A comparative study of shoot and root development of interior and coastal Douglas-fir seedlings

PETER FIELDER

British Columbia Ministry of Forests, Research Branch, 1320 Glyn Road, Victoria, B.C., Canada V8Z 3A6

AND

JOHN N. OWENS

Department of Biology, University of Victoria, Victoria, B.C., Canada V8W 2Y2

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Bud development and root elongation of coastal and interior seedlots of Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) were compared from September through March after 4 months of growth under Styroblock container nursery production conditions. Apical development of vegetative buds was described in terms of morphology, mitotic index, apical dimensions, and cell numbers. An estimate was made of the number of leaf primordia produced on the embryonic shoot. Morphological and anatomical stages of bud development in the interior variety occurred about 4 weeks in advance of those in the coastal variety. Cell divisions ceased in apices in November and December in interior and coastal varieties, respectively, and resumed in mid-February in both varieties, so all apices were undergoing cell division by March 1. Flushing was variable but occurred earlier in the interior variety. Numbers of white root tips were highest during October and March in both varieties. Root elongation tended to follow the pattern of bud development in each variety, so the interior variety had greater numbers of white root tips >0.5 mm long about 2 weeks earlier in the fall than the coastal variety. Apical activity and peak numbers of root tips >0.5 mm long were synchronized in both varieties by the following March. In the interior variety white root tips were absent during December and January whereas some were present throughout the sampling period in the coastal variety. The interior variety produced a larger bud and entered dormancy earlier than the coastal variety when cultivated under coastal conditions. Differences in phenology between the two varieties are sufficient to justify the development of more specific nursery cultural practices to achieve maximum seedling quality.

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Le développement des bourgeons ainsi que l'élongation des racines de lots de graines de Sapin de Douglas (*Pseudotsuga menziesii*) (Mirb.) Franco) provenant des zones côtières et de l'intérieur de la Colombie-Britannique ont été comparés de septembre à mars après 4 mois de croissance en Styroblocks dans une pépinière. Le développement apical des bourgeons végétatifs a été décrit quant à la morphologie, à l'indice mitotique, aux dimensions apicales et au nombre de cellules. Une évaluation a été faite du nombre des primordia foliaires produits sur les pousses embryonnaires. Les phases morphologiques et anatomiques du développement des bourgeons de la variété de l'intérieur sont survenues environ 4 semaines plus tôt que celles de la variété côtière. La division des cellules a cessé dans les apex en novembre et décembre, respectivement, dans les variétés de l'intérieur et côtière, et a repris à la mi-février dans les deux variétés, de telle sorte que tous les apex étaient en voie de division cellulaire le 1er mars. Le débourrage était variable, mais il est survenu plus tôt dans le cas de la variété de l'intérieur. Le nombre des extrémités blanches racinaires était le plus élevé durant octobre et mars dans le cas des deux variétés. L'élongation racinaire tendait à suivre la marche du développement des bourgeons dans chaque variété, de telle sorte que la variété de l'intérieur possédait le plus grand nombre d'extrémités blanches racinaires >0,5 mm de longueur environ 2 semaines plus tôt à l'automne que la variété côtière. L'activité apicale et le nombre maximal d'extrémités racinaires (>0,5 mm de longueur) étaient synchronisés dans les deux variétés durant le mois de mars suivant. Pour la variété de l'intérieur, les extrémités blanches racinaires étaient absentes en décembre et janvier, tandis que certaines étaient présentes durant toute la période d'échantillonnage pour la variété côtière. La variété de l'intérieur a produit de plus gros bourgeons et est entrée en dormance plus tôt que la variété côtière lorsque cultivée dans un milieu à climat côtier. Les différences de phénologie entre les deux variétés sont suffisantes pour justifier l'élaboration de protocoles de culture en pépinière plus spécifiques afin d'obtenir des semis de la meilleure qualité.

[Traduit par la revue]

Introduction

Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) exists as two varieties (Little 1953): *glauca*, which is distributed in the interior, from 55°N throughout the Pacific Northwest along the Rocky Mountains, well into Mexico (19°N) (Silen 1978), and *menziesii*, which grows on the islands and in coastal regions. Both varieties are subject to variation within their ranges of distribution (Christophe and Birot 1979; Wright *et al.* 1971).

Seedlings of both varieties are grown in coastal nurseries of the Pacific Northwest under different cultural regimes.

Maintenance of seedling quality in these large centralised nurseries is difficult because seedlots of different ecotypes may respond to unique sets of environmental cues. Coastal conditions may not be those of the seedlot's origin or of its final destination. Thus, detailed information is required about developmental characteristics of conifer ecotypes for the best reforestation success (Duryea 1985).

Bud development is important to survival and vigour of seedlings. The number and size of predetermined stem units may be affected by cultural conditions in the fall growth phase (Clements 1970), which in turn affects shoot growth

and subsequent yield the following year (van den Berg and Lanner 1971; Kozlowski 1971; Colombo 1982; Lavender and Stafford 1985). Studies of the annual cycle of bud development of the Abietoideae include mature Douglas-fir trees (Sterling 1946; Owens and Molder 1973a, 1979) and Douglas-fir seedlings (Allen 1947), white spruce (*Picea glauca* (Moench) Voss), red spruce (*P. rubens* Sarg.), black spruce (*P. mariana* (Mill.) B.S.P.), Norway spruce (*P. abies* (L.) Karst.), and balsam fir (*Abies balsamea* Mill.) (Jablanczy 1971). There has been no comparative study of bud development of the varieties of Douglas-fir seedlings.

Bud development of mature Douglas-fir is characterised by a period of initiation of bud scales followed by preformed leaf initiation. The dormant bud of Douglas-fir consists of numerous bud scales enclosing the embryonic shoot, which possesses all the leaf primordia for the following season (Owens and Molder 1973a). These findings confirmed those of Sterling (1946) and Allen (1947) for Douglas-fir and Parke (1959) for white fir (*A. concolor* Lindl. & Gord.). Studies of bud development in mature trees generally define the dormant period as an absence of cell division in the apical meristem.

Shoot and root apical development has been reviewed by Romberger (1963) and Torrey and Feldman (1977). Root development has been reviewed by Sutton (1980). Root morphology and its relationship to shoot development have been described throughout the annual cycle for Douglas-fir by Kreuger and Trappe (1967) and Riedacker (1976). The physiological relationship between the two has been studied in Douglas-fir by Zaerr and Lavender (1974), Lavender et al. (1970), and Lavender and Hermann (1970). No study has described the activity of elongating and newly initiated roots in relation to apical development in the fall and spring in Douglas-fir. A developmental study of root apices in relation to bud development was made for white spruce seedlings (Johnson-Flanagan 1985).

This paper presents a comparative anatomical study of bud development in relation to root development of Douglas-fir seedlings from interior and coastal seedlots grown under nursery operational conditions.

Methods and materials

In 1982 two seedlots of Douglas-fir seedlings, interior dry[1] and coastal,[2] were grown from seed in PSB 313 Styroblock containers in a polythene shelter house at the British Columbia Forest Service Nursery, Duncan (latitude 48′80″N). Three Styroblock containers of seedlings from each seedlot were kept in the Duncan Nursery shelter house from May 1982 to March 1983. All cultural practices, fertilising, and watering were carried out according to the nursery operational schedule (Matthews 1981).

The interior variety was grown under an 18-h photoperiod until mid-July and then transferred to natural photoperiods. The coastal variety received natural photoperiods for the entire growing season and a 2-week period of full drought stress from July 30. Shoot growth, root collar diameter, and shoot and root dry weight curves of this coastal seedlot and of an interior seedlot,[3] which was grown with the interior seedlot used in this study, were prepared by the British Columbia Ministry of Forests, Silviculture Branch (file No. 955-20-6).

Ten seedlings of each variety were selected at random every 2 weeks between September 1982 and March 1983, placed in plas-

[1]B.C. For. Serv. Reg. No. 3040/82L2/B5/1207/0.884
[2]B.C. For. Serv. Reg. No. 1070/92J09/B3/3442/0.80.
[3]B.C. For. Serv. Reg. No. 3040/82M04/B5/2064/1.01.

tic bags, and transported to the University of Victoria. The bud scales were removed from the terminal vegetative buds. The embryonic shoots were excised just below the bud and fixed in formalin – acetic acid – alcohol (Sass 1958). Shoot tips were fixed by midmorning on each collection date to avoid the confounding effects of possible diurnal fluctuations in mitotic index (Kawazawa et al. 1970). The material was dehydrated in a tertiary butyl alcohol series (Johansen 1940) and embedded in Tissue Prep. Serial longitudinal sections of embryonic shoots were cut at 6 μm and stained with safranin and hematoxylin.

Median sections were selected for apical measurements (Owens et al. 1977). The mitotic index (MI), the percentage of cells in division (Owens and Molder 1973a; Carlson et al. 1980; Carlson 1985), cell number, apical height and width, stage of apical development, and the number of needle primordia along the flanks of the apex were determined from the median section of each apex. Mitotic index was determined from counts of all dividing cells in the portion of the embryonic shoot above the last preformed needle primordia. Early and late bud scale initiation was distinguished by observing the number of bud scales removed during dissection. Early performed leaf initiation was the period during which the first two-thirds of all leaf primordia were initiated.

After excision of the shoot tips of seedlings for fixation during 1982, the soil mix was carefully removed from their root plugs and the white root tips were counted. The white root tips were classified into three length classes: >0–4.9, 5–9.9, and >10 mm. An increase in the number of while root tips >5 mm long was assumed to have occurred by elongation of previously suberized or existing white root tips of long laterals. While root tips <5 mm long were assumed to have arisen from elongation of suberized laterals or newly initiated lateral roots.

Variances between varieties and dates were tested with the F_{max} test ($\alpha = 0.05$) (Bliss 1970) and were found to be homogeneous in all data. Data were subjected to a two-way analysis of variance ($\alpha = 0.05$), using the GLM procedure for unbalanced sample sizes (SAS Institute Inc. 1982). Data employing percentages (mitotic index) were normalised using an arcsine transformation (Zar 1974).

Results and observations

The seedlings underwent a free growth phase of about 4 months after germination (B.C. Ministry of Forests, Silviculture Branch, file No. 955-20-6). The phenology of the two varieties differed in that morphological events in apices from the interior preceded those in apices from the coastal variety by 4–6 weeks under nursery growing conditions. A two-way analysis of variance indicated that the main effects of variety and date and their interaction were significant ($\alpha = 0.05$) for all morphological and anatomical changes in apices.
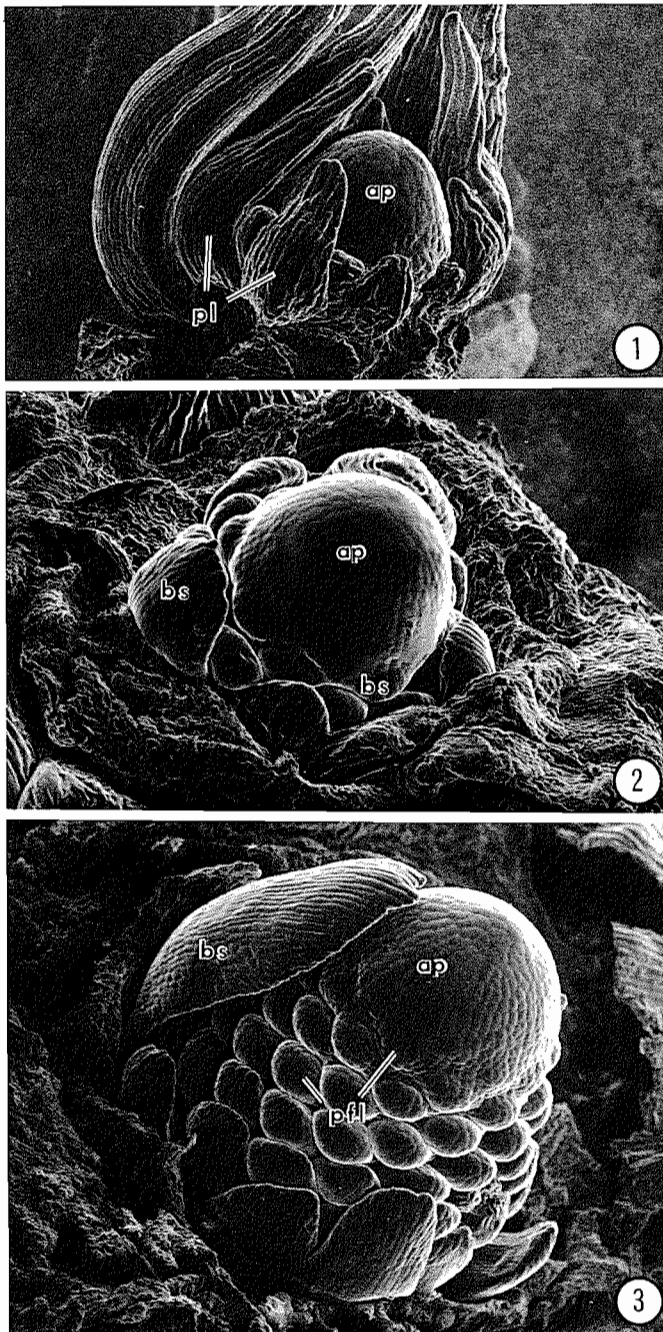
Bud scale and preformed leaf initiation in the coastal variety

On early collection dates there was considerable variation in the stages of bud development. The change from primary (neoformed) leaf initiation (Figs. 1, 4) to bud-scale initiation (Figs. 2, 5) was estimated to have occurred during the last 2 weeks of August. In early September apices were initiating either primary leaves or bud scales (Fig. 10) and MI was 0.95 (Fig. 11A). By the last 2 weeks of September, apices were initiating late bud scales or early preformed leaf primordia (Figs. 3,6,12,10) and MI was more than 2.0 (Fig. 11A). The MI was greatest during early preformed leaf initiation when analysed by stage (Fig. 10). By early October, apices of all seedlings were initiating preformed leaf primordia (Fig. 10) and apical zonation was most conspicuous (Fig. 6). By mid-October, apices had reached their maximum size and number of cells (Fig. 11B).

FIGS. 1–3. Scanning electron micrographs of dissected living shoot tips of coastal Douglas-fir. Fig. 1. Apex (*ap*) collected in early August during primary leaf (*pl*) initiation. ×95. Fig. 2. Apex collected in early August during bud scale (*bs*) initiation. ×95. Fig. 3. Apex collected in early September during performed leaf (*pfl*) initiation. ×76.

The increase in apical size appeared to result from cell divisions in the rib meristem and peripheral zones (Fig. 6). Cell divisions in the rib meristem were chiefly responsible for the rapid increase in apical width during growth of the shoot tip (Figs. 13A, 13B). A mammillary apex (Allen 1946) was observed in which the mitotically less active apical initials and central mother cells formed a prominent tip on the apex, with a constriction between these zones and the subtending peripheral zone (Fig. 6). Apices having a similar form were not found in the interior variety because apical

development had passed that stage when the first collection was made (Fig. 12).

Preformed leaf initiation occurred from the peripheral zone and proceeded rapidly up the flanks of the apex. The rate of preformed leaf initiation was constant from September until it ceased in mid-November (Fig. 12). Apices continued to increase in cell number (Fig. 11B) and size (Figs. 13A, 13B) until mid-October. In late October, MI and apical growth decreased (Fig. 11A). Apical size began to decrease as the rate of preformed leaf initiation exceeded apical growth and preformed leaf primordia encroached upon the apical dome. Mitotic activity (Fig. 11A) and preformed leaf initiation continued at an increasing rate until early December, when all preformed leaf primordia had been initiated (Fig. 12).

Bud scale and preformed leaf initiation in the interior variety

Comparable stages of bud development are estimated to have occurred about 1 month earlier in the interior variety than in the coastal variety. Figure 14 estimates the timing of the earlier stages of bud development. In early September, apices were observed in both early and late preformed leaf initiation (Figs. 6, 7, 10) and MI was highest, at 2.3 (Fig. 11A). By the beginning of October, most apices had completed preformed leaf initiation (Fig. 10) and MI had decreased to 0.7 (Fig. 11A). During September, apical width, height, and cell numbers decreased to a minimum (Figs. 11B, 13A, 13B). Apical zonation was initially distinct but became less so during late September and October (Figs. 7, 8). Mitotic activity continued at a low level throughout October, and apical width, height and cell numbers showed no significant change (Figs. 11B, 13A, 13B).
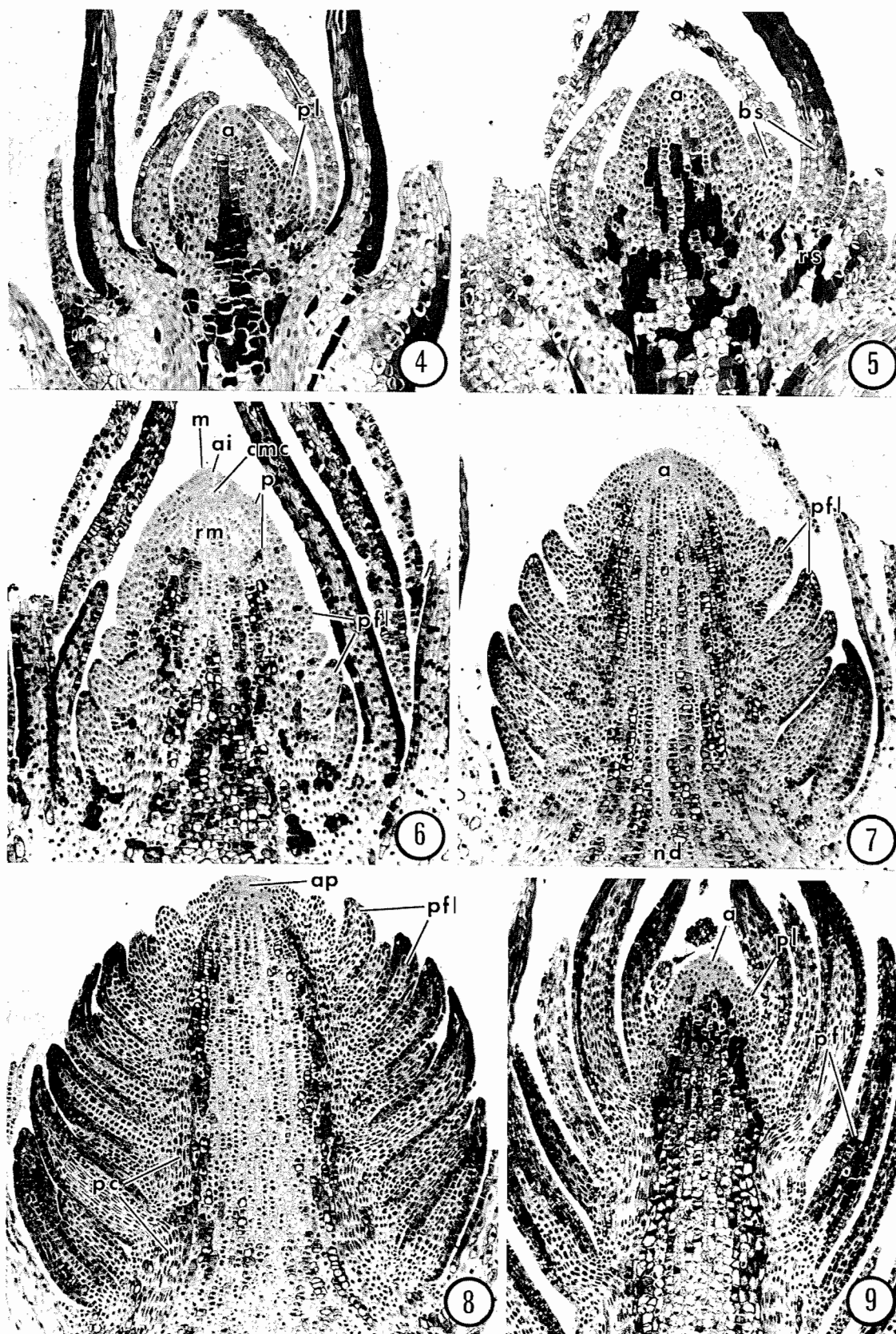
Nodal diaphragm development

A nodal diaphragm (crown region) began to develop at the base of the embryonic shoot during early preformed leaf initiation in both varieties and became clearly visible during late preformed leaf initiation (Fig. 7). Differentiation began late in September and was completed late in October in the coastal variety. The interior variety had already begun nodal diaphragm development in early September and this was completed by October. In both varieties a plate of thick-walled cells differentiated at the base of the embryonic shoot and extended to the edge of the cup-shaped receptacle bearing the bud scales. It was interrupted by the anastomosing eustele of procambial strands. By late November, a cavity formed immediately below the crown in both varieties, and remained until bud break in March.

Cessation of mitotic activity

No mitotic activity was found in apices of the coastal variety between mid-December and March (Fig. 11A), nor was there any change in apical dimensions (Figs. 13A, 13B). However, mitotic activity was observed at a low level in the subtending preformed leaf primordia until mid-January (Fig. 11A). In the interior variety, apices ceased mitotic activity in mid-November and remained inactive until March (Fig. 11A). Apical dimensions remained unchanged from October to March, and mitotic activity ceased in the subtending preformed leaf primordia in mid-November (Figs. 11A, 13A, 13B).

Considerable variation existed in the time when mitotic activity ended in buds of each variety (Fig. 10). Some individuals of the interior variety had ceased mitotic activity as

FIGS. 4–9. Median longitudinal sections of the developing embryonic shoots of coastal Douglas-fir seedlings. Fig. 4. Apex collected in early September during primary leaf (*pl*) initiation, showing the apical zone (*a*). ×60. Fig. 5. Shoot tip during bud scale (*bs*) initiation. ×60. Fig. 6. Embryonic shoot during early preformed leaf (*pfl*) initiation, showing the mammillary apex (*m*) and its zonation into apical initials (*ai*), central mother cells (*cmc*), peripheral zone (*p*), and rib meristem (*rm*). ×60. Fig. 7. Embryonic shoot during late preformed leaf initiation, showing the formation of the nodal diaphragm (*nd*). ×50. Fig. 8. Dormant embryonic shoot showing the apex (*ap*) and partially elongated preformed leaf primordia with procambial strands (*pc*). ×50. Fig. 9. The expanding shoot collected in March before flushing, showing the formation of current-year leaf primordia (*pl*). ×50.
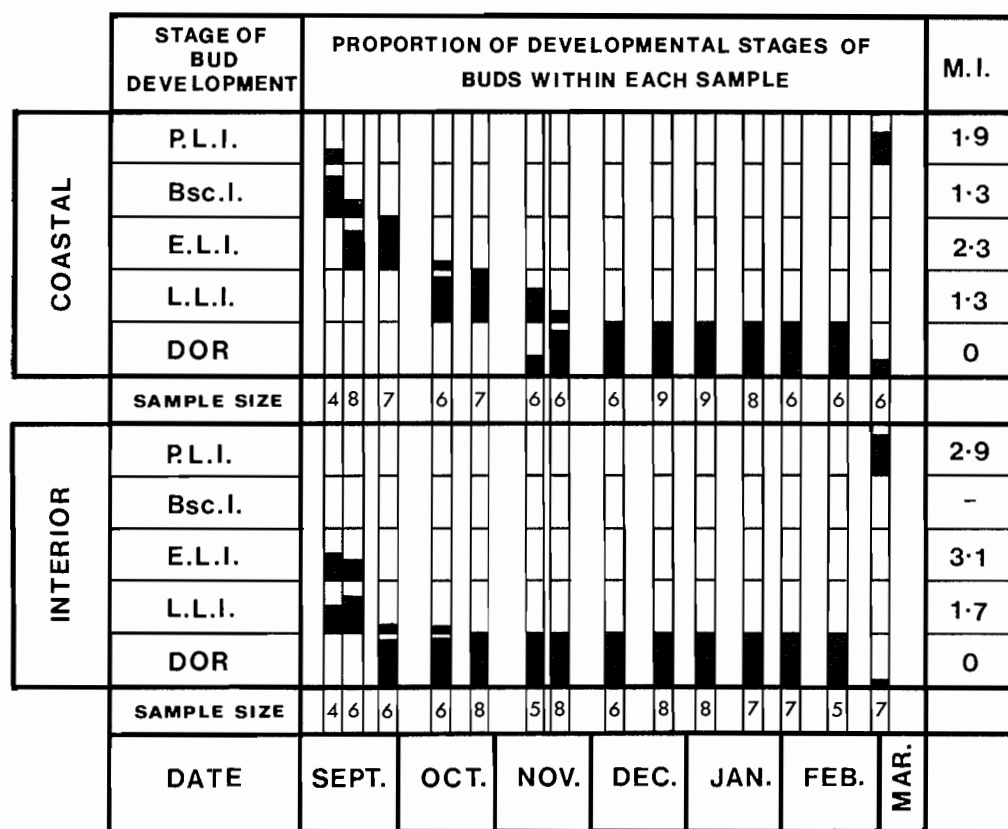
	STAGE OF BUD DEVELOPMENT	PROPORTION OF DEVELOPMENTAL STAGES OF BUDS WITHIN EACH SAMPLE	M.I.
COASTAL	P.L.I.		1·9
	Bsc.I.		1·3
	E.L.I.		2·3
	L.L.I.		1·3
	DOR		0
	SAMPLE SIZE	4 8 7 6 7 6 6 6 9 9 8 6 6 6	
INTERIOR	P.L.I.		2·9
	Bsc.I.		–
	E.L.I.		3·1
	L.L.I.		1·7
	DOR		0
	SAMPLE SIZE	4 6 6 6 8 5 8 6 8 8 7 7 5 7	
	DATE	SEPT. OCT. NOV. DEC. JAN. FEB. MAR.	

FIG. 10. Proportion of apices at different stages of bud development per collection date and mean average mitotic index (MI) for all apices at each stage of development. Stages of bud development are primary leaf initiation (P.L.I.), bud-scale initiation (Bsc.I.), early leaf initiation (E.L.I.), late leaf initiation (L.L.I.), and dormant (DOR).

early as late September and those of the coastal variety by early November (Fig. 10).

The dormant apex was a low dome in both varieties, although the coastal variety had consistently wider apices and 17% fewer preformed leaf primordia along the flanks than the interior variety.

Resumption of bud activity following dormancy

Mitotic activity began in the preformed leaf primordia of buds in mid-February in coastal and interior varieties. There was a delay of about 2 weeks after apical mitotic activity resumed before an increase in apical dimensions was observed. A sharp increase in mitotic activity occurred in the apex (Fig. 11A) and throughout the embryonic shoot in March, when shoot elongation began (Fig. 9). Preformed leaf primordia and embryonic shoots elongated (Fig. 9) causing bud swelling. Apices of elongating shoots initiated primordia (Fig. 9) that had the appearance of leaf primordia rather than of bud scales. MI exceeded 1.5 in both varieties (Fig. 11A). Bud scales lightened in colour from their usual bark tan, and enlarged for a time to accommodate the expanding shoot. Bud swell began in mid-February in some individuals of both varieties and continued until late March. By mid-March about 50% of coastal seedlings were undergoing bud swelling, whereas there was bud swelling in all interior seedlings. Variation in the onset of bud swelling was greater among individuals within a variety than between varieties; however, in both years development in the majority of interior seedlings was about 1 week in advance of that of the coastal variety.

Root development

Root development was heterorhizic. Roots longer than 5 mm consisted of long laterals (elongating roots) only. Roots shorter than 4.9 mm were composed of short laterals and some elongating roots produced by reactivation of suberised long laterals. Short laterals were produced in large numbers on secondary or tertiary laterals near the junction of root and shoot.

Root development of both varieties had two active periods, one in the fall and the other in early spring (Figs. 15A, 15B). Root elongation (white root tips >0.5 mm long) of coastal seedlings reached a maximum about 2 weeks later in the fall and was less vigorous than in interior seedlings. The elongation of newly initiated long and short laterals and existing short laterals was highest in both varieties during September and October. Root elongation of interior seedlings ceased in early December (Fig. 15B), but that of the coastal variety (Fig. 15A) did not stop during the winter although elongation slowed considerably.

Root elongation occurred in late January to February in coastal seedlings (Fig. 15A) but not until early March in the interior variety (Fig. 15B). Elongation of long laterals was at a maximum in both varieties in early March. Both varieties showed a decrease in numbers of white root tips in late April, indicating a reduction in both elongation and initiation of long and short lateral roots.

Discussion

Seedlings and mature Douglas-fir differ in their phenology with respect to shoot elongation and the duration of apical developmental stages (Fig. 16). Seedlings undergo a variable
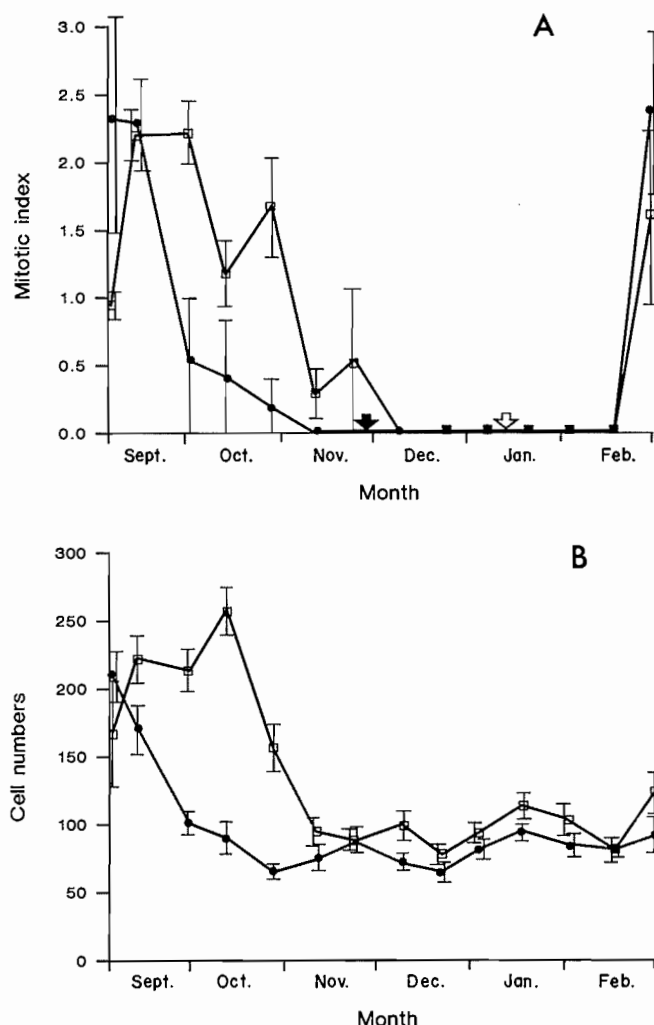
FIG. 11. The average mitotic index (A) and average number of cells (B) per median section, based on four to nine apices per collection of coastal (□) and interior (●) Douglas-fir. Cessation of cell division in subtending leaf primordia is indicated by an open arrow for the coastal variety and a solid arrow for the interior variety. Vertical bars indicate ±1 standard error.
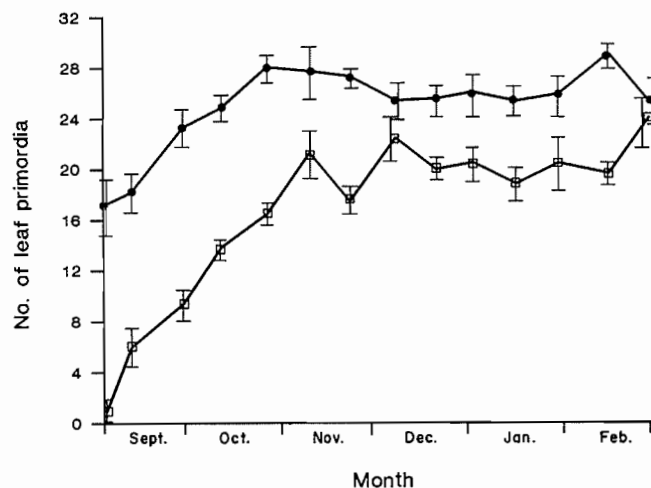

FIG. 12. The average number of leaf primordia along the flanks of the median sections of embryonic shoots of coastal (□) and interior (●) Douglas-fir seedlings based on four to nine apices per collection. Vertical bars indicate ±1 standard error.
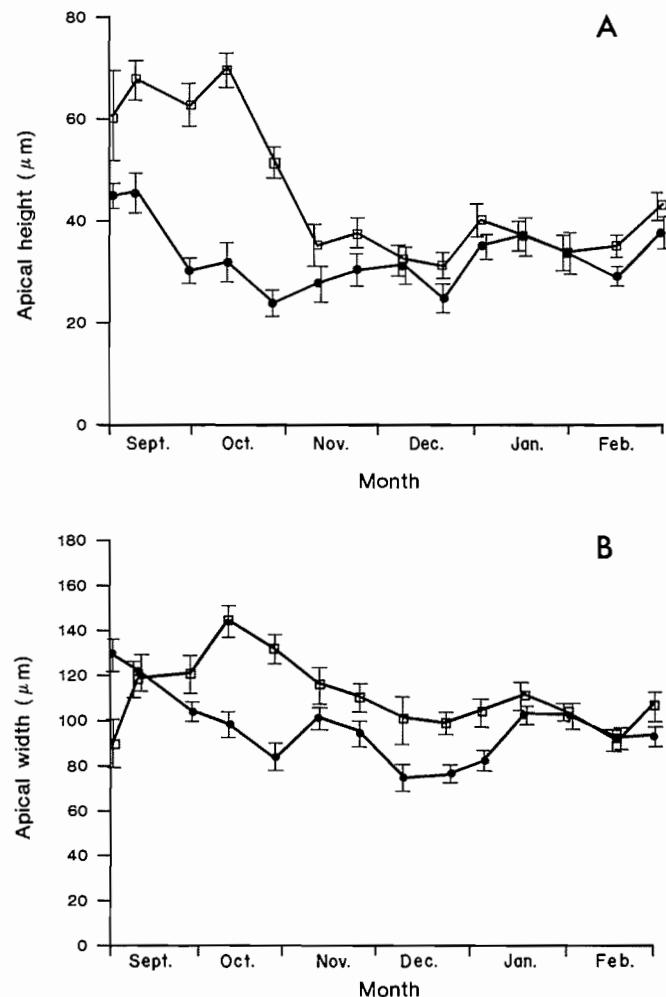

FIG. 13. The average apical height (A) and width (B) per median section of coastal (□) and interior (●) Douglas-fir seedlings, based on four to nine apices per collection. Vertical bars indicate ±1 standard error.

period of free growth during the 1st year, before bud development begins (Jablanczy 1971; Pollard *et al.* 1975; von Wuhlisch and Muhs 1986). In most conifer seedlings (Lines and Mitchel 1966; Jablanczy 1971) the potential for free growth diminishes with age, ceasing after 5–10 years (Pollard and Logan 1976; von Wuhlisch and Muhs 1986). In the present study, once free growth had ended, bud development in seedlings was similar to that in mature trees (Fig. 16). In most mature members of the Abietoideae the overwintering bud is completely preformed, which partly determines the capacity for shoot growth in the following year. Most do not undergo free growth during shoot elongation. However, a few retain to some extent the capacity for free growth. Western hemlock may initiate a few leaf primordia in some lateral buds before bud scale initiation begins in the spring (Owens and Molder 1973*b*).

Phenological differences between coastal and interior seedlings

The two varieties differ with respect to the length of the free growth period and the duration of bud development (Fig. 14). Free growth had ended in both varieties when sampling began in September, therefore no direct evidence is available for the end of free growth. However, when

FIG. 14. A comparison of stages of apical development of coastal and interior Douglas-fir seedlings in the 1st year of growth. Dotted lines indicate estimated stages of apical development that occurred before sampling began.

sampling began, the interior variety was already undergoing preformed leaf initiation, whereas the coastal variety had only initiated a few bud scales, indicating that the interior variety was probably the first to begin bud development. Height growth curves for the two varieties obtained from repeat measurements on the same trees by the B.C. Ministry of Forests bear out some of the morphological differences observed. Both varieties underwent a rapid period of free growth lasting about 4 months. The interior variety essentially ceased height growth about 3 weeks after the completion of this rapid phase, but the coastal variety continued to slowly increase in height for about another 7–8 weeks. Other evidence suggests that in the interior variety the period of free growth is shorter and bud development can be induced earlier by inductive short days (8 or 9 h) applied before the fall (Irgens-Moller 1968; Lavender and Overton 1972).

During bud development, the interior variety produced more leaf primordia and produced them more rapidly, and the apex had a higher MI than the coastal variety at comparable morphological stages. The later decrease in leaf primordia production and MI was also more rapid in the interior variety. Similar differences in primordia production were found between northern and southern provenances of white spruce (Pollard et al. 1975). The northern provenance produced primordia much earlier and more rapidly than the southern, although the southern produced primordia over a longer period.

Differences in bud development between the two varieties result partly from genetic responses to environmental cues and partly from environmental differences experienced at comparable morphological stages and as a result of imposed

cultural treatments. Genetically fixed responses to environmental cues, such as photoperiod (Lavender and Overton 1972), enable the interior variety to complete bud development earlier than the coastal variety and avoid low late summer precipitation (Irgens-Moller 1968) and the short frost-free season (Irgens-Moller 1967; Griffin and Ching 1977) of the continental climate.

Temperature and moisture also affect bud development (Pollard and Logan 1977). During early preformed leaf initiation daily temperatures in that study (in late August) were higher for the interior variety than for the coastal variety in September. Warm temperatures increased rates of needle initiation of black spruce and jack pine (Colombo 1982), and Macey (1982) showed that MI of white spruce seedlings was higher in a warm regime than in a cool one during bud development. Higher day and night temperatures in August, when the interior variety underwent the most rapid stage of bud development, probably contributed to the developmental differences observed at comparable stages in the two varieties (Fig. 10).

The coastal seedlings were drought stressed operationally to "induce dormancy", the criteria for dormancy in the nursery being the cessation of height growth and the formation of a terminal bud. Pollard and Logan (1977) found temperature to have a greater effect on bud development in Sitka spruce than moisture stress did. However, drought stressing has been shown to delay bud development in mature Douglas-fir trees (Owens et al. 1985) and decrease numbers of leaf primordia formed in seedlings (J.E. MacDonald, personal communication). The interior variety was not drought stressed. Thus, it is possible that
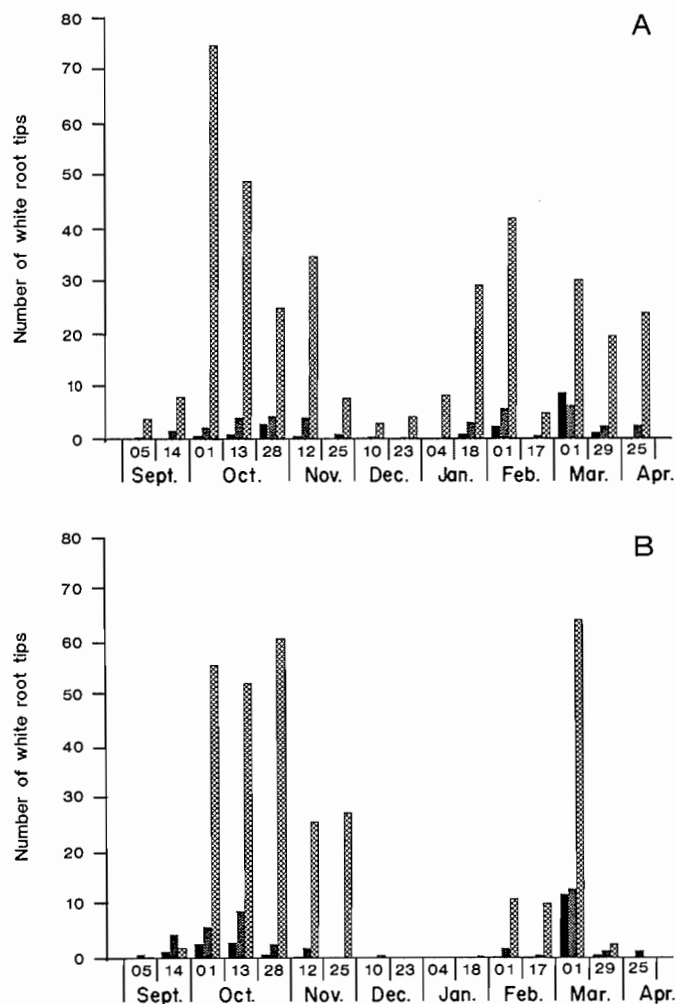
FIG. 15. Mean numbers of white root tips in three length classes of white root tips of 10 root systems of coastal (A) and interior (B) Douglas-fir seedlings per collection date. Vertical bars indicate the average frequency of white roots >0–4.9 ⊠), 5–9.9 (▨), and >10 mm (■) per root system for each date.

drought stress reduced the final number of leaf primordia formed in the coastal variety by inhibiting bud development and, in effect, reducing the ability of this variety to exploit the warm fall coastal environment. Environmental conditions during bud development affect shoot growth in the following year (Kozlowski 1971). However, spruce (Macey and Arnott 1986) and Douglas-fir (Carlson et al. 1980) seedlings were able to compensate through free growth for reduced predetermined foliage after replanting.

The coincidence of nodal diaphragm formation and the decrease in the rate of leaf initiation and MI suggests that this structure has some causative role. Such a role in restricting movement of nutrients and growth-promoting substances through the pith was suggested by Owens and Molder (1979). A detailed developmental study of nodal diaphragm development in coastal Douglas-fir (Krasowski and Owens 1988) showed no clear anatomical evidence to indicate that the flow of substances was restricted during dormancy development, as symplastic continuity was maintained by penetration of the nodal diaphragm by the procambial strands and by penetration of plasmodesmata between living cells of the nodal diaphragm. However, those workers suggested that in the spring, the formation of the cavity

beneath the nodal diaphragm may ensure that the movement of substrates between the embryonic shoot and subtending tissues is controlled until the vasular connections to the embryonic shoot can become conductive. The nodal diaphragm functions as a kind of apoplastic "filter." Water may move freely through the nodal diaphragm when ice forms in the cavity below.

Dormancy

In mature Douglas-fir, buds were considered dormant (Owens 1968; Owens and Molder 1973*a*) when all cell divisions ceased in the shoot apices. If this anatomical criterion for dormancy is applied to seedlings, the coastal and interior varieties entered dormancy in December and November, respectively, after a period of embryonic shoot development, and ended dormancy at essentially the same time in March. Bud dormancy has also been associated with the cessation of DNA synthesis in the apex following shoot elongation in mature Douglas-fir (Owens and Molder 1973*a*). Other workers concerned with the histochemistry of shoot apices have associated dormancy with a limited ability to duplicate DNA in potato buds (Tuan and Bonner 1964) and with a relatively low DNA level in ash (Cottignies 1983). There is also evidence in coastal Douglas-fir nursery seedlings that the cessation of mitotic activity in the apices coincides with the period of maximum resistance to stress during handling (Carlson *et al*. 1980).

The classical definition of dormancy is any case in which a tissue predisposed to elongate does not do so (Doorenbos 1953). However, dormancy based on the criterion of regrowth under prescribed conditions has several weaknesses. Physiological states within the embryonic shoot change and overlap between bud set and subsequent shoot elongation the following spring. Thus, the dormant period is rather vaguely delineated and depends on the physiology of the plant and the environmental conditions. Furthermore, the embryonic shoot is a complex organ containing several meristems, each having unique developmental characteristics. It was for these reasons that the more precise anatomical definition of dormancy was used in this study.

Shoot elongation following dormancy

Cell divisions were first observed in several individuals of both varieties on 14 February. This was approximately 2 weeks before measurable shoot elongation had begun in some individuals. Owens *et al*. (1985) showed that in mature Douglas-fir buds, cell division existed as a fairly distinct stage preceding cell elongation and that 10% of shoot elongation occurred before flushing.

Both cell division and cell elongation are important in timing of flushing. Intravarietal and intervarietal flushing were variable, but the interior variety generally flushed earlier than the coastal variety even though both varieties resumed growth at the same time. The postdormancy cell division phase was possibly shorter in the interior variety. Interior seedlings have been shown to elongate more rapidly than the coastal variety (Campbell and Sugano 1975). Differences in response to environmental cues in the spring would be expected in interior and coastal varieties of Douglas-fir because the interior variety has a shorter period in which to complete shoot elongation (Sorensen 1983).

The occurrence of cell division and cell elongation as two independent processes during early shoot elongation limits the use of flushing as a criterion for the end of dormancy.
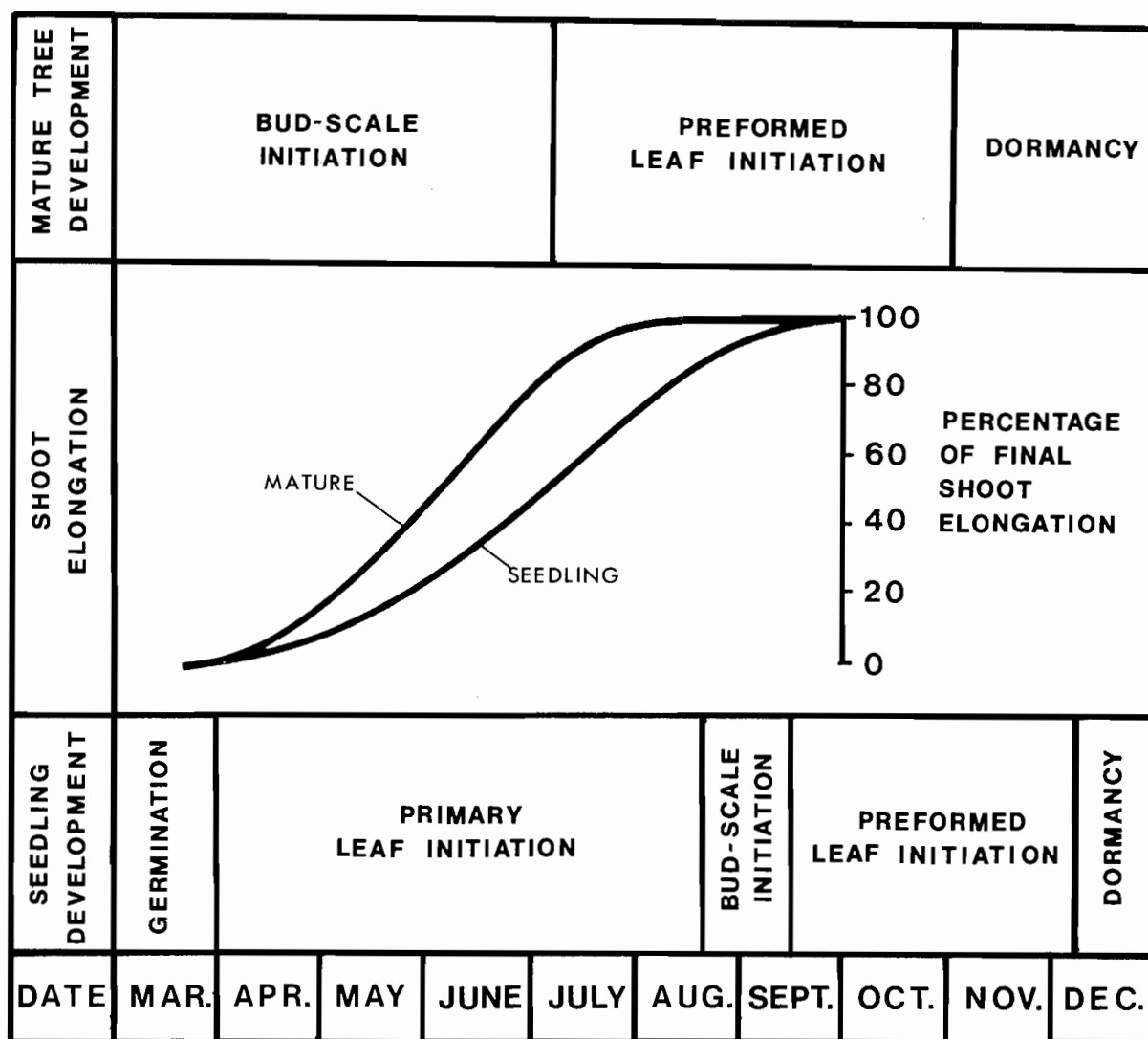
FIG. 16. Phenology of lateral bud development and lateral shoot elongation in mature trees, and terminal bud development and primary shoot elongation in seedlings of coastal Douglas-fir. Data for bud development and shoot elongation for mature coastal Douglas-fir were taken from Owens et al. (1985). Data for seedling bud development were those of the coastal Douglas-fir in this study and data for shoot elongation were taken from file No. 955-20-6, B.C. Ministry of Forests Records, Silviculture Branch.

Many physiological studies have used flushing as the criterion for the end of dormancy (Kreuger and Trappe 1967; Lavender et al. 1973; van den Driessche 1975) without considering how treatments affect both resumption of cell division and the extent of cell elongation before flushing occurs.

Shoot–root relationships

Root elongation and shoot apical mitotic activity follow similar patterns (see Figs. 11A, 15) and may be correlated in Douglas-fir seedlings, as has been shown in broad-leaved trees (Thielges and Beck 1976). However, no causal relationship can be assumed from the results of this study. In the spring, root elongation in interior Douglas-fir increased markedly from a low level on the same sampling date (1 March) as that on which shoot apical mitotic activity increased. Root elongation then decreased during the early stages of shoot elongation, as shoot axis MI decreased (Owens et al. 1985). This trend was not as strong in the coastal variety, where root elongation before 1 March was greater and more variable, and the increase in March was smaller. However, it should be noted that root elongation

and cell division in leaf primordia occurred even in January in the coastal variety. Possibly the same substances (hormones or carbohydrates) postulated by Lavender and Wareing (1972) and Lavender and Hermann (1970) are produced in large enough quantities in the shoot to maintain the processes of root elongation and cell division. The lack of shoot cell divisions in the interior variety, however, precludes root elongation. This hypothesis is supported by the present observation that root elongation increases markedly when rapid cell division occurs in apices. Past workers have reported that root elongation precedes visible shoot elongation in coastal Douglas-fir (Kreuger and Trappe 1967) and *Cedrus atlantica* (Riedacker 1979). These results, which appear to contradict those from the present study, may be accounted for because none of these workers used the onset of cell division in the apex as the beginning of shoot growth; visible bud swelling or flushing was used instead. This also means that flushing is not a good criterion for the onset of shoot growth.

In the fall there was an increase in root elongation and numbers of short roots in both varieties. This occurred

slightly later in the coastal variety than in the interior variety. During this phase of root development, embryonic shoots were undergoing late preformed leaf initiation in both varieties, but as in roots, stages of bud development were somewhat later in the coastal variety. The difference in timing of fall root development between the two varieties may depend on their differential response to environmental cues. Lavender and Hermann (1970) showed no evidence of stimulation of either root formation or root elongation by materials exported from nondormant buds or exogenously applied growth regulators in coastal Douglas-fir seedlings. However, the similar timing of root elongation and bud development in each variety in this study suggests some correlative influence between the root and the shoot tip.

The interior Douglas-fir seedlings ceased shoot elongation, underwent bud development, and became dormant about 4 weeks before the coastal variety. They also produced 17% more leaf primordia and a greater maximum mitotic index than the coastal variety. These differences in phenology and development between coastal and interior varieties of Douglas-fir warrant more specific containerized nursery cultural practices to obtain maximum quality of both varieties of seedlings.

Acknowledgments

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