AN ABSTRACT OF THE THESIS OF

Jeff S. Graham for the degree of Doctor of Philosophy in Forest Science, presented on April 4, 1989.

Title: The Control of Bud Development in Douglas-fir Seedlings by Photoperiod, Flurprimidol, and Endogenous Gibberellins and Abscisic Acid

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The objective of this thesis was to evaluate the hypothesis that bud development of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seedlings is controlled by endogenous gibberellins or abscisic acid and that cultural treatments affect bud development by altering levels of these growth regulators. To test this hypothesis, three experiments were conducted which measured bud set, bud flush, or endogenous growth regulators following treatment by photoperiod or flurprimidol, a synthetic growth regulator.

In experiment I, 8 or 17 hour photoperiods were applied to actively growing, one-year-old seedlings. Bud development, foliar gibberellin activity (dwarf rice bioassay), and abscisic acid levels (gas-liquid chromatography) were measured on treatment days 0, 4, 18, and

62. Compared to seedlings under 17 hour photoperiods, seedlings under 8 hour photoperiods developed resting buds, and had elevated gibberellin-like activity. Abscisic acid levels increased under 17 hour photoperiods.

In experiment II, seedlings with terminal buds that were given flurprimidol (10 mg per seedling) and held under photoperiods promotive for bud flush (15 or 17 hours) were compared with untreated controls. Bud flush was tallied periodically; foliar gibberellin activity and abscisic acid levels were measured 10 weeks after treatment; and post-dormancy shoot growth was examined. Bud flush and post-dormancy shoot growth were reduced by flurprimidol. Both gibberellin activity and abscisic acid levels were lower after flurprimidol treatment.

In experiment III, flurprimidol (5 mg per seedling) was applied to seedlings with terminal buds which were then held under 17 hour photoperiods. Overwintering bud morphology, and post-dormancy shoot growth were compared with untreated seedlings grown under either 17 or 8 hour photoperiods. Overall, flurprimidol did not counteract 17 hour photoperiods for either bud morphology or post-dormancy shoot growth.

It was concluded that both photoperiod and flurprimidol (at 10 mg per seedling) can control bud development for Douglas-fir seedlings. Abscisic acid, a

growth inhibitor, was positively correlated with shoot growth cessation and bud formation and could not be functioning as an inhibitor. However, when considering previous reports on gibberellin metabolism, gibberellin-like activity was affected in a manner which can explain treatment effects.

THE CONTROL OF BUD DEVELOPMENT IN DOUGLAS-FIR SEEDLINGS BY PHOTOPERIOD, FLURPRIMIDOL, AND ENDOGENOUS GIBBERELLINS AND ABSCISIC ACID

by

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INTRODUCTION

Nursery growing regimes for conifer seedlings in the Pacific Northwest must both promote and limit growth. Following germination, growth is promoted by fertilization and irrigation (Duryea 1984). Achieving adequate size is important for outplanting success (Zaerr and Lavender 1976). However, by mid-summer, nurseries must restrict height growth and promote resting bud set. Although mild water stress is the recommended procedure to stop height growth (Lavender and Cleary 1974, Duryea 1984), it is not always effective. Unwanted second flushes are common for seedlings in Northwest nurseries (Meyers 1984). Failure to obtain timely bud set will delay development of dormancy and cold hardiness and lead to poor plantation performance (Lavender 1984).

This thesis examines the physiological and cultural control of bud set and bud flush of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) seedlings. The hypothesis was that bud development is under endogenous gibberellin and/or abscisic acid control, and that the effects of cultural treatments on bud development are

mediated by these growth regulators. Three experiments were performed to assess this hypothesis (figure 1). In experiment I, the relationships between photoperiod controlled bud development and endogenous gibberellins and abscisic acid levels were examined. In experiment II, the relationships between the effects of the plant growth retardant flurprimidol, bud development, and endogenous gibberellin and abscisic acid levels were evaluated. Flurprimidol was used because it has been reported to be effective on conifers (Hare 1984) and also to inhibit gibberellin biosynthesis (Lilly Res. Labs. 1983). In experiment III, the ability of flurprimidol to counteract the effects of long photoperiod was investigated.

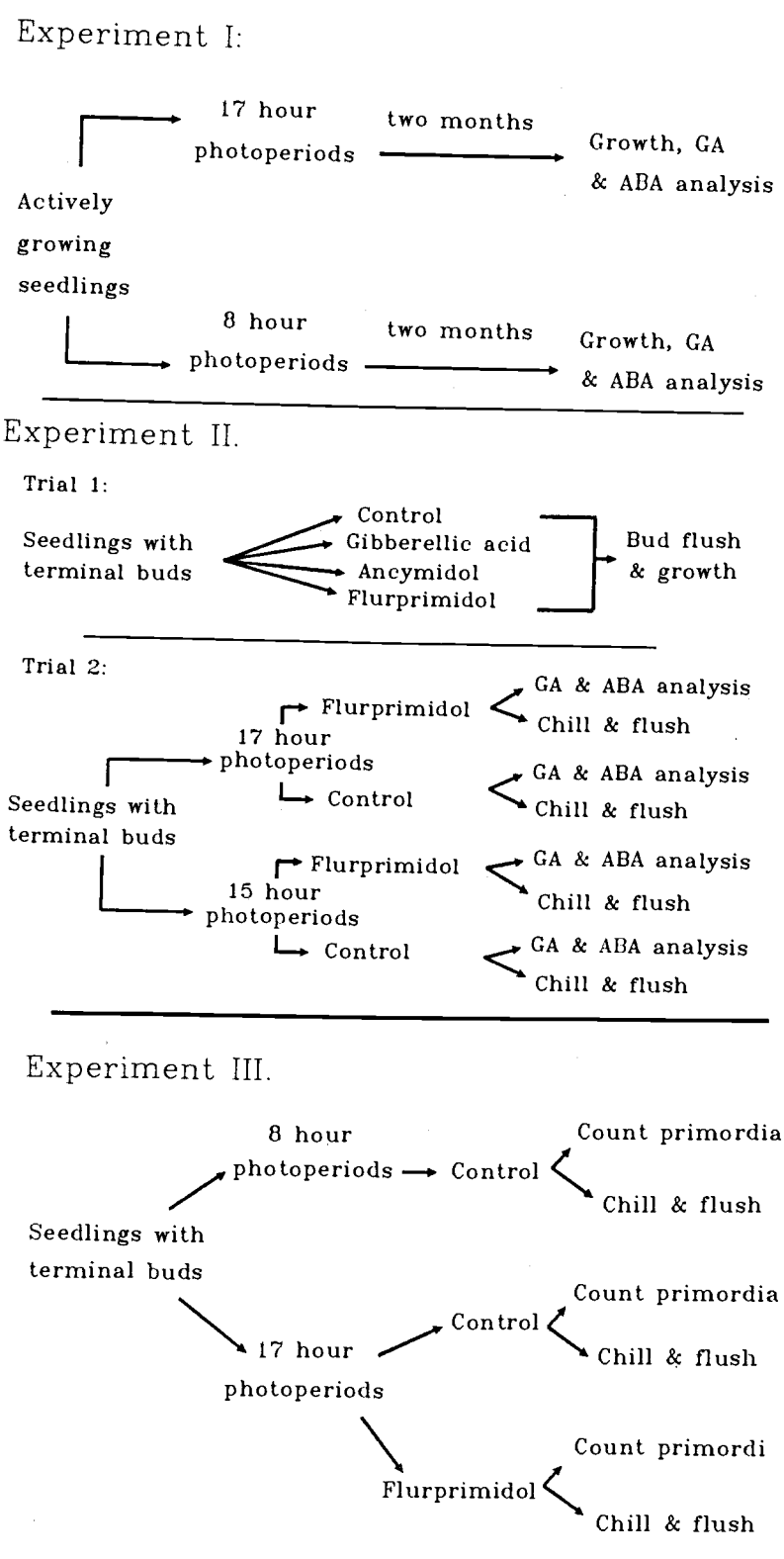

Figure 1. Schematic outline of the treatment applications and seedling manipulations.

LITERATURE REVIEW

Woody plants in the north temperate climate do not elongate throughout the entire year, but only during the spring and early summer (Romberger 1963). Even in the tropics, elongation is not continual but episodic with growth alternating with periods of rest (Kramer and Kozlowski 1979). However, in the tropics, rest periods do not follow annual climate cycles (Kramer and Kozlowski 1979).

The cessation of terminal growth is accompanied with the enclosure of the apical meristem within scales to form a bud. Until growth resumes, the shoot is in a condition broadly termed dormancy (Romberger 1963). This follows from the classical definition of dormancy "any case in which a tissue predisposed to elongate does not do so" (Doorenbos 1953). Although dormancy is often considered the lack of physiological activity, this is not the case. Cell division and primordia production continue within the bud until limited by cold temperatures (Owens 1968). Furthermore, shortly after bud set, elongation can be re-induced by defoliation or relief of the stimulus which caused bud set (Nitsch 1957, Wareing and Phillips 1981).

Induction and release from dormancy has been the

subject of physiological investigation for many years. The general observation for woody plants is that dormancy is induced by short photoperiods or mild water stress, and that dormancy is released by chilling (Lavender 1981, Wareing and Phillips 1981). This has also been shown for Douglas-fir. For first-year Douglas-fir seedlings, short photoperiods strongly promote bud formation (Lavender et al. 1968, McCreary et al. 1978). Mild water stress will also induce bud formation (Lavender and Cleary 1974, Lavender 1984). Twelve weeks of chilling (Wommack 1964) or long photoperiods (Lavender 1981) will release Douglas-fir from dormancy.

The mechanism by which these environmental signals release buds from dormancy and allow flushing is not fully understood. Bud flush, as used by Kramer and Kozlowski (1979), is the opening of the bud scales by elongation of the preformed shoot. Of the well characterized growth regulators, gibberellins (GAs) have been most clearly implicated. Exogenous application of GAs stimulate seed germination and bud flush in many species (Marth et al. 1956, Nooden and Weber 1978). Moreover, endogenous GA activity has been found to rise in response to chilling for buds of sycamore maple (Acer pseudoplatanus L., Eagles and Wareing 1964), cranberry (Vaccumium macrocarpon Ait., Eady and Eaton 1974), balsam poplar (Populus balsamifera L., Bachelard and Wrightman

1974), and foliage of Sitka spruce (Picea sitchensis (Bong.) Carr., Lorenzi et al. 1975). Furthermore, Lavender et al. (1973) provided evidence that spring bud flush in Douglas-fir is regulated by GAs and that these were supplied by the roots. Bioassays of xylem sap showed that GA activity rose as buds began to swell in the spring. Cold soils delayed bud swell but a GA_3 spray overcame this delay. This and other experiments leads to the hypothesis that chilling removes a block to GA biosynthesis, and that subsequent warming allows GA production (Wareing and Saunders 1971, Powell 1987). Upon translocation of GAs to buds, flushing is stimulated.

Although this model of dormancy release is attractive, it is almost assuredly too simple. For example, GA activity has not always been found to rise in the xylem sap of flushing Douglas-fir trees (Loferski 1981). Additionally, growth inhibitors including abscisic acid (ABA) have been found to decline in seeds and buds during chilling (Wareing and Phillips 1981). Specifically, ABA decline during chilling has been reported for buds of common birch (Betula verrucosa Ehrh., Harrison and Saunders 1975), and grape (Vitis vinifera L., During and Bachman 1975), and xylem sap of peach (Prunus persica L., Davidson and Young 1974), and willow (Salix viminalis L., Alvium et al. 1976). However, ABA is currently believed

to affect primarily stomatal movement during water stress (Mansfield 1987).

The physiological control of bud formation is also of practical concern and perhaps less understood. Early work with sycamore maple suggested that inhibitors are produced in the leaves in response to short photoperiods (Eagles and Wareing 1968). These materials were thought to induce bud formation by inhibiting internode elongation. However, later studies on other woody angiosperms, which specifically examined ABA levels under short or long photoperiods, did not find a correlation with bud set (Lenton et al. 1972, Loveys et al. 1974, Alvium et al. 1976, Powell 1976). Nonetheless, seasonal changes in ABA levels have been found to follow dormancy patterns for Douglas-fir (Webber et al. 1979). Moreover, moisture stress, which enhances bud formation, also stimulates increased ABA levels for Douglas-fir seedlings (Blake and Ferrell 1977).

In contrast to ABA, GA levels have provided a more consistent explanation for bud set. Seasonal decreases in GA activity during dormancy induction have been reported for hairy birch (Betula pubescens Ehrh., Digby and Wareing 1966), willow (Salix viminilis L., and walnut (Juglans regia L., Langrova and Sladky 1971). Additionally, photoperiod treatments have been found to affect GA activity in leaves or shoots of several species including

spinach (Spinacia oleracea L., Radley 1963, Zeevaart 1971), potato (Solanum andigena L., Railton and Wareing 1973), and peas (Pisum sativum L., Proebsting et al. 1978). Moreover, photoperiod has been found to control GA metabolism, particularly in the 13-hydroxlation pathway, GA_{53} -> GA_{44} -> GA_{19} -> GA_{20} -> GA_{29} (Jones and Zeevaart 1980, Metzger and Zeevaart 1980, Metzger and Zeevaart 1982, Davies et al. 1986). For spinach, long photoperiods allow the conversion of GA_{19} to GA_{20} which is blocked under short photoperiods (Metzger and Zeevaart 1980, Gilmour et al. 1986). It is uncertain if GA_{20} is the active growth regulator or if it is converted to another GA, for growth promotion. Phinney (1984), working with dwarf mutants, provided evidence that only GA_1 is active in shoot growth of corn (Zea mays L.). However, GA_1 has not been detected in spinach (Graebe 1987).

If bud set and bud flush are under GA control, then the application of GA biosynthesis inhibitors, such as growth retardants, should affect bud development. Early reports stated that growth retardants were inactive in gymnosperms (Cathey 1964). However, subsequent reports showed that growth retardants were active on a variety of conifers. Pharis at al. (1967) found that Arizona cypress (Cupress arizonica Greene) and coastal redwood (Sequoia sempervirens D. Don Engl.) were both stunted when treated

with AMO-1618 and B-995. The stunting was partially counteracted by an exogenous application of GA_3. Although Coulter pine (Pinus coulteri D. Don) was not stunted, Douglas-fir developed resting buds in response to AMO-1618. Dunberg and Eliasson (1972) and Dunberg (1974) reported growth reducing effects on Norway spruce (Picea abies Karst) from a number of chemicals. Again the reduction was partially counteracted by GA_3.

Recognizing their potential value to seedling production, Cheung (1975) tested several chemical retardants on Western hemlock (Tsuga heterophylla (Raf.) Sarg.) grown in British Columbia nurseries. Although bud set was obtained, no subsequent research reports were issued. Weston et al. (1980) tested several retardants, including ancymidol, on container-grown lodgepole pine (Pinus contorta Englem.) and white spruce (Picea glauca (Monench) Voss). Significant growth reduction was obtained as well as reduced shoot-root ratios. Unfortunately, they did not evaluate the effect following dormancy, but they speculated that the effect would be temporary.

As new growth retardants are discovered and tested, the possibility of useful applications has increased. Hare (1984) reported successful growth reduction for young loblolly (Pinus taeda L.) and slash (Pinus elliottii Englem.) pines in a seed orchard. Furthermore,

a new compound, flurprimidol, was highly effective in preventing second flushing while producing no unwanted side effects. Although the dosage Hare (1984) used was far above the suggested rate, tissue death did not occur. Similarly, Wheeler (1987) applied paclobutrazol, a related compound, to seedling and seed-orchard Douglas-fir and loblolly pine and reported significant stunting. Of these two recently developed compounds, flurprimidol appears to be the more potent retardant on woody plants (Sterrett and Tworkoski 1987).

Following the discovery that growth retardants could be partially counteracted by exogenous GA_3, it was thought that the mechanism of action was inhibition of gibberellin biosynthesis. Initial confirmation of that mechanism was provided by Dennis et al. (1965) working with AMO-1618 and wild cucumber (Echinocytis macrocarpa Greene). With this system, the growth retardant inhibits the cyclization of geranylgeranyl pyrophosphate to copalyl pyrophosphate (Dennis et al. 1965). Kuo and Pharis (1975) extended support of this mechanism to conifers by showing that GA-like substances were reduced to undetectable levels in Arizona cypress treated with AMO-1618. Ancymidol also inhibits gibberellin biosynthesis but the primary point of inhibition is the oxidation of kaurene to kaurenol (Coolbaugh and Hamilton

1976). The precise site of action of flurprimidol has not been determined, but the similarity of structure with ancymidol suggests a common mechanism.

Growth retardants have also been found to inhibit sterol production in tobacco (Nicotiana tabacum L., Douglas and Paleg 1978), and abscisic acid biosynthesis in the fungus cercospora (Cerospora rosicola passerini, Norman et al. 1986). Although the steps in the biosynthesis of abscisic acid have not been conclusively identified (Walton 1987), ABA, sterols, and GAs are all terpenoids and share common precursors up to farnesyl pyrophosphate (Goodwin and Mercer 1983). Consequently, the inhibition of gibberellin biosynthesis cannot be considered the only mechanism of action for growth retardants.

The major concern about the use of chemical retardants in seedling production is their potential effect on dormancy and subsequent growth. Lavender et al. (1973) provided evidence that dormancy release of Douglas-fir is brought about by gibberellins supplied by the roots. Therefore, inhibitors of gibberellin biosynthesis may prolong dormancy and lengthen the chilling requirement that many conifers possess. When the chilling requirement has not been fully satisfied, stunting, abnormal shoot growth, and delay in the onset of growth may occur (Romberger 1963). Additionally, persistent gibberellin de-

pression may reduce shoot growth even if growth resumption is not affected. The involvement of gibberellins in the shoot growth of conifers has been thoroughly reviewed (Pharis and Kuo 1977).

In addition to inhibiting bud flush, growth retardants may mimic other aspects of short photoperiods. It is generally accepted that bud formation by mid-summer is important to prepare buds for winter chilling (Lavender 1984). Lavender and Stafford (1985) have shown that long photoperiods in late summer result in poorly formed winter buds for Douglas-fir. Moreover, flushing and survival of such seedlings was markedly impaired following an eight week chilling period. Growth retardant application may counteract the effect of long photoperiods in late summer. As a result, otherwise poor shoot growth could be near normal. A counteracting effect may result either from increased primordia production in the overwintering bud or from enhanced free growth in the spring.

Free growth, as defined by Lanner (1976), is the simultaneous initiation and elongation of new stem units. Stem unit was defined by Doak (1935) as an internode plus its appendage (i.e. a needle). Free growth is a common occurrence for conifer seedlings (Jablanczy 1971), and is it known to vary with provenance (Pollard and

Logan 1974). Furthermore, free growth is influenced by both environmental conditions during bud flush (Pollard and Logan 1974) and earlier bud development treatments (Macey and Arnott 1986). However, the effects of growth retardants applied during bud development on subsequent free growth have not been reported.

EXPERIMENT I. THE EFFECTS OF PHOTOPERIOD ON BUD DEVELOPMENT AND ENDOGENOUS GIBBERLLINS AND ABSCISIC ACID OF DOUGLAS-FIR SEEDLINGS.

INTRODUCTION

Short photoperiod is the principal environmental stimulus for bud development in many temperate woody plants (Wareing 1968, Nooden and Weber 1978). For Douglas-fir, the critical photoperiod is about 14 hours (Downs 1962, Lavender 1981), although the response varies with provenance (Vaartaga 1959). Consequently, photoperiod control has become an important tool in greenhouse production of reforestation seedlings (Arnott and Mitchell 1981).

Research into the mechanism of photoperiodism has examined GA metabolism and has primarily used herbaceous flowering plants (Zeevaart 1971a, Railton and Wareing 1973, Proebsting et al. 1978, Jones and Zeevaart 1980). For spinach, short photoperiods inhibit the formation of GA_{20} with a resulting increase in GA_{19}, the immediate precusor of GA_{20} (Metzger and Zeevaart 1980, Gilmour et al. 1986). Although Douglas-fir has not been studied as intensively, breaking of dormancy by chilling has been found to affect GA metabolism (Lavender et al 1978). Furthermore, an association between endogenous GA levels and

genetic differences in growth rate for Douglas-fir has been reported (Crozier et al. 1970).

In addition to gibberellins, ABA has been associated with the dormancy cycle of Douglas-fir and may influence bud development (Webber et al. 1979). Moreover, application of ABA has been observed to speed dormancy development for Douglas-fir (Zaerr et al. 1973). However, for woody angiosperms, short photoperiods have not increased endogenous ABA levels (Powell 1982, Wareing and Phillips 1983). The present study was conducted to examine endogenous GA and ABA of Douglas-fir seedlings under long and short photoperiods. The hypothesis was that bud development is under growth regulator control; hence photoperiod induced bud development is associated with altered endogenous GA or ABA levels.

MATERIALS AND METHODS

Seedling treatments

In mid-winter, two-hundred one-year old Douglas-fir seedlings of a southern Oregon seed source were obtained from the USDI Bureau of Land Management nursery near Merlin, OR. The seedlings had been raised in Ray-Leach supercells (164 cm^3 capacity) and had a mean shoot height of 24 cm. Seedlings were transplanted into sphagnum peat

moss in compressed fiber pot (4 l capacity), five per pot. The pots were then placed in a growth room under 16 hour photoperiods of 120 uE m^{-2} sec^{-1} photosynthetically active radiation (PAR) and a 22°C/18°C temperature cycle. The pots were watered every other day without fertilization.

All seedlings promptly flushed, and in June the pots were randomly assigned to either an 8 or 17 hour photoperiod. Photoperiod treatments were applied in separate growth rooms under the same PAR and temperatures as before. On four dates, samples of at least 10 seedings per treatment were randomly selected, measured for height, and had their foliage removed. The foliage was immediately weighed, placed in a plastic bag and quickly transferred to a -60°C freezer. The sampling dates were at the beginning of the photoperiod treatments (day 0), and on days 4, 18, and 62.

Growth regulator analysis

The procedure for growth regulator analysis was adapted from Reeve and Crozier (1978), Jones et al. (1980), and Loferski (1981). All solvents were HPLC grade or distilled prior to use. The foliage samples were ground under liquid nitrogen with a mortar and pestle. The resulting frozen powder was placed in 100 ml methanol, and left standing overnight at -20°C. Forty mg diethyldithiocarbamic acid (Sigma) were added as an

antioxidant.

The methanol was filtered and the foliage residue ground and filtered twice again with 100 ml methanol. Thirty ml of 5M phosphate buffer pH 9.2 was added and the solution reduced to the aqueous phase with a rotary evaporator at 33°C. The pH of the aqueous solution after methanol removal was 8.0 or less. The sample was then centrifuged at 2000 rpm to precipitate the chlorophyll and hydrophobic materials. The pellet was then washed twice with 0.5 M phosphate buffer, pH 8.0.

The combined supernatants were partitioned three times against equal volumes of hexane and the hexane discarded. The aqueous phase was loaded onto a 3 x 13 cm column containing 30 ml PVP (Polyclar) and eluted by applying pressure from compressed air. The PVP was washed with 50 ml 0.5 M phosphate buffer, pH 8.0, and the solutions combined. The extract was next adjusted to pH 3.0 with HCl and loaded in stages onto a 1.5 x 10 cm column containing 5 ml charcoal:celite (1:2). The charcoal column was washed with 25 ml 20% acetone and the growth regulators were eluted with 100 ml 80% acetone. After the acetone was removed under vacuum, the sample was partitioned 5 times against ethyl acetate. The ethyl acetate fractions were combined and frozen at -20°C. Any ice was filtered off, 1 gm Na_2SO_4 added for final drying,

and the sampled filtered again. The ethyl acetate was removed under vacuum, and the sample was stored at -60°C until high performance liquid chromatography (HPLC).

A chromatograph (Varian 5000) with a reverse-phase octadecyl silica column (C_{18}, 250 by 4.6 mm i.d., Beckman) was operated at 1 ml per min with a linear gradient of 10 to 100% methanol in 1% acetic acid over 23 minutes. The samples were injected in 50 ul methanol and 1 ml fractions collected from 0 to 30 minutes. Each fraction was dried and stored at - 60°C until analysis.

Preliminary testing found that ABA eluted in fractions 18 and 19. These sample fractions were methylated with ethereal-diazomethane and analyzed by gas chromatography (Varian 3700) with a ^{63}Ni electron capture detector and a glass column (180 x 0.2 cm i.d.) packed with 2% OV-101. The injector temperature was 210°C, column temperature 145°C, and detector temperature 260°C. Nitrogen carrier gas was used at a flow rate of 35 ml per min. The sample was diluted with methanol and triplicate analyses of 8 ul were made. Quantification was by peak height interpolation from a curve prepared using an authentic ABA standard (Sigma).

Gibberellin analysis was by dwarf rice (Oryza sativa L. cv Tan-ginbozu) bioassay (Murakami 1968). The rice seed was allowed to germinate in water for three days at 32°C, and 230 uE m^{-2} sec^{-1} PAR. The germinates

were rogued for uniformity and transplanted into clear plastic trays (12 x 12 x 2.5 cm) containing 250 ml of 0.7% agar, 80 to 100 seedlings per tray. The trays were sealed and the rice allowed to grow one additional day before bioassay. Fractions 5 through 30 (except 18 and 19) were diluted with 20 ul 50% ethanol and 1 ul aliquots applied to the rice seedlings. Eight to 10 rice seedlings were assayed for each fraction. The trays were placed into 310 x 160 x 8 cm clear plastic boxes containing 0.5 cm water, and the boxes sealed with clear plastic food wrap. After 3 days at 32°C, 230 uE m^{-2} sec^{-1} PAR, and 100% relative humidity, the total shoot length of each seedling was measured. The GA quantity was interpolated from a standard curve prepared using GA_3 (Sigma). A standard curve was assayed with each sample.

Abscisic acid recovery was estimated by adding a known ABA quantity to a separate Douglas-fir foliage extract with predetermined ABA content and analyzing for total ABA. Gibberellin recovery was estimated by subjecting a solution of known GA_3 content to the extraction procedure and bioassaying the recovered material. Abscisic acid and GA_3 recoveries were calculated to be 88 and 66%, respectively.

Statistical analysis

Treatment differences for this experiment were analyzed by analysis of variance using a general linear models procedure (SAS Institute Inc. 1985). The experiment was considered a completely randomized design with sample date and photoperiod as treatments. Height was analyzed separately for each date. Because the variance of growth regulator analysis was proportional to the mean, $\log_{10}$ transformations were computed before analysis of variance (Zar 1974). Log_{10} transformations were also computed for least significant differences (LSDs) between GA bioassays and bioassay controls, but means and LSDs were not transformed for graphical presentation.

RESULTS

Seedling growth

Dormant seedlings promptly flushed when placed in the growth room under a 16 hour photoperiods. From March to June, seedlings mean height increased from 24 to 49 cm. Following the initial flush, many seedlings set bud and some flushed a second time.

Seedlings transferred to the 8-hour photoperiod chamber continued elongation of the current flush, but did not reflush. Seedlings under the 17-hour photoperiod continued to reflush up to final harvest. Consequently,

the seedlings under different photoperiods diverged in height over the two month period (figures 2 and 3). Analysis of variance showed a significant difference in height ($p \leq 0.001$) on day 64, but not on days 4 or 18 ($p=0.462$ and $p=0.419$, respectively).

Abscisic acid quantification

Reverse phase HPLC of the acidic ethyl acetate fraction of the foliage extract had considerable uv absorbance (figure 4). When full samples were injected, a substantial portion of the chromatogram was above 2 absorbance units.

Experimentation with the analytical standard found the ABA eluted in HPLC fraction 18 or 19. Hence, these fractions were both used for ABA quantification and the result summed for each sample. Experimentation with the methylated standard showed a retention time (R_t) of 6.43 minutes on the gas chromatograph. The electron capture detector appeared very selective toward methylated ABA. Generally, the methylated plant extract had few peaks and the peak corresponding to the R_t of ABA was quite clear (figure 5). To verify that the assumed ABA peak in the plant extract was authentic ABA, a sample of methylated extract was spiked with standard methylated ABA and chromatographed (figure 6). Only one peak near the R_t of 6.43 was obtained and it closely reflected the summed peaks of

the separate samples.

Under 17 hour photoperiods, the ABA content increased over the two month period. (figure 7). Seedlings under 8 hour photoperiods had no apparent increase in ABA with time. Comparing the seedlings under the photoperiod treatments, analysis of variance revealed a significant interaction for date x photoperiod (table 1). Adjusting for recovery the average ABA levels on the final harvest were 60.1 ng/g and 202 ng/g for 8 and 17 hour photoperiods respectively.

Gibberellin quantification

Preliminary experimentation with the dwarf rice bioassay suggested a non-linear relationship between rice height and $\log_{10}$ GA_3 (figure 8). Hence, a regression was fitted to the standard curve with the following form:

$$Y = aX^2 + bX + C.$$

The dependent variable (Y) is rice height and the independent variable (X) is $\log_{10}(GA_3)$. The minimum amount of GA_3 detectable with the assay depended on sample size, but was generally about 0.1 ng/ul using 8 rice seedlings. However, for quantification a standard curve was developed for each sample, and each fraction was interpolated regardless of amount.

Bioassay of the HPLC fractions revealed several fractions with activity (figure 9). However, one fraction, number 24, had the most consistent and greatest

activity. Also, fraction 24 was significantly greater than the bioassay control for seedlings under either photoperiod (figure 9). Other fractions showing significant activity were 9, 22, and 26 through 29. The time sequence of fraction 24 activity showed generally higher levels under 8 hour photoperiods with a peak on treatment day 4 (figure 10).

Analysis of variance of fraction 24 activity indicated a significant difference between photoperiods (table 1). However, neither the date nor the date x photoperiod interaction were significant. Correcting for recovery, the average GA_3 equivalents were 0.179 ng/g and 2.05 ng/g for 8 and 17 hour photoperiods, respectively.

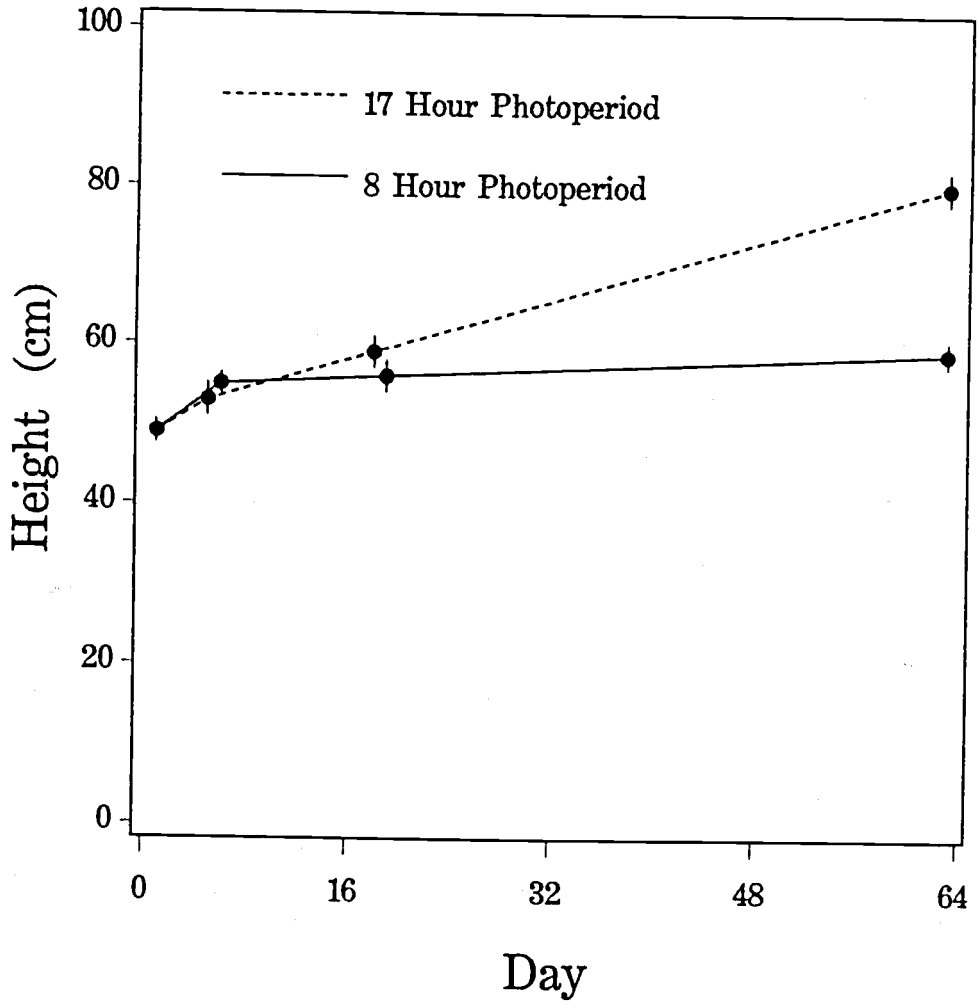

Figure 2. The height growth of one-year-old Douglas-fir seedlings maintained under 8 or 17 hour photoperiods. Vertical lines represent one standard error.

Figure 3. Representative second year Douglas-fir seedlings grown for two months under a 17 photoperiod (left) and an 8 hour photoperiod (right).

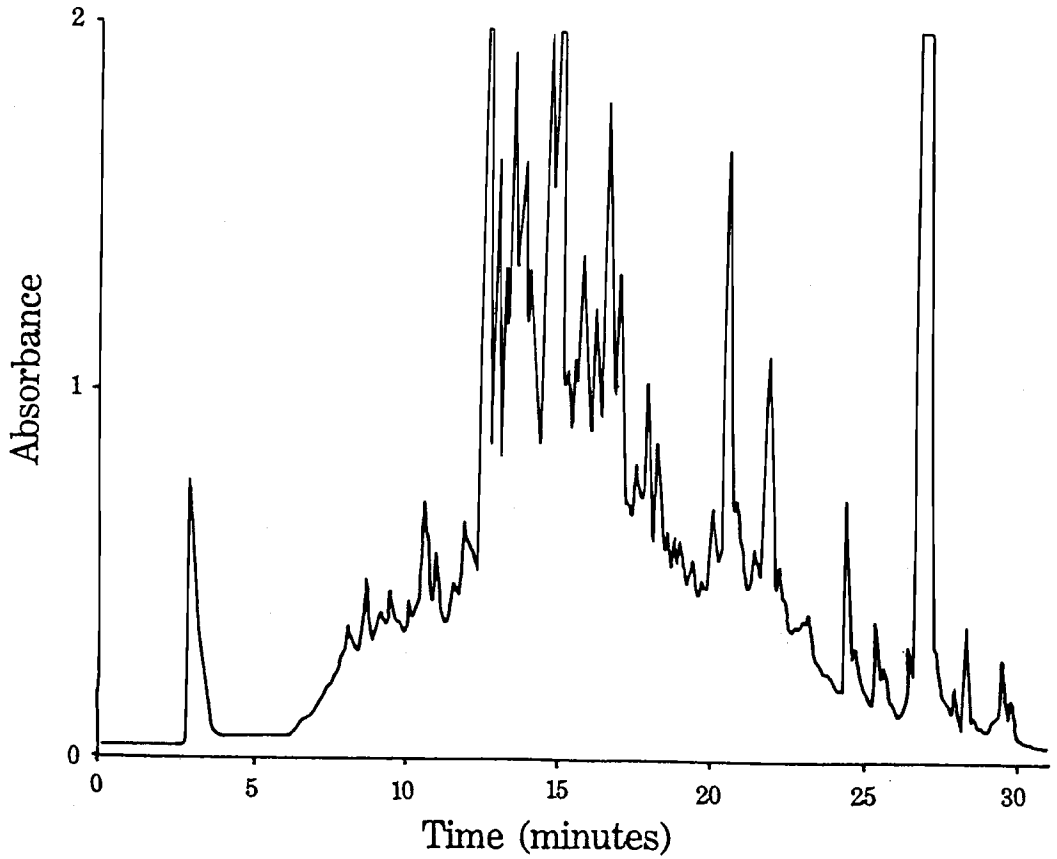

Figure 4. Representative HPLC chromatogram with uv absorbance detector (254 nm) of Douglas-fir foliage extract following solvent partitioning and PVP and charcoal filtration.

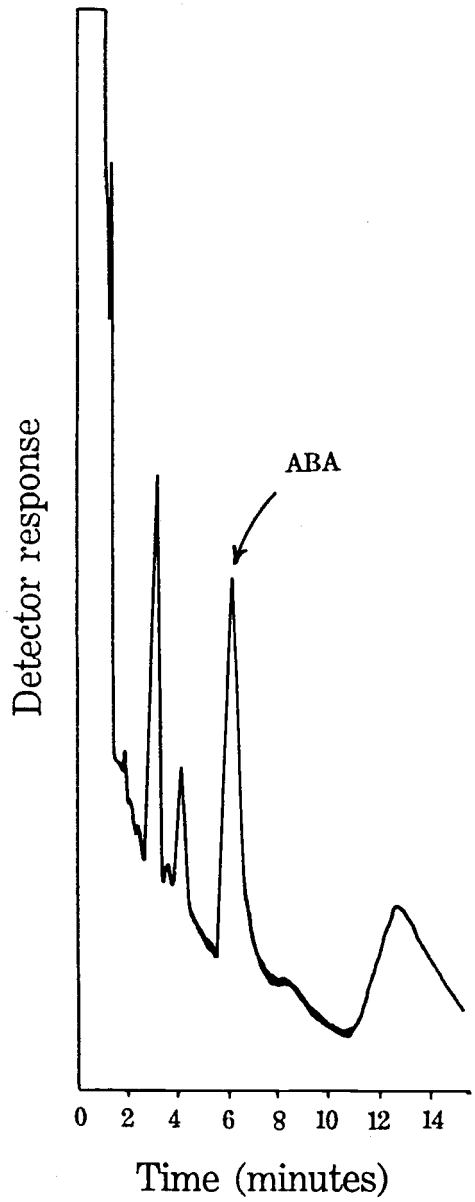

Figure 5. Representative gas chromatogram with electron capture detector of methylated fraction 18 from HPLC purification of Douglas-fir foliage extract. The peak corresponding to the retention time of abscisic acid standard is shown.

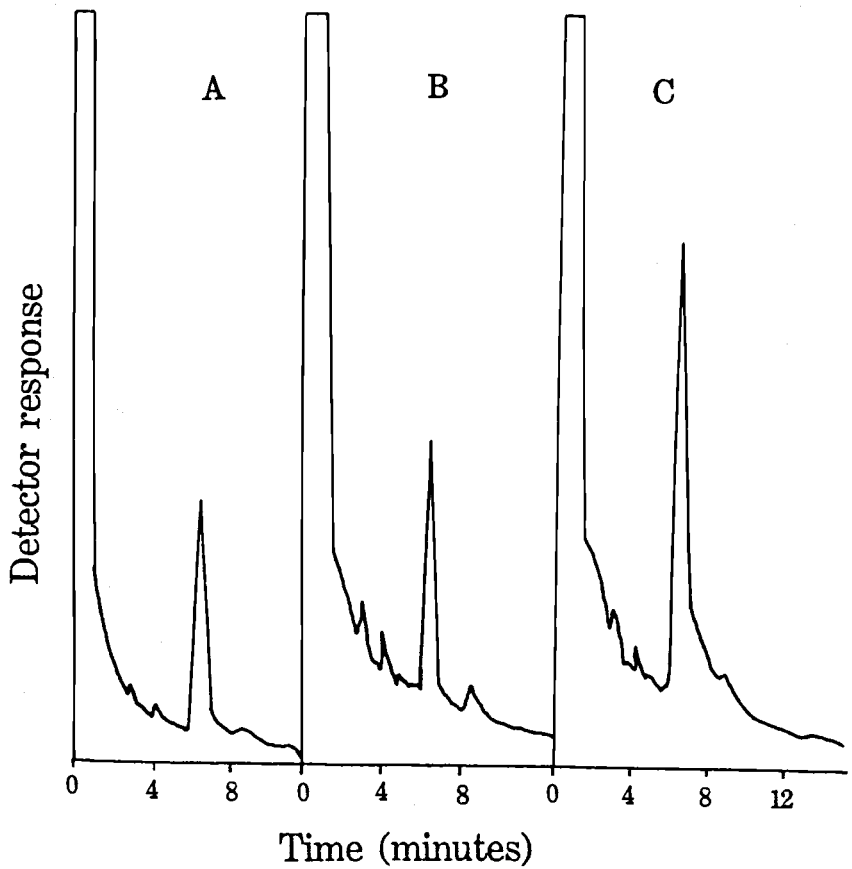

Figure 6. Gas chromatogram of methylated abscisic acid standard (A), purified, methylated Douglas-fir foliage extract (B), and combination of foliage extract and standard (C).

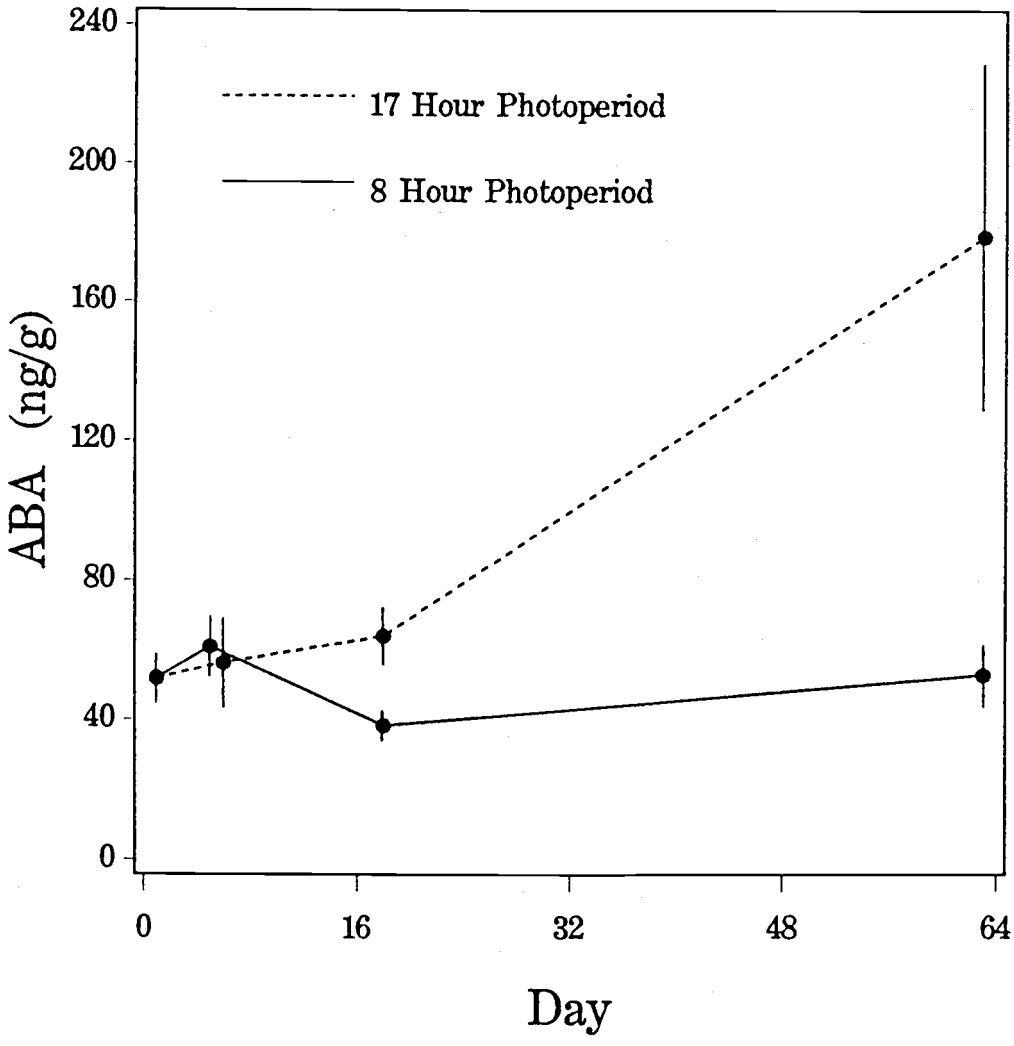

Figure 7. Abscisic acid concentrations for foliage extracts from Douglas-fir seedlings as they were grown under two photoperiods. Vertical lines represent one standard error.

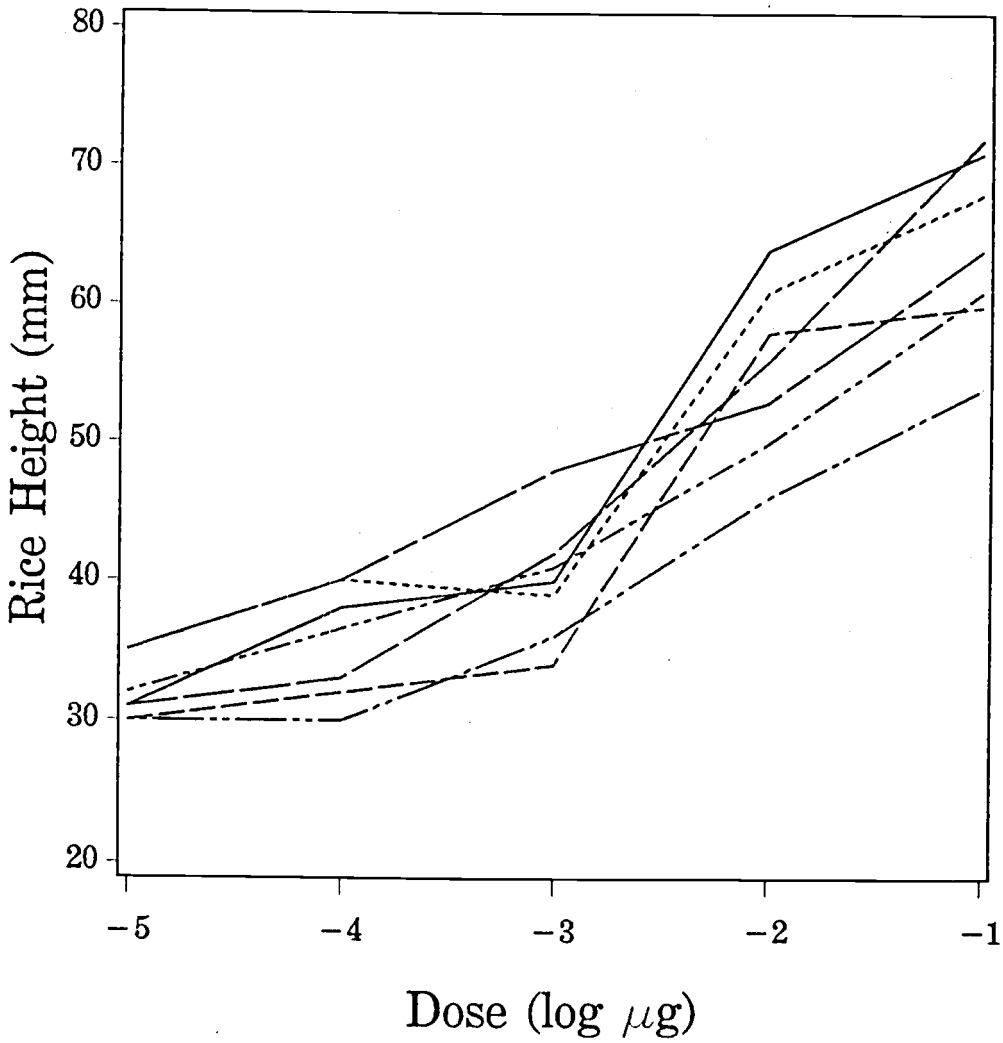

Figure 8. Standard curves of Tan-ginbozu rice response to GA_3 obtained during the development of the gibberellin bioassay. Each line is a separate assay.

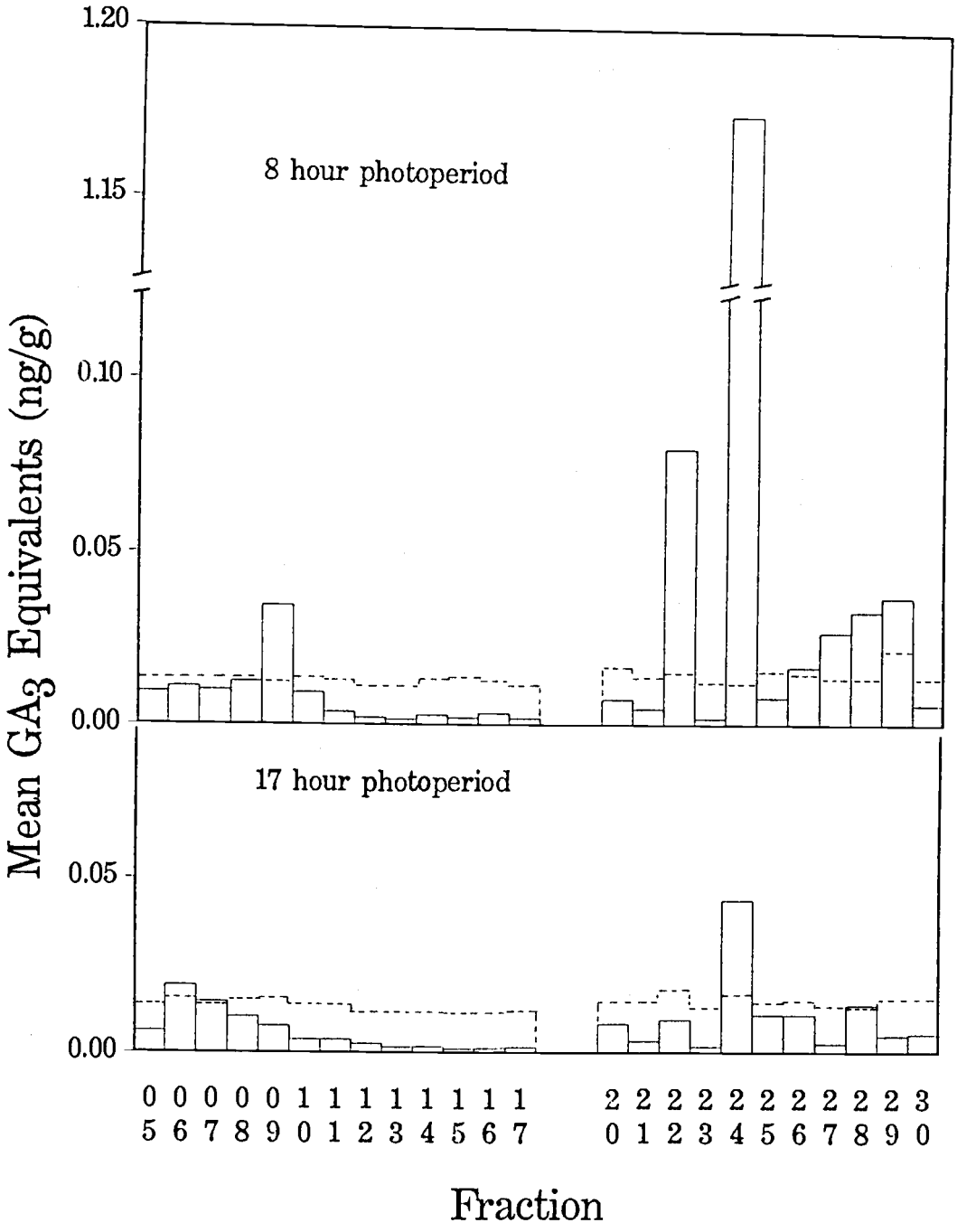

Figure 9. Mean bioassay measured gibberellin activity for HPLC fractionation of Douglas-fir foliage extract from seedlings held under 8 hour and 17 hour photoperiods. The dotted line is the 0.05% least significant difference level above the bioassay control.

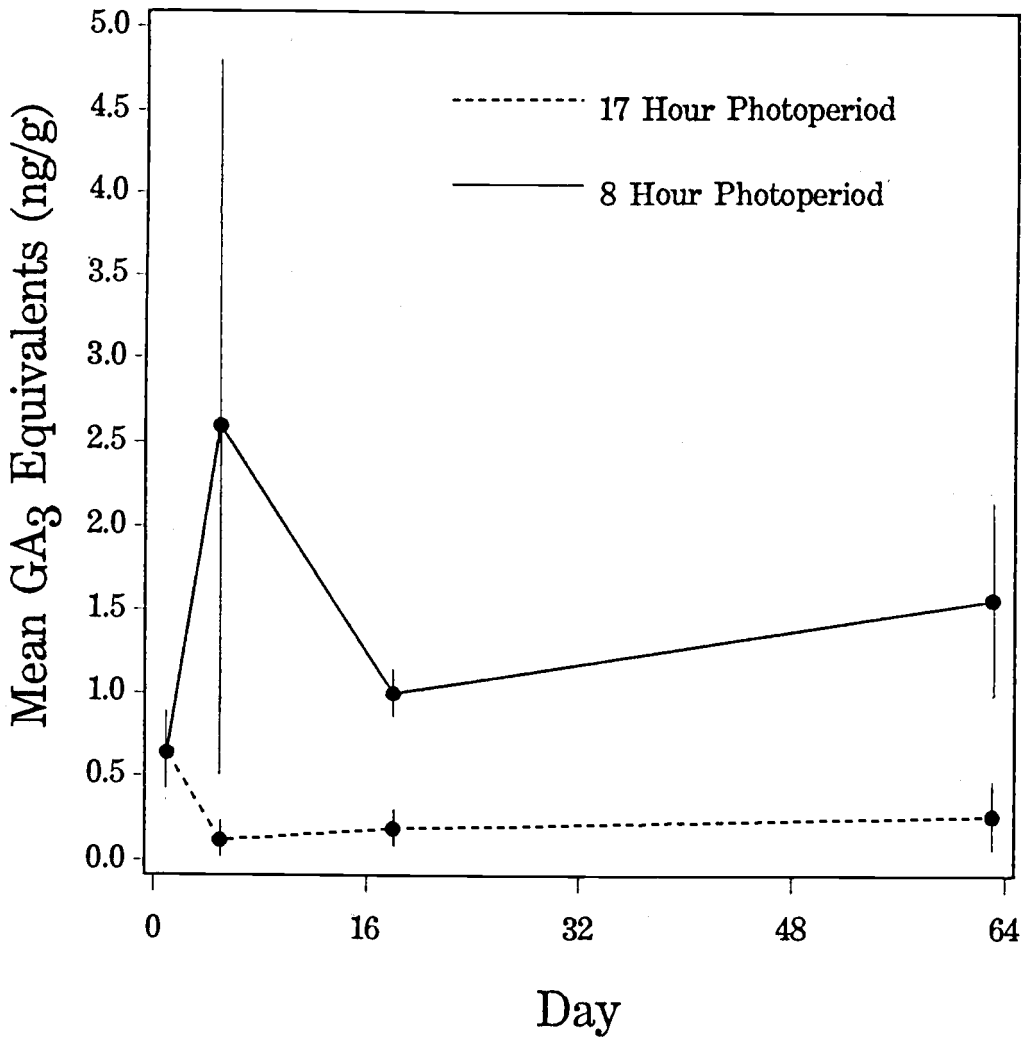

Figure 10. Bioassay measured gibberellin activity for fraction 24 of foliage extracts from Douglas-fir seedlings as they were grown under two photoperiods. Vertical lines represent one standard error.

Table 1. Analysis of variance for photoperiod and date effects on gibberellin-like activity and abscisic acid levels for Douglas-fir seedlings.

	Probability > F	
Treatment	Abscisic acid	Gibberellin
Date	0.006	0.938
Photoperiod	0.007	0.006
Date x Photoperiod	0.008	0.926

DISCUSSION

Height growth

Douglas-fir seedlings clearly responded to short photoperiods by height growth cessation and resting bud development. This observation agrees with earlier reports of photoperiodism in Douglas-fir (Vaartaga 1959, Downs 1962, McCreary et al. 1978). However, it should be noted that large differences in photoperiod do not mimic natural environments. Lavender et al. (1968) showed that cool nights or dry soil can be more important for dormancy induction than moderate changes in photoperiod. Furthermore, shortening photoperiods to 8 hours in greenhouses during summer is technically difficult (McCreary et al. 1978).

Photoperiods and ABA levels

Abscisic acid levels for Douglas-fir were higher under long photoperiods rather than short photoperiods. Given these results, it is not likely that ABA functioned as an inhibitor.

Early bioassay experiments with sycamore maple and hairy birch found higher levels of endogenous inhibitors under short photoperiods (Eagles and Wareing 1964). Purification of the inhibitory fraction revealed phenolic substances and abscisic acid, which was the most active inhibitor on a per weight basis (Cornforth et al. 1965).

The logical next question was whether ABA was the substance inducing dormancy. (Wareing and Phillips 1983). However, work with several species has repeatedly shown that ABA does not increase under short photoperiods (Zeevaart 1971b, Lenton 1972, Loveys et al. 1974, Zeevaart 1974, Powell 1976). On the contrary, most of these reports show a 2 to 3 fold increase in ABA under long photoperiods. Thus, ABA was considered to be unrelated to bud formation. Interestingly, the 4 fold increase observed in the present study agrees with these earlier reports. However, in Douglas-fir, the time required for the rise in ABA under long photoperiods was somewhat longer than for other species.

The reason for increased ABA under long photoperiods is unclear. Abscisic acid is well known for its role in stomatal closure during water stress (Davies et al. 1981). As Zeevaart (1971b) speculated, the increase in ABA under long days may be due to depressed leaf water potentials. The response of ABA is quite rapid, and diurnal fluctuations have been observed in Douglas-fir (Murphy and Ferrell 1982). Hence, despite being well watered, mild water stress could result from longer transpiration periods as well as larger seedlings occupying the same rooting volume.

Photoperiod and gibberellin-like activity

Many bioassays for gibberellin-like activity have been used (Reeve and Crozier 1976). Although all bioassays are criticized because they do not have the chemical specificity that gas chromatography--mass spectrometry (GC-MS) provides (Dunberg and Oden 1981), the dwarf rice bioassay is still widely used (Pharis et al. 1987). Dwarf rice has the broadest spectrum of response to gibberellins 1 to 38 of the bioassays reported by Reeve and Crozier (1976). More-over, Tan-ginbozu is geneticially deficient in its ability to synthesize gibberellins, yet it maintains the ability to metabolically convert GAs once formed or applied (Reeve and Crozier 1976). Thus, only GAs are known to elicit a positive response (Reeve and Crozier 1976), although many substances in plant extracts can inhibit growth of dwarf rice and mask GAs (Murakami 1968). However, because the identity of the GA is unknown with any bioassay, and because of the possibility that positively interfering materials cannot be ruled out, results must be reported as "gibberellin-like".

The bioassay for endogenous GAs is subject to considerable variation. This arises from variation in the extraction procedure, microdrop application, microdrop absorption, growth of the individual rice seedlings, and how each rice seedling responds to endogenous gibberell-

ins. Furthermore, there must be variation in endogenous gibberellin levels of Douglas-fir under the same photoperiod. However, despite these complications, significant differences in extractable GA-like materials from seedlings under long and short photoperiods were obtained.

The effect of photoperiod on endogenous gibberellins appears to be a widespread occurrence among herbaceous plants (Hoad and Bowen 1968, Proebsting et al. 1978, Metzger and Zeevaart 1982). Evidence exists for a similar pattern in woody angiosperms (Digby and Wareing 1966, Junttila 1978, Proebsting 1983). For potato (Railton and Wareing 1973), sycamore maple (Lenton et al. 1972), and Agrostemma (Agrostemma githago L., Jones and Zeevaart 1980) long photoperiods result in greater GA activity than short photoperiods. However, for spinach, transfer from short to long photoperiods produces a drop in total GA activity (Radley 1963, Zeevaart 1971). Additionally, in seasonal studies of apricot (Prunus americana L., Ramsay and Martin 1970), walnut (Landrova and Sladky 1971), and Sitka spruce (Lorenzi et al. 1975), GA-like activity declined during bud development.

Beginning in 1971, Zeevaart and coworkers (Zeevaart 1971, Jones and Zeevaart 1980, Metzger and Zeevaart 1982, Gianfagna et al. 1983) have shown that changing photoperiods induces changes in GA metabolism,

particularly in the early 13-hydroxylated pathway (GA_{12} -> GA_{53} -> GA_{44} -> GA_{19} -> GA_{20}). For spinach, short to long day transfer results in a drop in GA_{19} and a rise in GA_{20}. Furthermore, short days result in depressed activity of enzymes oxidizing GA_{19} to GA_{20} (Gilmour et al. 1986). Similar GA metabolism occurs with G2 peas (Pisum sativum L.), although the control point is earlier in the pathway and the relationship to photoperiod is reversed (Davies et al. 1986). It is uncertain if GA_{20} is the active growth regulator, or if it is metabolized to GA_1 for activity. For corn, Phinney (1984) gives strong evidence from the use of dwarf mutants that GA_1 is the active gibberellin. However, GA_1 has not been found in either spinach (Metzger and Zeevaart 1980) or G2 peas (Davies et al. 1982).

Despite the metabolic connection, the endogenous level of GA_{19}, GA_{20}, and GA_1 have not been equal. For Sorghum (Sorghum bicolor L.) these quantities were 8.8, 1.5, and 0.7 ng/g dry weight for GA_{19}, GA_{20}, and GA_1, respectively (Rood et al. 1986). For willow (Salix dasyclados Wimm.) these quantities were 0.798, 0.086, and 0.124 ng/g fresh weight for GA_{19}, GA_{20}, and GA_1, respectively (Junttila et al. 1988). Consequently, for willow, GA_{19} was the major source of bioassay activity.

It is reasonable, therefore, that HPLC fraction 24 of the present study is either GA_{19} or another GA with a

similar precursor role in Douglas-fir as GA_{19} has in spinach. Thus, the rise of fraction 24 under short photoperiods would be a result of blockage of GA metabolism and the build-up of the GA before the blockage point. Earlier work by thin layer chromatography suggests that GA_3 is the major GA in Douglas-fir (Crozier et al. 1970). However, the pathway leading to GA_3 is primarily known from the fungus Gibberella fujikuroi (MacMillan 1984), and this pathway is not characteristic of higher plants (Graebe 1987). Moreover, McMullan (1980), working with Douglas-fir, used silica gel chromatography as means of separation and found most activity in the GA_9 zone with little activity in the GA_3 zone. Additionally, recent GC-MS analysis of Douglas-fir has found GA_9 and GA_4 (Patrick Dumas 1989, personal communication). Therefore, considering this GS-MS finding and the expected retention time of GAs (Koshioka et al. 1983), fraction 24 is most likely GA_9.

If GA metabolism for Douglas-fir is similar to spinach, then a rise in some GAs would accompany a fall in others. Although fraction 6 activity was greater under long photoperiods and became significantly different from the control, the increase was small and cannot account for the drop in fraction 24. The simultaneous increase in one chromatographic zone of GA activity and decrease in

another zone has been reported during photoperiod treatment of spinach (Zeevaart 1971) and in the annual cycle of sitka spruce (Lorenzi et al. 1975). However, other studies of photoperiodism (Proebsting et al. 1978, Jones and Zeevaart 1980) have not reported a simultaneous increase and decrease in chromatographic zones of GA activity. Rather, GA levels rose or fell equally in all zones showing activity.

Despite the similarity of the present study with the effects of photoperiod on GA levels of spinach, other possibilities for GA control of bud development must be considered. It is conceivable that fraction 24 is a deactivation product of other active GAs which are maintained at relatively low levels within the plant. If active GAs promote growth, than short photoperiods would be expected to convert active GAs to inactive catabolites. GA_{34} is an inactive catabolite of GA_4 (Graebe 1987), and GA_4 is believed to be endogenous in conifers (Dunberg and Oden 1983). Moreover, GA_{34} should have a similar retention time to fraction 24 (Koshioka et al. 1983), and dwarf rice responds, although weakly, to GA_{34} (Reeve and Crozier 1976).

Alternatively, before intensive GA research began, photoperiodism was explained by phytochrome conversion (Wareing and Phillips 1981). With this hypothesis, long nights allow substantial time periods where the unstable

but growth promotive $P_{far-red}$ is in the form of the non-promotive P_{red}. If this explanation is correct, than changes in GA levels may be incidental photoperiod effects and unrelated to photoperiod controlled growth. However, Reid (1983) postulates that GAs mediate phytochrome induced phenomenon. Similarly, Trewavas (1981) suggests that changes in tissue sensitivity, rather than growth regulator levels, control plant growth and development. Thus, photoperiod may alter the plants response to endogenous GAs, and the levels of GAs may only control the rate or intensity of a predetermined response.

Many earlier studies computed GA quantities on a dry weight basis. Therefore, comparison with the present study is difficult. Crozier et al. (1970) reported 115 ng/g dry weight GA_3 equivalents for rapidly growing Douglas-fir seed sources, and 1.2 ng/g dry weight for slowly growing seed sources. Assuming a fresh weight:dry weight ratio of 10:1, the GA quantities for the present study (0.192 ng/g and 1.75 ng/g) appear reasonable.

CONCLUSIONS

It is concluded that bud set for southern Oregon Douglas-fir is under photoperiod control. Foliar ABA

levels are affected by photoperiod. However, since ABA is a growth inhibitor, the increase in ABA under promotive photoperiods could not mediate bud development. The increase in gibberellin-like activity under short photoperiods for Douglas-fir is similar to the behavior of more thoroughly studied angiosperms. This suggests that GA metabolism is blocked prior to the biosynthesis of a physiologically active GA but after the biosynthesis of a GA which can elicit a response in the bioassay. Consequently, GA activity appears to explain some features of photoperiodism. Therefore, this experiment supports the hypothesis that environmental control of bud development is mediated by endogenous growth regulators.

EXPERIMENT II. THE EFFECTS OF GIBBERELLIN BIOSYNTHESIS INHIBITORS ON BUD DEVELOPMENT, SHOOT GROWTH, AND ENDOGENOUS GIBBERLLINS AND ABSCISIC ACID OF DOUGLAS-FIR SEEDLINGS.

INTRODUCTION

Dormancy induction is an important process in the production of high quality Douglas-fir seedlings for reforestation. However, limiting height growth and promoting bud development can be difficult. The plentiful water and nitrogen of the nursery environment can encourage multiple flushing and delay bud development of resting buds (Lavender 1984). Moreover, mid-summer, the period during which bud development is usually sought, has long photoperiods that promote flushing (McCreary et al. 1978).

The common practice is to induce bud set by mild water stress (Duryea 1984), but this does not always work. Late season growth flushes are common in Pacific Northwest nurseries. When summer rainfall is above normal, second flushing is almost assured. In this event more drastic measures such as undercutting have been used. Understandably, a wider array of techniques for encouraging bud development and controlling multiple

flushes is needed.

Several published reports have shown that chemical growth retardants are active in conifers (Pharis et al. 1967, Cheung 1975, Weston et al. 1980, Hare 1984, Wheeler 1987). However, these reports have largely studied shoot growth rather than bud set or bud flush. The first objective of this experiment was to assess the effects of two newly developed growth retardants, flurprimidol and ancymidol, for inhibiting second flushing and promoting bud development of Douglas-fir seedlings. The second objective was to determine if they affect post-dormancy growth. Additionally, the expected mode of action, altering endogenous GAs or ABA levels (Lilly Res. Labs. 1983, Norman et al. 1986), was examined. Furthermore, this experiment provided an additional test of the hypothesis that bud development is under control of endogenous GAs or ABA.

MATERIALS AND METHODS

Two trials assessing the effects of growth retardants on bud activity of Douglas-fir were made. The first trial was a preliminary test to determine whether flurprimidol or ancymidol show activity for inhibiting photoperiod-induced second flushing and if exogenous

gibberellic acid (GA_3) affects seedlings in an opposite manner. The objective of the subsequent trial was to examine growth retardant effects under a range of promotive photoperiods and to determine if endogenous GA-like activity or ABA levels are associated with any inhibition.

Trial 1

Douglas-fir seeds from a southern Oregon source were soaked overnight in mid-April 1986 and then stratified at $4^{o}C$ for six weeks. Seed was sown onto a 2:1-peat:vermiculite mixture in plastic tubes (60 ml capacity). Germination and early seedling growth occurred in a growth room under 16-hour photoperiods with 120 uE m^{-2} sec^{-1} photosynthetically active radiation (PAR), and a $22^{o}C/18^{o}C$ day/night temperature cycle. Fertilization began two weeks after emergence and was by nutrient solution made from a commercial mix (Peters Professional 20:20:20, W.R. Grace Co., Fogelsville, PA) Fertilization was at a concentration of 0.5 g/l. After establishment, the seedlings were transferred to a greenhouse and maintained under fluorescent lamps to provide a 16-hour photoperiod.

In late August, seedlings were transferred back to the growth chamber for dormancy induction. This time photoperiods were shortened to 8 hours and terminal buds began to appear within 2 weeks. By mid-October, terminal

buds were well formed and chemical treatments were begun. Seventy-five seedlings were selected from the initial stock and randomly assigned to one of four treatments. Aqueous solutions of gibberellic acid (GA_3, Sigma), flurprimidol (El-500: alpha- (1-methylethyl-alpha- (4-(tri-fluoromethoxy)phenyl)- 5-pyrimidine-methanol), and ancymidol (El-531: alpha- cyclopropyl-alapha- (4-methoxy-phenyl) -5-pyrimidinemethanol) were prepared. Five ml aliquots were applied by syringe to the rooting media at 3 day intervals until 13 mg GA_3, 5 mg flurprimidol, and 8 mg ancymidol had been administered. The dosages were a result of different solubilities of the respective chemicals. At the same time, an untreated control group was given a 5 ml injection of distilled water.

Following chemical applications, the seedlings were placed in a growth chamber under 16 hour photoperiods. Seedlings were monitored daily and the number of days to bud break recorded. By December 11, all seedlings had flushed and initiated a new bud. The length of the new flush was measured. Seedlings were then transferred back to the greenhouse and held under natural photoperiods where elongation of the new shoot continued. The final height was measured in mid-March.

Trial 2

Douglas-fir seed from the same southern Oregon

source used in trial 1 were stratified beginning in mid-December and otherwise grown as in trial 1. In early June, four months after germination, 200 seedlings were selected for uniformity and healthy appearance. These seedlings were randomly assigned to either a 15 or 17 hour photoperiods. Half the seedlings in each photoperiod were treated with flurprimidol while the remaining seedlings served as the untreated control. The two photoperiods were applied in separate growth rooms under mixed fluorescent and incandescent lamps. Photosyntheticially active radiation intensity was 120 uE m^{-2} sec^{-1} at plant height. Because the critical photoperiod for dormancy induction of Oregon Douglas-fir is about 14 hours (Downs 1962, Lavender 1981), these photoperiods were chosen to be either weakly or strongly promotive for height growth. A range of photoperiods was deemed important to enhance the expression of flurprimidol effects as well as to extend the inference of the experiment.

Since flurprimidol is rather insoluble in water and some precipitation occurred during the first trial, another application method was used. The flurprimidol was prepared by dissolving 0.5 gm of technical material in 95% ethanol and then diluting with distilled water to 0.5 liter. Five ml aliquots containing 5 mg of flurprimidol in 1% ethanol were applied by syringe to the rooting media. Two application were made to give approximately 10

mg per seedling. As a control, a 1% solution of ethanol was injected into the potting mix of the untreated seedlings.

Initial bud set was induced by the change in environment when the seedlings were transferred from the greenhouse to the artificially illuminated growth room. Seedling height, caliper, and bud flush were measured biweekly throughout the summer. In late August, 2 groups of 12 seedlings from each treatment combination (for a total sample size of 8) were harvested for growth regulator analysis. Harvesting consisted of removing the foliage, recording the foliage fresh weight, and quickly transferring the sample to a -60°C freezer. Growth regulator analyses were by the same procedure described in experiment I.

On October 15 seedlings were brought into one growth room and exposed to 8 hour photoperiods and a 22°/18°C temperature cycle. Starting in early November, seedlings were given a 12 week chilling period at 5°C. Following chilling, seedlings were placed under a 16 hour photoperiod and a 22°/18°C temperature cycle. The seedlings promptly flushed, and after two months the new growth was harvested. The length of the new shoot was recorded, the number of needles tallied, and the dry weight measured. The number and length of stem units for

the new flush were computed by considering each needle to represent a stem unit. A stem unit is defined by Doak (1935) as a leaf and internode.

Statistical analysis

Treatment differences for both trials were analyzed by analysis of variance using a general linear models procedure (SAS Institute Inc. 1985). Both trials were considered completely randomized designs. Separation of means were computed with Fishers protected least significant difference (Steel and Torrie 1980). Treatment differences for percent bud flush in trial 2 were assessed by chi-square (Zar 1974) with separate analyses for each photoperiod. As in experiment I, $\log_{10}$ transformations of growth regulator data were made prior to analysis of variance and computation of least significant differences (Zar 1974). Means and least significant differences were not transformed for graphical presentation (figure 16).

RESULTS

Trial 1

The growth retardant treatments did not prevent flushing. However, the progression of flushing after the application of the chemicals resulted in a clear pattern of delayed flushing (figure 11). Seedlings treated with

GA_3 tended to break bud earlier than the untreated controls while seedlings treated with growth retardants flushed later than the untreated controls (table 2). Furthermore, analysis of variance showed the average time to bud break to be statistically different for all treatments (table 3).

The pattern of height increment among treatments was similar to that of bud break (figure 12). Seedlings treated with GA_3 had twice the height growth of the untreated controls while the flurprimidol-treated seedling had half the height growth of the untreated controls (table 2). Growth of ancymidol-treated seedlings was between flurprimidol-treated seedlings and the untreated controls. Analysis of variance confirmed significant differences in growth among treatments (table 3).

Trial 2

From the results of trial 1, it was clear that both growth retardants were active on Douglas-fir seedlings, and that they act opposite to GA_3. Furthermore, flurprimidol had a greater effect than ancymidol. Therefore, only flurprimidol was used in trial 2.

All seedlings set bud when transferred to the growth room. Terminal buds were evident within two weeks. As expected, many seedlings under the longer 17 hour photoperiod second flushed. The progression of flushing

was not immediate but gradually continued over the summer (figure 13). Prior to winter chilling more than 80% of the untreated controls under the 17 hour photoperiod had flushed, while only 20% of the controls under the 15 hour photoperiod had flushed.

Similar to trial 1, flurprimidol produced a reduction in the rate of flushing compared to the untreated control. Although the dosage was higher in the second trial, flurprimidol did not completely eliminate second flushing. Fifty percent of the retardant treated seedlings flushed under the 17 hour photoperiod. At the time of sampling for growth regulator assay, chi square analysis indicated that bud flush was significantly associated with flurprimidol treatment under the 17 hour photoperiod (table 4). However, due to the reduced sample size at the end of the growth period, the association was not as strong as prior to harvest (table 4). Flurprimidol was not significantly associated with bud flush under 15 hour photoperiods for either date ($p > 0.10$).

Following transfer to the growth room, height growth was sharply reduced (figure 14). However, second flushing, particularly under the 17 hour photoperiod, produced a late season height gain. The treatment effects on height growth were similar to bud flush (table 5). Analysis of variance showed a significant flurprimidol-photoperiod interaction for height growth (table

6). Therefore, flurprimidol effects were significant under the 17 hour photoperiod but not under the 15 hour photoperiod (table 5). Diameter growth was significantly affected by flurprimidol but not by photoperiod (table 6). There was no flurprimidol x photoperiod interaction on diameter growth (table 6). Mean diameter growth was 1.42 mm and 1.31 mm for the untreated control and flurprimidol, respectively.

The most striking growth effects from flurprimidol appeared following chilling. Analysis of variance indicated a significant photoperiod-flurprimidol interaction for stem unit count, new shoot length, and new shoot dry weight (table 6). For the seedlings grown under 15 hour photoperiods, which generally had earlier and more normally timed bud set, flurprimidol caused a strong depression in post-chilling growth (table 7, figure 15). New shoot length was strongly affected and was only one-third that of the untreated control. For shoot growth components computed by ratios, analysis of variance confirmed significant treatment effects but not significant interactions (tables 6 and 8). Interestingly, although most components of growth were significantly reduced by flurprimidol, both weight to length ratio and the average stem unit dry weight were sharply increased (table 8). Thus, the growth retardant appeared to cause a

reallocation of growth away from elongation toward girth.

Growth regulator analysis

The bioassay of composited seedlings showed GA activity in several of the 30 HPLC fractions (figure 16). However, one fraction, 24, was the most consistent, and, for the control group, contained by far the majority of the GA activity. Fractions 8, 22, and 26 also showed occasional activity.

Flurprimidol treated samples were compared with the untreated controls for two categories of gibberellin activity : fraction 24 alone, and total activity for all fractions. For fraction 24, there was a sharp drop in GA activity for the treated seedlings (table 9) which analysis of variance found to be statistical significant (table 6). Total GA activity also had a large reduction, but statistically the difference was only weakly significant (table 9). The 15 and 17 hour photoperiod treatments had no discernible effect on gibberellin activity nor was there a significant interaction between flurprimidol and photoperiod.

Abscisic acid was found in either HPLC fraction 18 or 19. Both fractions were analyzed and summed for ABA quantitation. Similar to GA activity, analysis of variance showed a significant depression in ABA levels after flurprimidol treatment (tables 6 and 9). Photoperiod also had an apparent effect on ABA levels but was

only weakly significant. The flurprimidol x photoperiod interaction was not significant.

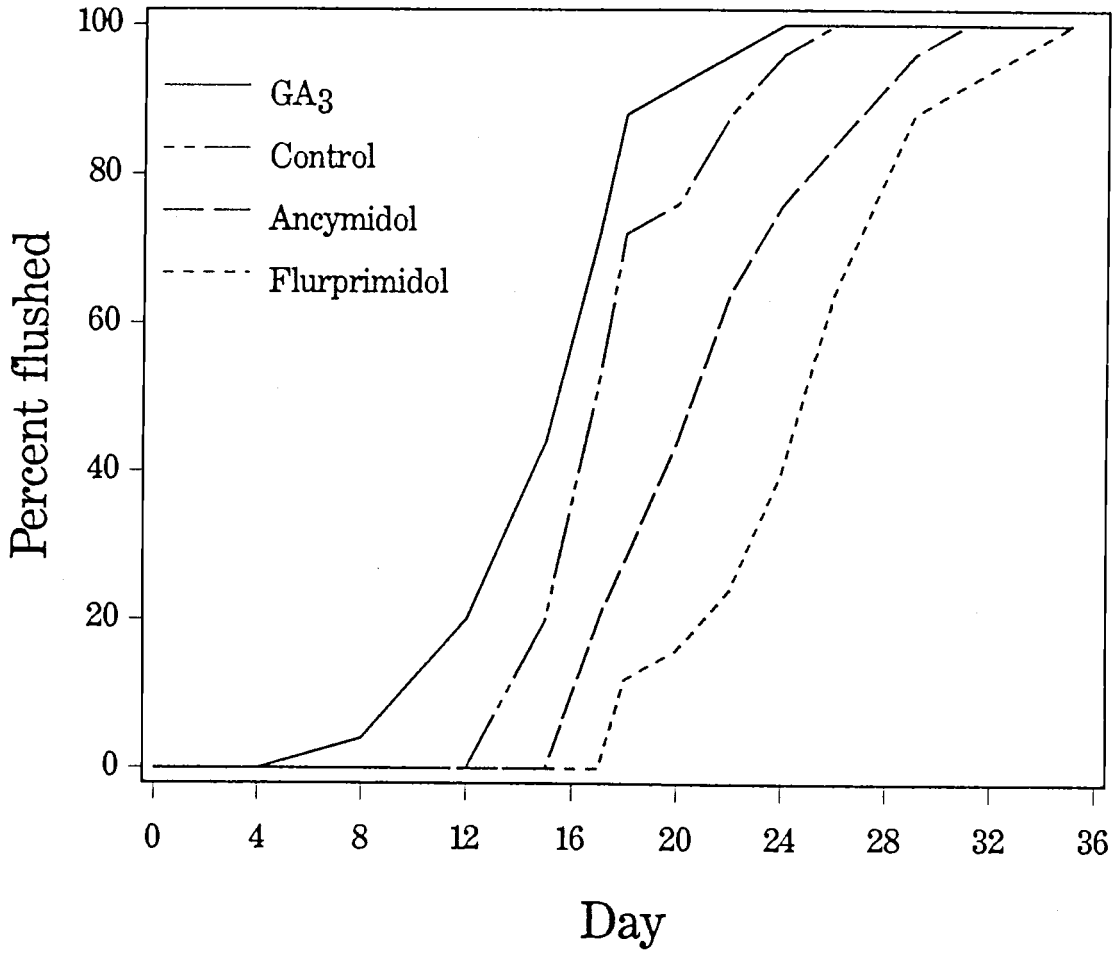

Figure 11. The progression of second flushing for Douglas-fir seedlings under a 16 hour photoperiod following growth retardant or gibberellic acid treatments (trial 1).

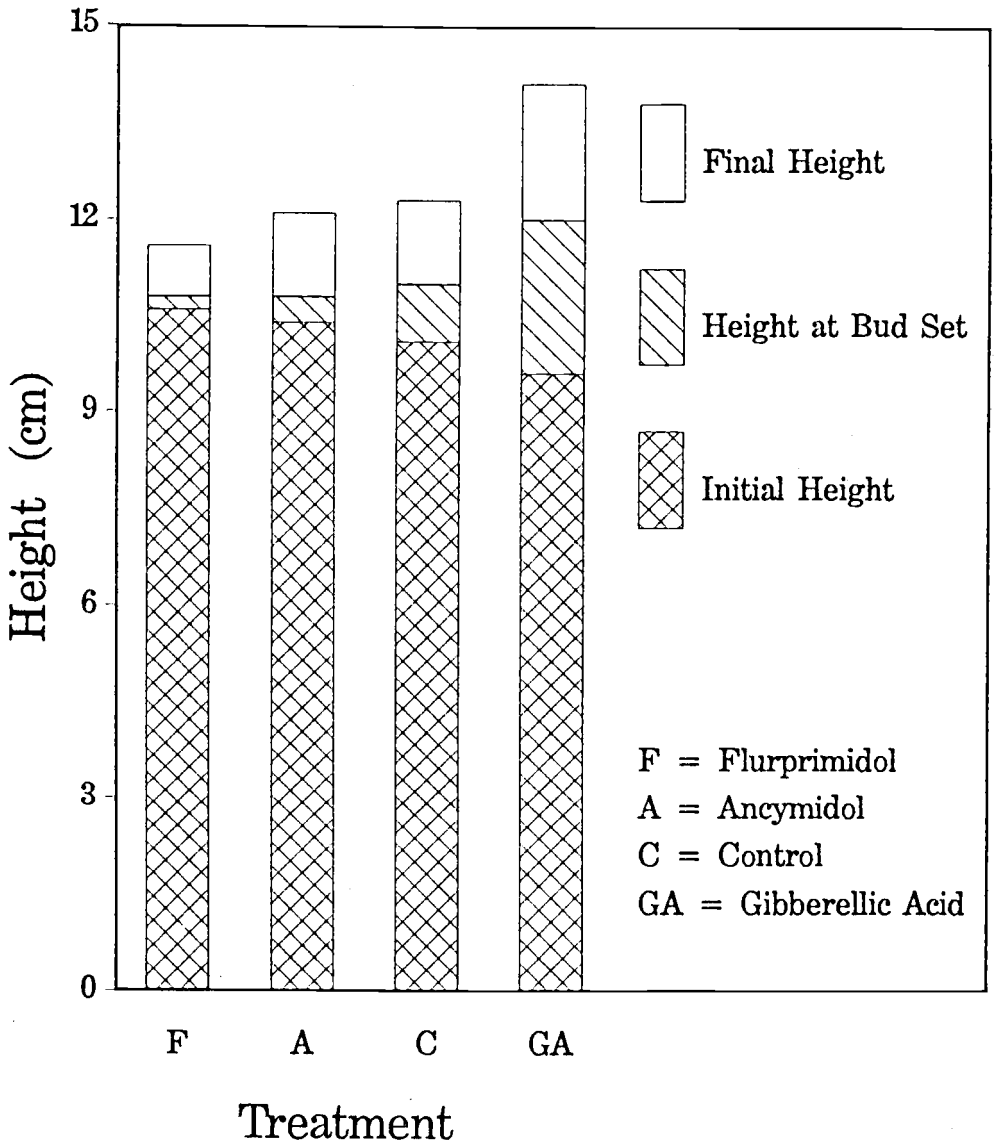

Figure 12. The mean height of Douglas-fir seedlings following chemical treatments and measured prior to treatment (initial height), after new buds became evident (height at bud set), and after growth had ceased (final height), (trial 1).

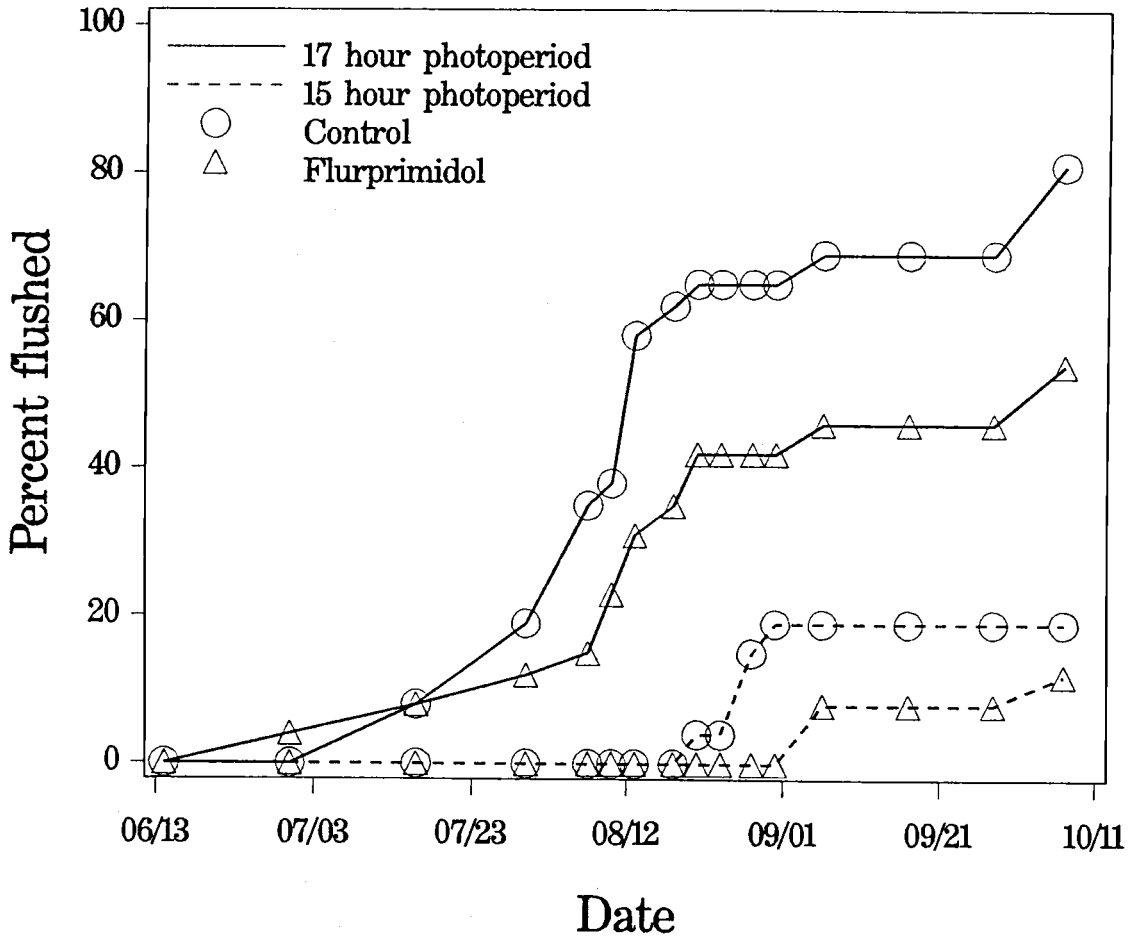

Figure 13. The progression of second flushing for Douglas-fir seedlings under a 15 or 17 hour photoperiod after flurprimidol treatment (trial 2).

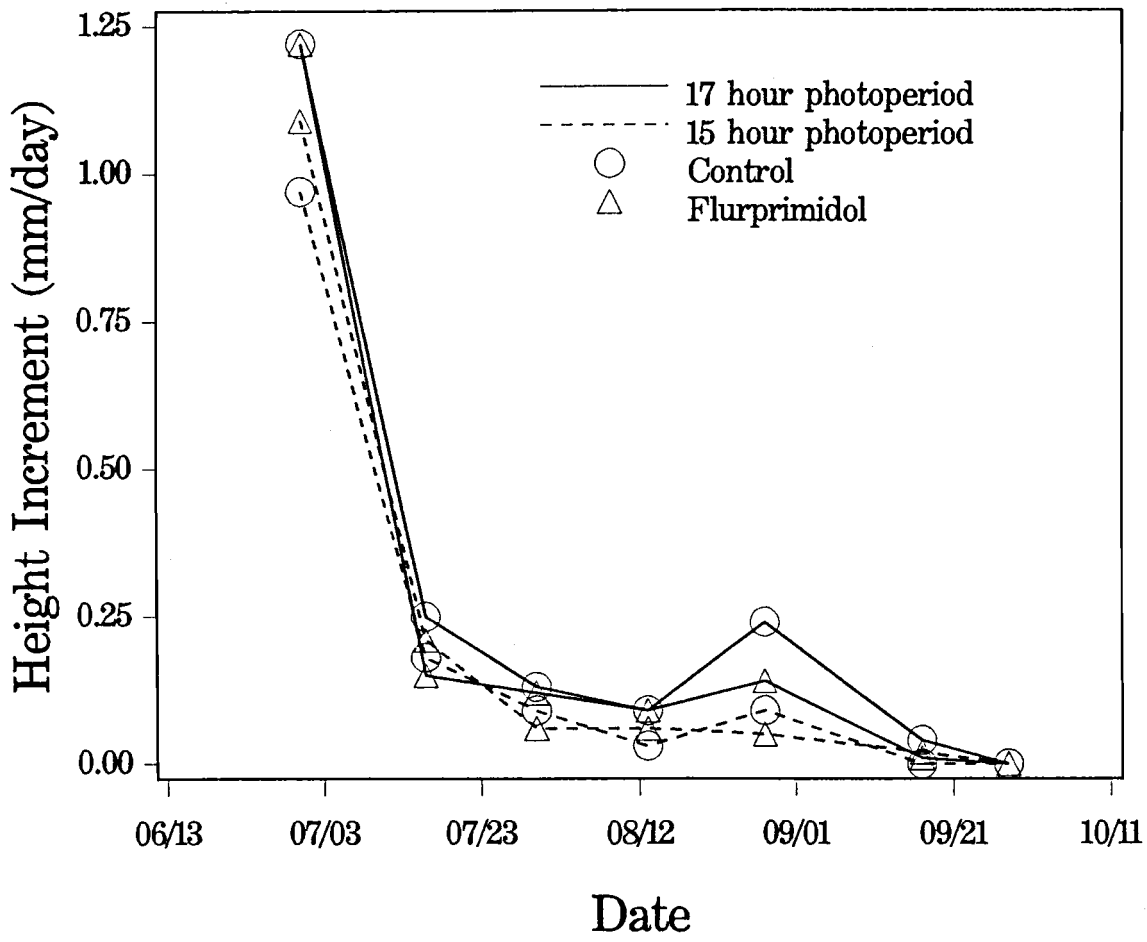

Figure 14. The change in height increment for Douglas-fir seedlings following the transfer from a greenhouse to a growth room (trial 2).

Figure 15. Flurprimidol treated (left) and untreated (right) Douglas-fir seedlings after a 12 week chilling period followed by a 2 month flushing period (trial 2).

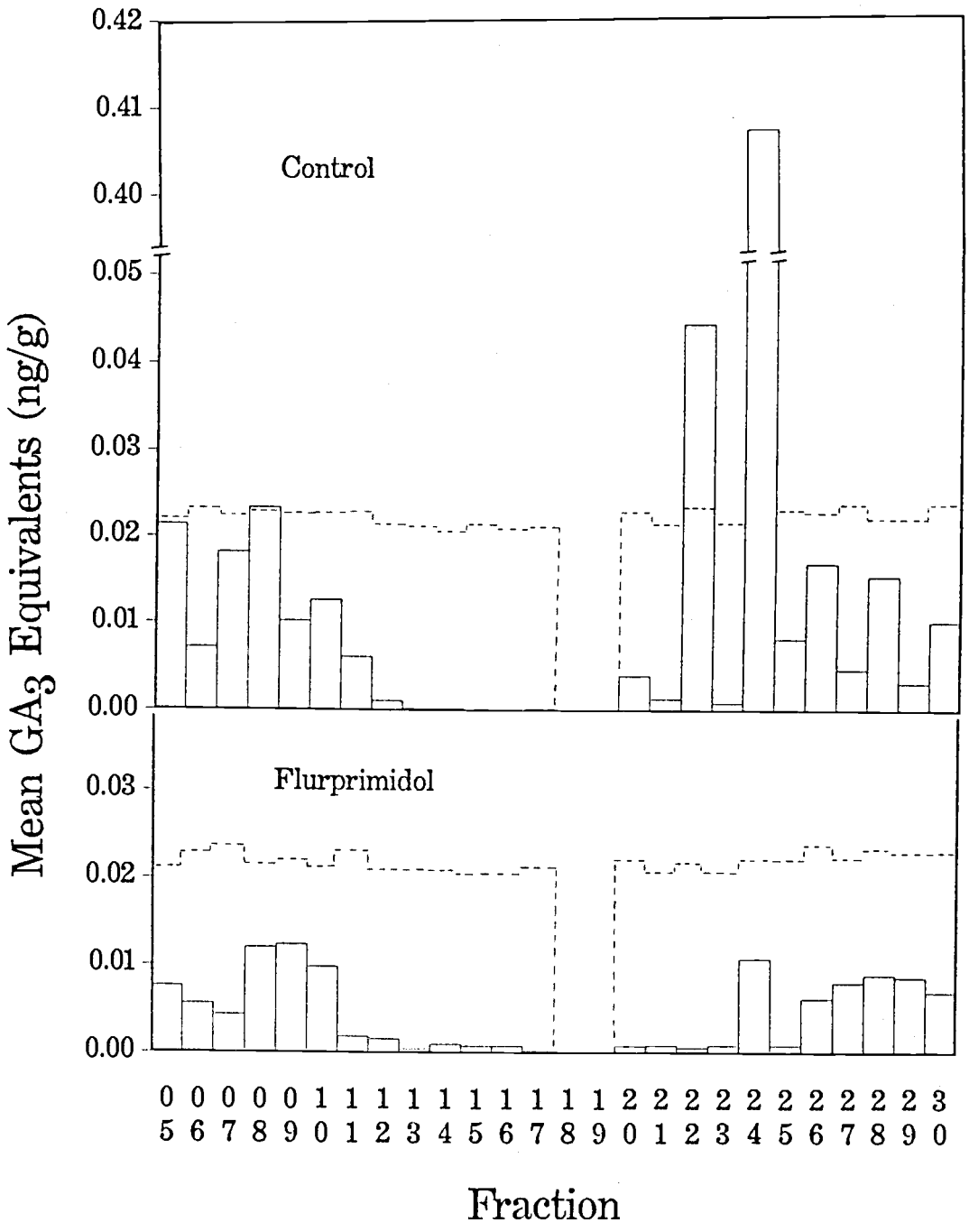

Figure 16. Mean bioassay measured gibberellin activity for HPLC fractionation of Douglas-fir foliage extract from seedlings treated or not treated with flurprimidol. The dotted line is the 0.05% least significant difference level above the bioassay control (trial 2).

Table 2. The mean flushing responses of Douglas-fir seedlings under 16 hour photoperiods following treatment with chemical growth retardants (trial 1). *

	Treatment			
Flushing response	GA_3	Control	Ancymidol	Flurprimidol
Days to flush	16 a	18 b	22 c	26 d
Initial growth (cm)	2.42 a	0.87 b	0.36 c	0.19 c
Growth after bud initiation (cm)	4.44 a	2.22 b	1.70 b	0.92 c
Final height (cm)	14.1 a	12.3 b	12.1 bc	11.6 c

* Means in the same row with different letters are significantly different at the 0.05% level.

Table 3. Analysis of variance for flushing responses of Douglas-fir seedlings under 16 hour photoperiods following four chemical treatments (trial 1).

Dependent variable	df error	mean square error	F value	Prob > F
Days to Flush	96	15.31	29.45	0.001
Initial growth (cm)	96	0.467	55.14	0.001
Growth after bud initiation (cm)	96	1.278	44.84	0.001
Final height (cm)	96	1.761	16.54	0.001

Table 4. The percentage of second flushing for flurprimidol treated and untreated Douglas-fir seedlings under 17 hour photoperiods and associated chi square probabilities (trial 2). *

Date of Analysis	Sample Size	Percent second flush		Chi Square probability
		Control	Flurprimidol	
August	100	35.0	18.0	0.001
October	52	80.8	53.9	0.039

*Chi square tests the probability of independence between second flushing and flurprimidol treatment.

Table 5. The height growth Douglas-fir seedlings under 17 or 15 hour photoperiods and treated or untreated with flurprimidol (trial 2). *

	17 Hour		15 Hour	
Seedling trait	Control	Flurprimidol	Control	Flurprimidol
Height growth (cm)	3.0 a	2.6 b	2.3 bc	2.1 c

* Means in the same row followed by different letters are significantly different at the 0.05% level.

Table 6. Analysis of variance for responses of Douglas-fir seedlings to photoperiod and flurprimidol treatments (trial 2).

Dependent variable	df error	Mean square error	Flurprimidol F value	Flurprimidol Prob > F	Photoperiod F value	Photoperiod Prob > F	Flurprimidol X Photoperiod F value	Flurprimidol X Photoperiod Prob > F
Initial growth								
Height (cm)	100	0.597	0.66	0.419	16.71	0.001	4.23	0.042
Diameter (mm)	100	0.071	5.39	0.022	0.07	0.797	0.03	0.854
Post-dormancy growth								
Stem unit count	90	155.05	1.79	0.184	55.66	0.001	5.44	0.022
Length (mm)	90	41.68	94.04	0.001	56.16	0.001	33.01	0.001
Dry weight (mg)	90	911.57	17.06	0.001	39.86	0.001	10.90	0.001
Stem unit length (mm)	90	0.0251	101.50	0.001	0.44	0.508	3.33	0.071
Weight to length ratio (mg/mm)	90	1.744	18.23	0.001	9.85	0.002	1.65	0.202
Dry weight per stem unit (mg)	90	0.261	21.24	0.001	10.80	0.001	2.57	0.112
Growth regulator analysis								
GA, Fraction 24 (ng/gm)	4	0.283	17.58	0.014	1.39	0.304	2.08	0.222
GA, total (ng/gm)	4	0.264	5.30	0.083	0.05	0.826	1.99	0.231
ABA (ng/gm)	4	0.012	32.17	0.005	4.19	0.110	2.37	0.199

Table 7. Mean post-chilling flush characteristics which had treatment interactions for Douglas-fir seedlings following photoperiod and flurprimidol treatments (trial 2). *

	Previous season photoperiod			
	17 hour		15 hour	
Flush trait	Control	Flurprimidol	Control	Flurprimidol
Stem unit count	23.6 a	25.7 a	49.3 c	39.4 b
Length (mm)	12.7 b	7.2 a	31.0 c	10.1 ab
Dry weight (mg)	26.5 ab	20.4 a	86.6 c	39.3 b

* Means in the same row followed by different letters are significantly different at the 0.05% level.

Table 8. Mean post-chilling flush characteristics that did not have treatment interactions for Douglas-fir seedlings following photoperiod and flurprimidol treatments (trial 2). *

	Retardant			Photoperiod (hrs)		
Flush trait	Control	Flurprimidol	P > F	15	17	P > F
Stem unit length (mm)	0.57	0.30	(0.001)	0.65	0.26	(0.508)
Weight to length ratio (mg/mm)	2.05	2.56	(0.001)	2.87	4.08	(0.002)
Dry weight per stem unit (mg)	1.13	0.80	(0.001)	0.98	1.65	(0.001)

* Probability values are the probability of no difference between the previous two means in the same row.

Table 9. Growth regulator analysis for Douglas-fir seedlings after flurprimidol and photoperiod treatments. Each mean is the average of four samples, and each sample was 12 composited seedlings. No statistically significant interactions occurred. Gibberellin is GA_3 equivalents (trial 2). *

Growth Regulator	Retardant			Photoperiod (hrs)		
	Control	Flurprimidol	P > F	15	17	P > F
GA, HPLC fraction 24 (pg/g fresh weight)	606	32.5	(0.014)	423	215	(0.304)
GA, total activity (pg/g fresh weight)	1200	296	(0.083)	549	947	(0.826)
ABA (ng/g fresh weight)	36.5	12.5	(0.001)	30.7	18.3	(0.110)

* Probability values are the probability of no difference between the previous two means in the same row.

DISCUSSION

Douglas-fir has previously been found responsive to the growth retardant paclobutrazol (Wheeler 1987) and possibly AMO-1618 (Pharis et al. 1967). The present experiment has shown that flurprimidol is also effective on Douglas-fir seedlings. Using loblolly pine and slash pine in a seed orchard, Hare (1984) found that flurprimidol was highly effective for reducing growth. Moreover, Hare (1984) reported complete elimination of second flushing when flurprimidol was used. However, in Hare's study the dosage of flurprimidol was far above that used in the present study.

The first trial showed that exogenous GA_3 also affected growth but opposite that of retardants. However, in this experiment, to conserve the supply of growth retardant, GA_3 was not applied to the same seedlings. Previous studies (Pharis et al. 1967, Dunberg and Eliasson 1972) have partially counteracted growth retardant effects on conifers by applying GA_3 to the same seedling. The ability of GA_3 to counteract retardants depended on species and the specific retardant.

The second trial confirmed the ability of flurprimidol to reduce second flushing. However, the effect on growth during the season of application was otherwise

limited. In contrast, Steffens (1988) has found that flurprimidol clearly inhibits the current years growth of apple (Malus domestica Borkh.). Moreover, inhibition was evident at 0.5 mg per plant which is 1/20 the dosage of the present study. If flurprimidol had been applied in the present study under higher light intensities, and thus higher growth rates, shoot growth inhibition prior to dormancy might have been stronger.

It was thought that chilling would remove the retardant effects and that normal growth would resume in the spring. However, the most striking effects of flurprimidol occurred after chilling and growth resumption. Similar effects were reported by Sterrett and Tworkoski (1987). They found that flurprimidol reduced the growth of black walnut (Juglans nigra L.) for up to 17 months. Furthermore, flurprimidol persisted in the soil for up to 1 1/2 years which may have caused the long-term growth effect. As a consequence, flurprimidol does not appear useful for reforestation seedling production where growth after outplanting must be maximized. However, as new growth retardants are developed, a compound which is potent yet not persistent may still find utility. Steffens (1988) noted that the important difference between the retardants paclobutrazol, flurprimidol, and XE-1019 was not potency but residual activity with XE-1019 being the most persistent.

The increase in the length to weight ratio of the new shoot was an unexpected result. However, this may be explained from the cellular mode of action of GAs. Like auxin, GAs are known to stimulate cell division and especially longitudinal elongation (Metraux 1987). Therefore, depressing the natural level of GA in a growing shoot by flurprimidol may reduce elongation more than division and thus produce a thicker stem.

Working with Arizona cypress, Kuo and Pharis (1975) first reported reduced endogenous gibberellin levels in conifers following growth retardant treatment. The results of the present study extend this relationship for flurprimidol on Douglas-fir. Abscisic acid levels in Douglas-fir were also reduced by flurprimidol. A similar phenomenon was reported by Norman et al. (1983) for growth retardants applied to the fungus Cercospora. Although these two growth regulators have opposing effects in some tissues (Jacobsen and Chandler 1987), ABA does not appear to have a regulatory role in shoot elongation (Powell 1982). Hence, ABA levels may be unimportant, and GA levels may be the controlling factor for growth retardant activity.

It has been accepted that most growth retardants act, in part, by restricting gibberellin biosynthesis (Graebe 1987). However, the inhibition of ABA biosyn-

thesis indicates that flurprimidol has broader effects. Similarly, Douglas and Paleg (1974) reported that AMO-1618 inhibits the biosynthesis of sterols in tobacco. They argue that sterol depression would reduce growth by affecting membrane function. Norman et al. (1986) showed that ancymidol inhibits terpenoid biosynthesis prior to farnesyl pyrophosphate (FPP) in Cercospora. Since FPP is a precursor to both ABA and GA, this may account for the depression of both ABA and GA and would also explain sterol depression. Furthermore, in immature wild cucumber endosperm, ancymidol can reduce the incorporation of mevalonic acid to kaurene, the precusor to gibberellins (Coolbaugh and Hamilton 1976). However, the inhibition of several oxidation steps in the metabolism of kaurene to GA_{12} by ancymidol is much stronger (Coolbaugh et al. 1978). The greater inhibition of GA than ABA in the present study suggests that the mechanism of action of flurprimidol in Douglas-fir is similar to ancymidol in cucumber endosperm. Consequently, gibberellin inhibition may be the primary means of affecting growth. However, the lack of specificity of some retardants and the apparent inhibition of early steps in terpenoid metabolism emphasize that non-gibberellin growth effects are possible.

CONCLUSIONS

Both flurprimidol and ancymidol are effective in delaying photoperiod induced bud flush of Douglas-fir seedlings. Exogenous GA_3 hastens photoperiod induced bud flush. Flurprimidol had a greater inhibitory effect under longer photoperiods than shorter photoperiods. Post-dormancy growth was reduced by earlier application of flurprimidol. Both endogenous ABA levels and GA-like activity were reduced following flurprimidol application. The depression in GA levels can explain the growth effects of flurprimidol.

EXPERIMENT III. SPRING GROWTH OF DOUGLAS-FIR SEEDLINGS FOLLOWING PHOTOPERIOD OR FLURPRIMIDOL TREATMENT.

INTRODUCTION

The spring height increment of conifer seedlings has considerable importance to reforestation. Rapid growth following outplanting enables seedlings to better compete with encroaching vegetation and escape browsing animals (Howard and Newton 1984). Furthermore, increased root growth and drought avoidance are associated with vigorous and early shoot growth (Heiner and Lavender 1976).

For analytical purposes growth can be divided into fixed and free growth (Jablanczy 1971, Pollard and Logan 1974). Fixed growth is a shoot increment that contains only stem units held in the overwintering bud, whereas free growth is an increment that contains stem units initiated after growth resumption (Lanner 1976). A stem unit, as defined by Doak (1935), is an internode plus its appendage. In addition, growth may be partitioned into total stem unit number and average stem unit length (Macey and Arnott 1986).

The magnitude of the spring growth flush of con-

ifer seedlings can be considered from several viewpoints. One approach is to regard shoot growth as a function of seedling quality (Burdett 1983). Thus, new growth is regulated by physiological parameters such as dormancy, mineral nutrition, water status, and carbohydrate reserves (Ritchie 1984). Physiological impairment can lead to reduced growth or no growth (Romberger 1963, Lavender and Stafford 1985).

A second approach has been to consider spring growth as the expansion of a preformed shoot. For trees, the correlation between height increment and bud size has been known for some time (Clements 1970, Kozlowski et al. 1971, Garrett and Zahner 1973). For seedlings the relationship is less precise because free growth can occur, especially under the age of 5 (Jablanczy 1971). However, for black spruce (Picea mariana (Mill.) B.S.P.), Columbo (1986) reported greater fixed growth but not greater free growth for seedlings with superior bud development. For Douglas-fir, percent bud break (Thompson 1982) and thus possibly field survival increase with bud size.

Short photoperiods provide a strong stimulus for bud development (Lavender 1981). Consequently, long photoperiods impair bud development, and, if applied late in the growing season, can reduce post-dormancy growth (Lavender and Stafford 1985). A current hypothesis for

photoperiod control of bud formation is altered GA metabolism (Experiment I). Therefore, growth retardants, which inhibit GA biosynthesis (Experiment II), should counteract the effect of long photoperiods and allow normal spring growth. The objective of this experiment was to assess the ability of the growth retardant flurprimidol to counteract the effects of long photoperiod prior to dormancy. The effects on both overwintering bud morphology and post-dormancy shoot growth were examined.

MATERIALS AND METHODS

Douglas-fir seed from a southern Oregon seed source were soaked overnight in mid-December 1986 and then stratified at 4°C for six weeks. Seed were allowed to germinate at 22°C in a heated greenhouse. Following radicle emergence, the seed was hand placed onto a 2:1 peat vermiculite potting mix in Ray-Leach single cells (60 ml capacity). A course grade industrial sand was used as a seed covering.

Both germination and early growth were conducted in the greenhouse under fluorescent lamps to provide photoperiod extension up to 16 hours. During the first several weeks of growth, the seedlings were watered daily

and fertilized weekly. Fertilization was by nutrient solution made from a commercial mix (Peters Professional 20:20:20, W.R. Grace Co., Fogelsville, PA) at a concentration of 0.5 gm per 1.

In early June, four months after germination, approximately 200 seedlings were selected for uniformity in size and a healthy outward appearance. These seedlings were randomly assigned to either an 8 or a 17 hour photoperiod. It was expected that this would produce seedlings with early and late bud set, respectively. The photoperiod treatments were applied in growth rooms with mixed fluorescent and incandescent lamps providing 120 uE m^{-2} sec^{-1} photosynthetically active radiation (PAR) at seedling height. The temperature was set for a 22°C/ 18°C day/night cycle. Fertilization continued as before, but watering was reduced to every other day.

Initial bud set was induced by the change in environment when the seedlings were transferred from a greenhouse to an artificially illuminated growth room. At the time of transfer, 40 seedlings under the long photoperiod regime were given 5 mg of flurprimidol (El-500: alpha-(1-methylethyl- (4- (triflоromethoxy)phenyl)- 5-pyrimidine-methanol). Flurprimidol was prepared by dissolving 0.5 gm of technical material in 5 ml of 95% ethanol. This solution was then diluted to 500 ml with

distilled water. Five ml aliquots containing 5 mg flurprimidol in 1% ethanol were then injected into the rooting medium by syringe. Flurprimidol was readily soluble in 95% ethanol but formed a cloudy suspension upon dilution with water. During injection some flurprimidol precipitated on the syringe and the walls of the dilution flask; however, most entered the potting mix as a finely divided suspension.

On October 1, the 17 hour photoperiod was reduced to 8 hours. This prevented further flushing and promoted bud development. Also, several weeks of short photoperiods is necessary if growth is to resume following chilling (Lavender and Stafford 1985). After three weeks of the 8 hour photoperiod treatment, both groups of seedlings were combined, and the growth room temperature was lowered to 4^{o}C for winter chilling.

At the beginning of the chilling period, 20 seedlings from each group (8 hour photoperiod, 17 hour photoperiod, and 17 hour photoperiod with flurprimidol) were randomly selected and frozen for subsequent bud examination. The number of needle primordia in the terminal bud of each seedling were counted under a dissecting microscope. The counting technique involved removing the bud scales with tweezers, and then moistening the apex

with tween-20 (Baker). The primordia were then carefully removed, spread out, and tallied. This technique was judged more accurate than counting the number of spirals (parastiches) and estimating the average number of primordia per spiral.

In early February, after 14 weeks of chilling at a constant 4°C, the remaining seedlings of each treatment were randomly assigned to two groups. One group was placed in an 8 hour photoperiod, 22°C growth room. The other group was placed in a 16 hour photoperiod, 22°C growth room. The 8 hour photoperiod has been reported to prevent free growth in spruce seedlings, while the 16 hour photoperiod promotes free growth (Pollard and Logan 1974, Macey and Arnott 1986).

After two months at the warmer temperature, all seedlings had flushed, set a bud, and the new growth appeared lignified. The new growth was then harvested, the total length recorded, and the number of needles tallied. The stem and foliage were then oven dried and the dry weight measured. Free growth was estimated by subtracting the mean primordia count from the mean stem unit count. Each needle was considered to represent a stem unit.

The treatment differences in this study were analyzed by analysis of variance using a general linear

models procedure (SAS Inst. Inc. 1985). The experiment was considered a completely randomized design with unequal replications. Separation of means was computed by Fisher's protected least significant difference (Steel and Torrie 1980). The association between flushing and flurprimidol treatment was assessed by chi square analysis similar to experiment II. The statistical significance of free growth was assessed by comparing the mean primordia count with the mean stem unit count by Student's t-test. Each treatment was analyzed separately for free growth.

RESULTS

The change in environment when seedlings were transferred to the growth room induced initial bud set under both photoperiods. As expected, most seedlings under the long photoperiod had a second and sometimes a third flush (table 10). Seedlings under the 8 hour photoperiod did not resume growth. This produced a striking difference in bud size and development (figures 17 and 18).

As anticipated, and later confirmed by analysis of variance, the primordia count differed significantly

between photoperiod treatments (figure 19, table 10). However, the primordia count did not differ significantly between the flurprimidol treated and untreated seedlings within the same photoperiod. Furthermore, chi square analysis did not show a significant association ($p > 0.05$) between flushing and flurprimidol treatment under the 17 hour photoperiod. Following the chilling period, all seedlings flushed within two weeks. There was no apparent difference in the number of days to bud break between treatments. Curiously, analysis of variance did not show a significant difference in either stem unit count or flush length between the 8 and 16 hour photoperiod flushing environments (table 11). However, the dry weight of the new growth was significantly greater under the 16 hour photoperiod. Consequently, these seedlings had significantly higher dry weights per stem unit, and higher dry weight per unit length (table 11).

In contrast to the effects of flushing environment, analysis of variance for the bud set treatments revealed significantly different flush characteristics (table 12). The 8 hour photoperiod produced a significantly higher mean flush length, stem unit count, and strikingly greater dry weight (figure 20). Furthermore, both the mean stem unit length and mean stem unit dry weight were higher for early bud set seedlings. There was

no statistically significant interaction ($p > 0.05$) between the bud set treatments and the flushing photoperiod treatments.

Under the 17 hour photoperiod bud set treatment, the flush for the flurprimidol treatment was very similar to the untreated control (table 12). Only for the dry weight per unit length was there a significant difference. Moreover, for this parameter, the flurprimidol treatment was not statistically different from the early bud set (8 hour photoperiod) seedlings.

Free growth was estimated by subtracting the primordia count from the stem unit count (table 13). This difference was statistically significant for both the late bud set (17 hour photoperiod) and the flurprimidol treated seedlings, but not for the early bud set seedlings. Combining the two flushing environments, free growth constituted 29% and 41% of the total growth for the late bud set and flurprimidol treatments, respectively.

Figure 17. Characteristic external bud appearance of Douglas-fir seedlings with early bud set (upper) and late bud set (lower).

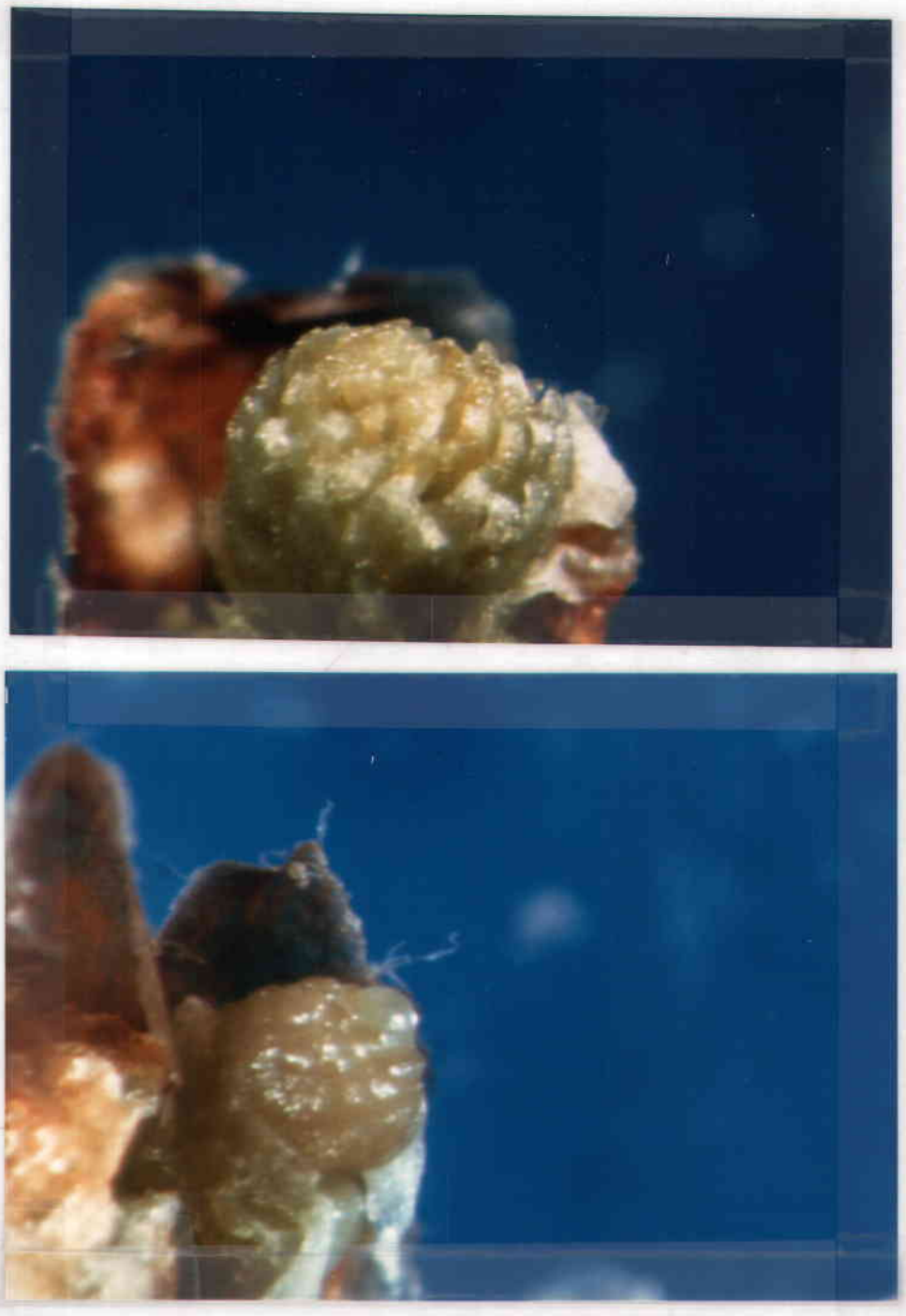

Figure 18. Characteristic internal bud appearance of Douglas-fir seedlings with early bud set (upper) and late bud set (lower).

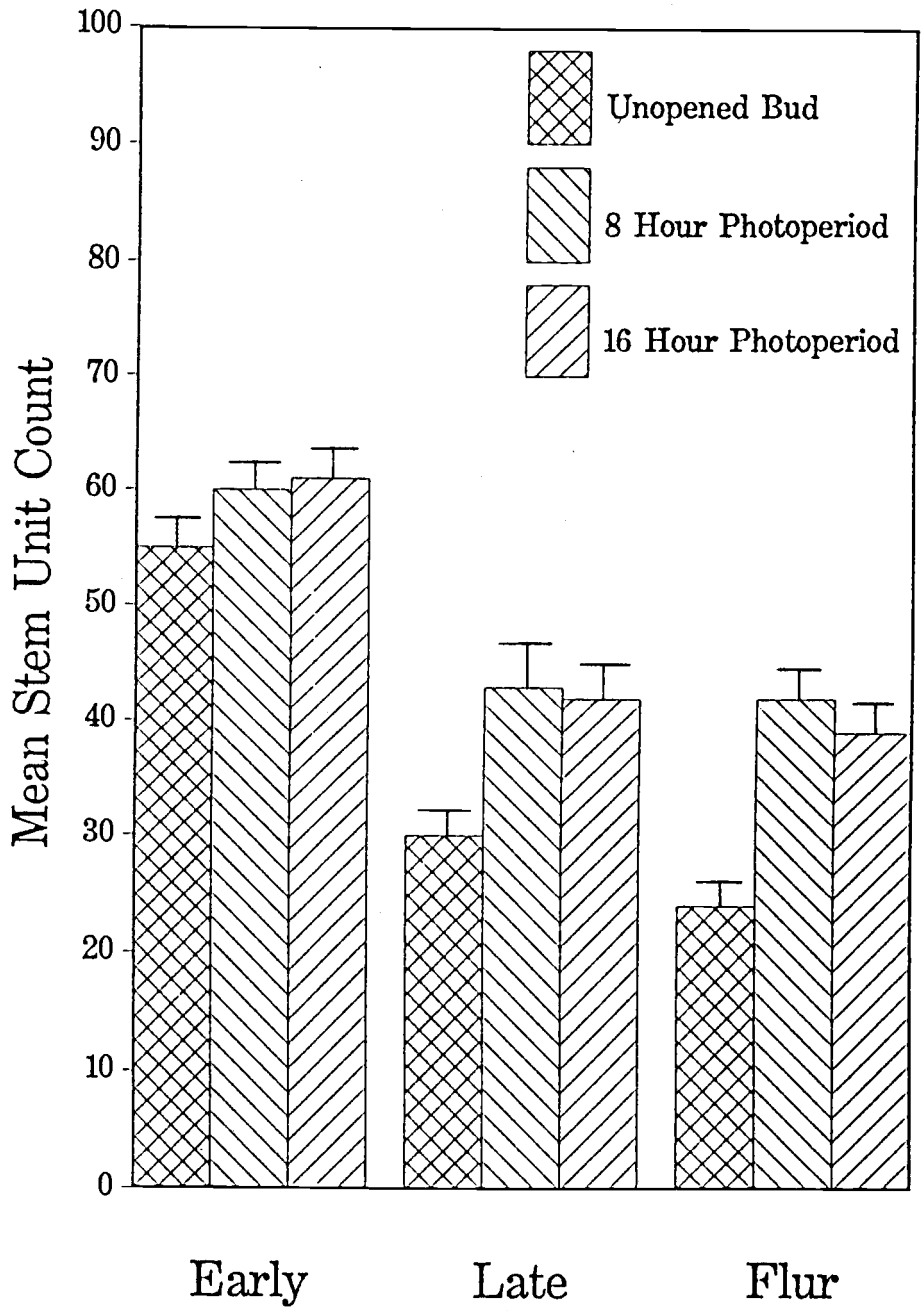

Figure 19. The mean stem unit count for Douglas-fir seedling buds and new shoots under an 8 or 16 hour photoperiod following three bud set treatments: early bud set (early), late bud set (late), or late bud set with an earlier application of flurprimidol (Flur). Vertical lines are one standard error.

Figure 20. Post chilling response of Douglas-fir seedlings following previously early bud set (early), late bud set (late), or late bud with 0.5 mg flurpimidol (EL-500).

Table 10. The bud development response of Douglas-fir seedlings to photoperiod and flurprimidol treatments. *

Bud set treatment	Percent % second flush	Average primordia count
8 hour photoperiod	0	55.0 a
17 hour photoperiod	80	30.0 b
17 hour photoperiod with flurprimidol	63	23.8 b

* Means in the same column with different letters are significantly different at the 0.05% level.

Table 11. Mean flush characteristics under 8 and 16 hour photoperiods of post-dormancy Douglas-fir seedlings. *

Seedling attribute	Flush photoperiod 8 hour	16 hour	P > F
Stem unit count	51.3	50.6	(0.55)
Flush length (mm)	27.5	30.2	(0.33)
Flush dry weight (mg)	89.6	125.5	(0.01)
Average stem unit length (mm)	0.514	0.563	(0.09)
Dry weight per unit length (mg/mm)	3.04	3.84	(0.01)
Dry weight per stem unit (mg)	1.59	2.21	(0.01)
Sample size	91	98	

* Probability values are the probability of no difference between means the same row.

Table 12. Mean flush characteristics of Douglas-fir seedlings after 8 hour photoperiod, 17 hour photoperiod, or 17 hour photoperiod with flurprimidol bud set treatments. *

	Bud set treatment		
	8 Hour photoperiod	17 Hour photoperiod	
Seedling attribute	Control	Control	Flurprimidol
Stem unit count	60.4 a	42.2 b	40.5 b
Flush length (mm)	41.1 a	17.9 b	14.7 b
Flush dry weight (mg)	165.2 a	46.6 b	59.1 b
Average stem unit length (mm)	0.69 a	0.36 b	0.42 b
Dry weight per unit length (mg/mm)	3.93 a	2.35 b	3.85 a
Dry weight per stem unit (mg)	2.66 a	1.01 b	1.40 b
Sample size	94	55	40

* Means in the same row followed by different letters are significantly different at the 0.05% level.

Table 13. Estimated free growth (stem unit count) for Douglas-fir seedlings under two flush photoperiod regimes following three bud set treatments. *

	Flush Photoperiod			
	8 Hour		16 Hour	
Bud set treatments	Free growth	P > T	Free growth	P > T
8 hour photoperiod	5.0	(0.10)	5.8	(0.07)
17 hour photoperiod	13.1	(0.01)	11.7	(0.01)
17 hour photoperiod with flurprimidol	18.3	(0.01)	15.1	(0.01)

* Probability values are the probability of no difference between samples from which free growth was computed.

DISCUSSION

Douglas-fir seedlings are known to be sensitive to photoperiod (Downs 1962, Lavender et al. 1968). The primary effect when the photoperiod is less than 14 hours is cessation of height growth and initiation of a resting bud (McCreary et al. 1978). However, short photoperiods are also known to promote cold hardiness (van den Driessche 1975) and prepare the apical meristem for subsequent chilling (Lavender and Stafford 1985). In the present study, 17 hour photoperiods were effective in promoting growth while 8 hour photoperiods maintained resting bud set. Furthermore, these treatments produced dramatic differences in bud size and morphology prior to winter chilling.

The ability of flurprimidol to compensate for delayed bud set was limited. There was no discernible affects on bud morphology or free growth. Only in the dry weight to length ratio of the post-chilling flush was there a significant difference. The treated seedlings produced a stouter, more massive flush than the untreated controls. Surprisingly, for this ratio the treated seedlings were comparable to the early bud set control. Thus, flurprimidol acted to partially compensate for reduced bud size by producing a flush with a more normal dry

weight to length ratio. However, this compensatory effect was small and should not be substituted for early bud set.

The environmental control of bud morphogensis has been studied in pines (Clements 1970, Garrett and Zahner 1973), and spruces (Pollard and Logan 1977, Pollard and Logan 1979, Columbo et al. 1982, Macey and Arnott 1986). Although moisture stress (Carlson et al 1978) as well as short photoperiods (Lavender et al. 1968) are known to initiate bud set in Douglas-fir, the environmental regulation of bud morphogensis of this species has been less well defined. For black spruce, primordia production generally stops six weeks after bud initiation (Pollard 1974); however, temperature can greatly modify the rate of primordia production and final bud size (Pollard and Logan 1977). A similar trend may occur with Douglas-fir where the size of the apical dome within the bud appears to control the number of primordia (Owens 1968).

The chilling a bud receives during winter is essential to normal growth resumption (Romberger 1963). For Douglas-fir the chilling requirement varies with ecotype (Wells 1979, Lavender 1981) but 12 weeks at a constant $4^{o}C$ is normally satisfactory (Wommack 1962). To ensure high vigor during bud flush, the present study

used a 14 week chilling period at constant 4°C with a daily 8 hour photoperiod. It was intended that the stimulatory effect of a long chilling period would promote the expression of free growth.

Numerous investigations of conifer shoot growth have found that spring height growth increment is related to bud size and development (Clements 1970, Kozlowski1 et al. 1973, Garrett and Zahner 1973, Kremer and Larson 1982, Columbo 1986). Although these studies examined a variety of species and age classes, the present study supports this relationship for Douglas-fir seedlings. Seedlings which set bud earlier, and thus had larger buds, produced significantly more stem units, and had strikingly greater length and dry weight in their spring flush. Hence, the results of this study support the belief that bud induction date and bud size are important attributes that reflect seedling quality.

For many conifer species, free growth in the seedling stage is a common occurrence (Jablanczy 1971, Pollard and Logan 1974). Kaya (1987) observed free growth for Douglas-fir seedlings in their second year and found that it was related to provenance. However, the present study found significant free growth only for late formed buds. The late formed buds had large apical domes that indicates incomplete bud morphogensis (Owens 1968).

Hence, free growth occurred when development during the previous season left space on the apical dome. Apparently, this space was reactivated for primordia initiation which resulted in free growth. Since the fully formed buds did not initiate primordia upon growth resumption, the free growth of the poorly developed buds partially compensated for the differential development. This study, therefore, agrees with Macey and Arnott (1986) who found that free growth of white fir can compensate for differential bud development. Carlson's (1978) results for Douglas-fir may also be explained by compensatory free growth. In contrast, Columbo (1986) did not observe differential free growth for black spruce following three bud development treatments. However, substantial free growth was reported (an average of 118 needles).

Photoperiod, in addition to regulating dormancy, has been reported to control free growth in conifers (Pollard and Logan 1974, Macey and Arnott 1986). Unexpectedly, the present study found that free growth was unaffected by photoperiod. However, since dry weight was affected, photoperiod did have a physiological effect, probably through photosynthesis. Earlier studies used either outdoor conditions (Pollard and Logan 1974) or a

24 hour photoperiod (Macey and Arnott 1986). Thus, either higher light levels or very long photoperiods may be necessary for free growth to occur on well developed buds.

Also in contrast to previous studies, the present study found significant free growth under 8 hour photoperiods but only for weakly developed buds. The long chilling period, being highly promotive, may have overridden the inhibiting effect of short photoperiods and allowed free growth to occur.

CONCLUSIONS

Douglas-fir bud development can be induced by 8 hour photoperiods. Overall, flurprimidol, at 5 mg per seedling, cannot compensate for the growth promoting effects of 17 hour photoperiods. Only for the dry weight to length ratio of the spring shoot flush was flurprimidol able to compensate for long photoperiods. Seedlings with early bud set had more primordia and greater shoot growth following chilling than seedlings with late bud set. Free growth is greater for poorly developed buds but cannot fully compensate for otherwise reduced growth.

CONCLUSIONS

The objective of this thesis was to investigate the physiological and cultural control of bud development in Douglas-fir seedlings. The hypothesis was that bud development is under endogenous gibberellin or abscisic acid control and that cultural treatments affect bud development by altering these growth regulators. Short photoperiod has been regarded as a strong stimulus for bud development in temperate conifers. This was confirmed by experiment I. However, large differences in photoperiod without other environmental changes do not mimic natural environments and should not be extrapolated outside growth room conditions.

The increase in gibberellin activity under short photoperiods for Douglas-fir is similar to the effect on more thoroughly investigated angiosperms. This suggests that short photoperiods block gibberellin metabolism in Douglas-fir which results in bud development. Thus, the results from gibberellin assays in experiment I support the thesis hypothesis. Abscisic acid levels were also affected by photoperiod. Moreover, the pattern of increasing levels of abscisic acid under long photoperiods conforms to several previous studies with angiosperms. However, since ABA is a growth inhibitor, this does not

provide a satisfactory explanation for bud development.

Plant growth retardants inhibit gibberellin biosynthesis in several plant species. In experiment II, the growth retardant flurprimidol decreased gibberellin activity in Douglas-fir seedlings. Furthermore, photoperiod induced bud flush was inhibited by flurprimidol in two separate trials. Additionally, internode elongation following dormancy was inhibited by flurprimidol. However, flurprimidol also depressed abscisic acid levels suggesting a general depression of terpenoid metabolism. Therefore, although this experiment could not independently confirm the hypothesis, experiment II provides additional support for the thesis hypothesis.

In experiment III, flurprimidol was applied to counteract the effects of long photoperiod, and, therefore, provided a third test of the thesis hypothesis. However, the results were generally unsupportive. Flurprimidol did not counteract the effects of long photoperiods on either overwintering bud development or post dormancy shoot growth. Only for the dry weight to length ratio of the post-dormancy shoot growth did flurprimidol compensate for long photoperiods. Rather, bud development and subsequent growth were strongly affected by the date of bud set, as determined by photoperiod.

It is concluded that both photoperiod and flurprimidol are effective in controlling shoot growth and bud development of Douglas-fir seedlings. The discrepancy between experiments II and III for flurprimidol effects may be explained by the different dosages applied. It is further concluded that gibberellin activity is likely a major cause of bud and shoot behavior in Douglas-fir seedlings. However, further research is needed to identify the specific gibberellins of Douglas-fir as well as their metabolic pathways.

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APPENDIX

APPENDIX 1. BIOASSAYS FOR GIBBERELLIN ACTIVITY FROM HPLC CHROMATOGRAMS FOLLOWING PHOTOPERIOD OR FLURPRIMIDOL TREATMENTS.

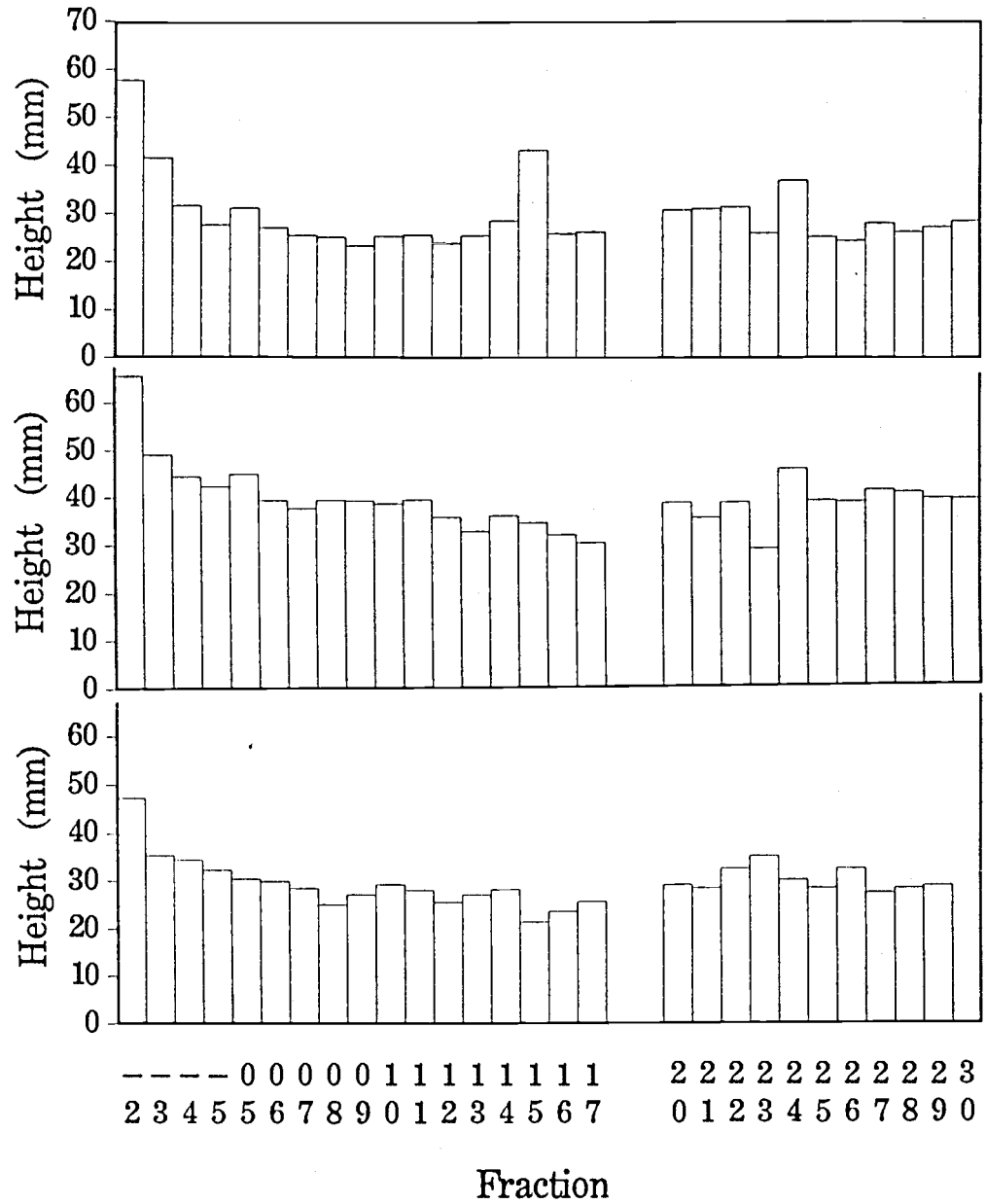

Figure 21. Gibberellin bioassays of HPLC chromatogram for seedlings prior to photoperiod treatment (experiment I).

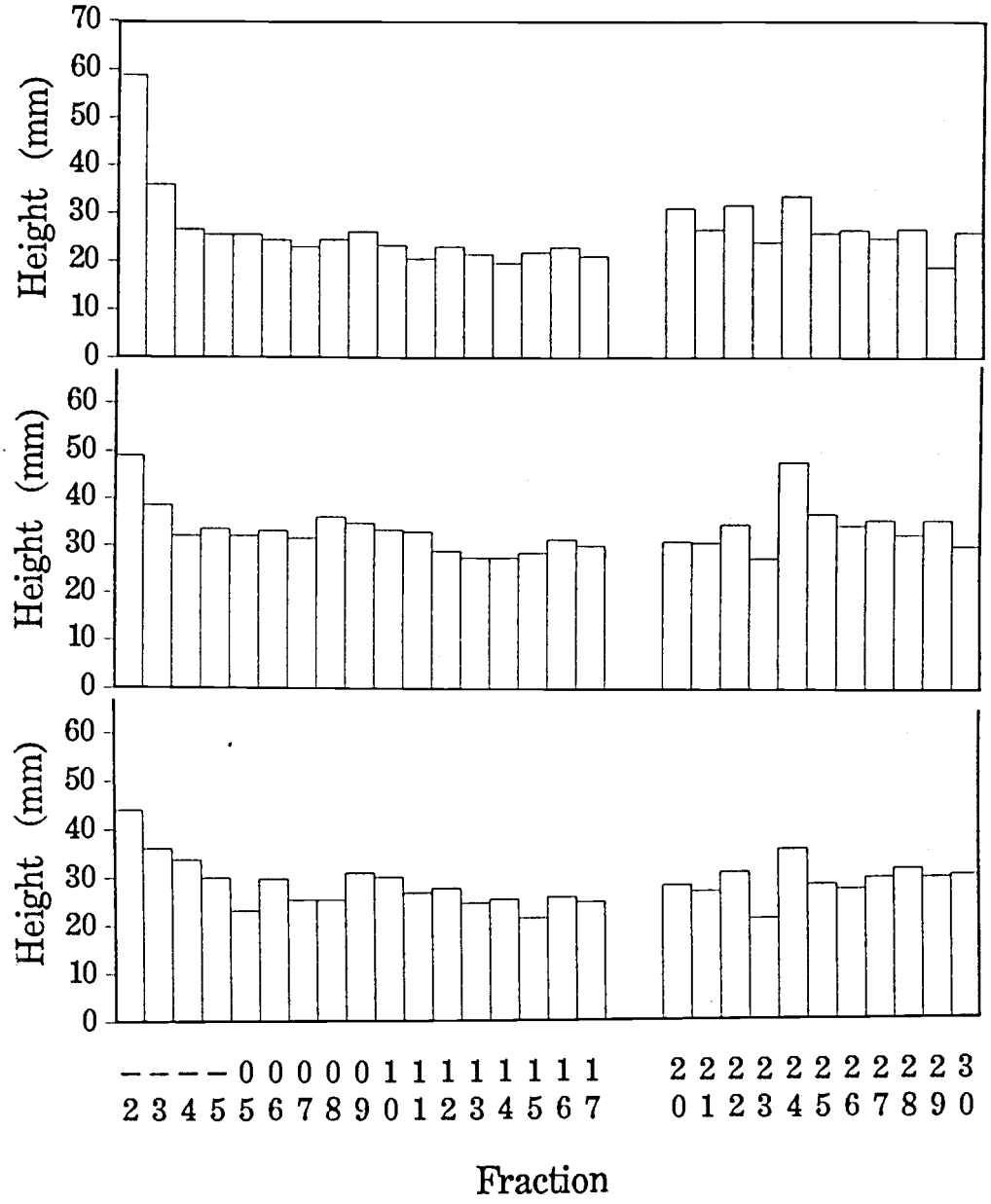

Figure 22. Gibberellin bioassays of HPLC chromatogram for seedlings given an 8 hour photoperiod for 4 days (experiment I).

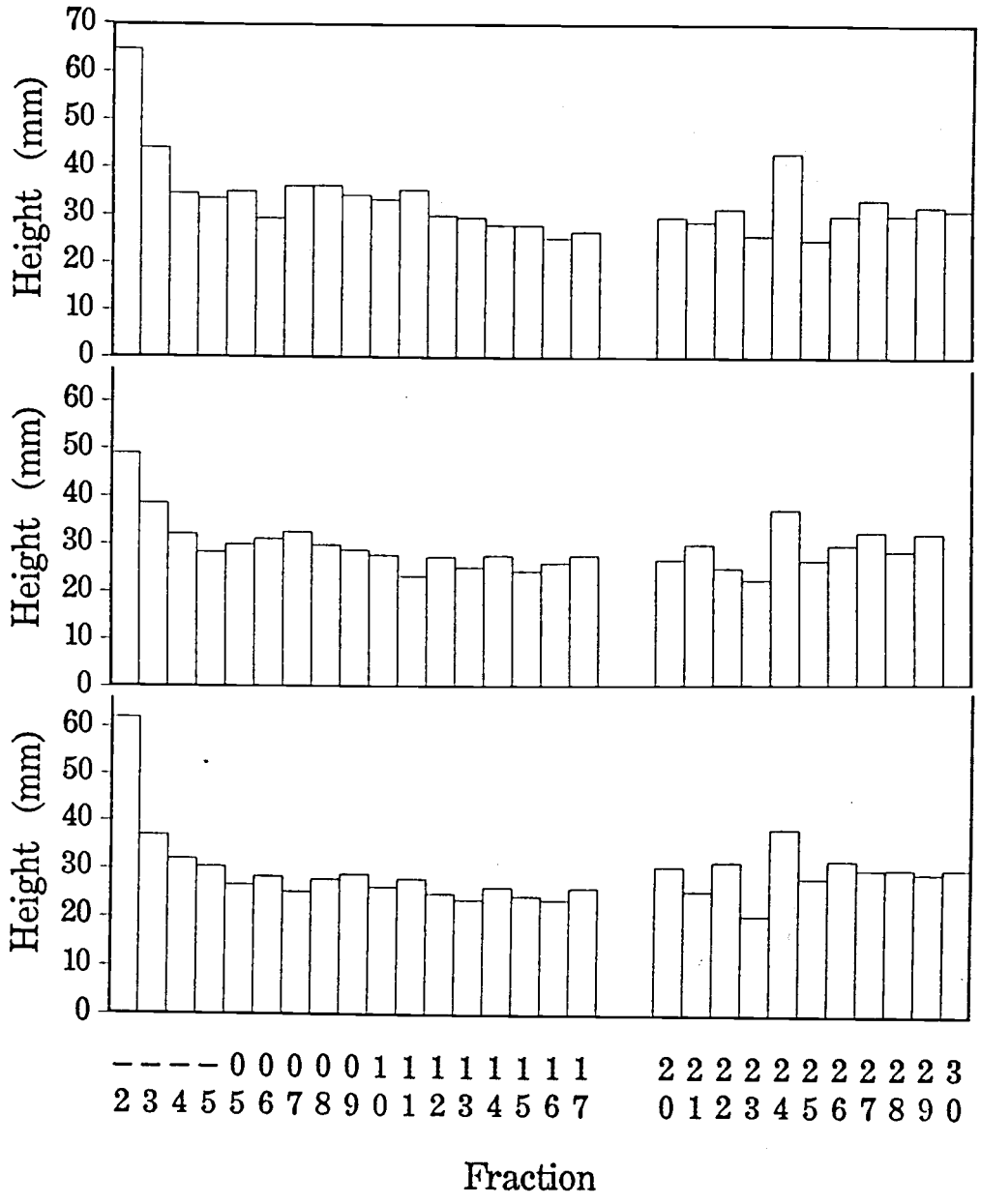

Figure 23. Gibberellin bioassays of HPLC chromatogram for seedlings given an 8 hour photoperiod for 18 days (experiment I).

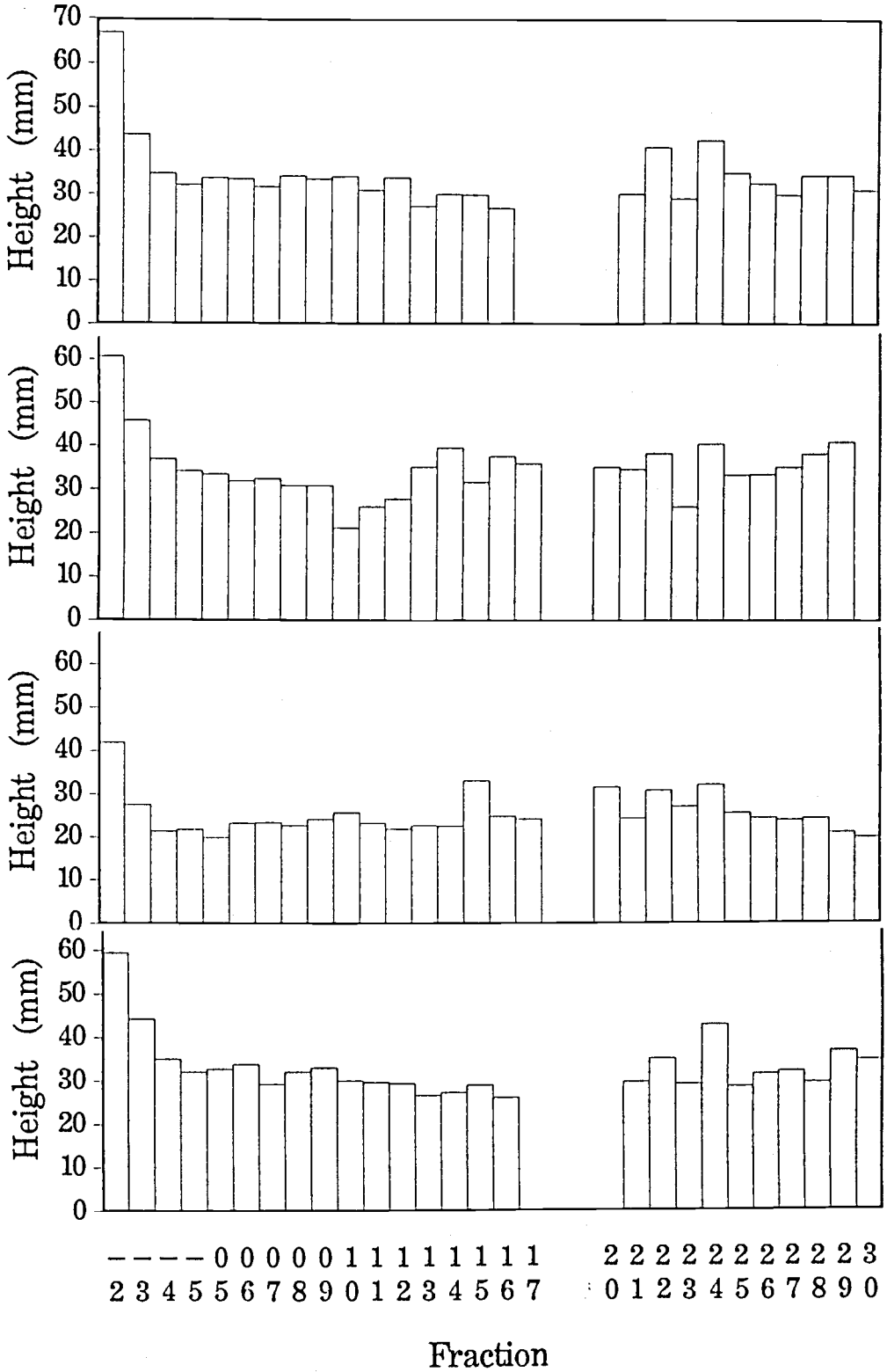

Figure 24. Gibberellin bioassays of HPLC chromatogram for seedlings given an 8 hour photoperiod for 62 days (experiment I).

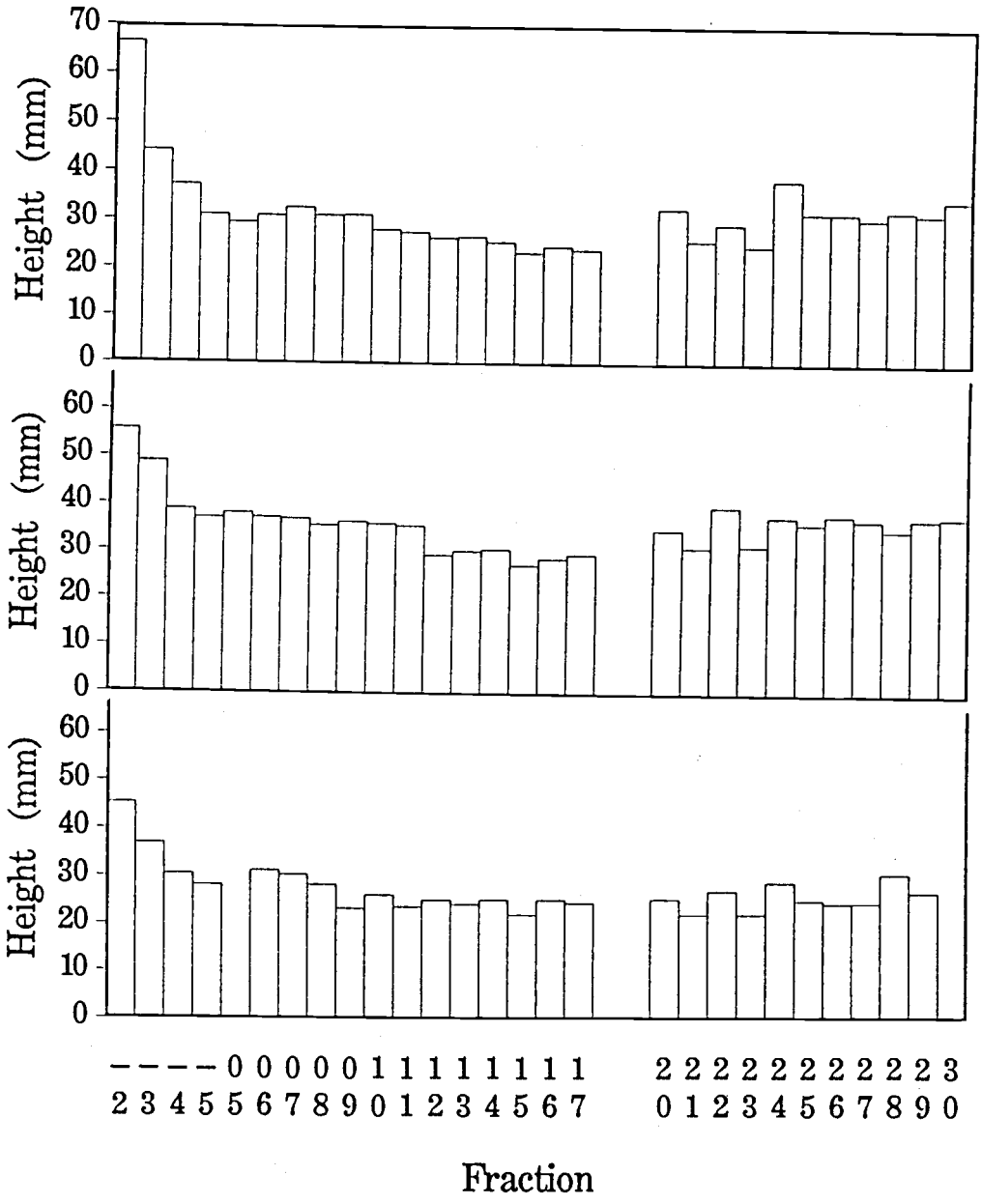

Figure 25. Gibberellin bioassays of HPLC chromatogram for seedlings given a 17 hour photoperiod for 4 days (experiment I).

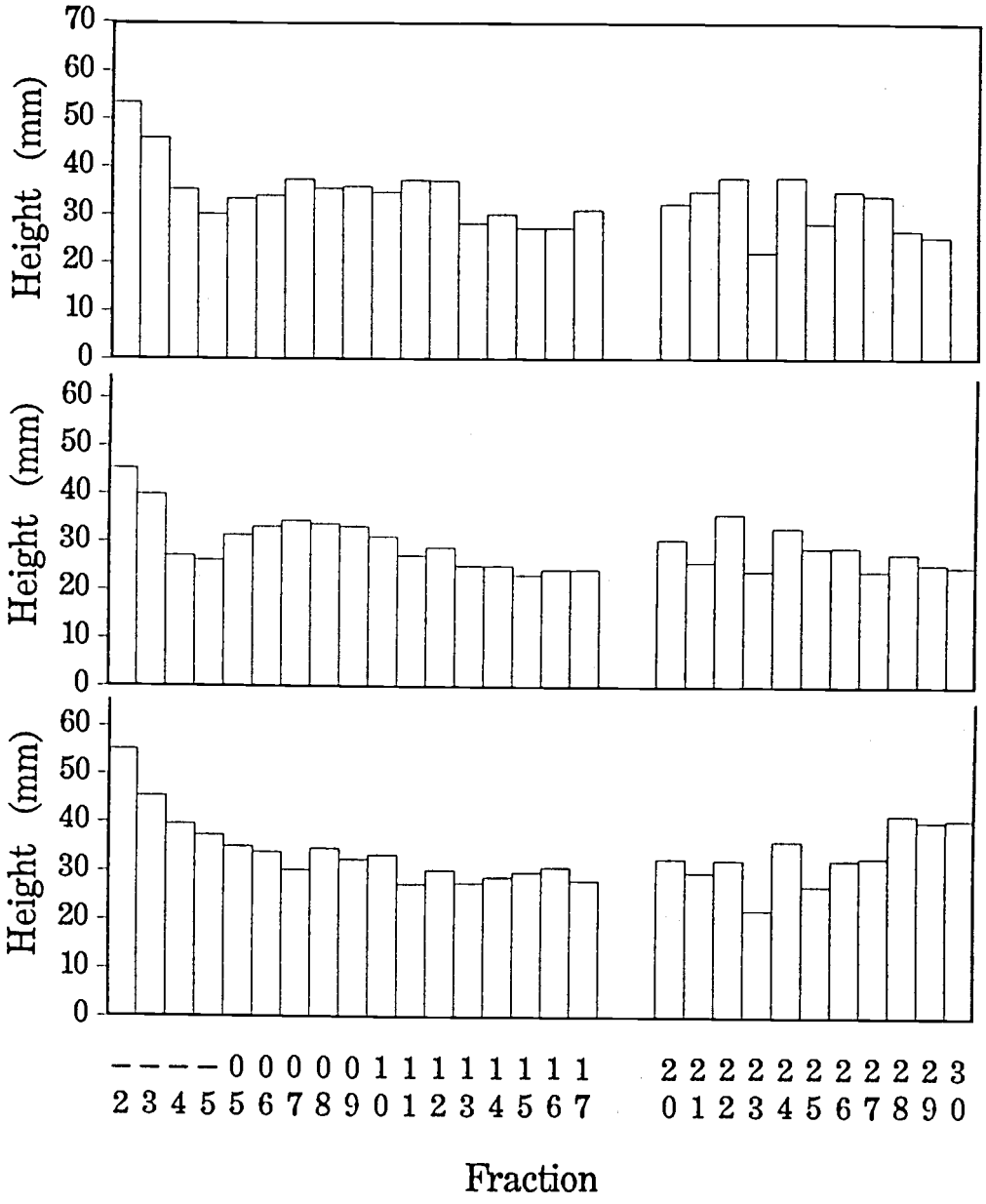

Figure 26. Gibberellin bioassays of HPLC chromatogram for seedlings given a 17 hour photoperiod for 18 days (experiment I).

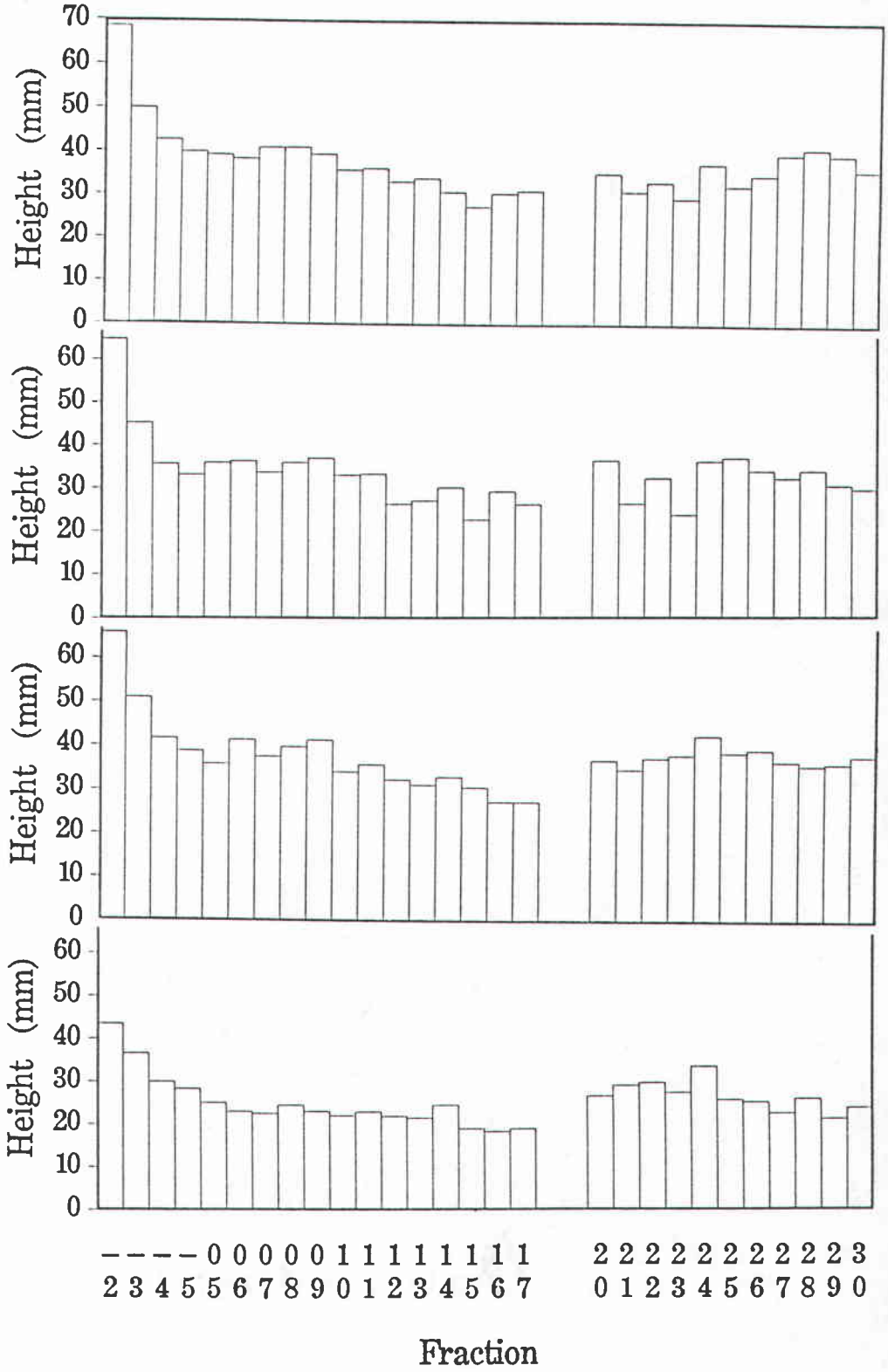

Figure 27. Gibberellin bioassays of HPLC chromatogram for seedlings given a 17 hour photoperiod for 62 days (experiment I).

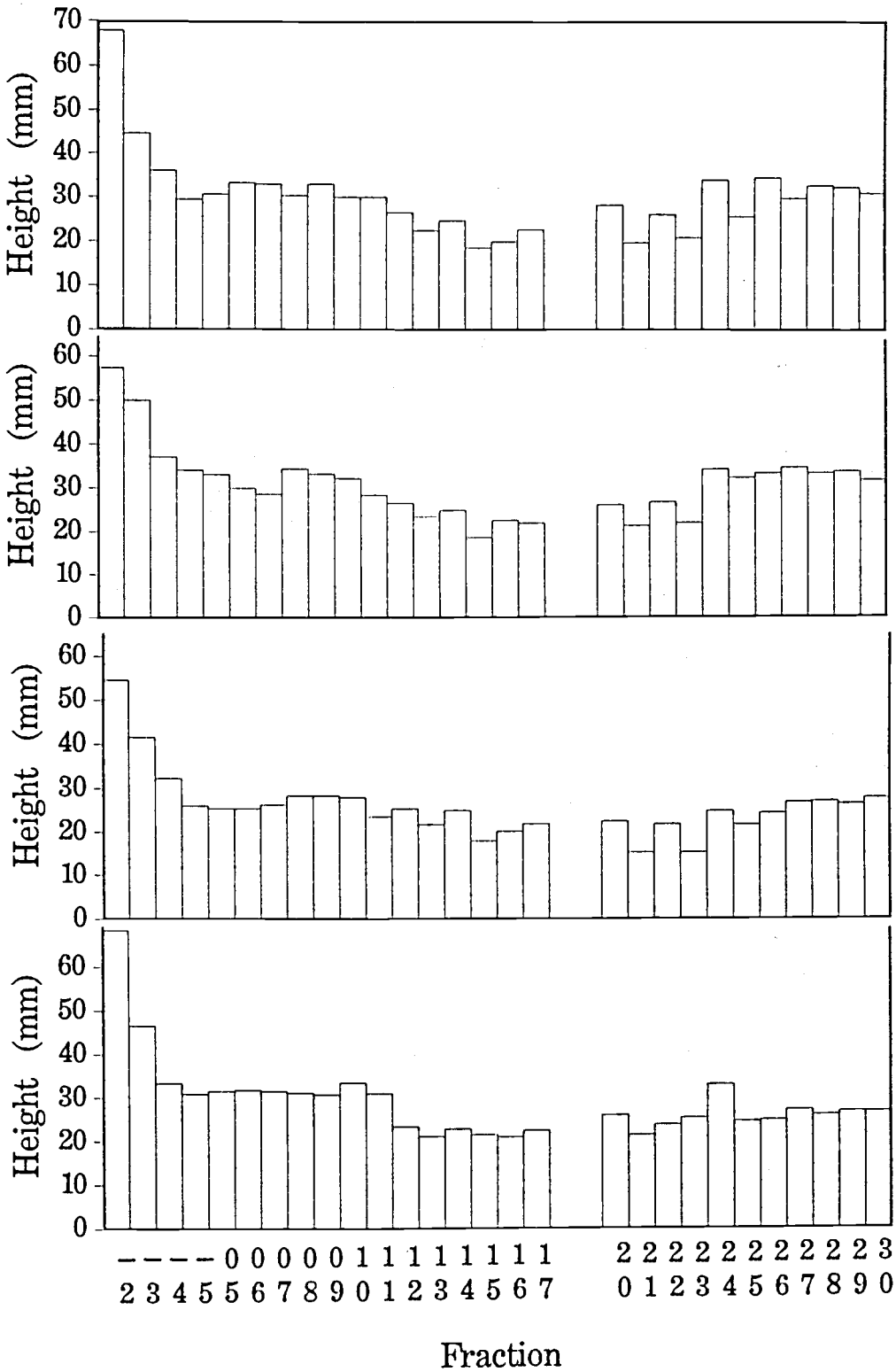

Figure 28. Gibberellin bioassays of HPLC chromatogram for flurprimidol treated seedlings (experiment II).

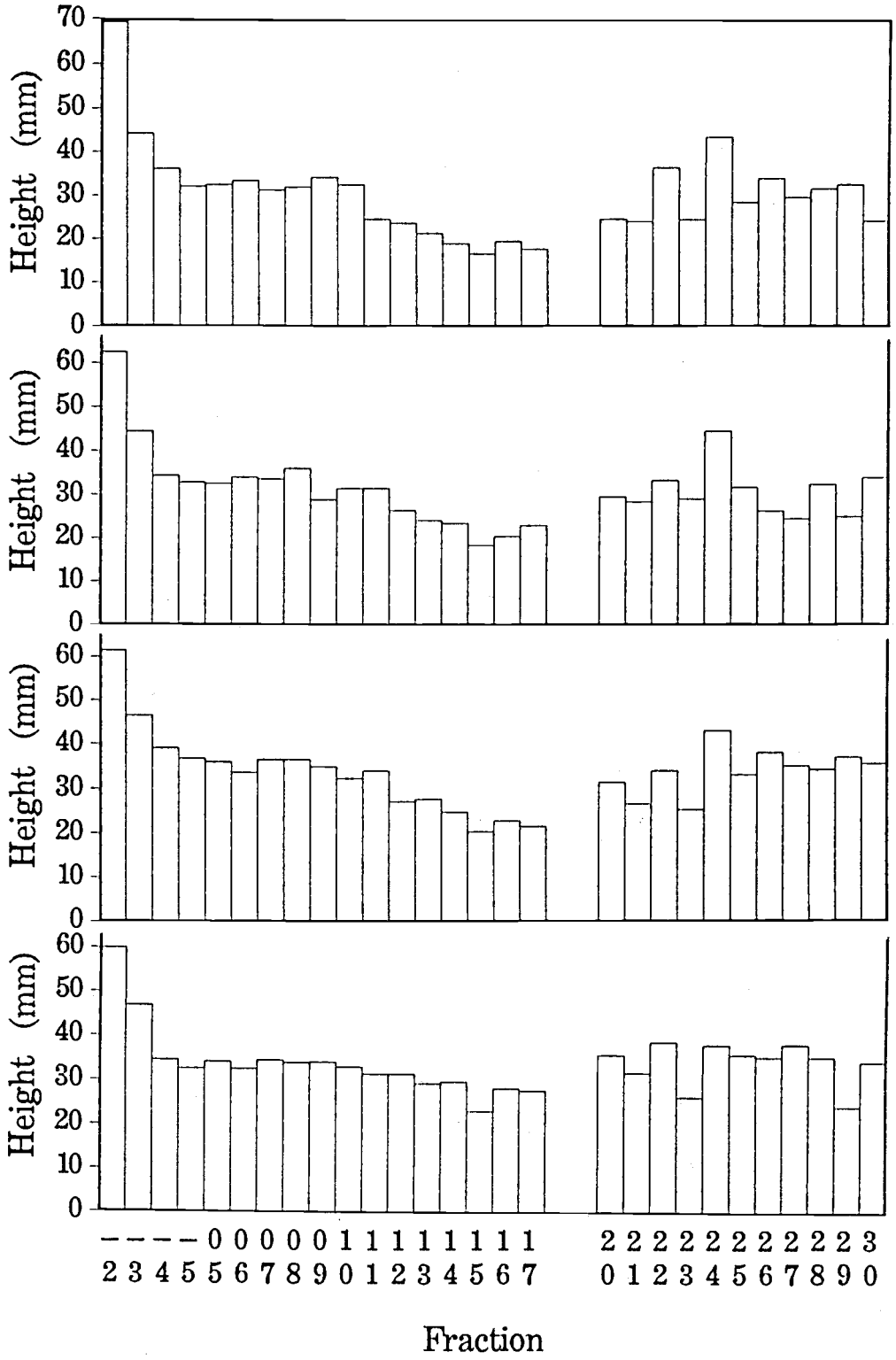

Figure 29. Gibberellin bioassays of HPLC chromatogram for control seedlings not treated with flurprimidol (experiment II.)