EFFECTS OF CONTAINER DENSITY ON GROWTH

AND COLD HARDINESS OF DOUGLAS-FIR SEEDLINGS [1]

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Abstract.--Douglas-fir [Pseudotsuga menziesii (Mirb) Franco] seedlings were grown in 2.7 cubic inch plastic containers for five months at densities of 25, 50, 75 and 100 ft^2 in a greenhouse. Low densities produced shorter, thicker-stemmed plants with greater shoot and root dry weights, lower shoot-root ratios, and a greater degree of cold hardiness. Ability to harden at low temperature was also increased significantly at the 100 ft^2 density by artificially raising the plant water stress range from 4-10 atm to 8-17 atm during the growth period. Morphology and physiology of seedlings are discussed in relation to measured microenvironmental changes associated with density variation.

INTRODUCTION

Although considerable work has been carried out to determine the effect of density on growth (van den Driessche, 1971; Wilson and Campbel, 1972), nutrition (Switzer and Nelson, 1963) and field performance (Baron and Schubert, 1963; Shipman, 1966) of bare-root coniferous seedlings, we cannot apply this information directly to the production of containerized seedlings. These seedlings have only limited space for root extension, a short production period and a faster growth rate. Effects which are not noticeable in the environmentally more complex nursery bed may become rather important in a tightly controlled greenhouse environment intended to maximize growth.

We have found, for example, that seedlings grown under such conditions have taken substantially longer to attain a desired level of hardiness under cool, short days in the fall even though they had reached a desirable size and state of bud maturation. This led us to examine the effect of container density and associated microclimatic changes on seedling growth and cold hardening ability.

MATERIALS AND METHODS

Stratified Douglas-fir seeds from near Aberdeen, Washington (coastal 500 ft elevation), were sown into 2.7 cubic inch plastic tubes containing 1:1 peat-vermiculite mix (v/v) in May 1973, and grown at densities of 25, 50, 75 and 100 per square foot in a greenhouse (spacing between plants was 6, 4.3, 3.5 and 3 cm respectively). The treatments were replicated eight times in a completely randomized design.

Beginning one month after sowing, the germinants were fertilized weekly with 3 ml of a 5X full strength modified Hoagland's nutrient solution (Hoagland and Arnon, 1950). The pH of the growing medium ranged between 5 and 6. Greenhouse temperature was maintained at 25 degrees C from 06:00 to 20:00 hours and 15 degrees C from 20:00 to 06:00 hours PST for the first 4 months. During the last month (October) cool outside air was drawn through the greenhouse and heaters were used to maintain temperature above 0 degrees C. The greenhouse was under natural photoperiod throughout the experimental period.

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Heights and root collar diameters were measured on October 27 on 20 5-month-old seedlings selected at random from each replicate. Shoots and roots were then dried at 70 degrees C for 48 hours and weighed.

GROWTH RESPONSE

The most densely grown seedlings were 50 percent taller and 13 percent thinner at the root collar than the least dense (fig. 1). Those grown at 50 or 75 ft^{-2} were intermediate in height and diameter.

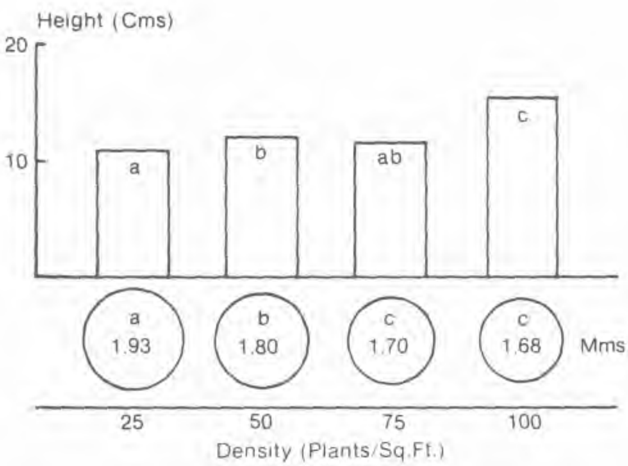

Figure 1.--Shoot heights (bars) and root collar diameters (circles) of 5-month-old Douglas-fir seedlings at four densities. Each value is an average of 160 observations. Bars or circles bearing the same letter are not significantly different (p=0.05) by Tukey's test.

Seedling height was inversely related to dry weight (fig. 2). That is, shoots and roots of the shortest seedlings grown at 25 ft^{-2} were the heaviest (significant at 5%). Differences in weights among the remaining three treatments were relatively small and were not significant statistically. The shoot-root ratio of 100 ft^{-2} seedlings (2.3) was significantly higher than for other treatments (fig. 2). Seedlings at 25 ft^{-2} had the smallest shoot-root ratio (1.5).

COLD HARDINESS

Injury after an artificial freezing test at -9° C was used to compare hardiness among the four treatments on November 10. Plant tops were cooled conductively at 5° C/hr to -9° ± .25° C where they were left for 2 hours, then warmed at 20° C/hr. The roots were

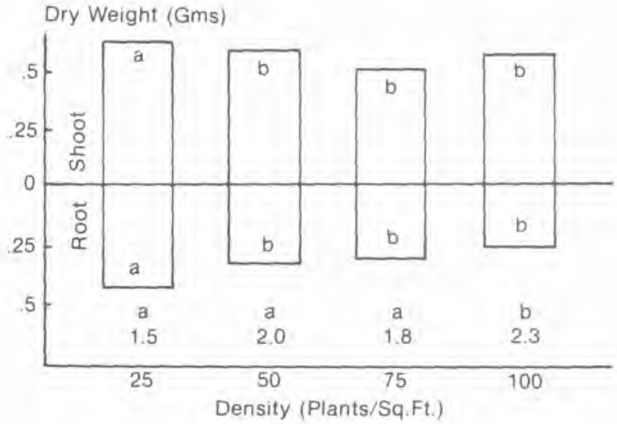

Figure 2.--Shoot and root dry weights (bars) and shoot-root ratios (lower numerals) at four densities. Each value is an average based on 8 bulked samples each of 20 seedlings. Values bearing the same letter are not significantly different (p = 0.05) by Tukey's test.

protected from freezing by a surrounding layer of vermiculite. Seedlings were assessed one month later and classified as dead if the browning of bark tissues had girdled the lower half of the stem, or if 50% of the needles were injured.

Mortality was directly related to seedling density (fig. 3). Nearly twice as many plants from 100 ft^{-2} density (60%) were killed as were those from 75 ft^{-2} (34%) due primarily to cambial damage at the base of

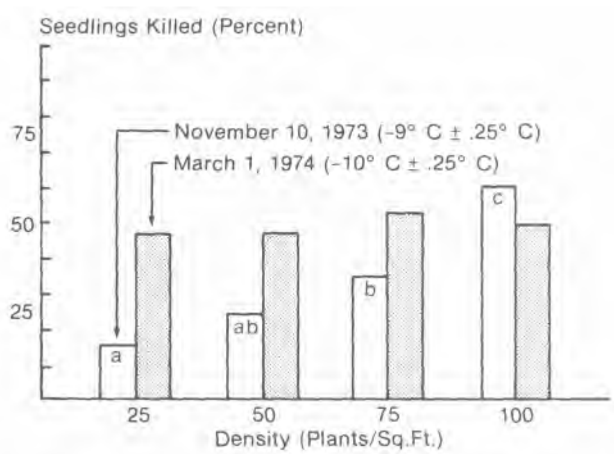

Figure 3.--Percent Douglas-fir seedlings killed in fall and spring laboratory freezing tests. Each value is based on 60 observations. Bars bearing the same letter are not significantly different (p=0.05) by χ^2 test.

the main stem; survival was further improved at densities of 50 ft^{-2} (24% killed) and 25 ft^{-2} (16% killed). Injury to needles, however, was not affected by seedling density. All hardiness differences had disappeared by the time of a second freezing test (-10 degrees+ 25 degrees C) in March, 1974, when plants were at a similar level of hardiness, but in the dehardening part of their cycle.

Water content, which is frequently related to both hardiness and growth rate, was measured shortly after the November freezing test on 80 seedlings randomly selected from each treatment, and is expressed in figure 4 on a fresh weight basis. Hardier seedlings produced under low densities contained less water than those less hardy seedlings from higher densities. Percent water contents ranged from 63.5 (25 ft^{-2}) to 68.6 (100 ft^{-2}). The differences among the four treatments, though relatively small, were all significant at 5% level.

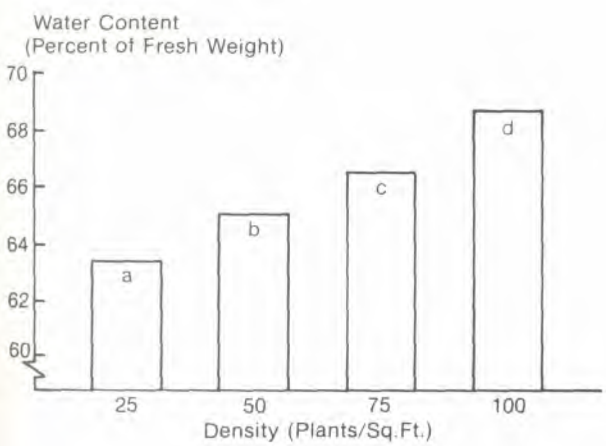

Figure 4.--Percent water content (based on fresh weight) of 5-month-old Douglas-fir seedlings at four densities. Each value is an average of 80 observations. All differences were statistically significant (p=0.05) by Tukey's test.

MICROENVIRONMENT

The substantial growth and hardiness differences noted above are presumably the result of microenvironmental differences between seedlings grown at different densities. Measurements of temperature, light intensity and quality taken at different positions in the seedling canopy provide some indication of these differences.

Temperature was measured using thermocouples at the seedling tip, the stem base, and in the soil at a depth of 6 cm. Average daytime temperature at each of the three positions was computed from 16 readings (8 per replication) made at 1-hour intervals between 08:30 and 15:30 hours PST. Incident radiation in the range 380-775 nm was measured with an ISCO spectroradiometer at the base of the seedlings in the lowest and highest density treatments only. These environmental measurements were made only after plants had entered their second growing season and initiated new growth (May 10) in a different greenhouse, and under partly cloudy conditions. Nevertheless, they indicate the type of differences which existed in the latter half of the previous growing season prior to growth and hardiness measurement.

Visible radiation reaching the bottom of the plants (expressed as a percentage of the energy above the plants at the same wavelength) was 10 times higher at the lowest density (fig. 5). Higher proportions of red light (680-775 nm) as compared to other wavelengths penetrated the canopy under both densities. The effect of this spectral change on seedling growth is not known.

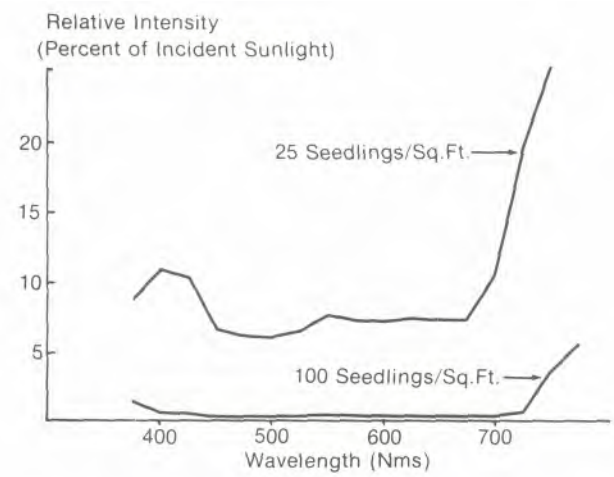

Figure 5.--Proportion of incident solar energy reaching the soil surface at two seedling densities.

Average daytime temperatures were highest at the tip of the seedlings, intermediate at their base, and lowest in the root medium in all four density treatments (fig. 6). At the base of the seedlings and in the root medium temperature in the lowest density treatment was more than 3 degrees C higher than that at the highest density due presumably to greater penetration of infrared radiation; at these same locations, temperatures were intermediate in the 50 ft-2 and 75 ft^{-2} densities. We have no simple explanation for the lower temperatures observed above the seedlings of the 50 ft^{-2} density treatment. Perhaps the medium contained a greater amount of water on that occasion and the air above experienced evaporative cooling.

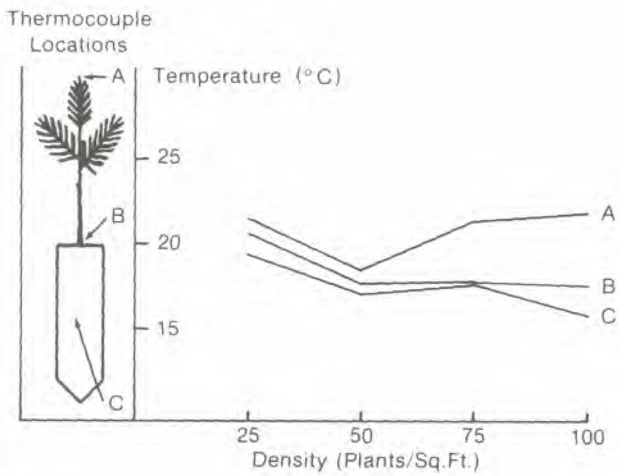

Figure 6.--Temperature differences at four seedling densities. Each point is an average of 16 observations (8 per container) made at one-hour intervals between 08:30 and 15:30 hours PST, May 10, 1974.

WATER STRESS AND HARDINESS AT A SINGLE DENSITY

One expected consequence of the greater penetration of radiant energy, the higher air and soil temperatures, and the greater facility of air movement among plants at lower densities is higher transpiration. This would cause low density seedlings to be under greater average water stress during their growing season. In a concurrent study conducted under similar greenhouse conditions, but using somewhat smaller containers (2 cubic inch), water stress levels were artificially increased in densely grown seedlings (100 ft^{-2}), and their effect on cold hardiness observed.

Water stress treatments were begun eight weeks after sowing and terminated at the 16th week in one group; in another group they ran from the 12th to the 21st week. Thereafter, the seedlings were placed in the humid cold room at 2° C on an 8-hour photoperiod of 500 foot-candles with mixed incandescent and fluorescent light. Plant water stress was controlled within two fairly broad ranges (4-10 atm and 8-17 atm) by an automatic mist sprinkler system based on the weight of a sample block of 50 plants and a previously determined relationship between container plant weight and pre-dawn plant water stress. Achieved water stress was recorded on samples throughout the study and used as a basis for periodic recalibration of the automated system.

Samples of 30 seedlings from each treatment and age group were measured for hardiness (1) immediately following greenhouse growth, (2) after 5.5 weeks of cold treatment, and (3) after 11.5 weeks of cold treatment. The freezing tests were conducted as described earlier, but at three temperatures (using different sub-samples of plants) so that a "50% kill" temperature (T_{50}) could be interpolated from the mortality/temperature curve.

The higher water stress during the growing period significantly increased the seedlings' subsequent ability to harden off at low temperature, although the hardiness measured immediately after the stress treatment was not improved (fig. 7). This increase was most noticeable after approximately 3 months of cold treatment, at which time it amounted to a difference in T_{50} of 4 or 5 centigrade degrees.

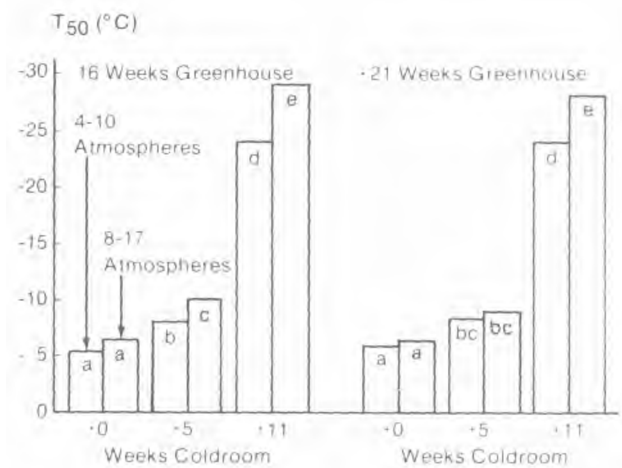

Figure 7.--Cold hardiness (measured as temperatures killing 50 percent of the seedlings) as affected by water stress during the growth period, and subsequent cold room treatment. Bars bearing the same letter do not differ significantly (p=0.05) by Duncan's test.

DISCUSSION

Morphological characteristics of Douglas-fir seedlings observed under four densities in the present investigation generally agreed with those reported by Harris et al. (1972) for container grown Betula verrucosa Ehrh., Eucalyptus sideroxylon A. Cunn., Dodonaea viscosa 'Purpurea' Jacq. and Liquidambar styraciflua L. Low density produced short seedlings with larger diameters and greater dry weights, while high density resulted in tall but thin-stemmed and succulent seedlings. Root dry weight significantly increased as density decreased even though root growing space was the same at all densities.

Various hypotheses analogous to those proposed for developing forest stands can account for the growth and morphology differences. We would expect total dry weight of a seedling to be higher at low densities because more than 90% of the incident photosynthetically active light is intercepted at both lowest and highest densities, but in the lowest density the intercepted light is distributed among only one quarter the number of plants. Moreover, the higher subcanopy temperature and (probably) greater CO_2 movement may mean that the pentrating light can be more effectively used.

The diversion of relatively large amounts of dry matter into diameter rather than height growth at low densities has, in forest stands, been the subject of at least three theories, any of which could conceivably be applied to seedling crops. (1) More active photosynthesis by the basal leaves may lead to more carbohydrate and/or hormone being available for cambial growth in the adjacent part of the stem; (2) due to greater evaporative water stress, there may be a preferential synthesis of xylem for water conduction and/or a reduction of cell enlargement at the stem apex; (3) the greater air movement among low density plants (from greenhouse fans) may lead to a stimulation of diameter growth as a result of mechanical bending stress.

The contribution by lower leaves to carbohydrate production in low density plants would be significant because 7% of the incident light reached the lower part of the plant. Only 25% of full sunlight (2500-3000 foot candles) is necessary to saturate photosynthesis in Douglas-fir seedlings (Brix, 1967; Kreuger and Ruth, 1969). Hence, on sunny days, available light for photosynthesis of lower leaves would be around 400-500 foot candles, about one-sixth of the saturation intensity (assuming 50% filtering by greenhouse glass), whereas at the base of the densest plants a negligible amount of light would be available for photosynthesis.

The contribution of water stress to reduced seedling height growth is well documented. Kramer and Kozlowski (1960) state that water stress reduces enlargement more than cell division or differentiation, resulting in seedlings with smaller, thicker and more heavily cutinized leaves, lignified stem and reduced height.

The greater proportion and total amount of dry matter in the root system at low densities may be a direct adaptation to avoid water stress by exploiting more soil or a result of the higher soil temperature. There is evidence in the literature for both effects, though not as part of seedling density studies.

The effect of mechanical action is less certain. Harris et al. (1972) postulated that hormonal control over apical growth caused by increased stem movement may be involved because Neel and Harris (1971) had observed that daily shaking of liquidambar trunks for 30 seconds reduced height growth to 20 to 30 percent of that in unshaken trees. Because of insufficient evidence, however, the hormonal hypothesis has met with opposition by Parkhurst and Pearman (1972) who emphasized that, among other mechanisms, water stress resulting from blocked vessels due to cavitation by shaking should be considered.

The increase in subsequent cold-induced hardening of densely grown seedlings at artificially high water stress supports the hypothesis that the greater hardiness of low density plants was at least partly a result of higher water stress during growth. It should be remembered that hardiness in the density experiment was also measured after a period of low temperature treatment, albeit by natural ventilation of the greenhouse during the fall. This result is in agreement with Levitt's conclusion (1956) that water stress during the hardening off period would not increase hardiness but that plants grown under drier conditions were subsequently able to cold harden more deeply. The ineffectiveness of water stress during the hardening off period as a factor for increasing hardiness in Douglas-fir has been demonstrated previously (Timmis, 1972).

The physical basis for improved hardening in such plants may be smaller cell size and lower content of vacuolar water. The lower water contents of hardier seedlings observed in the density study tend to support this view. Another mechanism may be a greater concentration of sugars in the vacuole, hence increased osmotic potential. The osmotic contribution to hardiness thus derived would be additive to the deeper hardiness levels achieved by other mechanisms.

In conclusion we note that a better seedling is produced at lower densities. It has a greater dry weight, smaller height-diameter ratio, smaller shoot-root ratio, lower water content and greater cold-hardening capability--all characteristics generally known to favor field performance of outplanted Douglas-fir. There are several microenvironmental changes which could account for these responses to seedling density. Further work is needed to determine exactly which factors are mainly responsible. This perhaps would then allow us to grow "low density" type seedlings by careful environmental control without the economic penalty of wider spacing. Regulation of water stress offers one such possibility.

ACKNOWLEDGEMENT

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Question: Are seedlings used in cold-hardiness studies, mycorrhizal?

Timmis: I don't think so. I didn't see any and we did not introduce mycorrhizae.