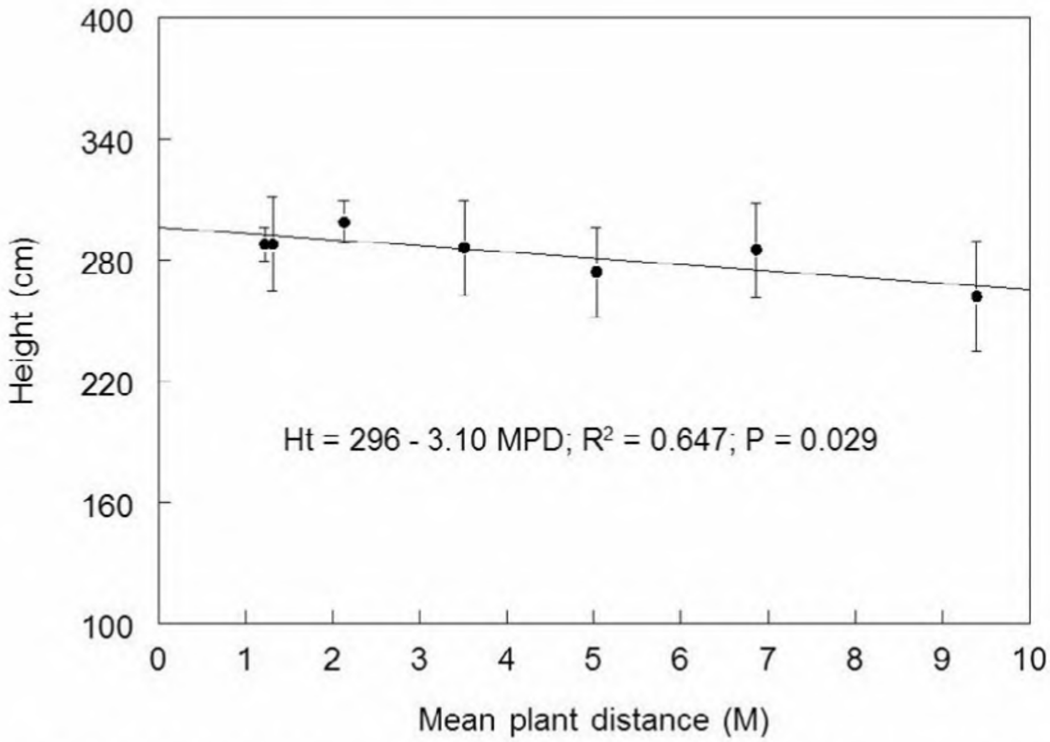

Figure 5—Total height of trees growing at different mean plant distances at the end of the third growing season in a Nelder plot. Each point is a mean (± 1 SE) of 12 trees.

Table 2—Chlorophyll fluorescence emissions parameters from 4-year-old Douglas-fir trees (3 years from planting) growing in a Nelder plot.

Mean plant distance	Fv/Fm	Y	qP	qN
(m)				
1.31	0.773	0.488	0.822	0.684
2.13	0.797	0.483	0.817	0.729
3.51	0.797	0.545	0.856	0.674
5.03	0.789	0.505	0.833	0.696
6.86	0.754	0.422	0.757	0.730
9.39	0.780	0.490	0.815	0.700

1 year earlier. The fact that we observed no differences in diameter is at variance with Scott and others (1998), who reported such differences in their study. However, their diameter measurements were taken at 5 or 6 years from planting—ours at only 3 years. Our results agree with those of Woodruff and others (2002).

The alteration in R/FR followed the hypothesized pattern—a large reduction in R/FR at the plot center (high planting density) that gradually became asymptotic with distance from the plot center. The trend was consistent throughout the growing season and along both a north-south and an east-west transect. During 2000, the curve reached a plateau about 6 m (20 ft) from the plot center; during the 2001 growing season, this plateau extended out to about 11 m (36 ft) from the plot center, or at a MPD of about 2.1 m (6.9 ft). This indicates that, as seedlings increase in height and above-ground biomass, they send stronger R/FR signals, as would be expected.

Another observation from this study, and that of Ritchie (1997) (fig. 5), is that the R/FR ratio across densities tended to be higher on overcast days than on clear days. We have no firm explanation for this, but speculate that it may relate to the diffuse rather than direct components of sunlight. Red light is always greatest in direct beam radiation. Almost any reflective surface will absorb at least a little of the red light. In most conditions, the more light that is reflected (that is, diffuse) and the less that is not reflected (that is, direct), the lower the proportion of red light to bands that are not absorbed, such as far red.

A reduction in R/FR can be brought about by: (1) a reduction of reflected red light owing to absorption of red by chlorophyll; (2) an increase in far red light owing to changes in canopy reflectance over time; or (3) some combination of both. We were able to shed some light on this question by examining the nature of the spectroradiometer outputs across densities. A typical output is shown for 24 May 2000 (fig. 6). Red light intensity is weakest at the plot center and increases outward. In contrast, far red light intensity tends to be roughly constant across the plot. This is consistent with the notion that the most important change as one moves from the inside to outside is that there is less direct beam radiation. This supports the view that the spectral alteration results from absorption of red light, rather than enhanced reflection of far red.

During 2001, when the change in R/FR with density became very strong (fig. 3), there was a clear trend for R/FR to decrease at all densities from 24 May to 30 July. One interpretation would be that during this period of active growth, as photosynthetic foliar mass increased, there was increasing red light depletion and depressed R/FR ratios. Another interpretation would be that sun angle changed from May to July. Since the integrating cylinder measured only radiation coming in horizontally, light registered in May would likely have a higher component of direct beam (hence red) radiation than July. So even if both times were sunny, the amount of red to far red would be lower in July simply because the proportion of diffuse (reflected) relative to direct radiation would be greater.

The increase in above-ground biomass with increasing density could reflect: (1) an allometric redirection of carbon from below-ground to above-ground structures; or (2) an increase in the rate or efficiency of photosynthesis. In the earlier greenhouse experiment (Ritchie 1997), seedling root-to-shoot ratios increased as growing density decreased, suggesting that the growth response involved changes in carbon allocation patterns. In the present study we examined photosynthetic efficiency of seedlings showing different growth rates during the third growing season using chlorophyll fluorometry. This analysis did not show any differences among trees growing at different rates (table 2). Woodruff and others (2002) found no differences in carbon isotope ratios of annual rings of Douglas-fir planted at different densities. Such differences would be expected if either photosynthetic capacity or stomatal conductance varied with density. These results further support the idea that changes in carbon allocation, rather than photosynthetic differences, underlie the apparent growth differences.

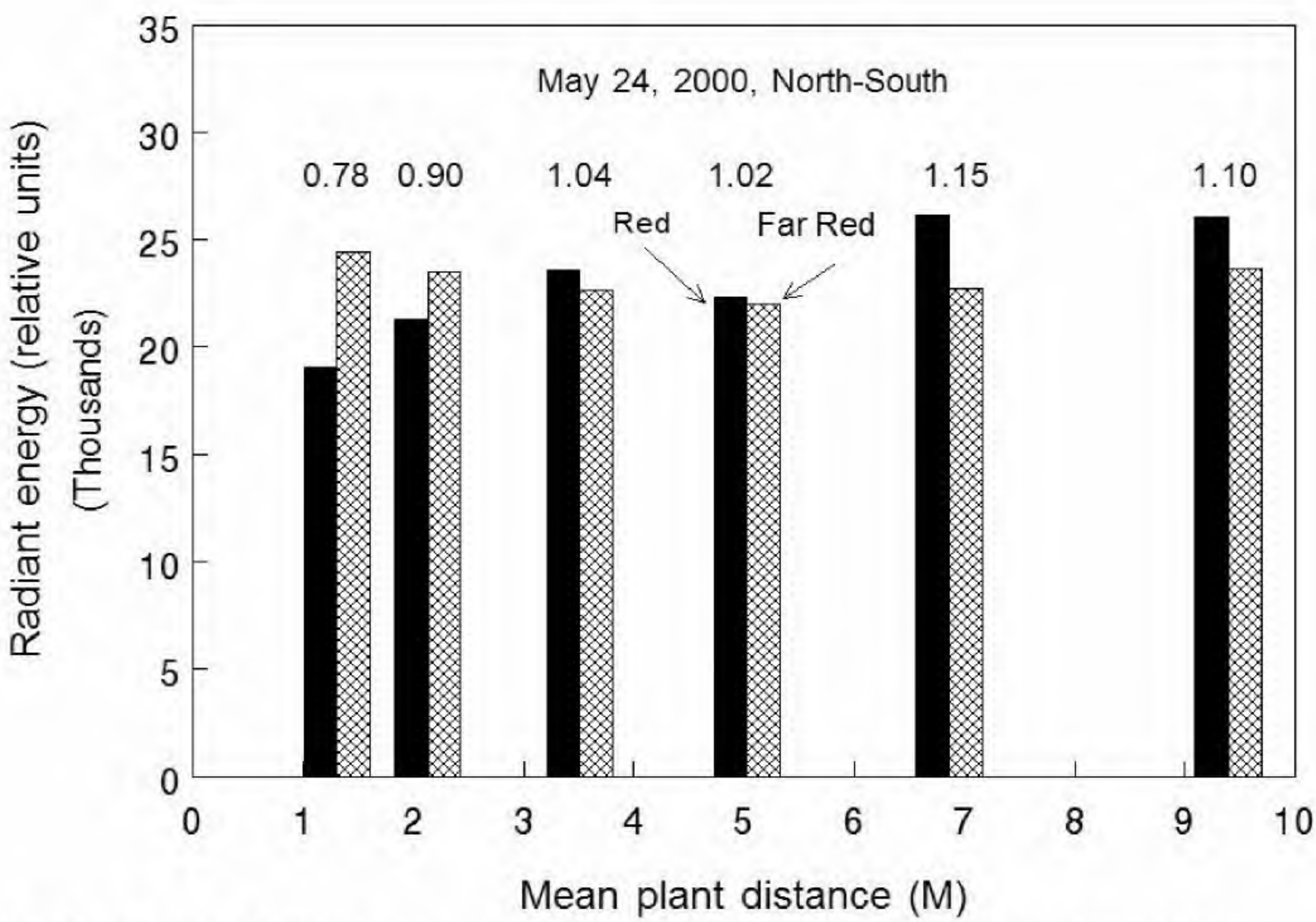

Figure 6—Red and far red light intensity measured at varying mean plant distances in a Nelder plot. Numbers above bars are R/FR.

Conclusions

Results of this study have extended the scope of inference of the earlier greenhouse experiment. We have shown that the R/FR ratio is strongly affected by planting density, that alterations of this ratio are propagated sufficient distances to be perceived by surrounding trees, and that the anticipated growth responses can be detected as early as the second growing season after planting. All are consistent with the R/FR signaling hypothesis for density-related early growth differences in young Douglas-fir plantations.

Although cause-effect was not demonstrated in this experiment, Ballaré and others (1987, 1990, 1995) have demonstrated cause-effect with annual plant species in experiments in which light spectra were artificially altered and produced the expected growth responses. Demonstration of cause-effect with field-grown Douglas-fir will require further research.

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