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ABSTRACT

Objectives:
- to study the effect of short- (SD) and long-day (LD) treatments under moisture stress or no stress conditions on shoot growth, morphology, bud development and cuticle and epicuticular waxes in greenhouse-grown western hemlock seedlings from two styroblock cavity sizes
- to examine the influence of lift date on bud morphogenesis

Methodology:
- treatments other than cavity size were applied for 1 month beginning mid-July
- weekly measurements of shoot growth were made from early July until growth cessation
- buds were sampled at 1-4 week intervals from early July, 1986 until mid-March, 1987
- bud development was followed and the apices squashed to estimate mitotic index (MI)
- bud development was studied also in seedlings placed in cold storage in November and January
- after growth cessation, records were made of root collar diameter, height, numbers of branches and needles, and needle lengths
- cuticle thickness was measured in needle cyrostat cross sections
- needle epicuticular waxes were studied using a scanning electron microscope

CONCLUSIONS/RECOMMENDATIONS:
- SD caused a rapid cessation of growth and transition to bud development, whereas free growth continued under LD

- SD reduced final height, whereas diameter growth was mostly influenced by moisture
- LD increased the total number of primary needles and rates of branching per cm
- LD increased stem unit and needle lengths in the proximal half of the shoot, while moisture stress reduced these values in the distal section of the shoot
- SD and moisture reduced MI and rates of apical expansion
- bud development began in late July after SD and about 1 month later after LD
- the longer period of needle primodium initiation in SD increased final needle numbers
- November-lifted seedlings were mitotically active during first month of storage, whereas the January-lift material was inactive
- no differences due to dormancy induction were detected in cuticle thickness and numbers of stomata
- fusion of wax rodlets in the stomata was less common in needles from the SD moisture stress treatment than others
- short days are an effective method of slowing elongation and stimulating bud development without the need for moisture stress
- our results suggest that SD treatments should begin at a later date than that tested here, but this aspect requires further study
- seedlings should not be placed in cold storage until after mid-December

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ACKNOWLEDGEMENTS

This research was carried out as part of a joint study on the effects of a number of greenhouse nursery regimes on the growth, development and physiology of container-grown western hemlock seedlings. The propagation, culture, and maintenance of the nursery material was carried out by the Canadian Forestry Service, Pacific Forestry Centre. The assistance provided by J.T. Arnott and G. Goodmanson of the CFS, and G. Dunsworth of MacMillan Bloedel Ltd., is appreciated.

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INTRODUCTION

Western hemlock (Tsuga heterophylla (Raf.) Sarg.) is an important reforestation species in coastal British Columbia. Because of the high survival and superior growth of container-grown seedlings (Arnott 1975), most interest has focused on this method of regeneration rather than using bare-root stock. About 8.25 million container-grown western hemlock seedlings were planted in 1981 (about 12.5% of provincial total) (Johnson 1982). Nevertheless, high mortality and poor growth, often due to drought stress, is common in container-grown western hemlock seedlings (Arnott 1975). There is much evidence that the use of specific "stock types" suited to various environments would increase outplanting success, thus reducing regeneration costs (Hobbs 1984). These different stock types may have different morphological and physiological attributes and are produced by changing or modifying nursery cultural practices. However, there is little information on how nursery cultural regimes affect physiological, developmental and morphological characteristics, other than those of seedling size and vigour (see Duryea 1985). Information is needed on the influence of nursery regime on seedling development in western hemlock. Developmental factors influence the physiological condition and morphological attributes of the seedlings. For example, the nursery environment has a marked effect on bud development which ultimatey influences field growth potential of conifer seedlings (Colombo 1986).

The purpose of this study was to investigate the effects of nursery practices, such as short- (SD) or long-day (LD) photoperiods, in combination with drought (D) stress or wet (W)/no stress conditions and styroblock cavity size on growth and development of buds and shoots of container-grown western hemlock seedlings. The effects of modifying these practices on seedling growth are discussed.

In another study, the influence of these regimes on physiological factors was examined (Arnott and Dunsworth, unpubl.[1]). The results of that study will be reported elsewhere.

MATERIALS AND METHODS

Western hemlock seeds of mid-elevation (British Columbia Ministry of Forests Registered Seed Lot No. 3907; 48°39'N, 123°39'W, elevation, 760 m) seedlot from Vancouver Island were stratified at 2°C for 4 weeks before sowing on February 12, 1986 in BC/CFS styroblocks (PSB) (Beaver Plastics Ltd., Edmonton, Alta.) of two different sizes, PSB 313 having a cavity diameter of 27 mm, a ribbed cavity volume of 57 cm^3, a spatial density of 932 cavities m^{-2}, and PSB 415B having a cavity diameter of 35.2 mm, a ribbed cavity volume of 102 cm^3, and a spatial density of 527 cavities m^{-2} in a Pacific Forestry Centre, Victoria, B.C. (48°28'N) greenhouse that was maintained at 24°/18°C (day/night), 50% humidity and an 18-h photoperiod. Natural day length was supplemented by high pressure sodium vapour lights to provide at least 500±100 lx (6 $\mu E \cdot m^{-2} \cdot S^{-1}$) at the seedling level. The styroblock cavities contained a 3:1 mixture of peat and vermiculite with 2.0 kg/m^{-2} dolomite lime (10 mesh and finer) added. The styroblocks were misted with water daily during germination. After germination, biweekly applications of 20N-20P-20K fertilizer with micronutrients (Green Valley fertilizer, Surrey, B.C. (500 mg/L) and a heptahydrate form of ferrous sulphate (155 mg/L) were made. Temperatures were reduced 5 days after germination to 21°/18° until September 15. Temperatures were 18°/15° until September 29, 15°/10° until October 27, 15°/5° until November 17, and 10°/5°, thereafter. Maximum daily

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[1] "Field performance of new stock types", a joint project between the Canadian Forestry Service (Project PC-61-03) /MacMillan Bloedel Ltd. (Project 312.12).

temperatures were about 2°C above programmed temperatures from late spring to late fall, whereas minimum tempertatures were seldom 1°C below their setpoints. Maximum temperatures deviated by up to 8-9°C during extremely warm weather in August and early September.

Nursery treatments

The seedlings from the two styroblock cavity sizes, PSB 313 (abbreviated S3) and PSB 415B (S4), were subjected to four dormancy induction regimes - long (18 h) or short (8 h) days under moisture stress or no stress conditions. The induction regimes began July 15, 1986 and ended 4 weeks later. The styroblocks in the stressed treatments were allowed to dry to 2 to 3 kg below saturated weight before re-watering to saturation, with the dry-down repeated 3 times during the 4 week dormancy induction period. Seedling water stress levels were monitored by predawn xylem water potential readings taken with a pressure bomb (PMS Inc., Corvallis, OR) on four single tree replicates from each treatment. Pre-dawn seedling water potentials ranged from -0.6 to -1.4 MPa before rewetting to saturation. The final treatment included three lift/storage treatment combinations. The first lift (lift one) was on November 10, 13, 1986, the second on January 14, 15 and the third on March 16, 17, 1987. The lift three material was moved to an unheated shelterhouse on February 16, 1987. The cold-stored seedlings were maintained at 1°C in cold rooms at the Pacific Forestry Centre, Victoria.

Nursery experimental design

The experiment was layed out in the greenhouse according to a modified split-split-plot design (Fig. 1). This modification was used because of the constraints imposed by having one study greenhouse only. Day length was randomized between halves of the greenhouse (main plots) and

moisture regime was randomized between quarters (sub plots) within each half greenhouse. Each quarter of the greenhouse was divided into eight strips (or blocks). Groups of styroblocks, two S4 to one S3, were randomly assigned to each strip, with three such groups per strip (Fig. 1). Each group was randomly assigned to a lifting date. In a separate study, no significant differences were found among positions within the greenhouse (Arnott, pers. comm.), and this was assumed to be the case in our study.

Observations and measurements

Shoot elongation and morphological data

Shoot elongation of five seedlings in a row plot per strip, each row selected at random in the eight major treatments (two stryoblock cavity sizes x four dormancy induction regimes) of those destined as lift three material were measured weekly from July 11, 1986 until October 10, 1986 (total of 320 seedlings). For ease of repeated measurement, seedlings were measured from the top of the styroblock to seedling tip or base of terminal bud (when visible).

In another subsample, final seedling height and other morphological information were determined from seedlings that were also used in assessing stages of bud development and mitotic activities. Data were collected between December 22, 1987 and March 11, 1987 from 576 seedlings, 192 from each lift/storage treatment combination (Table 1).

The morphological information collected included: (1) diameter at root collar; (2) height from root collar to base of terminal bud and height to cotyledon scars; (3) numbers of first-order (1°) and second-order (2°) or higher order branches; (4) numbers of needles in the upper and lower halves of the leader; (5) numbers of primary needles surrounding the terminal bud (terminal needles); (6) the lengths of 10 needles, five each taken at about 1 cm above

the cotyledons and about 1 cm below the base of the terminal bud. There were relatively few branches higher than 2°; these were included in the 2° data. The data collected in (4) and (5) were categorized according to half-leader portion so that before and after treatment effects in stem unit (SUL) and needle lengths could be assessed. This assessment was approximate for the distal section of the shoot because seedlings from some treatment combinations initiated a large percentage of their needles after the dormancy induction began (Fig. 2). New variables created from the above data included the ratios of 1°/2° branch numbers, 1° branch numbers/height, and 2° branch numbers/height. SUL per leader and per half leader height were calculated for each seedling. SUL of the proximal portion of the leader was half seedling height minus height to cotyledons divided by number of needles or stem units (NSU) in that section. SUL of the distal portion of the leader was half seedling height divided by NSU for that section. The terminal needles were not included in calculations of SUL.

Bud development

One seedling shoot tip from each of the eight strips per treatment combination were sampled at 1-4 week intervals, depending on the rates of development (Table 1). Sampling was taken equally across all predestined lifts until the first lift, in mid-November, 1986. Sample size was increased after each lift date as indicated in Table 1.

All shoot tips collected from the greenhouse material were placed in a cooler and processed within 5 h so as to minimize the possible effects of storage. This procedure involved sampling over 2 consecutive days. The seedlings removed from cold storage were stored at approximately the same temperatures as in the cold rooms, and were processed over 3-4 consecutive days, the first 2 days being the same as those for the greenhouse plants.

Terminal buds were dissected, numbers of bud scales and needle primordia determined, and fixed in McClintock's solution (Johansen 1940). Apical heights and widths were measured under a compound microscope equipped with a calibrated eyepiece micrometer disc. Squashes were made of all apices collected on dates at intervals of 2 weeks or more (Table 1). Therefore, every second sampling date only was used between August 18 and October 7, 1986.

The squash procedure was a modification of the Feulgen method outlined in Jensen (1962). Fixed specimens were placed in 1 N HCl at 63°C for 40 minutes, then stained overnight in the dark in Schiff's reagent. Shoot tips were stored in 45% acetic acid or distilled water until squashed. Shoot tips were placed on a slide and the apical meristem excised under a dissecting microscope using a microscalpel (Rudolph Beaver Inc., Waltham, MA) in a drop of 45% acetic acid. A cover slip was placed on the apex and tapped lightly with the tip of a pencil eraser. The squashed apex was viewed immediately under a compound microscope at 400X and the number of divisions determined. All stained cells were counted at 100X with the aid of a net micrometer disc. Each apex contained a number of cells for which mitotic figures and cell counts could not be determined (Fig. 16). These cells probably contained tannins, substances that are known to interfere with the Feulgen reaction (Greilhuber 1986). Our observations and those of Owens and Molder (1973) indicate that tannins occupy a similar percentage of the apex throughout the season and are restricted to the rib meristem and pith tissue where relatively few divisions occur. Mitotic index (MI) was calculated as the percentage of cells that were dividing in each apex.

Data analyses

An analysis of variance of the variables according to a split-split-plot design was used to test for treatment effects and their interactions. Variation among strips,

and/or strips by lift, within the daylength by moisture treatment combinations was used as the error term for testing daylength, moisture and/or lift effects (Tables 2-5 and 7)[2]. A number of variables such as apical size and primordium numbers were not always categorized by strip within treatment combination, although seedlings were always sampled across all eight strips. When strips were not a factor, data were analysed according to a completely randomized factorial design. Percentage data were normalized using an arcsine transformation (Zar 1984).

Cuticle thickness, epicuticular wax features and numbers of stomata

The thickness of the cuticle and the distribution of epicuticular waxes were separately studied in needles taken from S4 seedlings only. One needle from each of 24 seedlings in each of the four dormancy induction regimes were examined in each study. Needles were sampled from December to May from that section of the shoot that was produced most likely during the induction period. This included needles from the last 10% of shoot growth in the SD plants and 70 to 80% of growth in the LD plants (Fig. 2).

Cuticle thickness

Cryostat cross sections at 10 to 15 μm at the midsection of each leaf were made and stained in Sudan IV (Johansen 1940) for approximately 1 h at 0-4°C to minimize wax alteration (Reed 1982). The sections were washed in 60% alcohol at the same temperatures to remove excess stain, then mounted in Farrant's medium (BDH Inc., Toronto, Ontario). Staining with Sudan IV allows differentiation of the cuticular membrane or cuticle (Holloway 1982). The cuticular membrane includes epicuticular waxes, the cuticle proper and the cutinized cell wall. No attempt was made to measure the components of the cuticle.

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[2] The analysis of variance procedure was a modification of the standard split-split-plot design, prepared by Dr. R. Davidson of the Mathematics Department, and Mr. P. Konkin of Computing User Services, University of Victoria.

Cuticle measurements were made at the centre of one cell at each of two positions on the adaxial and abaxial surface of each needle, approximately halfway between the midrib and edge of needle. For this purpose, photographs were taken of cells at each position and measurements made from projections of the negatives (8500 X).

Epicuticular wax features and numbers of stomata

The scanning electron microscope (SEM) was used to observe the surface wax features of each needle. The midportion of each leaf was mounted on aluminum stubs with silver paste, gold coated for 5 min using a Technics sputter coater, then observed with a JOEL 35 J5M SEM operating at 15 kV. Photographs were taken of typical wax features on both leaf surfaces. Photographs (at 3000X) were taken of the adaxial surface, a stoma, between stomatal rows and along the midrib of the abaxial surface. The numbers of stomatal rows, stomata, and number of unoccluded stomata were recorded from photographs taken at 100X.

RESULTS AND OBSERVATIONS

Phenology of shoot growth

Differences in shoot length between cavity sizes were small before July 15, at the time the dormancy induction treatments began (Fig. 2). Although plants subjected to SD showed declining rates of elongation after this time, differences among treatments other than cavity size were not significant until mid-August. Seedlings exposed to SD ceased elongation by late August (just after the induction treatments ended), with no significant differences between moisture stressed and non-stressed seedlings. The LD seedlings ceased growth by October 10. Overall, day length was the only factor affecting the phenology of shoot growth whereas cavity size (and moisture stress in S4) influenced rates of growth.

Seedling morphology

Height, diameter, needle growth, and shoot length components

Treatments and some of their interactions significantly affected seedling height and root collar diameter (Table 2). Nevertheless, Figure 3 shows that day length had a relatively small effect on diameter. Mean diameters ranged from 2.3 mm (SDD) to 2.9 mm (LDW) in seedlings from S3, and similarly from 2.8 to 4.0 mm in S4. Mean seedling height ranged from 210 to 296 mm, and 244 to 389 mm, in seedlings from S3 and S4, respectively (Fig. 4). To examine further the effect of nursery treatments on height growth, NSU and SUL were analysed.

Numbers of stem units varied significantly due to day length, moisture, cavity size and some of their interactions (Table 3). Figure 5 shows that photoperiod was the most important factor affecting these numbers. However, variation in SUL also contributed because differences in seedling height were more than expected based upon NSU. For example, the mean height of seedlings from SDD-S3 was 54% of the mean height of seedlings from LDW-S4, whereas this figure was 64% for NSU.

Cavity size had the largest and most consistent effect on stem unit length in both portions of the leader (Table 3; Fig. 6). Average SUL was 0.2 mm larger in S4 than in S3. Day length, moisture stress and photoperiod by cavity size were significant factors affecting SUL in the proximal portion of the leader, although the levels of significance were generally low (Table 3). Differences among treatment combinations in SUL were small within a cavity size (Fig. 6). This result is not surprising as cavity size was the only factor expected to influence SUL in the proximal portion of the shoot as these stem units were initiated well before the dormancy induction treatments began (Fig. 2). Moisture stress was the dominant factor, other than cavity

size, affecting SUL in the distal section of the leader (Table 3; Fig. 6). SUL in this leader portion differed by 0.4 mm between seedlings from the SDD-S3 and the LDW-S4. As expected, average SUL per seedling gave similar results to those described for the distal portion of the leader (Table 3; Fig. 6).

Cavity size, day length and their interactions had the largest effect on needle lengths in the proximal portion of the shoot, although moisture and its interaction with day length was significant also (Table 4). Mean length of proximal needles ranged from 13.6 (SDD and SDW) to 14.9 mm (LDW) in S3 seedlings and 13.6 (SDD and SDW) to 15.8 mm (LDW) in S4 plants (Fig. 7). Length of distal needles was heavily influenced by moisture stress, although cavity size was a significant factor also (Table 4). Mean length of distal needles ranged from 11.1 mm (LDD-S3 and SDD-S3) to 12.5 mm (LDW-S4) (Fig. 7).

Branching characteristics

Day length, moisture stress, cavity size and most of their interactions greatly influenced 1° and 2° branching, numbers of 2° branches being especially sensitive to cavity size (Tables 5, 6). Numbers of 1° branches ranged from 13.9 to 21.1 in S3 and 16.8 to 29.0 in S4 (Table 6). Numbers of 2° branches had a larger range with the LDD-S4 plants having the greatest numbers. The ratio of 2°/1° branches gives an indication of the amount of 2° branching adjusted for the number of branches upon which they were borne. Short days and moisture stress tended to reduce these ratios, especially in S4 plants. Day length appeared to be the most important factor influencing 1° branch numbers per unit leader, although these values did not vary greatly (from 0.66 to 0.74/cm). The numbers of 2° branches per unit height ranged from 0.7 in the SDD-S3 plants to 1.6 in the LDW-S4 plants. However, these 2° values are confounded by

the influence of 1° branch numbers.

Bud development

The final number of leaf primordia was influenced little by lift date, indicating that most bud development was completed by first lift (Table 7). The low, but significant, effect of lift by day length reflected the lower values in plants from LD lifted in November than those remaining in the greenhouse or those lifted in January. Because this effect was so small, estimates of the final number of primordia presented in Figure 8 are averages of all three lifts on two sampling dates. However, dormancy induction treatments and cavity size had a large effect on the final number of primordia (Table 7). Numbers of bud scales and terminal leaves were influenced also by some of these treatments (Fig. 9).

Moisture stress and cavity size had the largest effect on final numbers of needle primordia, followed by day length by moisture interaction and day length (Table 7). The effect of cavity size by day length, although significant at a low level, had a minimal effect on these numbers. Moisture stressed plants had fewer primordia, especially under short-day conditions (Fig. 8). The larger cavity size and shorter days increased primordium numbers.

Day length alone had a significant effect on final bud-scale numbers (Table 7). Figure 9 shows that plants subjected to SD produced an average of 13 bud scales compared with 16 under LD.

The numbers of terminal leaves varied due to day length, moisture stress, cavity size and some of their interactions (Table 7). Seedlings subjected to short days had the most leaves (Fig. 9); moisture stress and the smaller cavity tended to reduce these numbers.

Differences among treatment combinations in the above variables can largely be explained by treatment effects on

the phenology of shoot growth and bud morphogenesis.

Phenology of apical activity, apical growth, bud-scale and leaf production before first lift

The seasonal pattern of mitotic activity was similar in all treatments, although there were differences in the overall rates and the time at which maximum rates were achieved (Fig. 10). There was a decline in MI in all treatment combinations in late July-early August. This coincided with a period during which maximum greenhouse temperatures were about 8-9°C above programmed temperatures. Mitotic indices were high from early September until mid-November. MI were lower in the SD treatments, and maximum rates were reached at an earlier date than in the LD treatments. The peak in MI approximately coincided with maximum apical size (Figs. 11, 12) in the LD treatments, whereas there was little or no increase in apical size in seedlings from SD.

Day length had a significant effect on apical size on most observation dates, while MI was significantly affected in mid-August to early September (Table 8). Moisture and cavity size had a relatively small effect on MI on most dates, although these factors often interacted with day length later in the season.

Apical height declined in the SD treatments in mid- or late August (Fig. 11). Apical width began to decline at a later date in these plants, in early to mid-September (Fig. 12). These declines were more rapid in the S3 plants. Rates of leaf production were high in the SD treatments during this period, especially in S4 seedlings growing under no moisture stress conditions (Fig. 8). This decline in apical size probably reflected the rapid encroachment of leaf primordia upon the apical dome. Rates of MI remained relatively high in the SD treatments until late October, whereas apical dimensions decreased continually from August onward. Few primordia were initiated after mid-November,

although apical activity continued.

In contrast to the SD treatments, those treated to LD showed a later increase in MI (Fig. 10), apical size (Figs. 11, 12), and leaf production (Fig. 8). Mitotic indices were relatively low in these treatments until mid- to late September, when rates of activity increased rapidly, but were not significantly higher than those from SD (Table 8). There was a large increase in apical size during this period (Figs. 11, 12), unlike the small increase observed in those from SD. Bud scales were first noted in late July in LDD treatments and in early August in the LDW. However, rates of bud-scale initiation were slow and these structures were found on few seedlings. Leaf initiation began in early September (Fig. 8), about 1 month before shoot growth cessation (Fig. 2). Mitotic indices were maximal in early October in the LD treatments, at about the time that apical size began to decline. Leaf production rates were very rapid in September, indicating that apical relative growth rates were high to compensate for the encroachment of primordia upon the apex. Differences in rates of apical growth due to moisture stress within the LD treatment were relatively small, although rates of leaf initiation differed (Fig. 8). Cavity size also had a small effect on these rates within a day length treatment.

Overall, day length was the major factor influencing the timing of bud development while the rates of activity were influenced by cavity size and moisture availability. Short days caused a rapid transition from free growth to bud formation, perhaps resulting in more terminal leaves with little internodal expansion. Short days may have caused the subsequent rapid transition from bud-scale production to leaf initiation, thus reducing bud-scale numbers. The relatively long period of leaf initiation in this treatment resulted in more leaf primodia, especially under no moisture stress conditions.

Mitotic activity and apical growth after first lift

Seedlings from all treatment combinations were mitotically highly active at the time of first lift (November 15, 1986), with rates being highest in the LD treatment (Fig. 10). After this time, seedlings remaining in the greenhouse showed declining rates of MI until reaching zero on January 12, 1987. Rates of activity fell rapidly after placement in cold storage, with all treatments showing a similar decrease (Fig. 13). Mitotic indices were approximately one-third of those in the greenhouse just three days after placement in storage. The cold-stored plants were inactive by December 22. Analyses of variance of MI during this period indicated significance due to day length and lift. There was a highly significant interaction between photoperiod and lift on the last two dates, reflecting the higher rates of MI of the LD treatment remaining in the greenhouse. All plants were mitotically inactive on January 12, just before the second lift. No mitotic activity was found in any cold-stored material on February 9 and March 9, 1987. The greenhouse plants (lift three) showed a slow increase in MI during this period, rates being highest in those from LD on March 9 (Figs. 10, 13, Table 9).

Apical size of seedlings in the greenhouse gradually declined from mid-November until late December, especially in the LD treatment (Figs. 14, 15). Apical dimensions remained about the same after this time, with similar among treatment differences as recorded in late December. The lift one cold-stored seedlings had larger apical dimensions, especially apical width, throughout the period of storage. Apices of the lift two cold-stored plants were about the same size as those from the greenhouse plants at that time. The analyses of variance results reflect these observations (Table 9).

Cuticle thickness, epicuticular wax features and numbers of stomata

Cuticle thickness

Photographs of typical views of the adaxial (Fig. 17) and abaxial (Fig. 18) surfaces of a needle are presented. No significant differences in cuticle thickness were found among treatment combinations of S4. Mean cuticle thickness was 4.4 (±0.09 SE) μm on the adaxial surface and 3.3 (±0.08) μm on the abaxial surface.

Epicuticular wax features and numbers of stomata

There were no significant differences among treatment combinations in the numbers of stomata, stomatal rows and frequencies of unoccluded stomata. On average, there were 11.2 (±0.19) rows of stomata and 96.6 (±1.89) stomata per mm^2; approximately 97% of these stomata were occluded. Fig. 19 is a typical example of such a leaf with most stomata occluded.

The morphology of the epicuticular waxes varied from rods (or tubules) or fibrils of wax (Figs. 20, 21) to amorphous layers (Fig. 22). Rods were often fused at their bases into tufts, sometimes coalescing into plate-like layers (Fig. 23). There was variation in wax morphology among positions within a leaf, although the morphological types found at one position tended to be similar on different needles. For example, the rods on the adaxial surface (Fig. 20) appeared shorter and less densely arranged than those in other positions (Figs. 21, 24).

The morphology of waxes at each leaf position was compared using an index, although in effect this was a new index for each position compared. The index was: (1) mostly rods; (2) mainly rods, often tufted and coalescing into small plate-like layers; (3) 25-50% of surface of plate-like waxes as well as some waxes of categories (1) and (2); (4) as in (3) but about 50-75% as plate-like layers; (5) as in (3) but more than 75% as plate-like layers; (6) amorphous layer with little evidence of rod-like structures.

A Mann-Whitney test was used to make pair-wise comparisons of index values for each treatment combination (Zar 1984). Values were generally lower in needles from the SDD treatment than in others, although these values were significant only for waxes occluding the stomata. Stomata in plants from SDD had significantly more rod-like waxes than stomata in needles from SDW and LDW ($p<0.05$). There was no significant difference between the SDD and LDD values. However, values for the LDD treatment were not significantly different from those in the SDW and LDW. Typical examples of the different types of stomatal wax morphologies are shown in Figures 24-26. A cross section through a stoma is shown in Figure 18.

No significant differences were detected between the LDD and other non-stressed treatments perhaps because it was difficult to locate needles that were most likely produced during the induction period. Plants from the LDD treatments underwent free growth during and after the induction period, whereas the SD ceased elongation during this period (Fig. 2).

DISCUSSION

Shoot growth and seedling morphology

All treatment combinations except lift date were shown to have a significant effect on seedling morphology. Cavity size had large and relatively consistent effect on all morphological variables. Seedlings grown in the larger container were taller, branchier and had a larger root collar diameter, more stem units, greater SUL and longer needles. Arnott and Beddows (1982) reported smaller differences in shoot length and stem diameter between seedlings from small and large cavities, although the growing season in that experiment was considered too short to allow seedlings from the larger cavities to reach their full potential. Wider spacing increased dry weight, root

collar diameter, and shoot height and affected the distribution of dry matter between needles, stems, and roots in 2+0 Douglas fir (Pseudotsuga menziesii (Mirb.) Franco) seedlings (van den Driessche 1982). A study of container grown Douglas fir seedling reported similar results to those of van den Driessche except for height growth, which was reduced by wider spacing (Timmis and Tanaka 1976). Because the effect of cavity size was so consistent in this study, it will not be discussed in most of the following discussion on seedling morphology.

Seedling height was influenced by all treatment combinations, day length being the most important factor. Long photoperiods prolong the free growth phase in western hemlock (Figs. 2, 4) (Cheung 1973), as was previously noted in white spruce (Picea glauca (Moench) Voss) and black spruce (P. mariana (Mill.) B.S.P.) (Pollard and Logan 1977) and Douglas fir (Lavender and Overton 1972). Root collar diameter, in contrast, was more heavily affected by moisture stress than photoperiod (Fig. 3). Cambial growth is known to be very sensitive to drought (Kramer and Kozlowski 1979), while the cessation of cambial activity appears to be influenced by the supply of growth substances from needles and developing buds (Larson 1969). Water stress may have reduced stem diameter through its influence on rates of cambial growth and indirectly through its effect on seedling size.

All treatment combinations had a large effect on seedling branching characteristics (Table 6). The higher levels of branching in some treatment combinations than others can be attributed largely to differences in height growth. The number of branches produced by a shoot is related to its rate of growth (Cannell et al. 1976). The potential multiplication rate of branches depends on factors regulating the numbers of lateral buds per unit length of shoot (Cannell and Bowler 1978), although little is known

about these effects in western hemlock. Shorter leaders produced fewer branches per unit length (Table 6; Fig. 4), in agreement with the findings for older trees of Picea and Larix (ibid.). However, the numbers of 1° branches per unit height differed somewhat (0.66 to 0.74/cm), whereas the number of 2° branches varied greatly mainly due to the multiplication effect. This multiplication was most pronounced at the wider spacing, presumably reflecting the heavy branching of lower shoots in seedlings from the larger cavities. The heavy branching in western hemlock seedlings may also be related to frequent shifts in apical dominance noted in this species (Hibbs 1981).

Most variation in shoot length is due to differences in NSU (Cannell et al. 1976), although SUL may also be an important factor especially under drought stress conditions (Garrett and Zahner 1973; Cannell et al. 1976). Western hemlock in this study followed this pattern, although the influence of SUL under no moisture stress was larger than expected, especially in the large cavities. Cavity size, day length and moisture influenced SUL in the proximal portion of the leader, although all of these stem units were produced well before the dormancy induction treatments were applied. Evidence from a study on clonal material of Picea engelmannii Parry (Owens and Simpson 1988) indicate that such elongation is likely due to cell expansion without cell division. It appears that cell elongation can occur over a long period and is responsive to environmental change such as day length and moisture. As expected, differences in SUL in the distal portion of the shoot was heavily influenced by moisture stress, while photoperiod was also a factor as an interaction with cavity size and moisture. Similar to our results, moisture stress and long days had a large effect on NSU in white spruce seedlings, but in contrast had no effect on SUL (Macey and Arnott 1986).

Needle growth showed a parallel pattern to that observed

for SUL, although day length had a much larger effect on growth of proximal needles than SUL for that section. These results are surprising because all needles measured except those from the distal positions under SD were initiated well outside of the dormancy induction period. Needle growth in western hemlock seedlings appears to be responsive to environmental conditions over a relatively long period. Results based on Picea and Abies (Cannell et al. 1976), which have a similar bud and shoot development cycle to Tsuga (Owens and Molder 1973), suggest that needle growth is determinate, and probably would not respond to treatments applied outside the elongation phase. Observations on needle growth in mature Douglas fir (Owens 1968) indicate that treatments applied here probably influenced cell expansion. However, needle growth in Pinus is highly responsive to moisture levels (Clements 1970; Garrett and Zahner 1973), perhaps because of the long duration of leaf meristematic activity in Pinus.

Bud development

Short days were effective in slowing shoot elongation and stimulating bud morphogenesis in western hemlock seedlings. In contrast, moderate moisture stress was effective in inducing bud development in Douglas fir (Lavender et al. 1968), blue spruce (Picea pungens Engelm) (Young and Hannover 1978), and white spruce (Macey and Arnott 1986) seedlings growing under long days. Water stress, commonly used to stimulate bud formation in container-grown seedlings of Picea growing in B.C. (Matthews, 1981), is not recommended for achieving this end in western hemlock seedlings.

Cheung (1973) noted that some buds were present on seedlings of western hemlock subjected to various lengths of drought stress while growing under long days (16 h), compared with the continued free growth observed in the

unstressed trees. All seedlings from treatment combinations receiving short days in Cheung's investigation produced buds. In this experiment, long day and moisture stress treatments were applied for 4 weeks only after which they were returned to natural day lengths. Perhaps the period of induction used here was too short to be effective such that natural photoperiod was probably the factor stimulating bud formation in seedlings previously receiving long days. The earlier initiation of bud scales in plants from LDD here indicated that bud development in western hemlock seedlings may be mildly responsive to moisture stress over a much longer period than white spruce (Macey and Arnott 1986).

Mild water stress applied to Douglas fir seedlings under long days had little effect (van den Driessche 1969), or enhanced frost hardiness (Timmis and Tanaka 1976). Blake et al. (1979) noted that mild water stress increased cold hardiness in Douglas fir seedlings when applied before naturally inductive short days. The mild levels of water stress applied to western hemlock here had little effect on frost hardiness (Arnott and Dunsworth, pers. comm.).

There was a rapid transition from free growth to bud morphogenesis under SD conditions. Figure 9 shows that seedlings from SD produced more terminal leaves, probably due to a rapid cessation of shoot elongation, similar to the accumulation of primary needles in a rosette noted in Scots pine (Pinus sylvestris L.) seedlings grown under short days and cool temperatures (Thompson 1981). Also, there was a rapid transition from bud-scale initiation to leaf initiation, which probably reduced bud-scale numbers (Fig. 9). In addition, apical size remained small during all bud development in seedlings from SD (Figs. 11, 12), perhaps due to the rapid rate of leaf initiation which quickly "used up" the apical dome, thus allowing little reinvestment in new apical tissue. Furthermore, rates of

mitoses were relatively low in plants from SD during the periods of most active bud development (Fig. 10), reducing further the potential growth of the small domes. Work on seedlings of Sitka spruce (Picea sitchensis (Bong.) Carr.) (Cannell 1978) has shown that apices have greater growth potential if they build up and maintain large dome volumes and produce small primordia. Larger apices produced significantly smaller primodia in Sitka spruce, but this relationship was not examined in our study. The greater number of needle primordia produced under SD conditions in this study was probably due to the longer period of bud development than under LD. In contrast to western hemlock, apices of Sitka spruce seedlings transferred to short days reinvested up to 30% of new apical tissues in the apical dome, resulting in rapid enlargement of the apex (Cannell and Cahalan 1979), similar to that noted under LD conditions in this study.

The final number of needle primordia might have been increased in the LD treatments by modifying the cultural conditions used here. Mitotic indices were relatively high and apical dimensions (especially width) large in late October in plants from LD, around the time that greenhouse temperatures were reduced considerably (15°/5°C), indicating that more primordia might have been formed had higher temperatures been maintained for 2 to 3 weeks longer. Temperature is known to have a large effect on the rates of primordium production (Pollard and Logan 1977). Alternatively, a later inductive period using SD, probably just before maximum apical size is achieved (Figs. 11, 12), might be more effective. This treatment might be applied in late August-early September when the natural photoperiod in Victoria is about 13.5 h, resulting in a rapid cessation of shoot elongation followed by a uniform transition to bud morphogenesis in all plants. Hardening-off temperatures could probably begin at about the times scheduled in the

present study. However, later SD starting dates need to be experimentally tested before definitive recommendations can be made.

There was interesting differences in the patterns of bud and shoot development between day length treatments. Under short days there was a rapid cessation of shoot elongation followed by bud formation, whereas under long days close to one-third of the needle primordia were present at cessation of shoot growth. The pattern observed under short days is similar to that described for mature trees (Owens and Molder 1973), where the time of growth cessation and bud-scale initiation coincide. Much of the elongation under LD conditions appears to be due to the continued expansion of many internodes and not solely the elongation of internodes between the most recently initiated primary needles (see Fig. 6). Similar to the seedlings from LD here, Macey and Arnott (1986) reported a 2-week lag between the time of shoot growth cessation and the beginning of bud-scale initiation.

Moisture stress and cavity size influence the rates of bud morphogenesis, but had no effect on its timing. The effect of moisture stress was most pronounced under short days, perhaps because needle initiation commenced during the 4 weeks of moisture stressing whereas this began well after the stress treatment ended under LD conditions. Macey and Arnott (1986) noted a similar effect on leaf production in white spruce seedlings. Water stress is known to reduce photosynthetic rates in seedlings of western hemlock (Brix 1979), reducing net assimilation rates and presumably reducing rates of primordium initiation.

Seedlings from S4 produced significantly more leaf primordia than those from S3. These differences can probably be explained by the larger (Figs. 3, 4) and branchier (Table 6) seedlings produced in the bigger cavities. Results presented by Pollard (1974) indicate that

apical growth is sensitive to seedling size, presumably reflecting the assimilatory capacity of the plant, and is in agreement with the findings presented here.

The final number of leaf primordia (Fig. 8) were unaffected by lift date, although this had a significant effect on MI and apical size for seedlings lifted in November (Figs. 13-15). Apical dimensions of seedlings lifted in November did not decline to the dormant size in cold storage, although MI had reached zero within a month. The period during which there is no apical mitotic activity corresponds with the period of deep dormancy and appears to be closely correlated with seedling resistance to stress (Lavender 1985). Early cold storage was shown to reduce height growth after outplanting in Douglas fir seedlings (Carlson et al. 1980). Therefore, lower seedling survival and performance can be expected in seedlings lifted in November, especially those grown under LD conditions. Seedlings lifted in mid-January were dormant, as defined by the absence of apical mitoses (Owens and Molder 1973), coinciding with the period (mid-December to mid-February) generally recommended for lifting of conifers in the Pacific Northwest (Lavender and Cleary 1974). However, a host of factors as well as dormancy status influence the potential survival of cold-stored material, as demonstrated for Douglas fir seedlings (Ritchie 1984).

Seedlings not placed in cold storage had broken dormancy between January 12 and February 9, although MI was very low during this period. Based on experience with Douglas fir (Ritchie 1984), such "hot-lifted" plants would be expected to have lower survival and growth than those lifted and placed in cold storage in January. A study of the growth and development of seedlings from all treatment combinations under field conditions is presently underway.

Cuticle and epicuticular wax features and numbers of stomata

Relatively small differences were detected in

epicuticular wax features in a small sample of needles from the four dormancy induction treatments, whereas there were no differences in cuticle thickness and numbers of stomata. Note that plants grown under controlled greenhouse conditions as in this study may have different cuticle and epicuticular wax characteristics than the same variety grown in the open (Martin and Juniper 1970; Jeffree et al. 1971).

Baig and Tranquillini (1976) noted a reduction in cuticle thickness in needles from mature trees of Picea abies (L.) Karst. and Pinus cembra (L.) growing at increasing elevations in Austria. They attributed these differences to the influence of the shortening growing season. The relatively mild levels of water stress applied over a 4-week period to western hemlock in this investigation may not have been sufficient to cause changes in cuticle thickness of the magnitude observed in the Austrian study. In addition, all other factors except photoperiod were the same for all seedlings in this study, unlike the changing temperatures, light intensities, wind exposure, and possibly genetic factors in the Austrian study. There are no comparable studies of cuticle thickness for seedling material. There is considerable controversy over the relationship between cuticle thickness and plant growing environment and its effect on water loss (Martin and Juniper 1970).

Fusion of wax rodlets in the stomatal complex, often into a solid plug, was less frequent under moisture stress conditions in this study. This observation is surprising as it might be expected that the plug would reduce transpiration rates while having a smaller effect on photosynthetic rates (Jefferee et al. 1971). However, as hypothesized by Riding and Percy (1985), the stomatal plug may equally function in keeping the stomatal complex open.

Treatment combination appeared to have little effect on epicuticular wax morphology in the present study, except for

those waxes occluding the stomata as discussed above. Similarly, Rook et al. (1971) found no differences in morphology of the surface waxes in seedlings of Pinus radiata D. Donn. grown at various temperatures and two moisture regimes (watered biweekly and daily). Also, Trimble et al. (1982) found no differences wax morphology between two clones of P. strobus (L.), although differences were noted in the chemical components of these waxes. Analyses were not made of the chemical components of the waxes in the present study or that of Rook et al.'s. Simini and Leone (1986) found differences in total waxes and their chemical constituents in 3-year old P. strobus and P. thunbergii (Parl.) seedlings grown at different temperatures and photoperiods. The alkane concentrations increased with increasing temperature and longer photoperiod while the total weight of wax decreased. Unfortunately, they did not examine epicuticular wax morphology. Although a relationship might be expected between wax morphology and chemistry (Baker 1982), apparently this is not always the case. The chemical and physical properties of surface waxes are known to have an effect on surface wetability (Leyton and Armitage 1968; Holloway 1970), light reflectance (Reicosky and Hannover 1978), and water relations (Leyton and Juniper 1963; Leyton and Armitage 1968; Jefferee et al. 1971) of conifer plants. Further detailed studies of the morphology, chemical composition and their effects on the physiology of growth under various moisture regimes, temperatures and photoperiods needs to be conducted for western hemlock seedlings.

CONCLUSIONS

Short days are an effective method of slowing shoot elongation and stimulating bud development in container-grown western hemlock seedlings. Moisture stress is not recommended for achieving this end. Our results suggest

that SD treatments should begin at a later date than that tested here, probably early September, but this requires further study.

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Table 1. Nursery sampling scheme for seedlings of western hemlock in 1986-1987.

Sampling Dates	Approx. Intervals (weeks)	No. Collection Dates	Treatments[1] Levels S	D	M	L	Combinations	No. Seedlings/date
July 14-Aug. 12	2	3	2	2	2	-	8	64
Aug. 18-Oct. 7	1	8	2	2	2	-	8	64
Oct. 21, Nov. 4	2	2	2	2	2	-	8	64
Nov. 18-Jan. 12/87[2]	2-3	4	2	2	2	2	16	128
Feb. 9[3]	3	1	2	2	2	3	24	192
Mar. 9[3]	4	1	2	2	2	3	24	192

[1] S = styroblock cavity size, D = day length, M = moisture, L = lift.
[2] After first lift in mid-November.
[3] After second lift in mid-January.

Note: The lift three material remained in the greenhouse until moved to a shelterhouse in mid-February. These plants were lifted after the final sampling, on March 9.

Table 2. Analysis of variance of treatment effects on diameter and final height of greenhouse-grown western hemlock seedlings.

Source	df	Diameter MS	Diameter F	Height MS	Height F
Day length (D)	1	1219	42***	746280	462***
Moisture (M)	1	5525	191***	269231	167***
DxM	1	2	0 NS	27736	17***
Error (Blocks (B) within DxM)	28	29		1614	
Styroblock Cavity (S)	1	7773	281***	401481	232***
SxD	1	198	7*	44785	26***
SxM	1	230	8**	21206	12**
SxDxM	1	55	2 NS	16033	9**
Error (SxB within DxM)	28	28		1732	
Sampling Error	512	30		1792	

*, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively.

Note: Analyses are based on 576 seedlings, 192 from each lift storage combination.

Table 3. Analysis of variance of treatment effects on numbers of stem units and stem unit lengths in greenhouse-grown western hemlock seedlings.

Source	df	Number of Stem Units		Stem Unit Lengths					
				Proximal		Distal		Average	
		MS	F	MS	F	MS	F	MS	F
Day length (D)	1	185187	516***	613	5*	343	4 NS	10	0 NS
Moisture (M)	1	13963	39***	684	6*	7579	80***	3204	52***
DXM	1	2248	6*	24	0 NS	69	0 NS	3	0
Error (Blocks (B) within DxM)	28	359		124		94		62	
Styroblock cavity (S)	1	22300	78***	3837	76***	7420	163***	5482	171***
SxD	1	1196	4 NS	525	10**	425	9**	474	15**
SxM	1	655	2 NS	34	0 NS	599	13**	230	7*
SxDxM	1	1202	4*	180	4 NS	41	0	98	3 NS
Error (SxB within DxM)	28	286		55		45		32	
Sampling error	512	301		119		90		70	

*, **, *** significant at 0.05, 0.01 and 0.001 levels, respectively.

Note: Stem unit lengths were calculated for the proximal and distal halves of the leader, as well as the shoot average for each seedling. Analyses are based on 576 seedlings, 192 from each lift/storage combination.

Table 4. Analysis of variance of treatment effects on needle lengths of greenhouse-grown western hemlock seedlings.

Source	df	Proximal MS	Proximal F	Distal MS	Distal F	Average MS	Average F
Day length (D)	1	243	51***	5	1 NS	80	35***
Moisture (M)	1	29	6*	90	22***	55	24***
DxM	1	34	7*	6	1 NS	17	7*
Error (Blocks(B) within DxM)	28	5		4		2	
Styroblock cavity (S)	1	52	21***	36	12**	44	27***
SxD	1	49	19***	1	0 NS	9	5*
SxM	1	3	1 NS	0.1	0 NS	0.5	0 NS
SxDxM	1	3	1 NS	0.7	0 NS	2	0 NS
Error (SxB within DxM)	28	3		3		2	
Sampling Error	512	3		3		2	

*, **, *** significant at 0.05, 0.01, and 0.001 levels, respectively.

Note: Needle lengths were seedling averages of five needles each from the proximal and distal halves of the leader, as well as the average of all 10 needles. Analyses are based on 576 seedlings, 192 from each lift/storage combination.

Table 5. Analysis of variance of treatment effects on numbers of first- (1°), and second-order (2°) branches, their ratios, and 1° and 2° numbers divided by seedling height in greenhouse-grown western hemlock seedlings.

Source	df	1°		2°		2°/1°		1°/height		2°/height	
		MS	F	MS	F	MS	F	MS	F	MS	F
Day length (D)	1	5968	327***	31447	111***	5	14***	27	26***	609	30***
Moisture (M)	1	1528	84***	17600	62***	10	26***	1	1 NS	544	26***
DxM	1	203	11**	3080	11**	0.1	0 NS	0	0 NS	17	0 NS
Error (Blocks (B) within DxM)	28	18		284		0.4		1		21	
Styroblock cavity(S)	1	2809	95***	59658	294***	57	169***	7	4*	3368	184***
SxD	1	374	13**	7014	35***	0.6	2 NS	1	1 NS	93	5*
SxM	1	117	4 NS	3917	19***	1	4 NS	0	0 NS	56	3 NS
SxDxM	1	156	5*	2576	13**	0.4	1 NS	1	0 NS	45	2 NS
Error (SxB within DxM)	28	30		203		0.3		2		18	
Sampling Error	512	23		343		0.5		2		32	

*, **, *** significant at 0.05, 0.01, and 0.001 levels, respectively.

Note: Analyses are based on 576 seedlings, 192 from each lift/storage combination.

Table 6. Number of first- (1°) and second-order (2°) branches, their ratios, and number of branches per unit height by treatment combination[1] in greenhouse-grown western hemlock seedlings.

Treatment Combination	1°	2°	2°/1°	1°/ height (cm)	2°/ height (cm)
SDD-S3	14(0.3)	15(0.8)	1.0(0.05)	0.66(0.012)	0.69(0.035)
SDD-S4	17(0.4)	27(1.7)	1.6(0.08)	0.69(0.011)	1.09(0.062)
SDW-S3	16(0.4)	20(1.2)	1.2(0.08)	0.68(0.015)	0.85(0.053)
SDW-S4	19(0.5)	34(1.9)	1.8(0.10)	0.68(0.016)	1.25(0.073)
LDD-S3	19(0.6)	22(1.6)	1.2(0.08)	0.70(0.017)	0.84(0.059)
LDD-S4	23(0.7)	40(2.5)	1.7(0.09)	0.73(0.017)	1.28(0.074)
LDW-S3	21(0.7)	28(2.1)	1.3(0.08)	0.71(0.013)	0.95(0.063)
LDW-S4	29(0.8)	65(4.0)	2.2(0.10)	0.74(0.014)	1.63(0.090)

[1]SDD = short-day dry, SDW = short-day wet, LDD = long-day dry, LDW = long-day wet, S3 = stryoblock cavity size PSB 313, S4 = stryroblock cavity size PSB 415B.

Note: Values are means based on a total of 72 seedlings per treatment combination (standard errors are in parentheses). There were few branches higher than 2°; these were included with 2° numbers. See Table 5 for analyses of variance results.

Table 7. Analysis of variance of treatment effects on final numbers of terminal leaves,[1] bud scales and leaf primodia in greenhouse-grown western hemlock seedlings.

Source	df	Terminal Leaves MS	Terminal Leaves F	Bud Scales MS	Bud Scales F	Needle Primordia MS	Needle Primordia F
Day length (D)	1	197	129***	969	78***	3480	9**
Moisture (M)	1	13	9**	22	2 NS	27676	73***
DxM	1	4	2 NS	19	2 NS	6386	17***
Error (Blocks(B) within DxM)	28	2		12		382	
Lift (L)	2	3	1 NS	12	1 NS	427	1 NS
LxD	2	2	0 NS	8	1 NS	1808	4*
LxM	2	3	1 NS	7	1 NS	28	0 NS
LxDxM	2	0	0 NS	3	0 NS	293	1 NS
Error (BxL within DxM)	56	3		10		443	
Styroblock cavity (S)	1	20	9**	11	1 NS	11310	23***
SxD	1	10	5*	18	2 NS	699	1 NS
SxM	1	0	0 NS	3	0 NS	482	1 NS
SxL	2	1	0 NS	4	0 NS	471	1 NS
SxDxM	1	12	6*	0	0 NS	1350	3 NS
SxLxD	2	1	0 NS	3	0 NS	1394	3 NS
SxMxL	2	6	3 NS	1	0 NS	628	1 NS
SxDxMxL	2	5	2 NS	10	1 NS	494	1 NS
Error (BxLxS within DxM)	84	2		9		488	
Sampling Error	192	4		10		573	

[1] Primary leaves surrounding the terminal bud that do not undergo internodal expansion.

*, **, *** significant at 0.05, 0.01, and 0.001 levels respectively.

Note: Analyses are based on 384 seedlings, 16 per treatment combination.

Table 8. Significant levels for analyses of variance by date before first lift of treatment effects on mitotic index (MI), apical height (AH), apical width (AW) and numbers of leaf primordia (LP) in greenhouse-grown western hemlock seedlings.

	July 14			July 29			Aug. 12				Aug. 18			Aug. 26				Sept. 2			Sept. 9				Sept. 16		
	MI	AH	AW	MI	AH	AW	MI	AH	AW	LP	AH	AW	LP	MI	AH	AW	LP	AH	AW	LP	MI	AH	AW	LP	AH	AW	LP
Day length (D)	NS	NS	NS	NS	**	NS	NS	NS	***	***	*	*	***	**	***	**	***	**	**	***	NS	***	NS	***	***	NS	***
Moisture (M)	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	*	NS	NS	N	*	*	NS	**
DxM	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	*	NS	NS	NS	*	NS	*	NS	**	NS	NS	**
Styroblock cavity(S)	NS	NS	NS	*	*	NS	NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS	*	NS	NS	**	NS	NS	NS	NS	NS	NS
SxD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	**	*	NS	NS	NS	NS	**	*	NS	**	**	NS	NS	NS	NS	NS
SxM	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SxDxM	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

	Sept. 23				Sept. 30			Oct. 7				Oct. 21				Nov. 4			
	MI	AH	AW	LP	AH	AW	LP	MI	AH	AW	LP	MI	AH	AW	LP	MI	AH	AW	LP
Day length (D)	NS	***	***	***	***	***	***	***	***	***	***	**	***	***	*	***	**	***	*
Moisture (M)	NS	NS	*	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	***	**
DxM	NS	*	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Styroblock cavity(S)	NS	NS	NS	NS	NS	*	**	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS
SxD	NS	NS	*	*	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SxM	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SxDxM	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

*, **, *** significant at 0.05, 0.01, and 0.001 levels, respectively.

Note: Analyses are based on 64 seedlings, 8 per treatment combination. See Figures 8, and 10-12 for plots of mean numbers versus date.

Table 9. Significance levels for analyses of variance by date after first lift of treatments effects on mitotic index (MI), apical height (AH) and apical width (AW) in greenhouse-grown western hemlock seedlings.

	Nov. 18[1]			Dec. 2[1]			Dec. 22[1]			Jan. 12[1]			Feb. 9[2]			Mar. 9[2]		
	MI	AH	AW	MI	AH	AW	MI	AH	AW	MI	AH	AW	MI	AH	AW	MI	AH	AW
Day length (D)	***	***	***	***	***	***	***	*	***	NS	***	***	NS	NS	*	**	***	**
Moisture (M)	NS	NS	**	NS	NS	NS	NS	NS	*	NS	NS	***	NS	NS	NS	NS	*	NS
DxM	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	*	**
Lift (L)	***	NS	NS	***	NS	NS	***	NS	***	NS	NS	NS	**	***	***	***	***	***
LxD	NS	NS	NS	***	NS	NS	***	NS	NS	NS	NS	NS	NS	NS	NS	**	**	NS
LxM	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
LxDxM	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Styroblock cavity(S)	*	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	**	NS	NS	NS	NS	NS	NS
SxD	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	*	NS	NS	NS	NS	NS	NS
SxM	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SxL	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	NS	NS	NS	NS	NS	NS	NS
SxDxM	NS	NS	*	NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS	**	NS
SxLxD	NS	NS	NS	NS	NS	*	NS	NS	*	NS	NS	*	NS	NS	NS	NS	NS	NS
SxMxL	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
SxDxMxL	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	**	**	NS	NS	NS	NS	NS

[1] Analysis includes lifts one and three. [2] Analysis includes lifts one, two and three.
*, **, *** significant at 0.05, 0.01 and 0.001 levels, respectively.

Note: Analyses are based on 128 seedlings, 8 per treatment combination (two lifts) from November 18-January 12, and 192 seedlings (tree lifts) on February 9 and March 9. See Figures 8. and 10-15 for plots of mean numbers versus date.

Figure 1. Experimental layout of the greenhouse according to a split-split-plot design for the study of western hemlock seedling growth.

Note: The day length and moisture stress treatments were for 4 weeks beginning in mid-July. Each block is divided into three sections that were randomly preassigned to a lift date (L1 = mid-November, 1986; L2 = mid-January, 1987; and L3 = mid-March). Each section was randomly assigned one styroblock of smaller cavity size (S3) to two styroblocks of larger cavity size (S4). The two S4 styroblocks were always placed together within a lift. The detailed layout is given for one-quarter of the greenhouse.

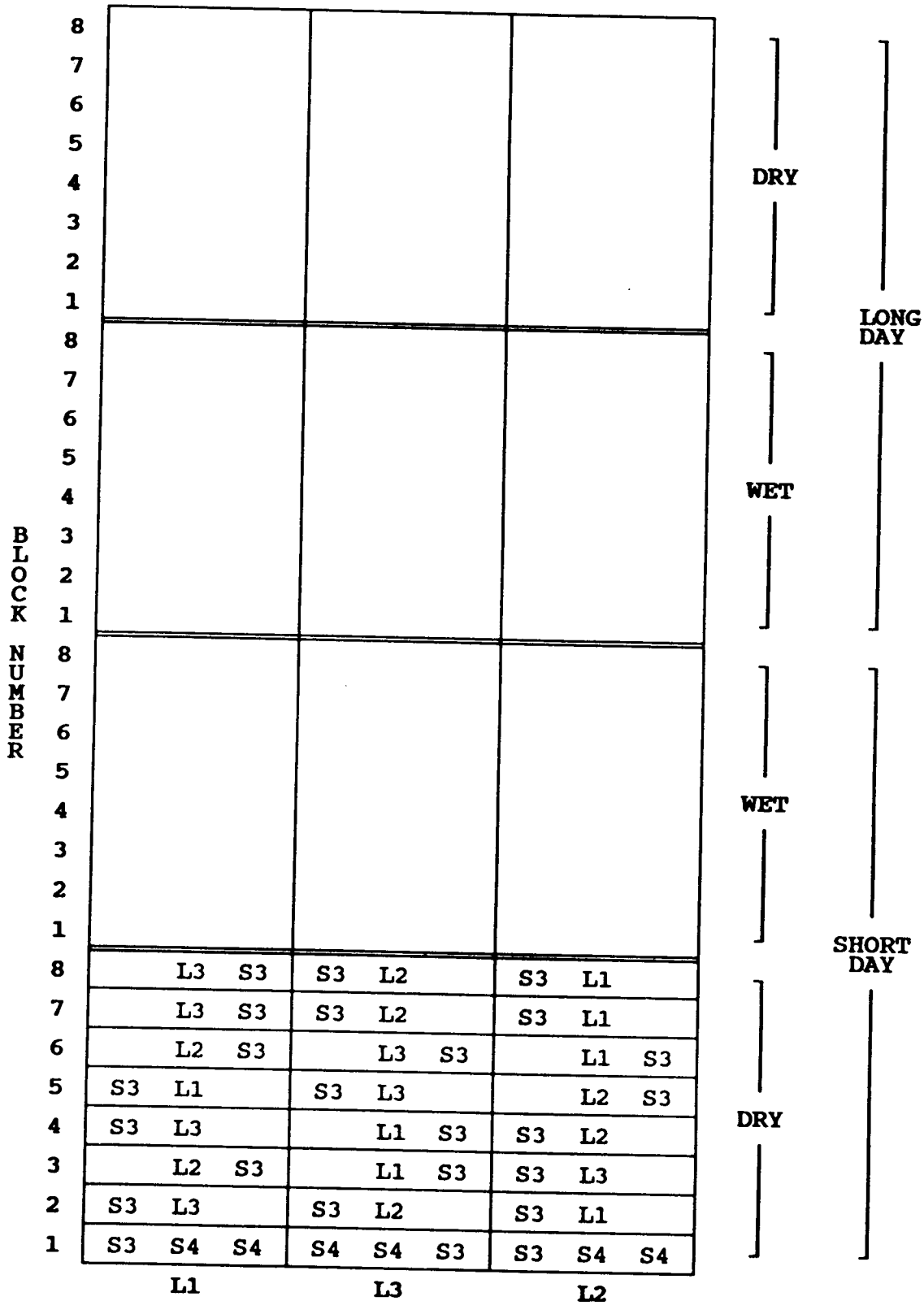
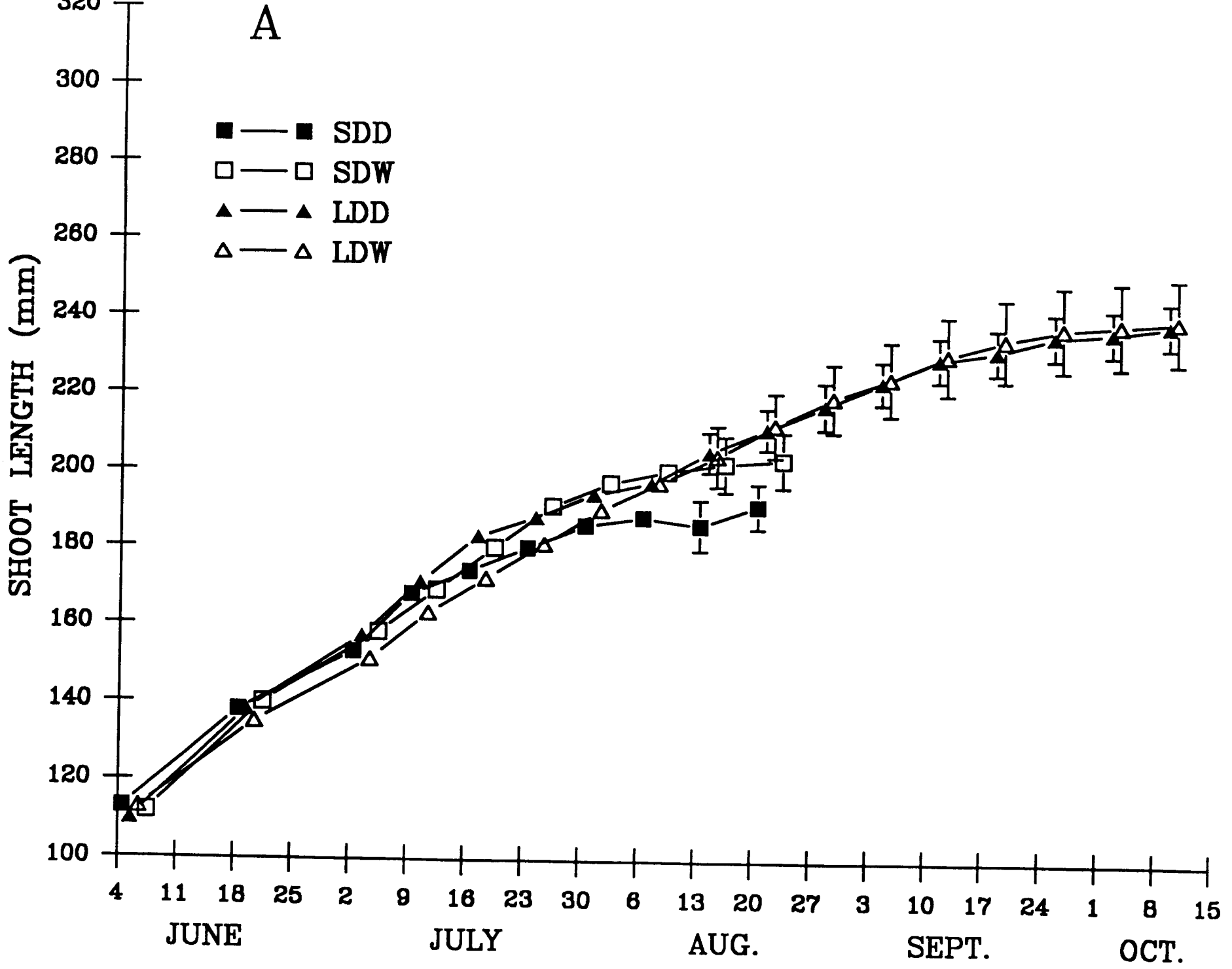

BLOCK NUMBER
8 7 6 5 4 3 2 1
8 7 6 5 4 3 2 1
8 7 6 5 4 3 2 1
8 7 6 5 4 3 2 1
L3 S3 S3 L2 S3 L1
L3 S3 S3 L2 S3 L1
L2 S3 L3 S3 L1 S3
S3 L1 S3 L3 L2 S3
S3 L3 L1 S3 S3 L2
L2 S3 L1 S3 S3 L3
S3 L3 S3 L2 S3 L1
S3 S4 S4 S4 S4 S3 S3 S4 S4
L1 L3 L2
DRY
WET
WET
DRY
LONG DAY
SHORT DAY

Figure 2. Shoot elongation versus date in western hemlock seedlings growing in a greenhouse in small (S3)(A) and large (S3)(B) styroblock container cavities subjected to 4 weeks of dormancy induction beginning in mid-July.

Note: SDD, SDW, LDD and LDW indicates short-day dry, short-day wet, long-day dry and long-day wet treatment combinations, respectively. Means are based on 40 seedlings per treatment combination (vertical bars indicate ± 1 SE). Points are staggered around sample date so that SE bars are visible. Analyses of variance showed significant differences ($p < 0.05$) between cavity sizes on all dates until August 8, and from August 29 onward within the LD treatments. Differences due to cavity size, day length and moisture were evident on August 15 and 22. Data collected before July was supplied by J.T. Arnott, Pacific Forestry Centre.


A
SDD
SDW
LDD
LDW
SHOOT LENGTH (mm)
320
300
280
260
240
220
200
180
160
140
120
100
4
11
18
25
2
9
16
23
30
6
13
20
27
3
10
17
24
1
8
15
JUNE
JULY
AUG.
SEPT.
OCT.

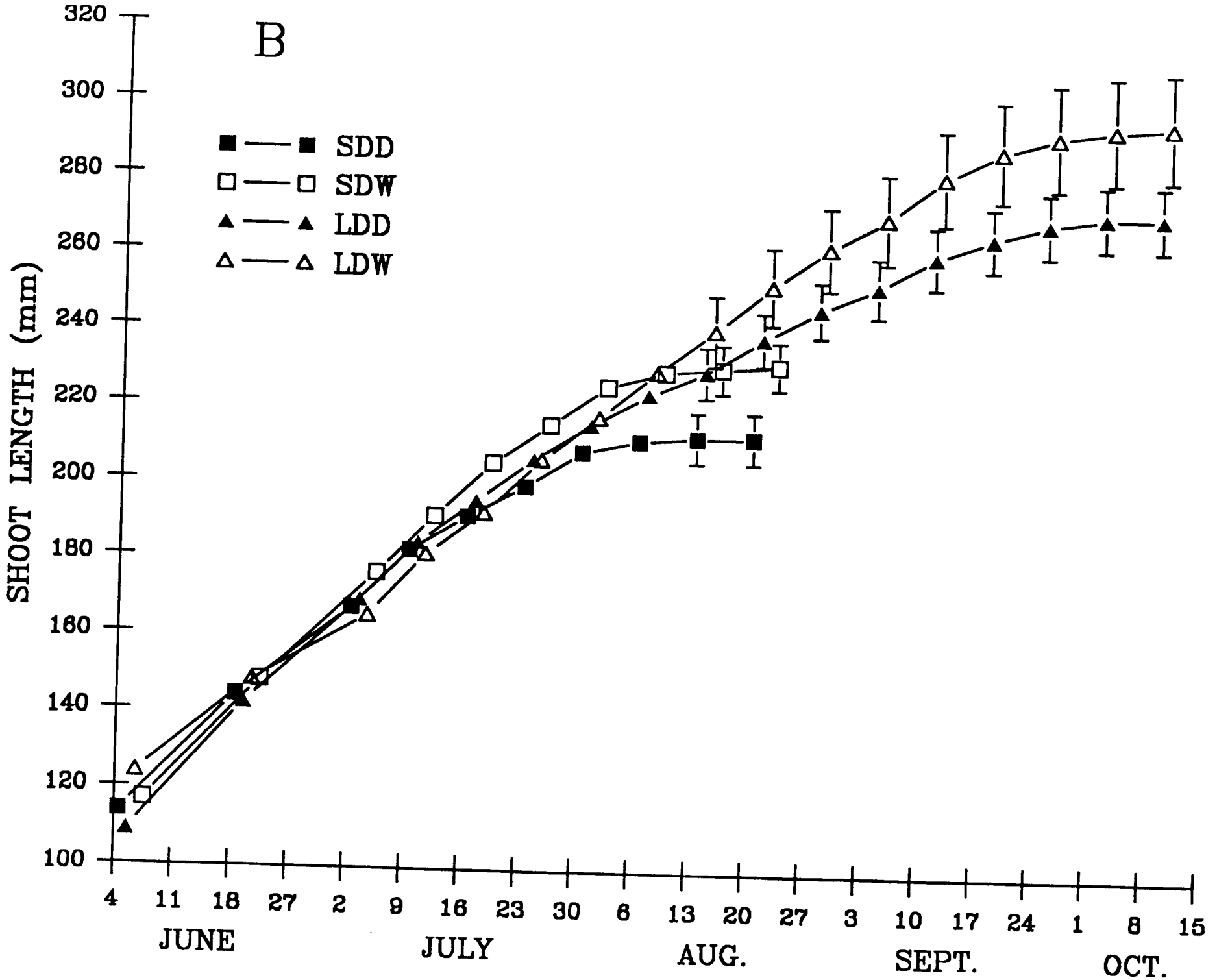

B
SDD
SDW
LDD
LDW
SHOOT LENGTH (mm)
320
300
280
260
240
220
200
180
160
140
120
100
4
11
18
27
2
9
16
23
30
6
13
20
27
3
10
17
24
1
8
15
JUNE
JULY
AUG.
SEPT.
OCT.

Figure 3. Root collar diameter of greenhouse-grown western hemlock seedlings from small (S3) and large (S4) styroblock container cavities subjected to 4 weeks of dormancy induction beginning in mid-July.

Note: SDD, SDW, LDD and LDW indicate short-day dry, short-day wet, long-day dry and long-day wet treatment combinations, respectively. Means are based on 72 seedlings per treatment combination (vertical bars indicate 1 SE). Analyses of variance results are presented in Table 2.

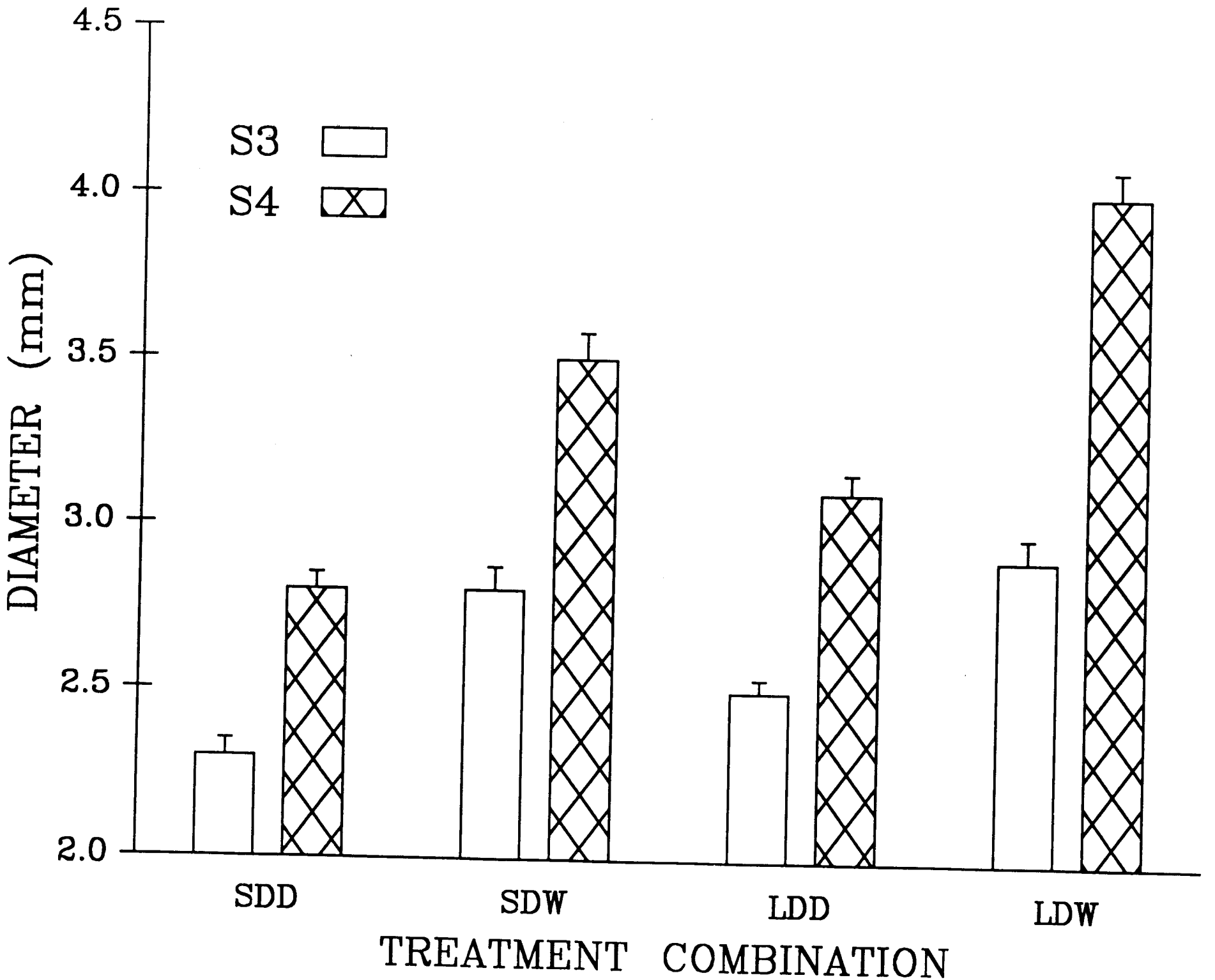

4.5
4.0
3.5
3.0
2.5
2.0
S3
S4
DIAMETER (mm)
SDD
SDW
LDD
LDW
TREATMENT COMBINATION

Figure 4. Final height of greenhouse-grown western hemlock seedlings from small (S3) and large (S4) styroblock container cavities subjected to 4 weeks of dormancy induction beginning in mid-July.

Note: SDD, SDW, LDD, and LDW indicate short-day dry, short-day wet, long-day dry and long-day wet treatment combinations, respectively. Means are based on 72 seedlings per treatment combination (vertical bars indicate 1 SE). Analysis of variance results are presented in Table 2.

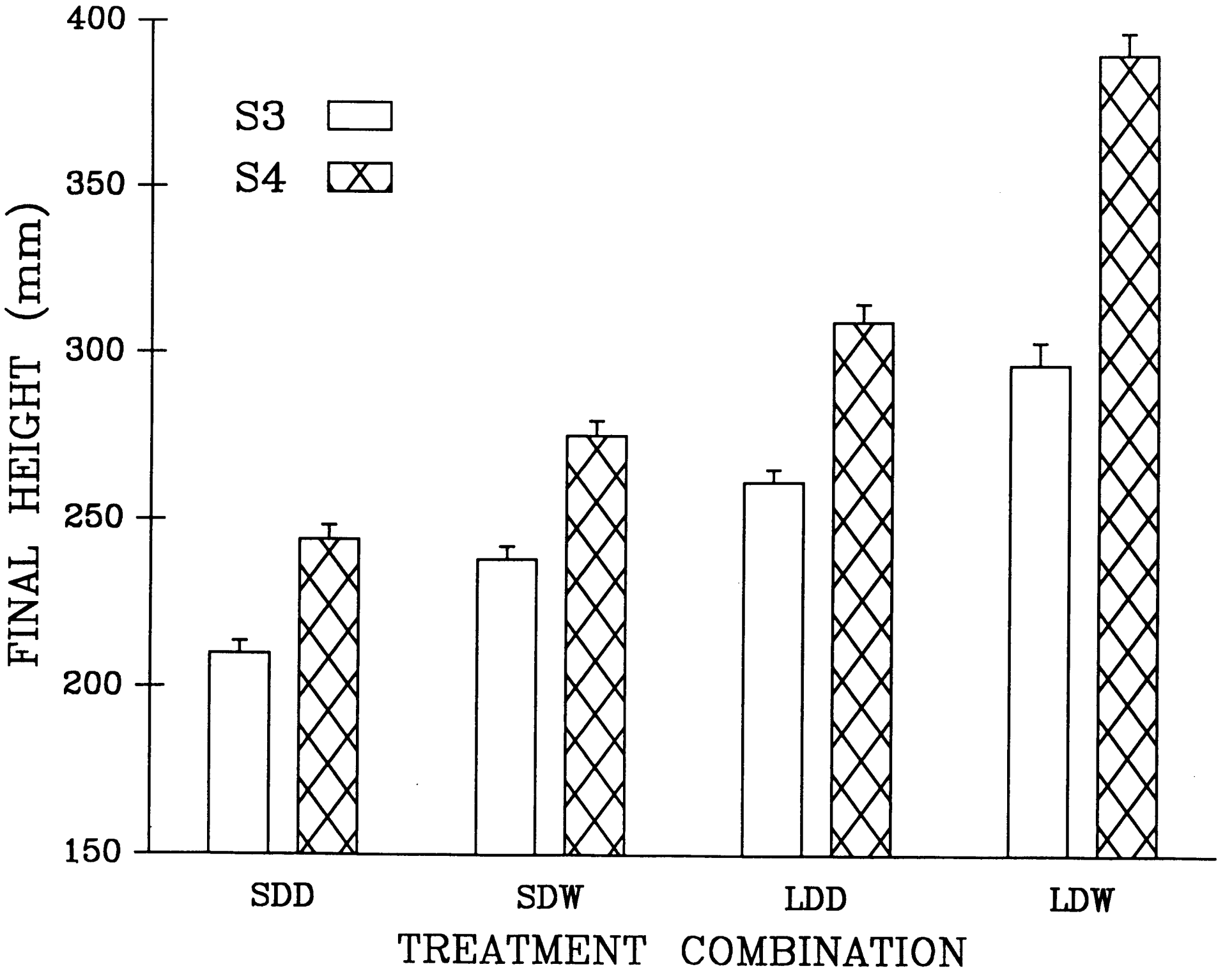

400
350
300
250
200
150
S3
S4
FINAL HEIGHT (mm)
SDD
SDW
LDD
LDW
TREATMENT COMBINATION

Figure 5. Numbers of stem units in the leader of greenhouse-grown western hemlock seedlings from small (S3) and large (S4) styroblock container cavities subjected to 4 weeks of dormancy induction beginning in mid-July.

Note: SDD, SDW, LDD, and LDW indicate short-day dry, short-day wet, long-day dry and long-day wet treatment combinations, respectively. Means are based on 72 seedlings per treatment combination (vertical bars indicate 1 SE). Analysis of variance results are presented in Table 3.

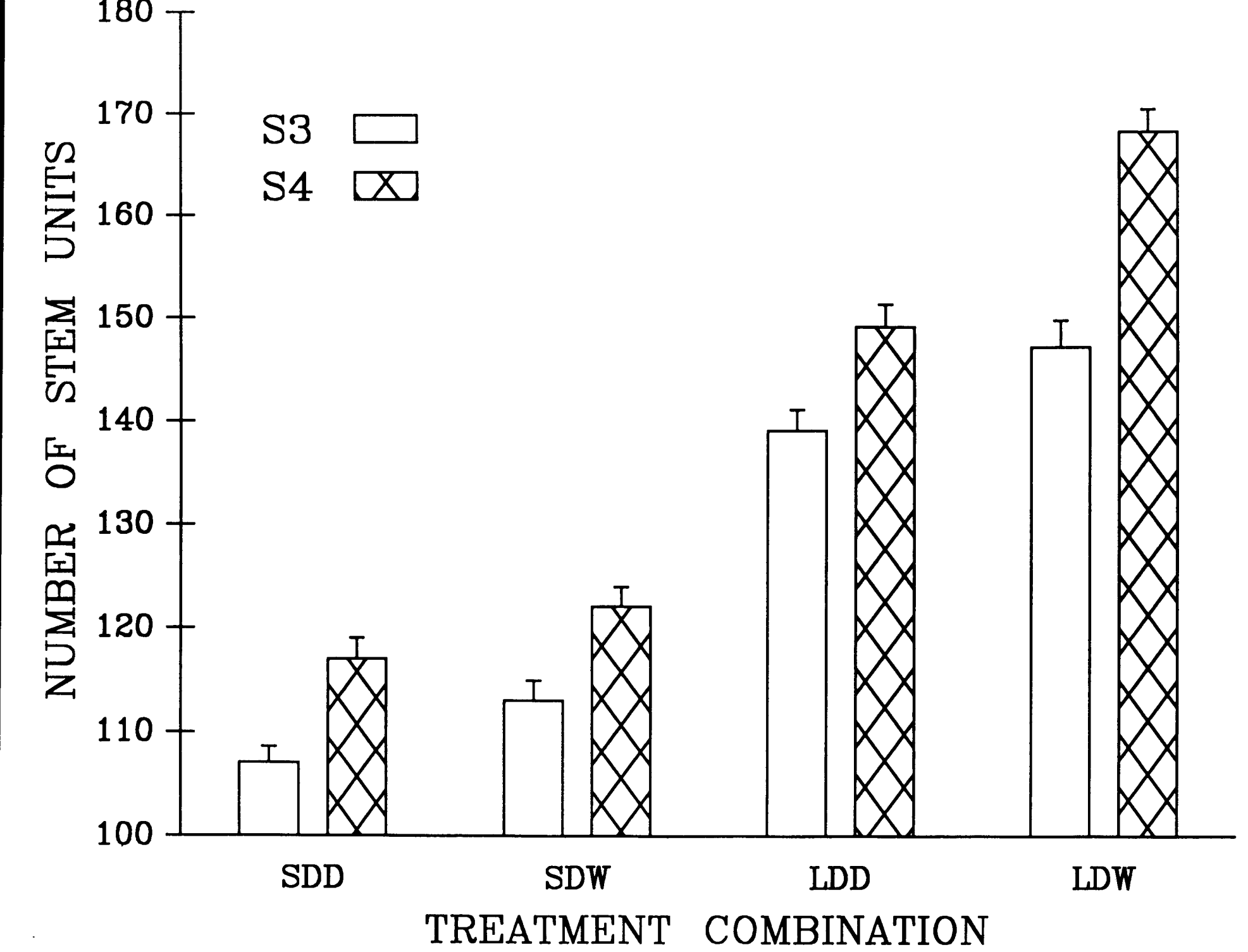

180
170
160
150
140
130
120
110
100
S3
S4
NUMBER OF STEM UNITS
SDD
SDW
LDD
LDW
TREATMENT COMBINATION

Figure 6. Stem unit lengths of the proximal (P) and distal (D) halves of the leader and shoot average (A) in greenhouse-grown western hemlock seedlings from small (S3) and large (S4) styroblock container cavities subjected to 4 weeks of dormancy induction beginning in mid-July.

Note: SDD, SDW, LDD, and LDW indicate short-day dry, short-day wet, long-day dry, and long-day wet treatment combinations, respectively. Means are based on 72 seedlings per treatment combination (vertical bars indicate 1 SE). Analysis of variance results are shown in Table 3.

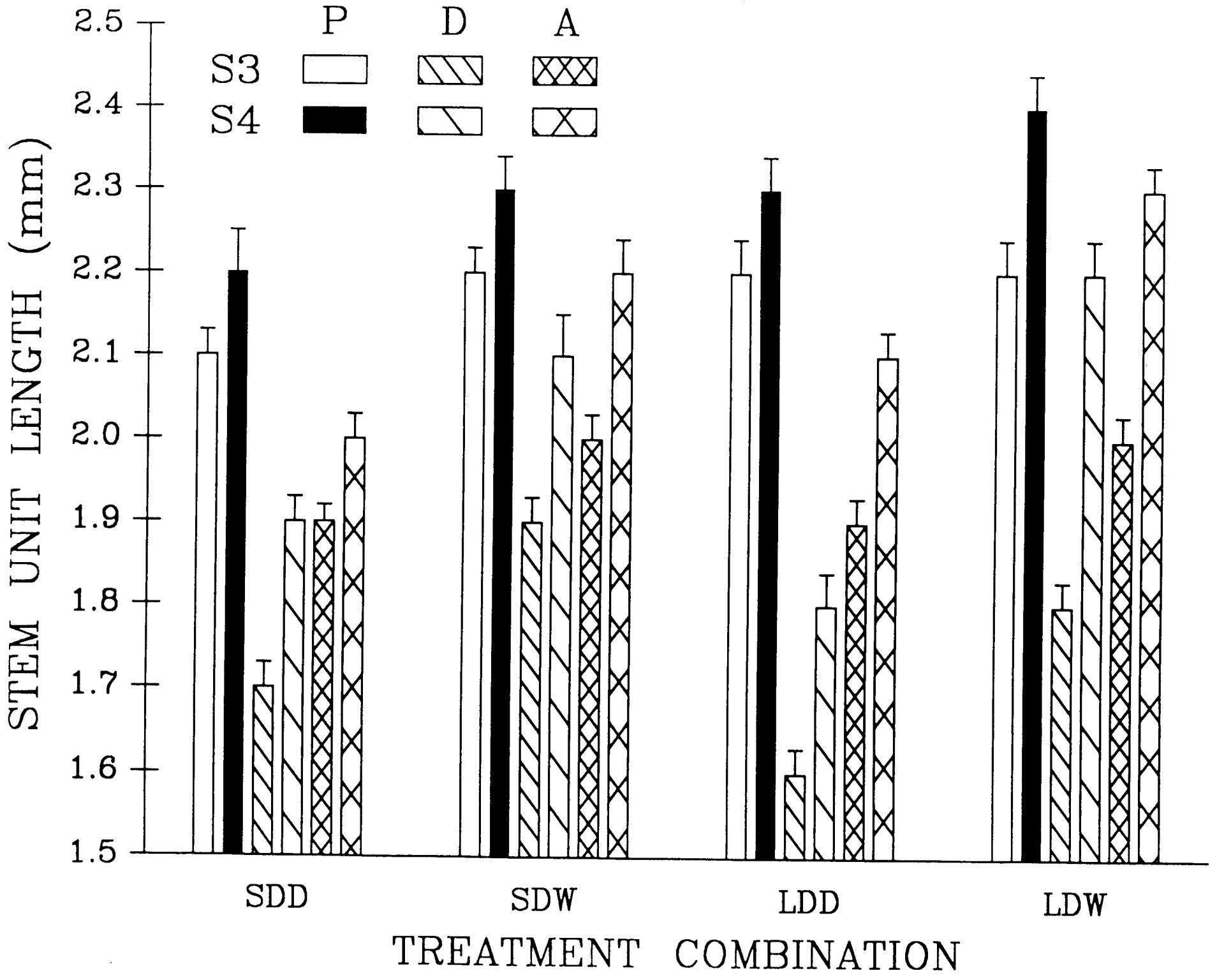

P
D
A
S3
S4
2.5
2.4
2.3
2.2
2.1
2.0
1.9
1.8
1.7
1.6
1.5
STEM UNIT LENGTH (mm)
SDD
SDW
LDD
LDW
TREATMENT COMBINATION

Figure 7. Needle length in the proximal (P) and distal (D) halves of the leader and plant average (A) in greenhouse-grown western hemlock seedlings from small (S3) and large (S4) styroblock container cavities after 4 weeks of dormancy induction beginning in mid-July.

Note: SDD, SDW, LDD, and LDW indicate short-day dry, short-day wet, long-day dry, and long-day wet treatment combinations, respectively. Needle length was the average measurement of 5 needles each taken about 1 cm above the cotyledons and 1 cm below the terminal bud in the proximal and distal sections of shoot, respectively. Means are based on 72 seedlings per treatment combination (vertical bars indicate 1 SE). Analysis of variance results are shown in Table 4.

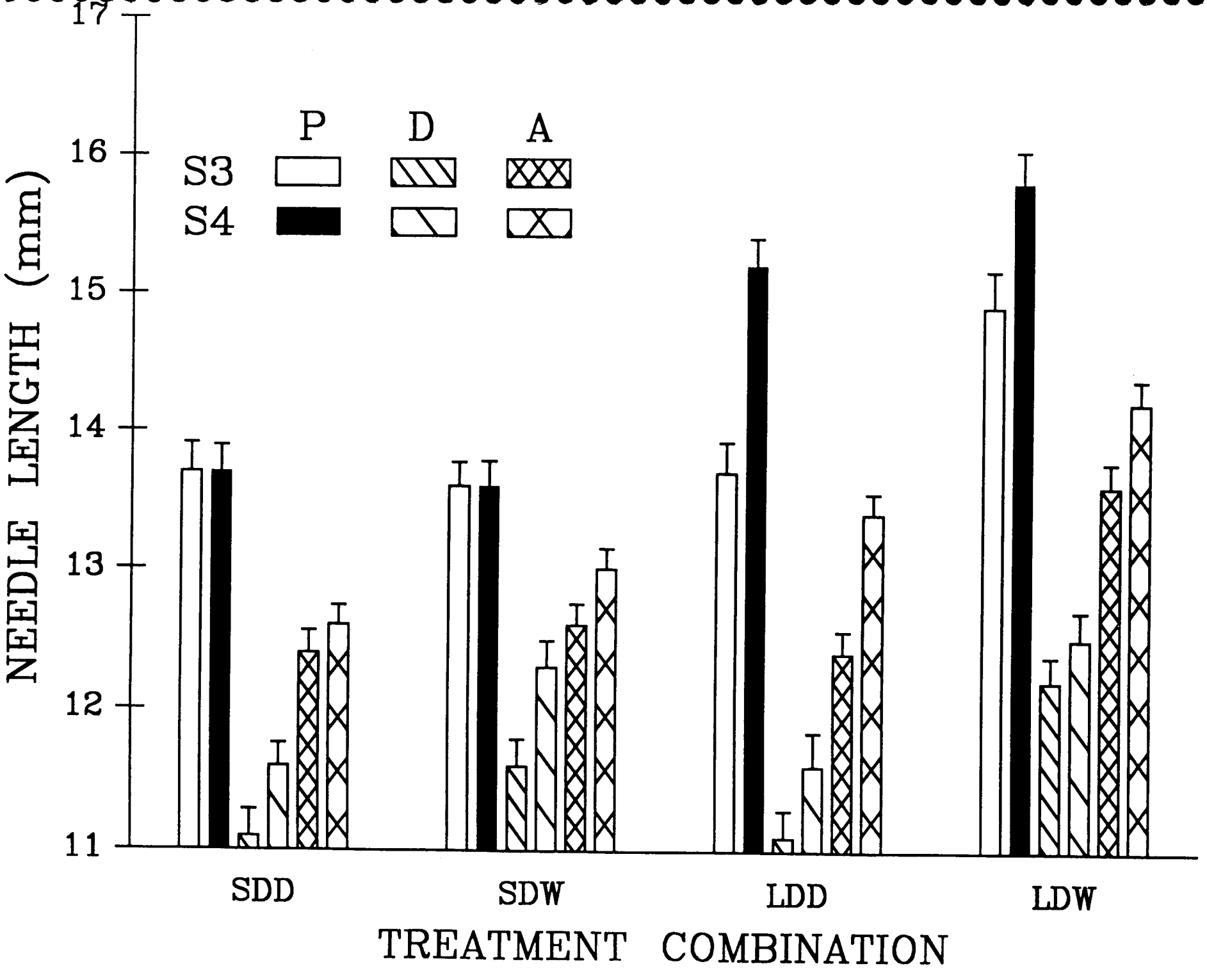
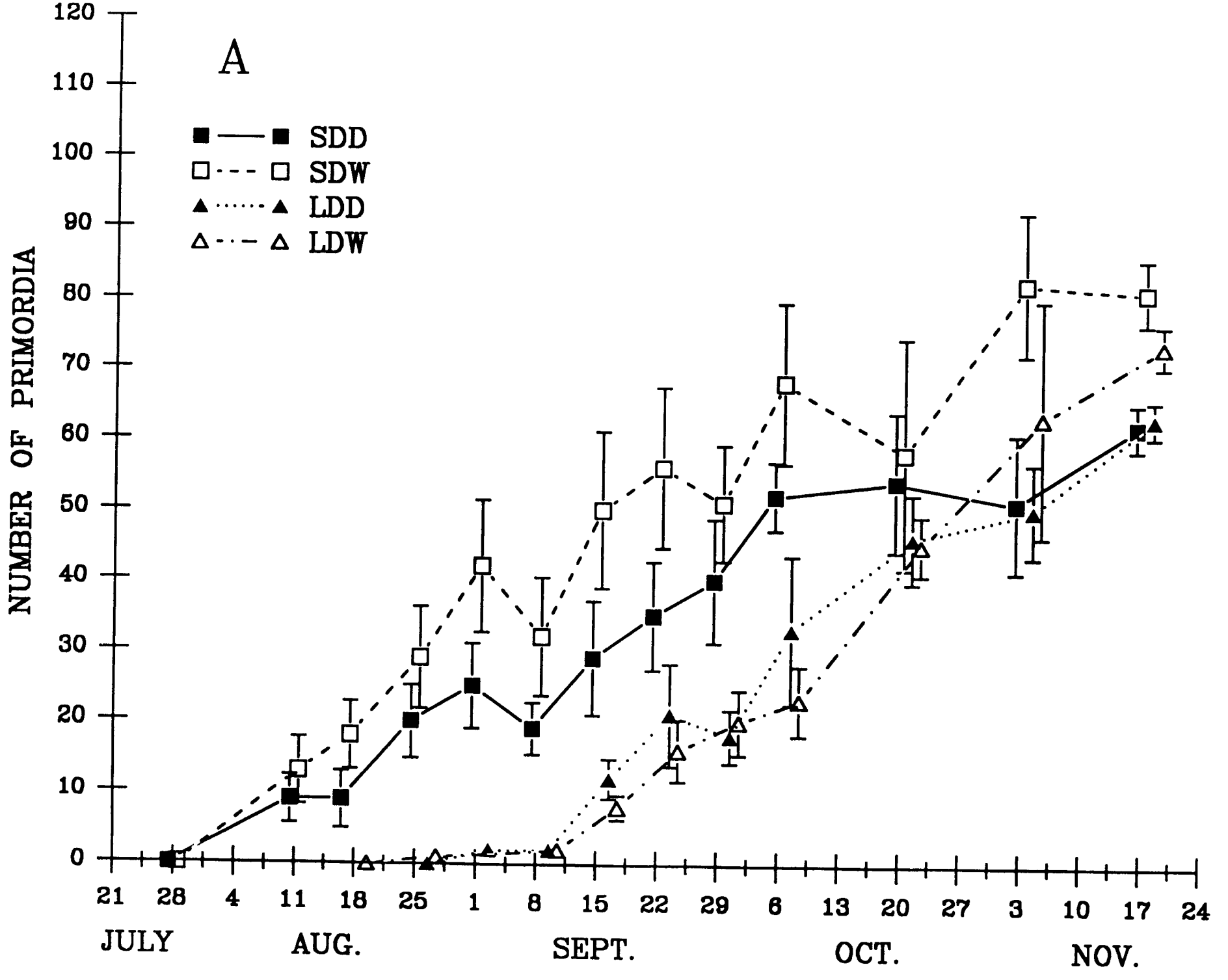

17
16
15
14
13
12
11
NEEDLE LENGTH (mm)
P
D
A
S3
S4
SDD
SDW
LDD
LDW
TREATMENT COMBINATION

Figure 8. Numbers of leaf primordia versus date in western hemlock seedlings growing in a greenhouse in small (S3)(A) and large (S4)(B) styroblock container cavities subjected to 4 weeks of dormancy induction beginning in mid-July.

Note: SDD, SDW, LDD, and LDW indicate short-day dry, short-day wet, long-day dry, and long-day wet treatment combinations, respectively. Means are based on eight seedlings per treatment combination on all except the last date. Final means were derived from 48 seedlings per treatment combination (vertical bars indicate ± 1 SE). Points are staggered around sample date so that SE bars are visible. As lift and sampling date had little effect on primordium numbers from November 18 onward, the final sample was based on data from two dates and three lifts (Table 7). Treatment effects were significant on all dates after August 12 (Tables 8, 9).


A
SDD
SDW
LDD
LDW
NUMBER OF PRIMORDIA
0
10
20
30
40
50
60
70
80
90
100
110
120
21
28
4
11
18
25
1
8
15
22
29
6
13
20
27
3
10
17
24
JULY
AUG.
SEPT.
OCT.
NOV.

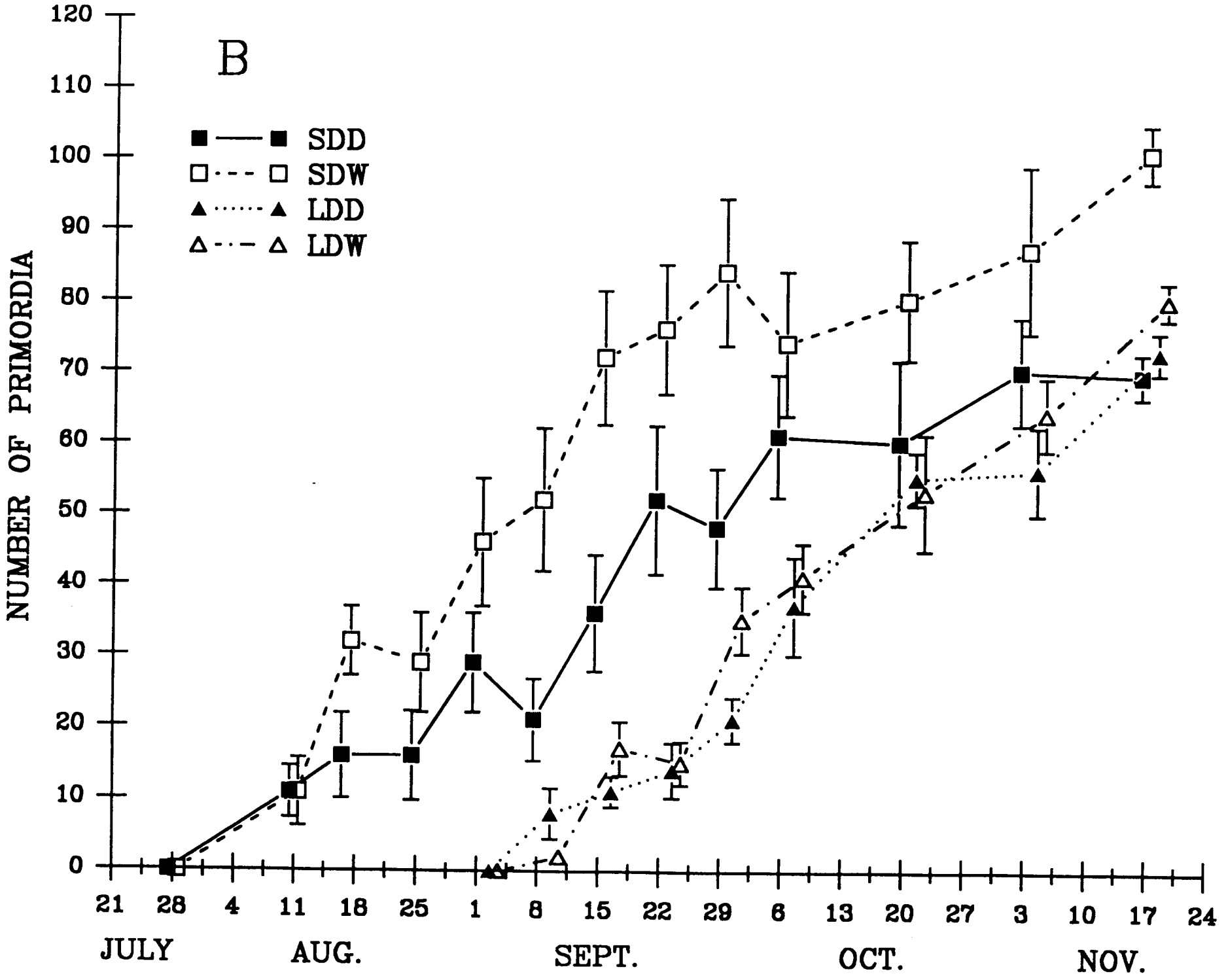

B
SDD
SDW
LDD
LDW
NUMBER OF PRIMORDIA
0
10
20
30
40
50
60
70
80
90
100
110
120
21
28
4
11
18
25
1
8
15
22
29
6
13
20
27
3
10
17
24
JULY
AUG.
SEPT.
OCT.
NOV.

Figure 9. Numbers of terminal leaves (TL) and bud scales (BS) in greenhouse grown western hemlock seedlings from small (S3) and large (S4) styroblock container cavities after 4 weeks of dormancy induction beginning in mid-July.

Note: SDD, SDW, LDD, and LDW indicate short day dry, short day wet, long day dry and long day wet treatment combinations, respectively. Means are based on 48 seedlings per treatment combination (vertical bars indicate 1 SE). Analysis of variance results are shown in Table 7.

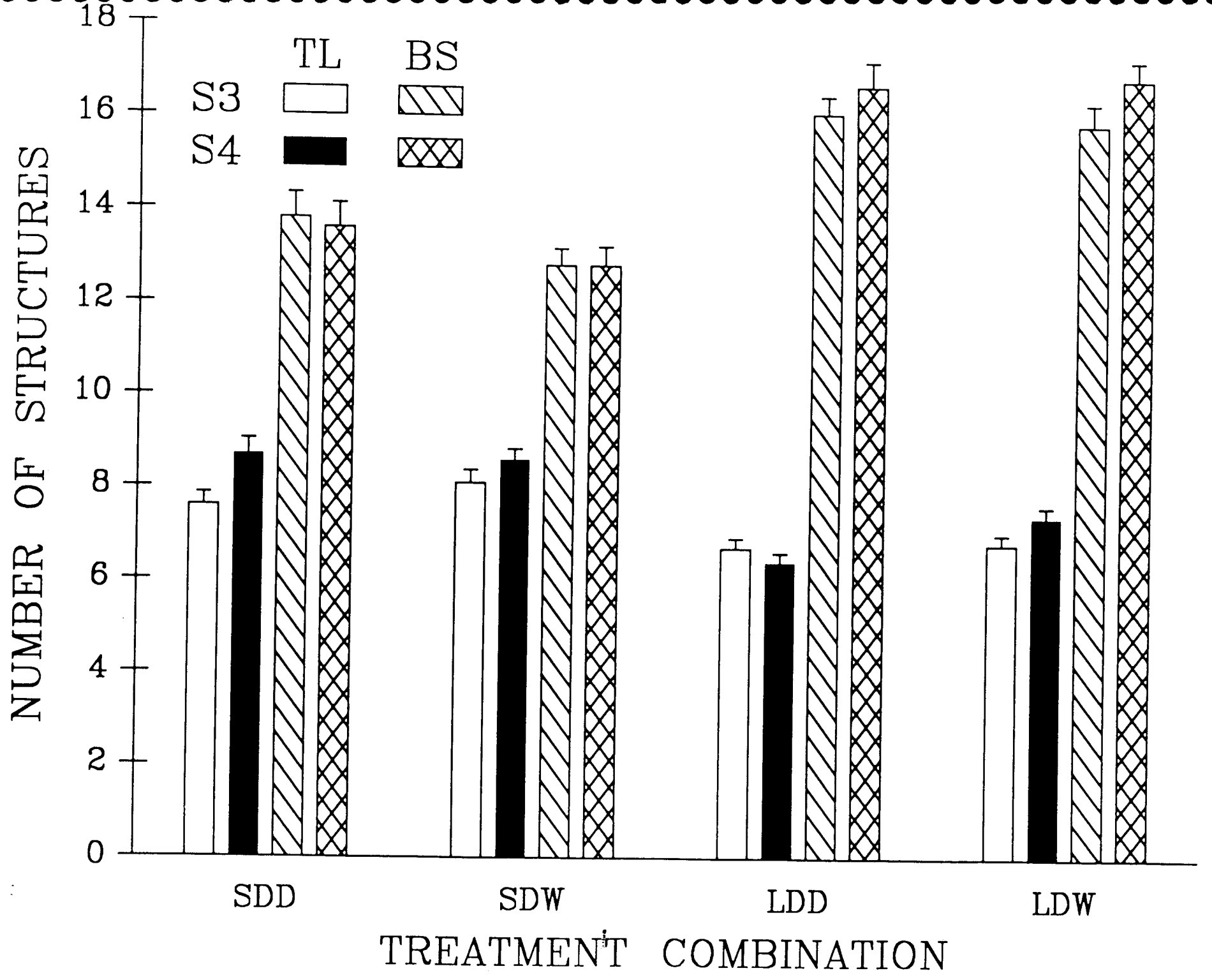
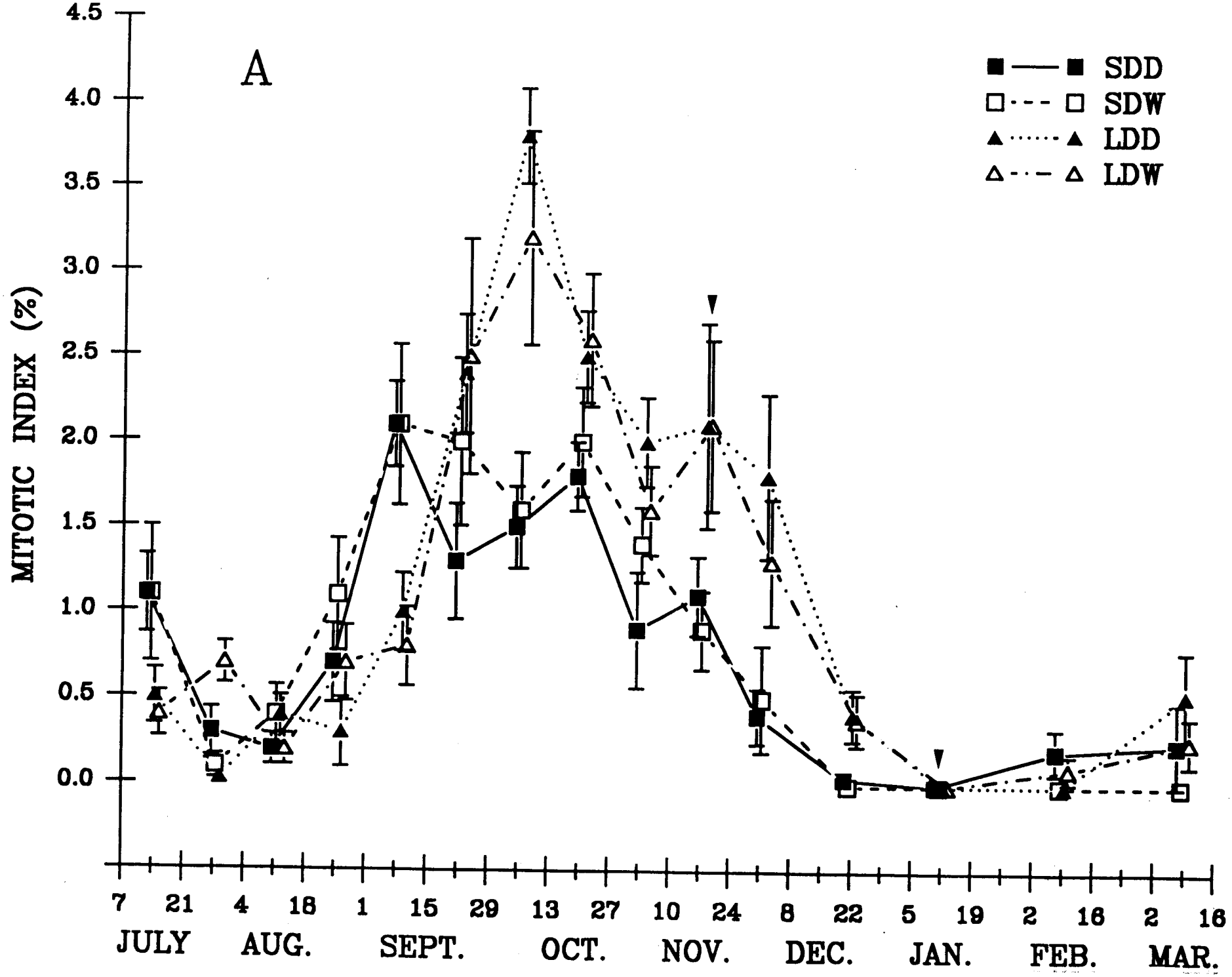

TL
BS
S3
S4
NUMBER OF STRUCTURES
0
2
4
6
8
10
12
14
16
18
SDD
SDW
LDD
LDW
TREATMENT COMBINATION

Figure 10. Apical mitotic index versus date in western hemlock seedlings growing in a greenhouse in small (S3)(A) and large (S4)(B) styroblock container cavities subjected to 4 weeks of dormancy induction beginning in mid-July.

Note: SDD, SDW, LDD, and LDW indicate short-day dry, short-day wet, long-day dry, and long-day wet treatment combinations, respectively. Means are based on eight seedlings per treatment combination (vertical bars indicate ± 1 SE). Points are staggered around sample date so that SE bars are visible. Seedlings were moved to a shelterhouse mid-February. Treatment effects were significant on most dates (Tables 8, 9). The dates of first and second lift are indicated (arrows).


A
SDD
SDW
LDD
LDW
MITOTIC INDEX (%)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
7 21 4 18 1 15 29 13 27 10 24 8 22 5 19 2 16 2 16
JULY AUG. SEPT. OCT. NOV. DEC. JAN. FEB. MAR.

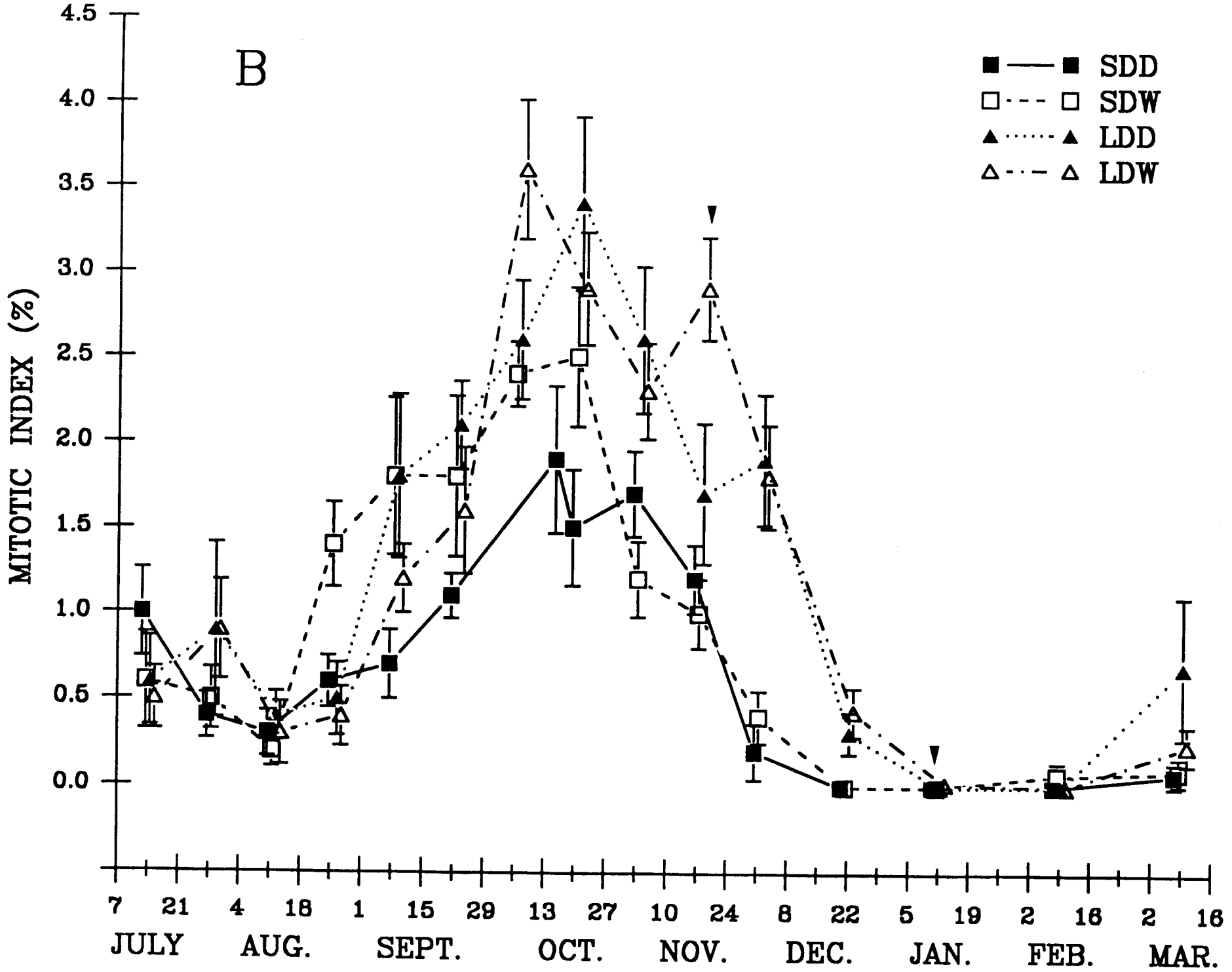
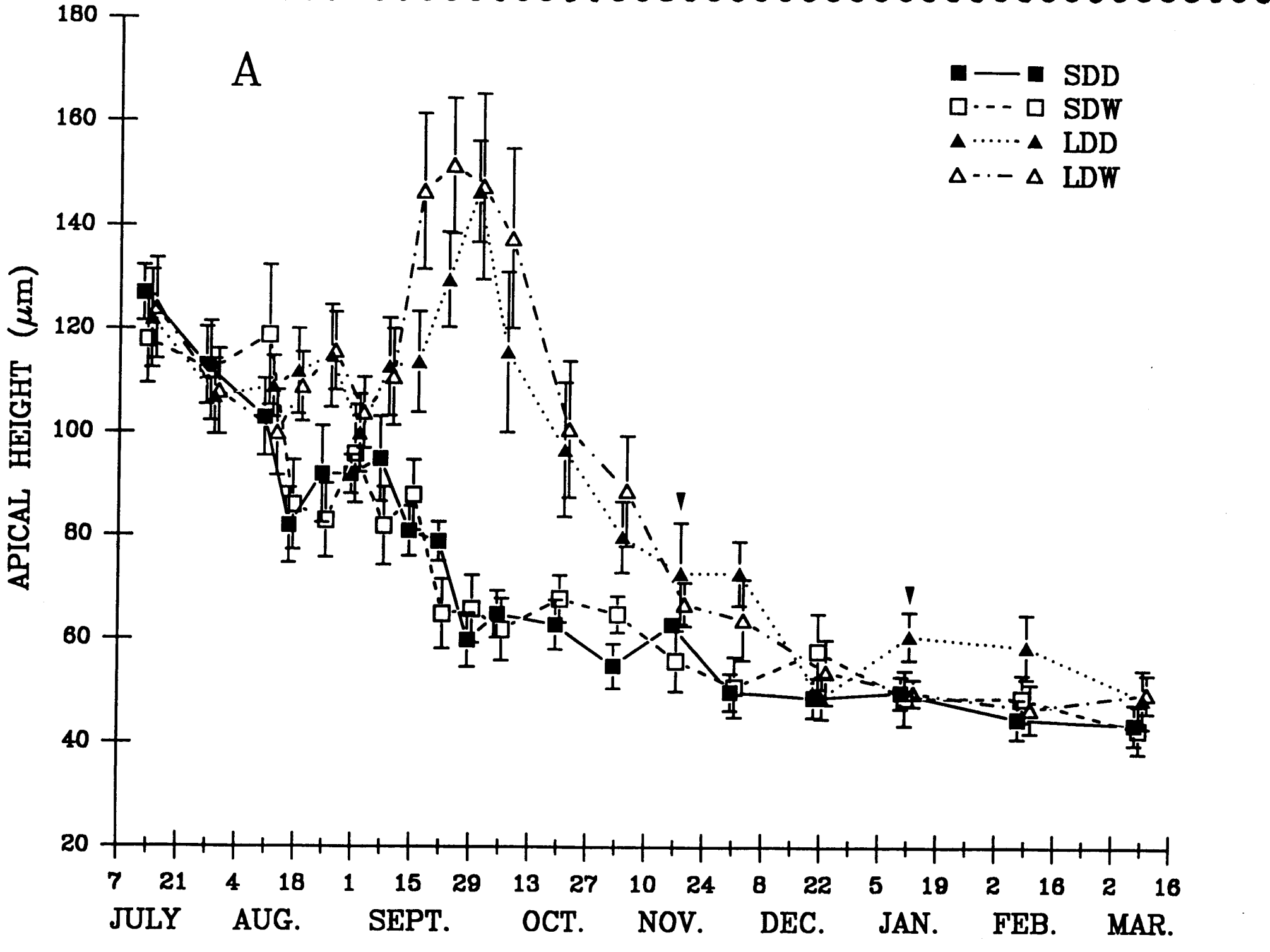

B
SDD
SDW
LDD
LDW
MITOTIC INDEX (%)
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
7 21 4 18 1 15 29 13 27 10 24 8 22 5 19 2 16 2 16
JULY AUG. SEPT. OCT. NOV. DEC. JAN. FEB. MAR.

Figure 11. Apical height versus date in western hemlock seedlings growing in a greenhouse in small (S3)(A) and large (S4)(B) styroblock container cavities subjected to 4 weeks of dormancy induction beginning in mid-July.

Note: SDD, SDW, LDD, and LDW indicate short-day dry, short-day wet, long-day dry, and long-day wet treatment combinations, respectively. Means are based on eight seedlings per treatment combination (vertical bars indicate ± 1 SE). Points are staggered around sample date so that SE bars are visible. Seedlings were moved to a shadehouse in mid-February. Treatment effects were significant on most dates (Tables 8, 9). The dates of first and second lift are indicated (arrows).


A
SDD
SDW
LDD
LDW
APICAL HEIGHT (μm)
180
160
140
120
100
80
60
40
20
7
21
4
18
1
15
29
13
27
10
24
8
22
5
19
2
16
2
16
JULY
AUG.
SEPT.
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.

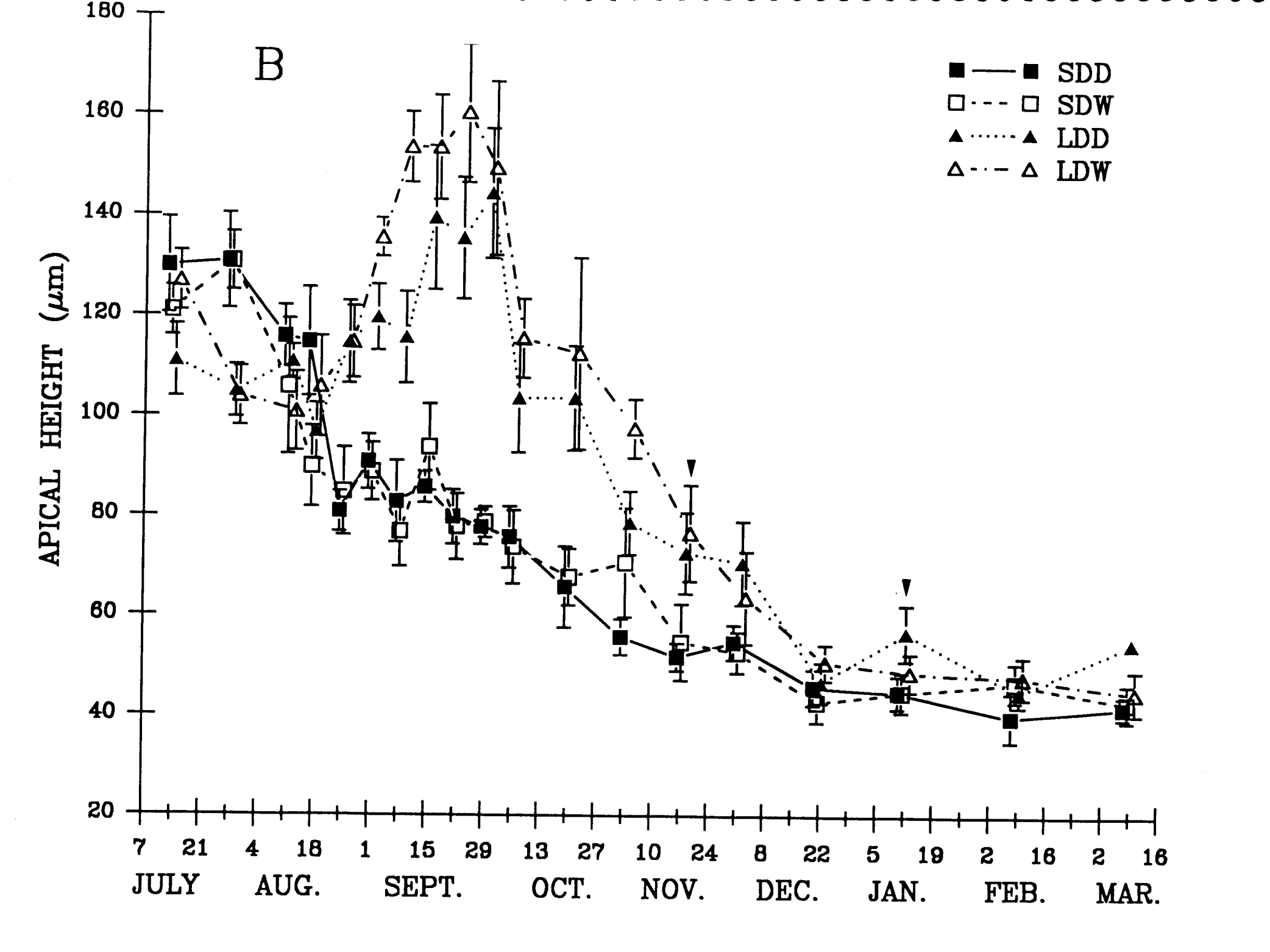
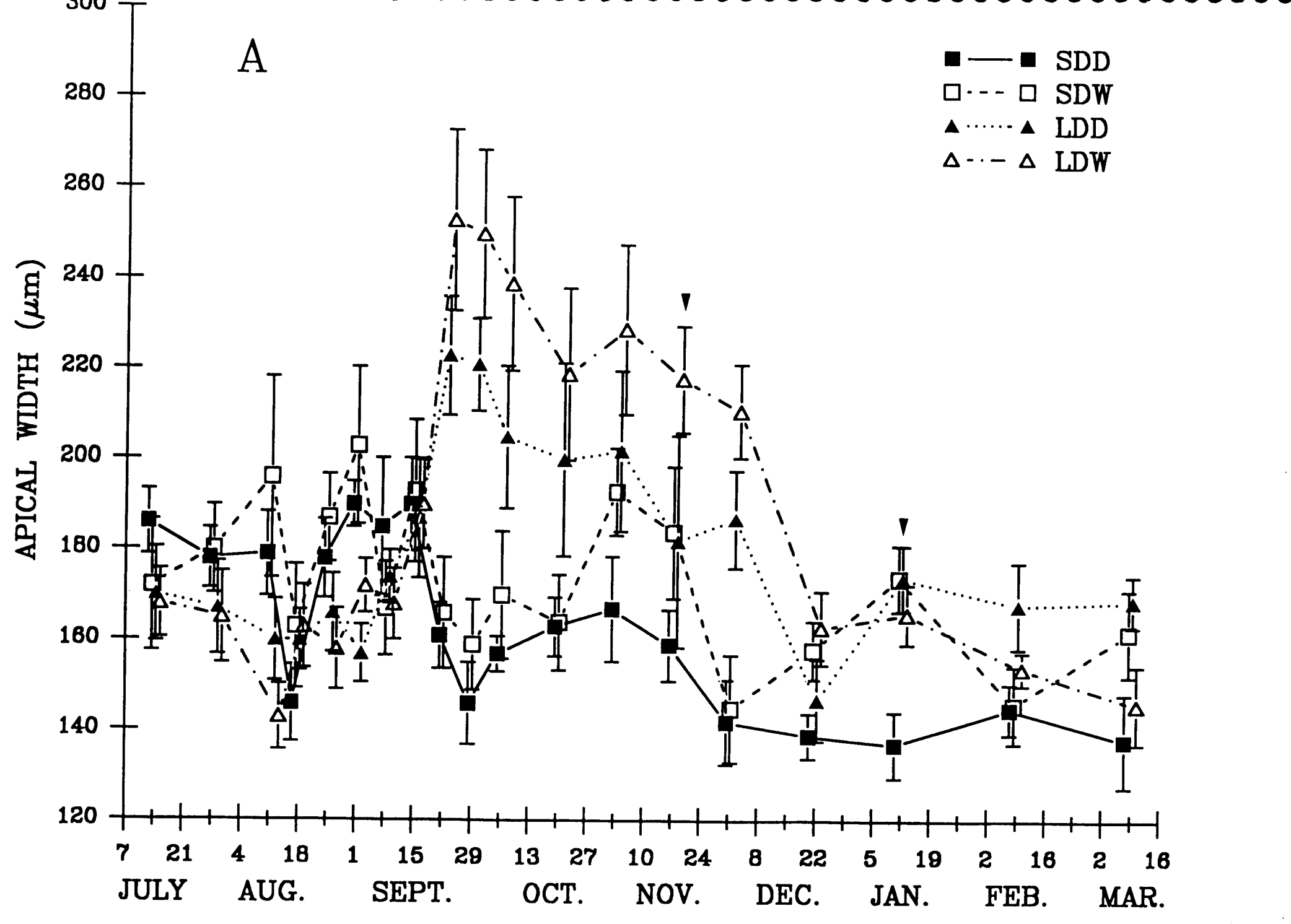

B
SDD
SDW
LDD
LDW
APICAL HEIGHT (μm)
180
160
140
120
100
80
60
40
20
7
21
4
18
1
15
29
13
27
10
24
8
22
5
19
2
16
2
16
JULY
AUG.
SEPT.
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.

Figure 12. Apical width versus date of western hemlock seedlings growing in a greenhouse in small (S3)(A) and large (S4)(B) styroblock container cavities subjected to 4 weeks of dormancy induction beginning in mid-July.

Note: SDD, SDW, LDD, and LDW indicate short-day dry, short-day wet, long-day dry, and long-day wet treatment combinations, respectively. These treatments were applied for 1 month beginning in mid-July. Means are based on eight seedlings per treatment combination (vertical bars indicate ± 1 SE). Points are staggered around sample date so that SE bars are visible. Seedlings were moved to a shelterhouse in mid-February. Treatment effects were significant on most dates (Tables 8, 9). The dates of first and second lift are indicated (arrows).


A
SDD
SDW
LDD
LDW
APICAL WIDTH (μm)
300
280
260
240
220
200
180
160
140
120
7
21
4
18
1
15
29
13
27
10
24
8
22
5
19
2
16
2
16
JULY
AUG.
SEPT.
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.

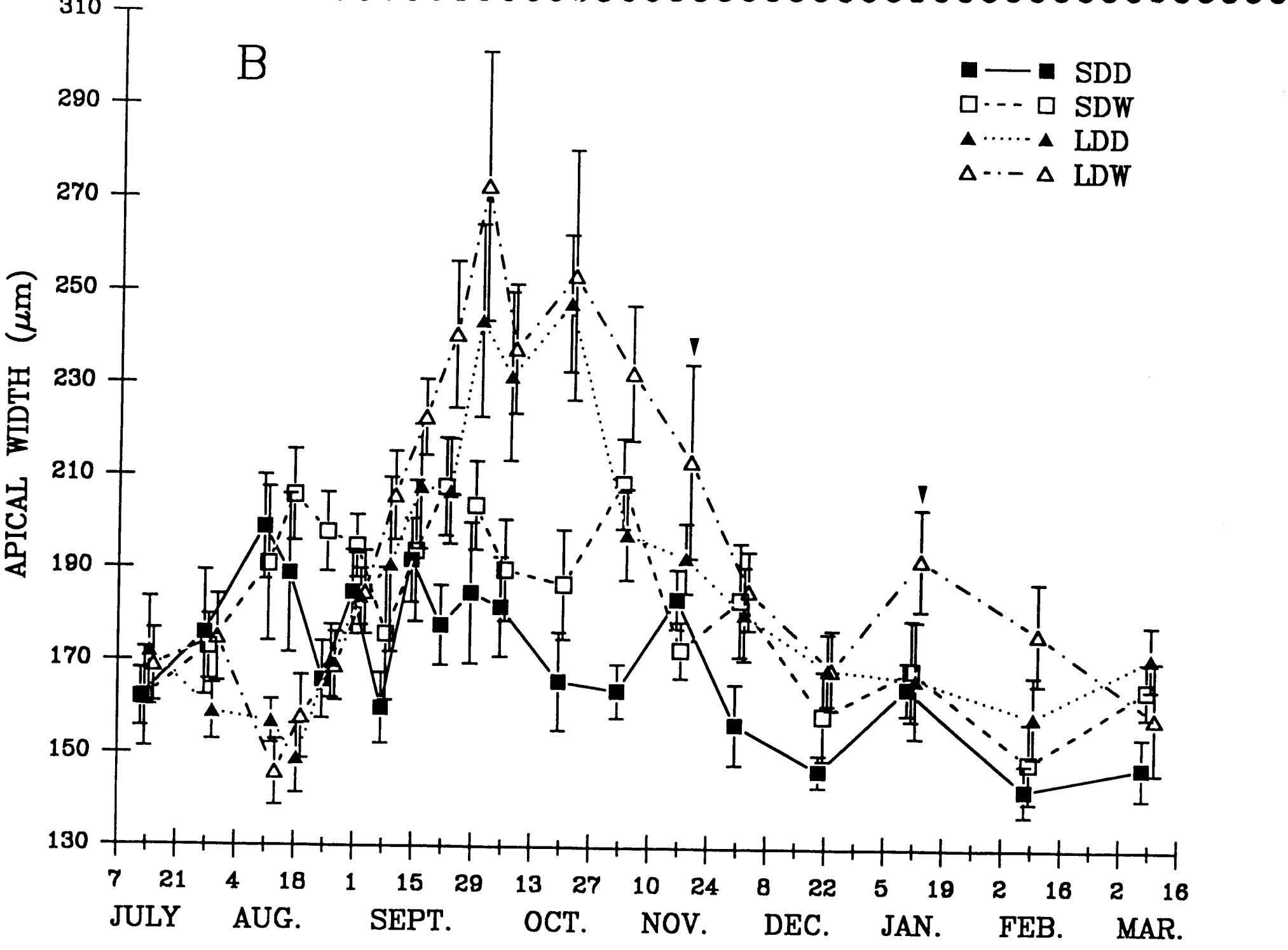

B
SDD
SDW
LDD
LDW
APICAL WIDTH (μm)
310
290
270
250
230
210
190
170
150
130
7
21
4
18
1
15
29
13
27
10
24
8
22
5
19
2
16
2
16
JULY
AUG.
SEPT.
OCT.
NOV.
DEC.
JAN.
FEB.
MAR.

Figure 13. Mean mitotic index versus date of western hemlock seedlings in cold storage and the greenhouse.

Note: L1 = seedlings lifted and placed in cold storage in mid-November. L3 = seedlings that remained in the greenhouse until they were moved to a shelterhouse in mid-February. Values are means of 64 seedlings per lift over all dormancy induction and styroblock cavity size treatment combinations (vertical bars indicate ± 1 SE). Points are staggered around sample date so that SE bars are visible. Seedlings lifted and placed in cold storage in mid-January showed no mitotic activity. Analysis of variance summary is shown in Table 9.

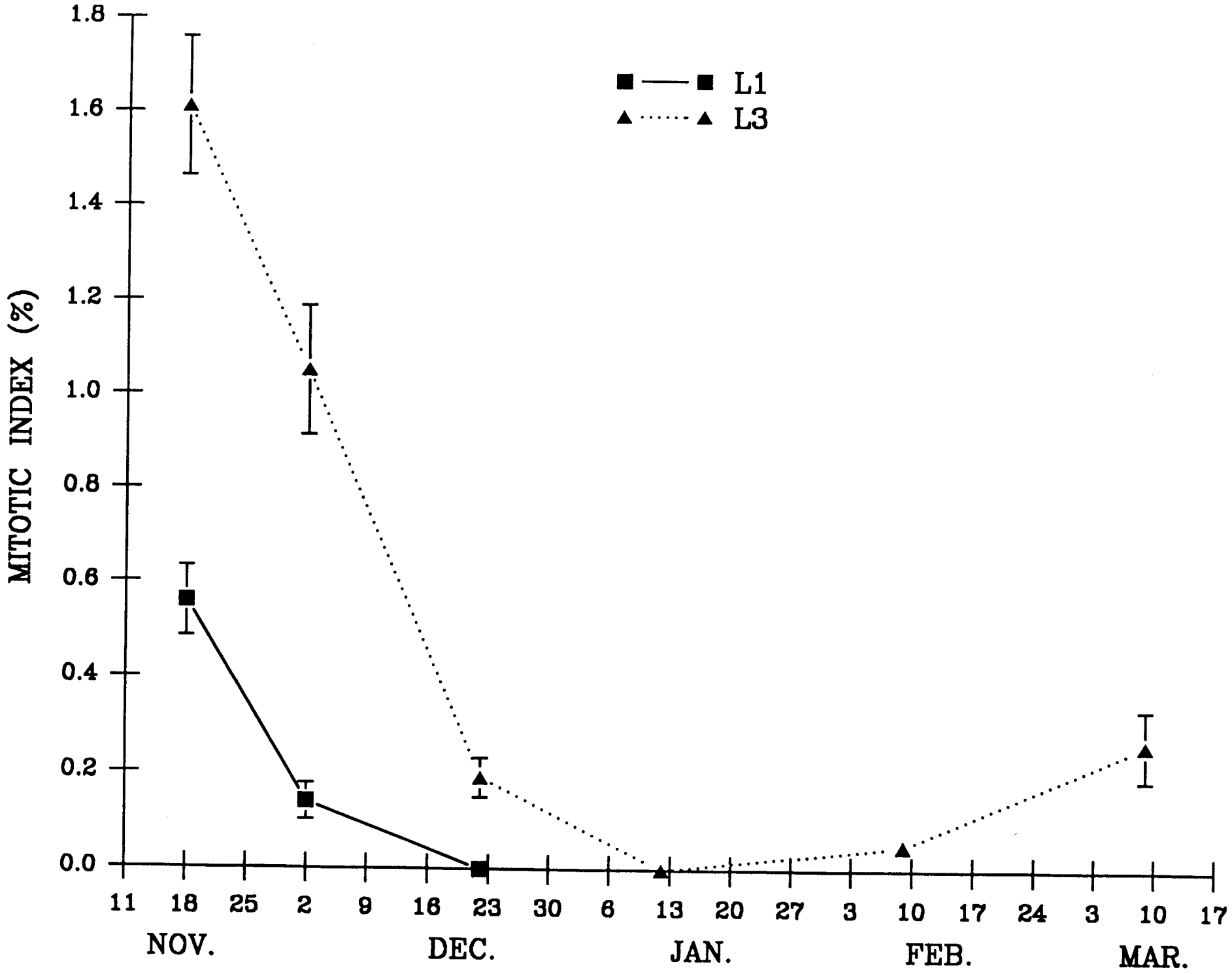

MITOTIC INDEX (%)
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
L1
L3
11
18
25
2
9
16
23
30
6
13
20
27
3
10
17
24
3
10
17
NOV.
DEC.
JAN.
FEB.
MAR.

Figure 14. Mean apical height versus date of western hemlock seedlings in cold storage and in the greenhouse.

Note: L1 = seedlings lifted and placed in cold storage in mid-November. L2 = seedlings lifted and placed in cold storage in mid-January. L3 = seedlings that remained in the greenhouse until moved to a shelterhouse in mid-February. Values are means of 64 seedlings per lift over all dormancy induction and styroblock cavity size treatment combinations (vertical bars indicate ± 1 SE). Points are staggered around sample date so that SE bars are visible. Analysis of variance summary is shown in Table 9.

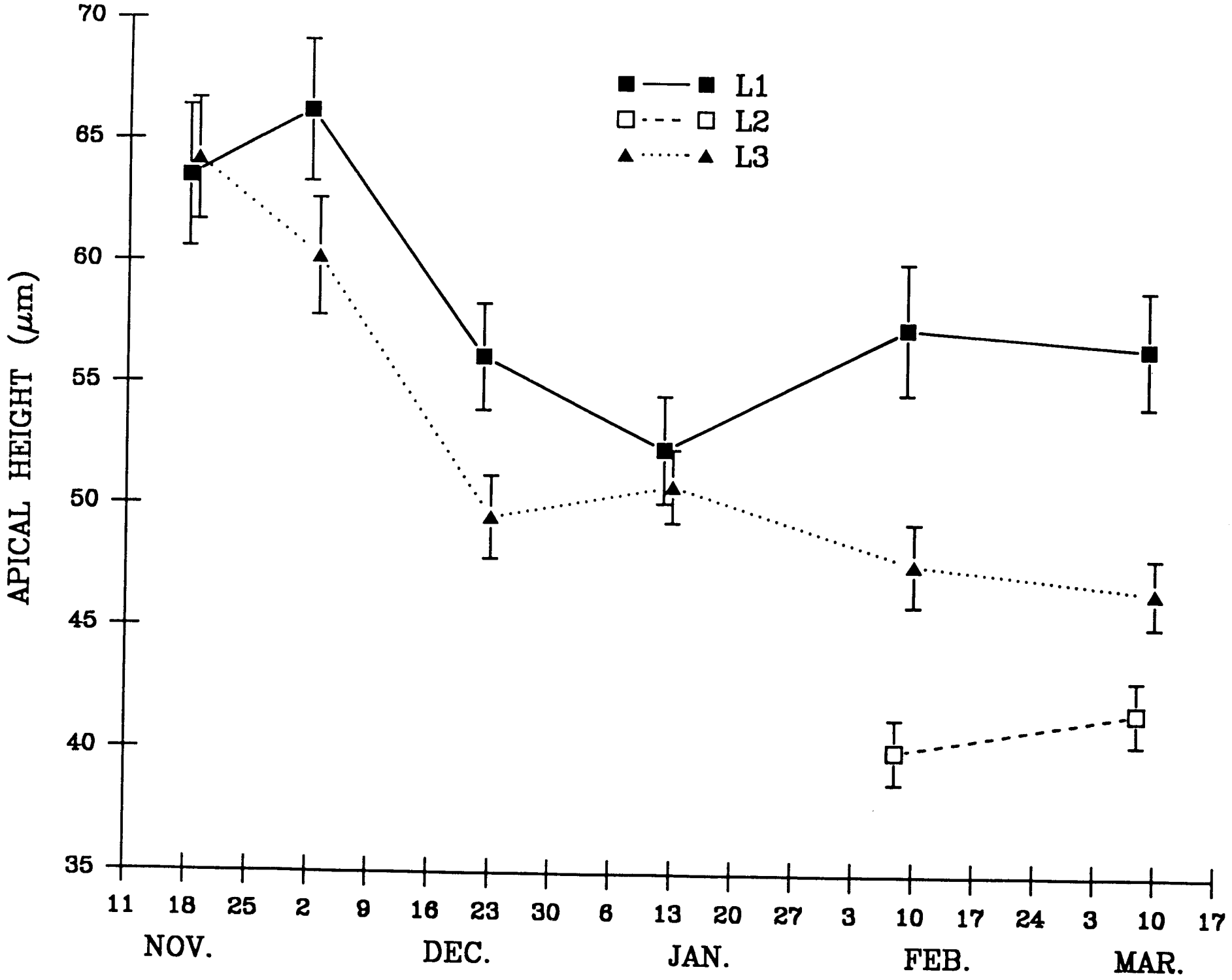
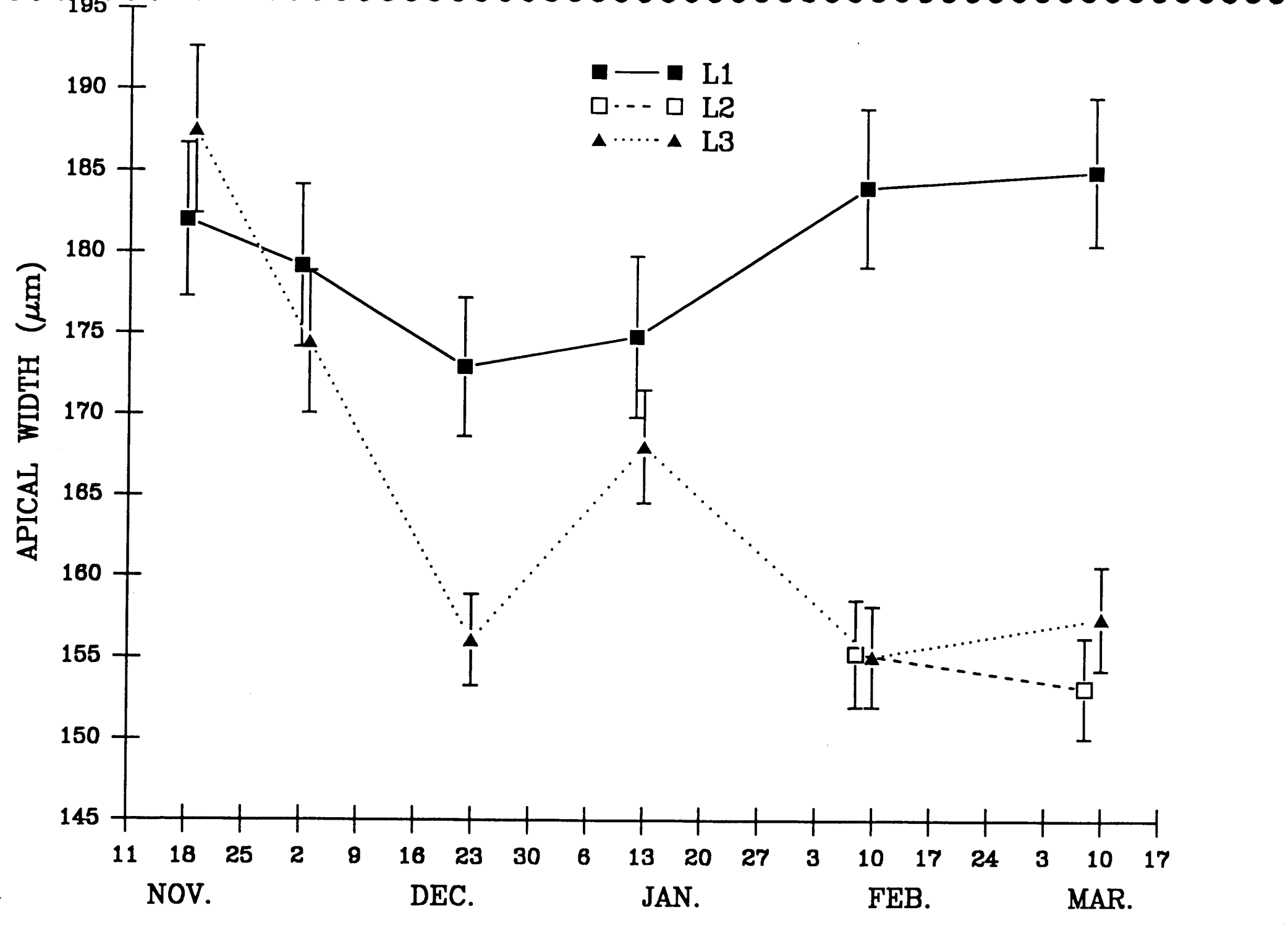

APICAL HEIGHT (μm)
70
65
60
55
50
45
40
35
L1
L2
L3
11
18
25
2
9
16
23
30
6
13
20
27
3
10
17
24
3
10
17
NOV.
DEC.
JAN.
FEB.
MAR.

Figure 15. Mean apical width versus date of western hemlock seedlings in cold storage and in the greenhouse.

Note: L1 = seedlings lifted and placed in cold storage in mid-November. L2 = seedlings lifted and placed in cold storage in mid-January. L3 = seedlings that remained in the greenhouse until moved to a shelterhouse in mid-February. Values are means of 64 seedlings per lift over all dormancy induction and styroblock cavity size treatment combinations (vertical bars indicate ± 1 SE). Points are staggered around sample date so that SE bars are visible. Analysis of variance summary is shown in Table 9.

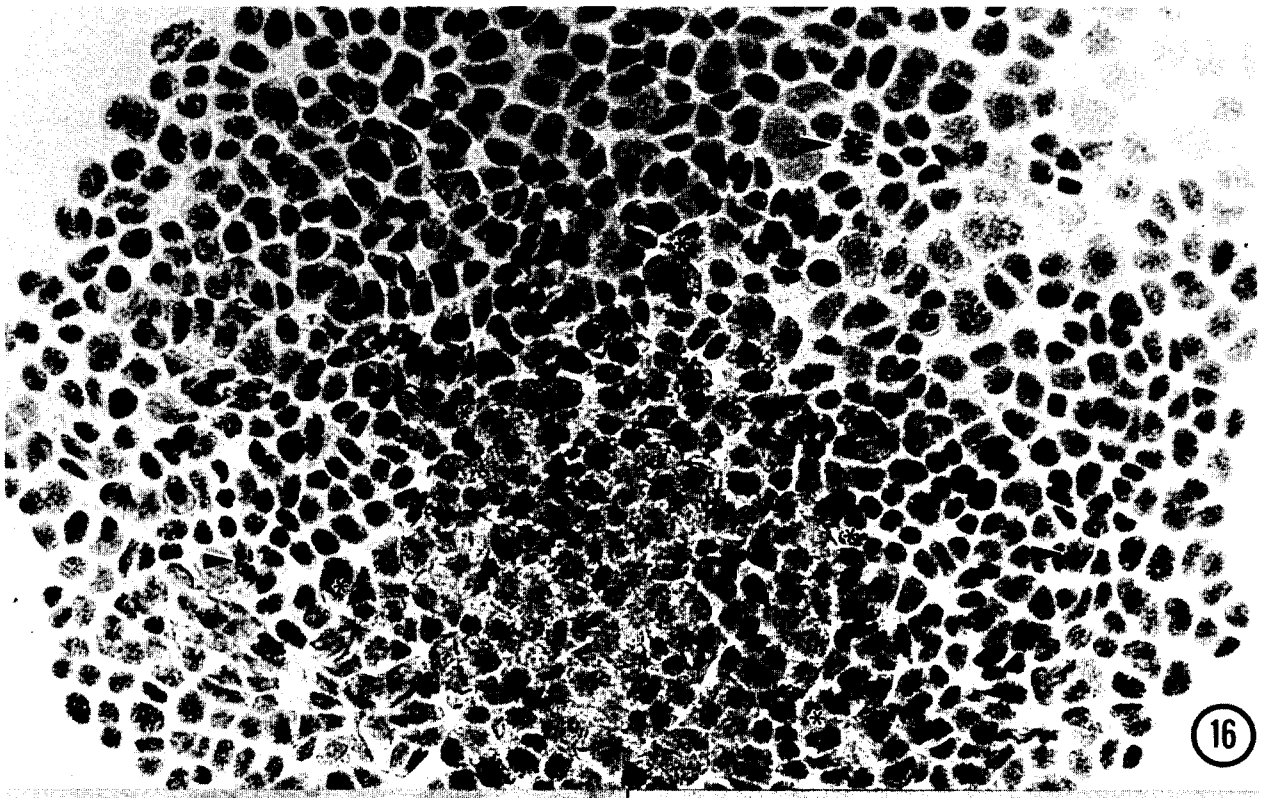

L1
L2
L3
APICAL WIDTH (μm)
195
190
185
180
175
170
165
160
155
150
145
11
18
25
2
9
16
23
30
6
13
20
27
3
10
17
24
3
10
17
NOV.
DEC.
JAN.
FEB.
MAR.

Figs. 16-18. Typical examples of a bud squash and the cuticle of leaves from seedlings of greenhouse-grown western hemlock seedlings.

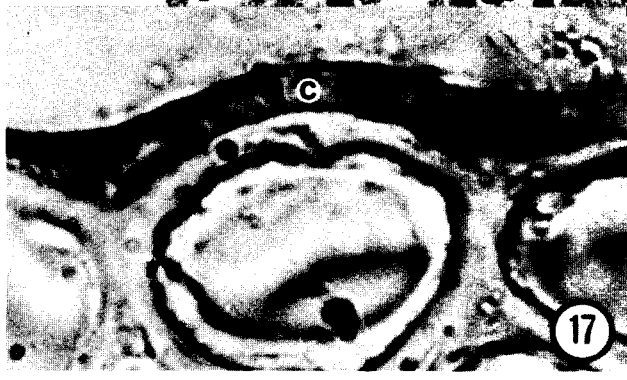
Fig. 16. A typical western hemlock bud squash showing a number of dividing cells (arrows). Mitotic figures could not be determined for cells within the rough circle outlined (*), probably because tannins interfered with the Feulgen reaction (see methods). X 227.

Fig. 17. Light micrograph of an epidermal cell with cuticle (C) on the adaxial surface of a western hemlock needle stained with Sudan IV. X 1667.

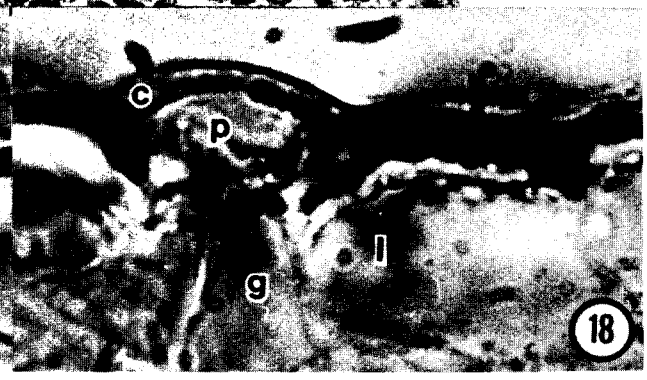
Fig. 18. Light micrograph of abaxial surface of western hemlock needle stained in Sudan IV showing the cuticle (C), and the polar subsidiary (P), lateral subsidiary (l) and guard (g) cells of the stomatal complex. X 1667.



16



17



18

Figs. 19-26. Typical epicuticular wax features on needles of greenhouse-grown western hemlock seedlings.

Fig. 19. Abaxial surface of needle showing stomata with wax occlusions. X 106.

Fig. 20. Adaxial surface of needle covered mostly with rods (or tubules) of wax. X 2900.

Fig. 21. Abaxial surface near stomata showing long rods (or tubules) of wax, mostly intermeshed into a dense covering of the needle. Compare Fig. 20. X 2900.

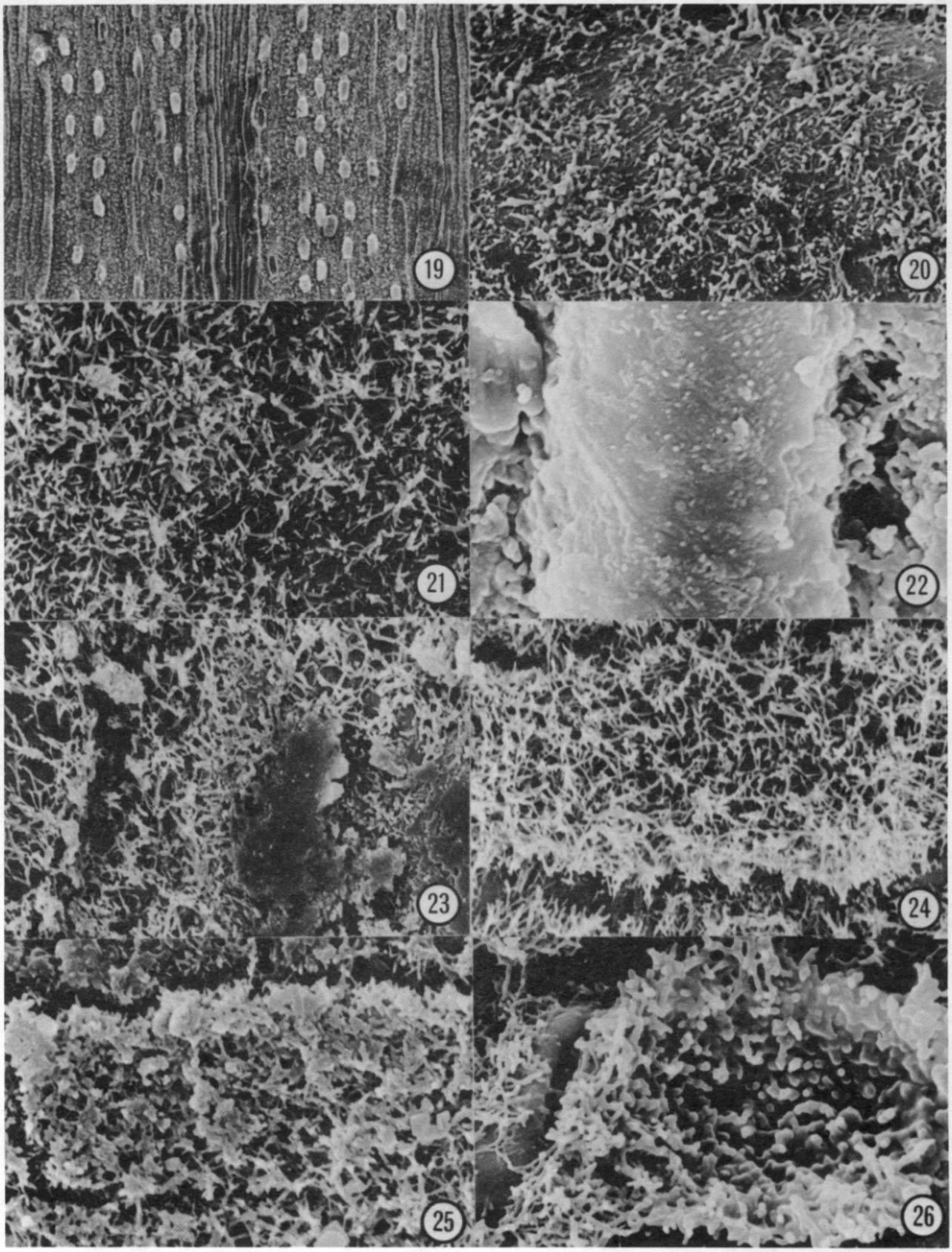
Fig. 22. Abaxial surface near stomata showing amorphous layer of wax. X 2900.

Fig. 23. Abaxial surface near stomata showing long rods, often fused and coalescing into plate-like layers. X 2900.

Fig. 24. Dense covering of intermeshing wax rods in stomatal opening. X 2900.

Fig. 25. As in Fig. 24, except that the rods have mostly fused into a series of small plates. X 2900.

Fig. 26. Waxes have fused into a solid plug in this stoma. X 2900.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20
21
22
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