DORMANCY INDUCTION IN COASTAL DOUGLAS-FIR SEEDLINGS: BUD DEVELOPMENT, DORMANCY DEVELOPMENT, AND DRY-MATTER ALLOCATION

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ABSTRACT

Bud development under controlled environment conditions in coastal Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii*) seedlings was investigated. Eight dormancy induction treatments varied type of short day (SD), temperature and moisture. Photoperiod was decreased to 8 h either abruptly (ASD) or gradually (GSD). Day/night temperature was either constant at a high temperature (HT), or gradually decreased to a low temperature (LT). Moisture was controlled to either result in no drought-stress (ND) or to cause drought-stress (D). Once the dormancy induction signal was perceived by seedlings, neoformed-leaf initiation stopped and bud development began. Bud development involved two stages of primordial initiation (bud-scale and preformed-leaf) separated by a transitional phase. The change from neoformed-leaf to bud-scale initiation was faster under ASD than under GSD, under HT than under LT, and under D than under ND. Bud-scale-complex development was faster under ASD than under GSD. Type of SD had a significant influence and moisture had a weakly significant influence on number of bud scales initiated. Fewer bud scales were initiated under ASD than under GSD and under ND than under D. The transitional phase was shorter and hence preformed-leaf initiation started earlier under ASD than under GSD and under ND than under D. Type of SD, temperature, and moisture had a significant influence on number of preformed-leaf primordia initiated. More preformed-leaf primordia were initiated under ASD than under GSD, under HT than under LT, and under ND than under D. Anatomy of the bud-scale receptacle

and crown region were distinctly different between ASD and GSD. Crown height was greater under ASD than under GSD. Crown width was greater under ND than under D.

Bud development, dormancy development, and dry matter allocation under commercial greenhouse conditions in coastal Douglas-fir seedlings were investigated. There were 3 dormancy induction treatments: SD without moisture stress (SD-MS), SD with moisture stress (SD+MS), and long day with moisture stress (LD+MS). The MS occurred during the first 2 weeks of SD+MS and LD+MS. There were 4 durations in SD: 3, 4, 5, and 6 weeks in SD (WK SD).

Within the first week in SD, neoformed-leaf initiation ended, bud-scale initiation began and ended, and rapid preformed-leaf initiation began. Rapid preformed-leaf initiation was completed by week 6, and slow preformed-leaf initiation was completed by week 10 for 6 WK SD and week 13 for 3, 4, and 5 WK SD. Number of leaves initiated ranged from 134.9 to 175.3. Duration in SD had a highly significant effect on number of preformed leaves initiated; significantly fewer leaves were initiated under 6 WK SD than under 3, 4, and 5 WK SD. As preformed-leaf initiation ended, mitotic index (MI) approached zero. After over-wintering, buds flushed 4.6 to 6.9 days after being placed under forcing conditions in March. Duration in SD had a highly significant effect on speed of bud break. Buds from 4 WK SD-MS flushed significantly faster than those from other SD-MS treatments; buds from 4 WK SD+MS flushed significantly faster that those from 3 WK SD+MS. Shoot diameter at the root collar and root dry weight ranged from 3.1 to 3.5 mm and 0.55 to 0.73 g, respectively. Duration in SD had a highly significant effect on root collar diameter. Root collar diameters of seedlings from 3 and 4 WK SD were significantly larger than those from longer durations in SD. There was a

highly significant moisture regime x duration in SD interaction for root dry weight.

Within the first week of LD+MS, neoformed-leaf initiation ended and bud-scale initiation began. After 3-4 weeks, bud-scale initiation ended and slow preformed-leaf initiation began. Rate of preformed-leaf initiation was slow until week 6, rapid during weeks 8-10, and then decreased slightly between weeks 10-13. Number of preformed leaves initiated averaged 159.4. By week 13, as preformed-leaf initiation slowed, MI was decreasing, but was not approaching zero. After over-wintering, buds flushed 11.0 days after being placed under forcing conditions in March. Root collar diameter and root dry weight were 3.2 mm and 0.62 g, respectively.

Both studies demonstrated that the sequence of stages in bud development is constant, but the phenology and characteristics of the stages vary with type of dormancy induction treatment. The relationship between bud development, mitotic activity of the apical meristem, dormancy development, and speed of bud break is presented and discussed. Terminology which better describes the active processes of dormancy induction and dormancy development is suggested. Recommendations concerning the use of SD for early dormancy induction in commercial greenhouse culture are made.

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TABLE OF CONTENTS

Abstract....ii

Table of Contents....vi

List of Tables....ix

List of Figures....xi

Acknowledgements....xiii

Chapter 1: Introduction....1

Chapter 2: Literature Review....4

2.1 Dormancy....4

2.2 Dormancy Induction and Dormancy Development 6
- 2.2.1 Photoperiod and Dormancy Induction....6
- 2.2.2 Temperature and Dormancy Induction....7
- 2.2.3 Growth Regulators and Dormancy Induction....8
- 2.2.4 Dormancy Induction in Nurseries....8

2.3 Breaking of Dormancy....9
- 2.3.1 Temperature and Breaking of Dormancy 9
- 2.3.2 Photoperiod and Breaking of Dormancy 10
- 2.3.3 Growth Regulators and Breaking of Dormancy 11

2.4 Bud Break 12

2.5 Description of First-Year-Seedling Shoot and Bud Development.12

2.6 Morphological Standards for Seedlings....14

Chapter 3: Controlled Environment Chamber Study 15

3.1 Introduction 15

3.2 Materials and Methods 16
- 3.2.1 Greenhouse Culture 16
- 3.2.2 Dormancy Induction Treatments 16
- 3.2.3 Experimental Design 17
- 3.2.4 Processing of Samples 20
- 3.2.5 Morphological and Anatomical Observations 20
- 3.2.6 Numbers of Bud Scales and Preformed Leaves 21
- 3.2.7 Statistical Analysis 21

3.3 Results 21
- 3.3.1 Bud-Scale Initiation 21
- 3.3.2 Bud-Scale-Complex Composition and Development 28
- 3.3.3 Transitional Phase 30
- 3.3.4 Preformed-Leaf Initiation 30

3.3.5 Bud-Scale Receptacle 35
3.3.6 Preformed-Leaf Primordium Development 38
3.3.7 Preformed-Shoot Height and Width 38
3.3.8 Crown Differentiation and Dimensions 40

3.4 Discussion 42
3.4.1 Bud Development 42
3.4.2 Effect of Type of SD, Temperature, and Moisture on Bud Development 43
3.4.3 Effect of Type of SD, Temperature, and Moisture on Numbers of Bud Scales and Preformed-Leaf Primordia Initiated 45
3.4.4 Functional Terminology 47

Chapter 4: Commercial Greenhouse Study 48

4.1 Introduction 48

4.2 Materials and Methods 49
4.2.1 Study 1 49
4.2.1.1 Seedling Culture 49
4.2.1.2 Dormancy Induction 50
4.2.1.3 Post-Induction and Over-Wintering Culture 51
4.2.1.4 Experimental Design 52
4.2.1.5 Sampling and Transport 52
4.2.1.6 Processing of Samples 53
4.2.1.7 Morphological and Anatomical Observations 53
4.2.1.8 Numbers of Bud Scales and Preformed Leaves ...54
4.2.1.9 Speed of Bud Break 55
4.2.1.10 Root Collar Diameter and Root Dry Weight 55
4.2.1.11 Presentation of Results and Statistical Analysis 56
4.2.2 Study 2 57

4.3 Results 58
4.3.1 Bud Development During and Following the SD-MS and SD+MS Treatments 58
4.3.1.1 Bud-Scale Initiation and Differentiation 58
4.3.1.2 Bud-Scale-Receptacle Development 61
4.3.1.3 Preformed-Leaf Initiation 63
4.3.1.4 Mitotic Activity During Bud Development 74
4.3.1.5 Crown Differentiation 78
4.3.1.6 Preformed-Shoot-Axis Height and Width 81
4.3.2 Speed of Bud Break After the SD-MS and SD+MS Treatments 83
4.3.3 Root Collar Diameter and Root Dry Weight for the SD-MS and SD+MS Treatments 86
4.3.4 Bud Development During and Following the LD+MS Treatment 88
4.3.4.1 Bud-Scale Initiation and Differentiation 88
4.3.4.2 Bud-Scale Receptacle Development ....9?

4.3.4.3 Preformed-Leaf Initiation 92
4.3.4.4 Mitotic Activity During Bud Development 93
4.3.4.5 Crown Differentiation 93
4.3.4.6 Preformed-Shoot-Axis Height and Width 93
4.3.5 Speed of Bud Break After the LD+MS Treatment 96
4.3.6 Root Collar Diameter and Root Dry Weight for the LD+MS Treatment 96
4.3.7 Comparison of Bud Development Under the SD and LD+MS Treatments 96
4.3.8 Comparison of Speed of Bud Break After the SD and LD+MS Treatments 97
4.3.9 Comparison of Root Collar Diameter and Root Dry Weight for the SD and LD+MS Treatments 97

4.4 Discussion 97
4.4.1 Bud Development During and Following the SD-MS and SD+MS Treatments 97
4.4.2 Bud Development During and Following the LD+MS Treatment 100
4.4.3 Bud Development During and Following the SD and LD+MS Treatments 101
4.4.4 Root Collar Diameter and Root Dry Weight 106
4.4.5 Dormancy Development as a Growth Process 108

Chapter 5: Summary and Conclusions 109

Literature Cited 112

LIST OF TABLES

Table 1. Percentage of apices initiating bud scales after 1-2 weeks under different dormancy induction treatments in Douglas-fir seedlings 25

Table 2. Percentage of apices initiating bud scales, in the transitional phase, and initiating preformed leaves after 3-5 weeks under different dormancy induction treatments in Douglas-fir seedlings 26

Table 3. Analysis of variance of dormancy induction treatment effects on number of bud scales initiated in Douglas-fir seedlings 27

Table 4. Numbers of bud scales and preformed-leaf primordia initiated under different dormancy induction treatments in Douglas-fir seedlings 27

Table 5. Percentage of bud-scale complexes (BSCs) in various stages of BSC development after 1-2 weeks and after 3-5 weeks under different dormancy induction treatments in Douglas-fir seedlings 29

Table 6. Analysis of variance of dormancy induction treatment effects on number of preformed-leaf primordia initiated in Douglas-fir seedlings 35

Table 7. Analysis of variance of dormancy induction treatment effects on final preformed-shoot height in Douglas-fir seedlings 39

Table 8. Final preformed-shoot height and width in mid-October under different dormancy induction treatments in Douglas-fir seedlings 39

Table 9. Analysis of variance of dormancy induction treatment effects on final preformed-shoot width in Douglas-fir seedlings 40

Table 10. Analysis of variance of dormancy induction treatment effects on final crown height in Douglas-fir seedlings 41

Table 11. Final crown height and width in mid-October under different dormancy induction treatments in Douglas-fir seedlings 41

Table 12. Analysis of variance of dormancy induction treatment effects on final crown width in Douglas-fir seedlings 42

Table 13. Percentage increases in number and size of pith and cortical cells by week during bud-scale-receptacle development under different dormancy induction treatments in Douglas-fir seedlings 62

Table 14. Numbers of preformed leaves initiated in buds of Douglas-fir seedlings under different dormancy induction treatments 75

Table 15. Analysis of variance of dormancy induction treatment and location in greenhouse effects on number of preformed leaves initiated in buds of Douglas-fir seedlings 75

Table 16. Crown height and width in buds of Douglas-fir seedlings from different dormancy induction treatments by early October 81

Table 17. Numbers of preformed-leaf primordia per preformed-shoot-axis flank and preformed-shoot-axis height and width in buds of Douglas-fir seedlings from different dormancy induction treatments by early October 82

Table 18. Analysis of variance of dormancy induction treatment, location in greenhouse, and location in controlled environment chamber effects on days to bud break in Douglas-fir seedlings 83

Table 19. Shoot diameter at the root collar and root dry weight in early October of Douglas-fir seedlings given different dormancy induction treatments 86

Table 20. Analysis of variance of dormancy induction treatment and location in greenhouse effects on shoot diameter at the root collar in Douglas-fir seedlings 87

Table 21. Analysis of variance of dormancy induction treatment and location in greenhouse effect on root dry weight in Douglas-fir seedlings 87

LIST OF FIGURES

Fig. 1. Schematic of dormancy induction treatments 18

Fig. 2-5. Light micrographs of median longitudinal sections of shoot tips of Douglas-fir seedlings 23

Fig. 6-9. Scanning electron micrographs of fresh preformed shoots of terminal buds of Douglas-fir seedlings from dormancy induction treatments 31

Fig. 10-13. Scanning electron micrographs of fresh preformed shoots of terminal buds of Douglas-fir seedlings from dormancy induction treatments 33

Fig. 14. Light micrograph of median longitudinal section of apical meristem of a Douglas-fir seedling initiating preformed-leaf primordia 36

Fig. 15-16. Light micrographs of median longitudinal sections of terminal buds of Douglas-fir seedlings 36

Fig. 17. Light micrograph of median longitudinal section of distal portion of a preformed shoot of a Douglas-fir seedling under abrupt short day 36

Fig. 18-19. Light micrographs of median longitudinal sections through the crown below preformed shoots of Douglas-fir seedlings in October 36

Fig. 20-25. Scanning electron and light micrographs of shoot tips of Douglas-fir seedlings during and following the short day treatments 59

Fig. 26. Number of preformed-leaf primordia per flank in median longitudinal sections through buds of Douglas-fir seedlings versus date 64

Fig. 27. Height of apical meristem in median longitudinal sections through buds of Douglas-fir seedlings versus date 66

Fig. 28. Width of apical meristem in median longitudinal sections through buds of Douglas-fir seedlings versus date 68

Fig. 29. Number of apical cells in median longitudinal sections through buds of Douglas-fir seedlings versus date 70

Fig. 30-31. Light micrographs of median longitudinal sections through buds of Douglas-fir seedlings during and following the short day treatments 72

Fig. 32. Mitotic index of apical meristem in median longitudinal sections through buds of Douglas-fir seedlings versus date 76

Fig. 33-35. Light micrographs of median longitudinal sections through the bases of preformed shoots during crown differentiation during and following the short day treatments 79

Fig. 36. Days to bud break in March of terminal buds of overwintered Douglas-fir seedlings from different dormancy induction treatments 84

Fig. 37-42. Scanning electron and light micrographs of shoot tips of Douglas-fir seedlings during and following the long day with moisture stress treatment 89

Fig. 43-45. Light micrographs of buds of Douglas-fir seedlings following the long day with moisture stress treatment 94

Fig. 46. Comparison of stages of apical activity/dormancy and of days to bud break in Douglas-fir seedlings after the short day and long day with moisture stress treatments 102

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Chapter 1

INTRODUCTION

The reforestation program of the British Columbia Ministry of Forests restocks currently denuded forest lands and the large backlog of areas that have not restocked satisfactorily. The commitment to reforestation was strengthened by amendments to the Forest Act in 1987. The number of seedlings of all species planted has increased dramatically in a decade, from 74 million in 1980 through 134 million in 1985 to a projected 335 million in 1990. Planting at the 1990 level will continue into the mid 1990's.

Seedlings must be of high quality to ensure survival and good growth initially and thus plantation success. Seedling quality integrates the physiological and morphological characteristics of a seedling. Aspects of seedling quality include bud dormancy, water relations, mineral nutrition, morphology, stress resistance, cold-hardiness, and root growth potential.

Presently in coastal British Columbia, forests at mid- to high elevations are being harvested. On these sites, snow pack delays spring planting to the extent that seedling root systems have insufficient time to become well-established prior to the annual summer drought. Consequently, seedlings can die from lack of water. Fall-planting offers better seedling survival because root growth in late fall and early spring result in well-established root systems prior to the summer drought.

Although all aspects of seedling quality are important, bud dormancy was the primary emphasis of this study. Seedlings which have developed bud dormancy prior to lifting, handling, transport and planting are likely to survive and perform well. The initiation of bud dormancy is readily manipulated by nursery practice. For spring-planting stock, moderate moisture stress is commonly used in nurseries as the dormancy induction treatment. And, for most nurseries, it continues to be used for

fall-planting stock even though survival of such stock is reduced because dormancy is not induced early enough. The use of short photoperiods shows greater promise for earlier dormancy induction. The specific problem addressed in this study was the development of early dormancy in containerized fall-planting stock.

Maximizing seedling photosynthesis is essential in nursery practice. This is especially important once desired seedling height has been attained because it is then that dry matter production increases in shoots and roots. Other than height, shoot diameter and root dry weight are the morphological characteristics used as standards for culling seedlings in British Columbia. Because short photoperiods reduce seedling photosynthesis, timing and duration of photoperiod control become very important.

Few nurseries in British Columbia have photoperiod control systems in their greenhouses because of the large capital investment required. The Angus P. MacBean Nursery of MacMillan Bloedel Limited installed photoperiod control systems in 1985 and began dormancy induction treatments using short days in that year. The two studies reported here were undertaken in co-operation with MacMillan Bloedel with the objective of refining dormancy induction treatments for fall-planting stock. The studies were conducted under controlled environment chamber and commercial greenhouse conditions. In both studies, commercially cultured seedlings were used; timing of the application of dormancy induction treatments was dependent upon attainment of desired seedling height and thus was under the grower's control.

The objective of the first controlled environment chamber study was to determine the effects of different dormancy induction treatments on bud development. In this study, the dormancy induction treatments did not simulate those used in commercial greenhouses. The objectives of the second commercial

greenhouse study were: (1) to determine dormancy induction and development under different short day durations with and without an initial period of moisture stress and to compare these treatments, (2) to determine dormancy induction and development under moisture stress alone and to compare this with that under short days with and without moisture stress, and (3) to determine the effect of different treatments on shoot diameter at the root collar and root dry weight.

Chapter 2
LITERATURE REVIEW

2.1 Dormancy

Numerous authors (e.g. Wareing and Phillips 1978, Cottignies 1987) have discussed the biological significance of dormancy in plants. In temperate regions, there are seasonal variations in climatic conditions that produce favourable and unfavourable seasons for plant growth. Hence, plants have evolved annual cycles of growth and dormancy. In woody plants, dormancy is recognized by the cessation of shoot elongation and the formation of over-wintering buds (Wareing 1950). Over-wintering buds offer two types of protection from unfavourable conditions. Scales of the over-wintering bud reduce water loss from the apical meristem during the winter, while dormant apical meristems are more resistant to cold damage (Wareing and Phillips 1978).

In the literature, there is confusion about what parts of a tree become dormant, and the difference between dormancy and cold-hardiness. Only shoot apical meristems become dormant: There is conflicting evidence about dormancy in root apical meristems and vascular cambia (Lavender 1980). Cold-hardiness represents resistance to cold that occurs, to some degree, throughout the entire tree (Lavender 1985). However, in a recent review (Cottignies 1987), it is clear that the difference between dormancy and cold-hardiness is not yet resolved.

Dormancy is defined physiologically as any case in which a tissue predisposed to elongate does not do so (Doorenbos 1953). Definitions of the kinds of dormancy abound, and a thorough review of the nomenclature and a table of equivalencies among terms has been presented by Romberger (1963). Recently, a reduced universal terminology for dormancy was proposed (Lang *et al.* 1985, Lang 1987).

However, the terminology *sensu* Doorenbos (1953) (which is in widespread use by physiologists) as defined by Romberger (1963) will be adopted in this paper. There are two types of dormancy under consideration: quiescence and rest. Quiescence is dormancy imposed by the external environment, and growth resumes as soon as environmental conditions are again favorable. Rest is physiological dormancy maintained within the organ itself, and growth resumes only after adequate cold treatment.

An anatomical determination of the dormancy status of buds has also been used. Owens and Molder (1973) proposed the use of mitotic activity of apical meristems to delimit dormancy, i.e. buds are dormant when there are no mitoses. On mature trees, they demonstrated that after shoot elongation ceased mitotic activity of the apical meristem continued for several months. Subsequently, mitotic activity was used to delimit dormancy in seedlings (Carlson *et al.* 1980, Fielder and Owens 1989).

The difference between anatomically-delimited and physiologically-defined dormancy has been discussed using coastal Douglas-fir as an example (Lavender 1985). Dormancy determined by mitotic activity lasts from early December to early March (Owens and Molder 1973); whereas, dormancy determined by lack of elongation lasts from early July through early April (Lavender 1985). That the apical meristem, within an apparently dormant over-wintering bud, remains active until leaf initiation is completed (Owens 1968) has been occasionally recognized in the physiological literature (Perry 1971, Lavender 1980, Carlson *et al.* 1980). In view of the observation that seedling resistance to stress seems closely correlated to mitotic activity of the meristem, anatomical determination of the dormancy status of seedlings may become a useful tool in assessing seedling physiology (Lavender 1985).

2.2 Dormancy Induction and Dormancy Development

For seedlings, it is accepted that dormancy starts as quiescence which is imposed directly by changes in the nursery environment (Cleary *et al.* 1978, Ritchie 1984a, Lavender 1985). During dormancy induction, it was thought that over-wintering buds were initiated and developed (Lavender and Cleary 1974). Dormancy induction is followed by dormancy deepening, by which time over-wintering buds were thought to be well-developed (Cleary *et al.* 1978). During dormancy deepening, there is development of rest (Cleary *et al.* 1978).

In physiological terms, for the Pacific Northwest and southwestern British Columbia, it was thought that dormancy induction occurred from mid-July to mid- or late September, and dormancy deepening occurred from mid- or late September to mid-November (Cleary *et al.* 1978). In reality, dormancy induction and deepening represent a developmental continuum. There is insufficient time for bud development to be completed during the dormancy induction phase; bud development would continue through the dormancy deepening phase and longer (for timing see Fielder and Owens 1989).

2.2.1 Photoperiod and Dormancy Induction

Wareing (1956) discussed the physiological effects of short days (SD) on dormancy induction. Under natural conditions, some species show photoperiodic control of dormancy induction; whereas, other species cease extension growth in the summer before appreciable photoperiodic reduction occurs. Some species from both categories show marked photoperiodic response under controlled experimental conditions. Seedlings often respond to photoperiodic control, whereas mature trees may not. Short days have been shown to induce dormancy under experimental conditions in seedlings of *Picea abies* (L.) Karst. (Dormling *et al.* 1968, Robak and

Magnesen 1970, Heide 1974), *P. glauca* (Moench) Voss (Pollard 1974a, Pollard and Logan 1977), *P. mariana* (Mill.) B.S.P. (Pollard and Logan 1977), *P. sitchensis* (Bong.) Carr. (Pollard *et al.* 1975), and *Pseudotsuga menziesii* (Mirb.) Franco (McCreary *et al.* 1978).

The locus of photoperiodic perception has been shown to be the active shoot apex or the mature leaves; it was postulated that a growth-inhibitor was produced in these organs during the dark period (Wareing 1954).

2.2.2 Temperature and Dormancy Induction

The effect of temperature on dormancy induction has been studied in combination with the effect of photoperiod. In *Picea sitchensis* seedlings, dormancy was induced under cool temperatures and long days (LD); whereas, SD were required to induce dormancy under warm temperatures (Malcolm and Pymar 1975). In *P. abies* seedlings, constant day/night temperatures did not appreciably change the photoperiod at which dormancy was induced (Heide 1974). However, for the same species, dormancy was induced under a cool day/night temperature and LD (Dormling *et al.* 1968) and under a warm day/cool night temperature and LD (Heide 1974). In *Pseudotsuga menziesii* seedlings under SD, cool night temperatures hastened dormancy induction as compared to a warm night temperature (Lavender *et al.* 1968). As the preceding examples indicate, the effect of temperature on dormancy induction is variable.

In general, decreasing temperatures contribute to dormancy induction. However, several authors point to the greater importance of photoperiod in dormancy induction. Dormling (1973) suggested that temperature affects dormancy induction only after a critical photoperiod has made plants receptive to the lower temperature. Heide (1974) also concluded that low temperature was not as

significant as short photoperiods in inducing dormancy.

2.2.3 Growth Regulators and Dormancy Induction

Abscisic acid (ABA) has been associated with dormancy induction. Higher ABA levels were present in mature leaves and apices of *Acer pseudoplantanus* L. (Phillips and Wareing 1959) and *Betula pubescens* Ehrb. (Eagles and Wareing 1964) seedlings transferred to SD as compared to those of seedlings maintained under LD. In *B. pubescens* seedlings, ABA was detected in mature leaves after 2 SD and in apices after 5 SD (Phillips and Wareing 1959). Higher amounts of ABA were extracted from *Fraxinus excelsior* L. buds that were in rest than from buds that had completed rest (Hemberg 1949).

Phillips and Wareing (1959) proposed that under SD, ABA was produced in the leaves and transported to the apex and thus was the cause and not the effect of dormancy induction. Eagles and Wareing (1963) suggested the term "dormin" to delineate the ABA responsible for dormancy induction as compared to ABA involved in general growth inhibition.

Abscisic acid has been applied to seedlings to induce dormancy with varying results. In *Picea abies* (Heide 1986) and *A. rubrum* L. (Perry and Hellmers 1973) seedlings grown under LD, application of exogenous ABA did not induce normal over-wintering buds. In contrast, in *B. pubescens* seedlings, grown under LD, application of ABA induced typical over-wintering buds (Eagles and Wareing 1964).

2.2.4 Dormancy Induction in Nurseries

In western North America, moderate moisture stress is commonly used to induce dormancy in bareroot (Cleary *et al.* 1978) and containerized (Matthews 1982) seedling nurseries. This treatment was adopted because it was noted that

under natural conditions, seedlings became dormant in response to mid-summer moisture deficits (Lavender *et al.* 1968). Another commonly used treatment in nurseries is nutrient stress (specifically, nitrogen stress) (Matthews 1982). The use of SD for dormancy induction in containerized nurseries is increasing, despite the substantial capital investment (A.H. Maher, pers. comm.).

The use of moisture stress to induce dormancy has been investigated in seedlings of *Pseudotsuga menziesii* (Lavender *et al.* 1968, Carlson 1978), *Picea glauca* (Macey and Arnott 1986), and *Larix occidentalis* Nutt. (Vance and Running 1985), but the mode of action of moisture stress was not investigated or discussed. Similarly, in an investigation of the effect of nutrient stress on dormancy induction in *P. glauca* (Macey and Arnott 1986), the mode of action was not addressed. Although the use of SD to induce dormancy is well-documented (see Section 2.2.1), its mode of action was only hypothesized (Wareing 1954).

2.3 Breaking of Dormancy

2.3.1 Temperature and Breaking of Dormancy

Rest must be "broken" by a period of cold (Samish 1954). The length of this period is referred to as the chilling requirement which is the number of hours at, or below, a threshold temperature required to break rest (Samish 1954). The threshold temperature varies with species (Samish 1954). Commonly suggested chilling temperatures are 7°C (Samish 1954) and 5°C (Perry 1971). It has been said that with the exception of very low temperatures, any temperature below the threshold will have a similar effect (Samish 1954). Indeed, sub-freezing temperatures accelerated the breaking of dormancy in *A. saccharum* seedlings (Olmsted 1951). In contrast, temperatures near 0°C are said to be not as efficient as

those near 5°C in breaking dormancy (Perry 1971).

Romberger (1963) discussed the effect of temperature during chilling. During the "depth of rest", growth is blocked at all temperatures. As the end of rest is approached, growth becomes possible in a narrow temperature range. Temperatures below the lower limit fulfill the chilling requirement; whereas, temperatures above the upper limit counteract the chilling requirement and a secondary dormancy develops that requires additional chilling hours. Similarly, van den Driessche (1975) demonstrated that interruption of chilling temperatures with high temperatures offset the effect of chilling.

Although knowledge of the chilling requirement of individual species can be used to determine time of lifting and duration of cold-storage of seedlings (Ritchie 1984a), the chilling requirement has only been determined for a few species. Two-year-old *P. glauca* (Nienstaedt 1966) and *Pseudotsuga menziesii* (van den Driessche 1975) seedlings have chilling requirements of 670-1345 h at 2-4°C and 2000 h below 4.4°C, respectively.

2.3.2 Photoperiod and Breaking of Dormancy

The effect of photoperiod on the breaking of dormancy varies with species, conditions during dormancy induction, and depth of rest. The chilling requirement of *A. saccharum* (Olmsted 1951), *Fagus sylvatica* L. (Wareing 1953), and *Picea glauca* (Nienstaedt 1966) seedlings was compensated for by LD. Under continuous LD, dormancy was broken in seedlings of *B. pubescens* and *L. decidua* Mill., but was not broken in seedlings of *A. pseudoplatanus* and *Robinia pseudoacacia* L. (Wareing 1954). In *P. abies*, dormancy was broken by exposure to LD on seedlings induced under 20°C; whereas, chilling was needed to obtain uniform breaking of dormancy in seedlings induced under 25°C (Dormling *et al.* 1968). In *Pseudotsuga menziesii*

seedlings, LD broke dormancy in seedlings lifted in November and December, but did not break dormancy in January- and February-lifted seedlings (Lavender and Hermann 1970).

Olmsted (1951) hypothesized that in species with a wide latitudinal range such as *A. saccharum*, long photoperiods influence dormancy breaking in the areas where there is insufficient chilling. However, Carlson (1985) demonstrated that the chilling requirement of *Pinus taeda* L. seedlings from Alabama and Mississippi provenances was less than that for *P. taeda* seedlings from North Carolina sources. Thus, the reported compensation of the chilling requirement by LD appears only to be a response under experimental conditions.

2.3.3 Growth Regulators and Breaking of Dormancy

Abscisic acid, gibberellic acid (GA), and cytokinins (CK) are thought to be involved in the breaking of dormancy. Abscisic acid content in buds in spring was minimal in *F. excelsior* (Hemberg 1949), *A. pseudoplatanus* (Eagles and Wareing 1964), and *Ribes nigrum* (L.) (Tinklin and Schwabe 1970). Gibberellic acid content in buds in spring was increased in *A. pseudoplatanus* (Eagles and Wareing 1964). Application of endogenous GA broke dormancy in *B. pubescens* (Eagles and Wareing 1964) and *R. nigrum* (Tinklin and Schwabe 1970). In *Pseudotsuga menziesii*, quantities of isopentyladenine-type (iP-type) CK were higher in dormant buds than in chilled buds (Pilate *et al.* 1989).

There is no consensus on the theory of hormonal control of dormancy breaking. According to Hemberg (1949), large quantities of growth inhibitor are present during rest, but disappear in spring. Eagles and Wareing (1964) suggested that during the breaking of dormancy, promoters overcome the effect of inhibitors. Vegis (1964) questioned the role of GA in the breaking of dormancy; he suggested

GA was involved in bud break after dormancy ended. Hewett and Wareing (1974) proposed that CK were involved in the breaking of dormancy. Pilate *et al.* (1989) suggested that levels of iP-type CK could be a growth-limiting factor.

2.4 Bud Break

The timing of bud break is thought to operate through the interdependent action of chilling, spring photoperiod, and spring temperatures (Heslop-Harrison 1964). This action has been clearly demonstrated in *P. menziesii* (Campbell and Sugano 1975). Both warm temperatures (Perry 1971, Wareing and Phillips 1978) and long photoperiods (Vegis 1964) are required for bud break.

Once rest has been broken by chilling, the bud is quiescent until environmental conditions permit bud break. In seedling quality assessment, a reliable physiological test of dormancy intensity is the speed of bud break under forcing conditions (Ritchie 1984a). As the chilling requirement accumulates, the intensity of dormancy weakens, and the speed of bud break increases (Ritchie 1984b). However, Owens *et al.* (1977) demonstrated that in mature trees under natural conditions, the breaking of dormancy determined by mitotic activity preceded that determined by bud break by several weeks. Consequently, Fielder and Owens (1989) commented that utility of the speed of bud break test as a criterion for the end of dormancy in seedlings was limited.

2.5 Description of First-Year-Seedling Shoot and Bud Development

As noted earlier, successful dormancy induction is indicated by cessation of height growth and appearance of over-wintering buds or "bud-set". Although bud-set is widely used by physiologists and growers, the term is unacceptable from a developmental perspective because it implies an end-point. Bud development,

which suggests a growth process, is a better term.

For conifers, several authors (Burley 1966, Jablanczy 1971) described first-year-seedling shoot and bud development in the framework established for mature trees (Korody 1937) while commenting, without discussion, that there were differences between seedlings and mature trees. Recently, shoot and bud development of seedlings and mature trees was compared (Fielder and Owens 1989). First-year-seedling shoot growth was awkwardly described by Jablanczy (1971) as free growth of non-preformed stems with needles formed *de novo*. For ease of description and understanding, the terminology for shoot growth proposed by Halle *et al.* (1978) is preferable.

Neoformation and preformation *sensu* Halle *et al.* (1978) can be used to describe first-year-seedling shoot development. At seed germination, initiation of primordia and supporting stem tissue by the shoot apical meristem begins. Once initiated, these products immediately develop into leaves and internodes. This process, known as neoformation, gives rise to the seedling shoot. In the Pinaceae, neoformation of the seedling shoot continues until a change in differentiation is cued by an environmental signal. Once neoformation stops, initiation of primordia and supporting stem tissue continues, but their differentiation changes. In the Abietoideae, bud scales and then preformed-leaf primordia differentiate. During preformed-leaf initiation, the supporting stem tissue does not expand vertically and results in a telescoped shoot. This process, known as shoot preformation, gives rise to the second-year-seedling shoot.

All stages of first-year-seedling shoot development have not been covered in any one paper. During germination and early growth, apical meristems of conifer seedlings have been investigated anatomically (Tepper 1964, Burley 1966, Riding 1972, Gregory and Romberger 1972), histochemically (Fosket and Miksche 1966,

Riding and Gifford 1973), and ultrastructurally (Cecich and Horner 1977). In addition, the volume of the apical dome and plastochron duration have been described (Gregory and Romberger 1972, Romberger and Gregory 1977, Cannell and Cahalan 1979). During terminal bud development, the following aspects have been investigated: apical volume and plastochron duration (Cannell and Cahalan 1979), duration of bud-scale initiation (Burley 1966, Pollard 1974a, Cannell and Cahalan 1979, Macey and Arnott 1986), and numbers of preformed-leaf primordia initiated (Pollard 1974 a,b, Pollard and Logan 1977, Macey and Arnott 1986).

2.6 Morphological Standards for Seedlings

Morphological characteristics of seedlings include shoot height, shoot weight, root weight or volume, shoot to root ratio, and shoot diameter at the root collar. Because these characteristics can be manipulated by nursery culture and are easily measured (Ritchie 1984a), they are used to define seedling quality. In British Columbia, the morphological characteristics used as planting stock standards are shoot height, root collar diameter, and root dry weight (Anon. 1986). During grading, seedlings that fail to meet these accepted standards are culled.

Morphological characteristics influence seedling tolerance to environmental stress after planting (Cleary *et al.* 1978). Consequently, morphological characteristics are important to seedling survival and field performance (growth) (Duryea 1984). This has been shown in study after study; however, it is not clear which characteristics ensure optimum performance (Iverson 1984). Presentation of these studies is beyond the scope of this paper, but good reviews are presented by Duryea (1984), Iverson (1984), Ritchie (1984a), and Thompson (1985). Furthermore, Cleary *et al.* (1978) and Greaves *et al.* (1978) present good syntheses of the ecophysiological considerations.

Chapter 3

CONTROLLED ENVIRONMENT CHAMBER STUDY

3.1 Introduction

Dormancy induction treatments stop height growth and cause terminal buds to form. During the past 20 years, the effect of dormancy induction treatment on terminal bud development has been examined at several levels of detail. Different dormancy induction treatments have been shown to alter the timing of height growth cessation and terminal bud visibility (Lavender *et al.* 1968, Dormling *et al.* 1968, Heide 1974, and Owston and Kozlowski 1981). Several studies have described differences in apical meristem activity and number of preformed-leaf primordia initiated under different dormancy induction treatments (Pollard and Logan 1977, Macey and Arnott 1986). One anatomical study has reported differences in phenology of bud development after moderate moisture stress of coastal and interior Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. *menziesii* and var. *glauca*) seedlings grown at a coastal nursery (Fielder and Owens 1989).

The objective of this study was to determine the effect of dormancy induction treatments under controlled environment conditions on terminal bud development, bud anatomy, and numbers of bud scales and preformed leaves initiated in one year-old (1-0) coastal Douglas-fir seedlings. This will provide information for a subsequent study aimed at achieving early dormancy under commercial greenhouse conditions.

3.2 Materials and Methods

3.2.1 Greenhouse Culture

Coastal Douglas-fir seeds from Vancouver Island (British Columbia Ministry of Forests, Registered Seedlot No. 9766; 50°10' N, 125°25' W, elevation 710 m) were stratified at 2°C for 4 weeks before sowing. Seeds were sown on April 8, 1985 in BC/CFS PSB 313A Styroblocks (Beaver Plastics Ltd., Edmonton, Alta.) at the MacMillan Bloedel Angus P. MacBean Nursery, Yellow Point, B.C.. Styroblocks were placed in a production polyethylene greenhouse maintained at 20°/20°C (day/night) at 90 % relative humidity.

Growing medium was a 2:1 peat:vermiculite mix. Nutricote Type 360 (16-10-10) (Chisso-Asahi, Tokyo, Japan) was incorporated into the mix at a rate of 1.3 kg/m^3. Styroblocks were misted up to ten times daily (depending on brightness of weather) during germination. Thereafter, styroblocks were watered on loss of 2 kg below saturated weight. After germination, Peters Conifer Starter (7-40-17) (W.R. Grace and Co., Fogelsville, PA) was applied with each watering during the first six weeks. Subsequently, Peters Conifer Grower (20-7-19) was applied with each watering during the growth period. Plant-Green iron chelate (13% Fe) (Westcan Horticultural Specialists Ltd., Calgary, Alta.) and Peters Soluble Trace Element Mix were applied when needed as indicated by foliar analysis. Peters Calcium Nitrate (15.5% N, 20% Ca) was applied as necessary to adjust the pH of the growing medium.

3.2.2 Dormancy Induction Treatments

On July 30, styroblocks were removed from the greenhouse, transported to the University of Victoria, Victoria, B.C., and kept outside until August 2 when they

were placed in controlled environment chambers (Conviron E15, Controlled Environment Ltd., Winnipeg, Man.). Both incandescent and fluorescent light was supplied at 3.2 E/s m^2. Relative humidity was ambient.

There were eight dormancy induction treatments varying three factors: type of short day (SD), temperature, and moisture (Fig. 1). Photoperiod was decreased from 15 h (natural photoperiod on August 2) to an 8-h SD either abruptly (ASD), or gradually (GSD). Under GSD, photoperiod was decreased by 15 minutes every 2 days, and thus represented an acceleration of the naturally shortening photoperiod. Under GSD, the 8-h SD was reached on September 27. For both treatments, the 8-h SD was maintained until the end of the study.

For each type of SD, day/night temperature was either kept constant at a high temperature (HT) of 25°/15°C, or decreased by 2° every 2 days to a low temperature (LT) of 15°/5°C, which was reached on September 30 and then maintained until the end of the study. HT remained constant throughout the study.

For each type of SD and temperature, seedlings were watered as neccssary either to result in no drought-stress (ND), or to cause drought-stress (D) in the range of -0.8 to -1.0 MPa (pre-dawn equilibrium water potential) as determined by the pressure chamber technique (Ritchie and Hinckley 1975). Drought-stress ended on September 30. Fertilizer was not applied to seedlings under ND or D.

3.2.3 Experimental Design

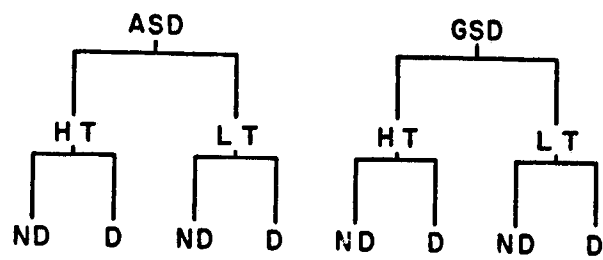
The experimental design was a modified split-plot. Type of SD was randomized among 4 controlled environment chambers. For each type of SD, temperature was randomized between 2 chambers. Moisture regime was assigned by plan between halves of each chamber. There were 2 styroblocks in each treatment.

Fig. 1. Schematic of dormancy induction treatments. Photoperiod was reduced to an 8 h short day (SD) either abruptly (ASD), or gradually (GSD). Temperature was either constant at a high temperature (HT), or decreased to a low temperature (LT). Moisture was controlled either to result in no drought-stress (ND), or to cause drought-stress (D).

TYPE OF SHORT DAY

TEMPERATURE

MOISTURE



3.2.4 Processing of Samples

Seedlings, which had well-formed terminal buds or lammas growth visible before treatment, were tagged to prevent subsequent sampling. Prior to treatment, seedlings were randomly sampled from 2 styroblocks; thereafter, seedlings were randomly sampled from one styroblock per treatment through mid-October. Neoformed leaves and/or bud scales were dissected away from shoot tips of 15 seedlings per styroblock. For each shoot tip, type of foliar organ being initiated was recorded, and when appropriate, stage of bud-scale-complex (BSC) development was described. A BSC comprised all of the neoformed-leaf-bud-scale transitional structures and bud scales.

Five of these shoot tips were sliced along both sides, fixed in formalin-acetic acid-alcohol (FAA), dehydrated in tertiary-butyl alcohol series (Johansen 1940), and embedded in TissuePrep (Fisher Scientific Co., Fair Lawn, NJ). Serial longitudinal sections were microtomed at 6 µm, and stained with safranin and hematoxylin.

3.2.5 Morphological and Anatomical Observations

Up to 5 median longitudinal sections were selected per treatment, and traced using a microprojector. Type of foliar organ, numbers of preformed-leaf primordia, bud-scale receptacle development, and crown differentiation were noted. For the mid-October sample, maximum crown height and width were measured, preformed-shoot height was measured vertically from a transverse line drawn between the bases of the first-initiated preformed-leaf primordia to the summit of the apical dome, and preformed-shoot width was measured along a transverse line between the bases of first-initiated preformed-leaf primordia.

In early November, bud scales were dissected away, and fresh preformed shoots, representative of each treatment, were observed and photographed using a

scanning electron microscope (JSM-35U, JEOL, Tokyo, Japan) operating at 10 kV.

3.2.6 Numbers of Bud Scales and Preformed Leaves

In early November, buds of 5 to 10 seedlings per treatment were dissected. Numbers of bud scales were counted and types of bud scales were described.

Seedlings were removed from controlled environment chambers, and over-wintered out-of-doors. In 1986, seedlings were maintained in styroblocks under a minimal watering/no-fertilizer regime until shoot elongation was completed. Then, 9 to 20 seedlings per treatment were sampled, and numbers of leaves on second-year preformed shoots were counted.

3.2.7 Statistical Analysis

For descriptive and numerical anatomical data, unequal sample size was due to differences in stage of bud development and to losses during the processing of specimens. Unequal sample size for numbers of bud scales and preformed-leaf primordia was due to a lack of material because of mortality from the first drought-stress for ASD-LT-D, and controlled environment chamber breakdown which reduced the space available for GSD-HT-ND. An analysis of variance for unequal sample sizes (GLM procedure) (SAS 1982) was used to test for treatment effects and their interactions.

3.3 Results

3.3.1 Bud-Scale Initiation

Prior to treatment, apical meristems appeared to be covered only by expanding neoformed leaves; no bud scales were visible. However, upon

microscopic dissection, differences in type of foliar organ being initiated were apparent. Thirty percent of apices were undergoing neoformed-leaf initiation (Fig. 2) and the remaining apices were initiating bud scales (Fig. 3). The transition from neoformed-leaf to bud-scale initiation was recent - only the last-initiated primordia were differentiating as bud scales (Fig. 3). Recently-initiated neoformed leaves and internodes were expanding (Fig. 3). The cause of this early transition from neoformed-leaf to bud-scale initiation may have been mild moisture stress during transport. Moisture stress of seedlings was not uniform, and consequently, there was a population of seedlings which did not receive this dormancy induction cue.

After 1 week, all apices under ASD were undergoing bud-scale initiation (Fig. 4, Table 1). After 2 weeks, most apices under GSD were undergoing bud-scale initiation (Table 1). More apices under HT were initiating bud scales than were those under LT, and more apices under D were initiating bud scales than were those under ND.

After 3 weeks, most apices under ASD had finished bud-scale initiation (Table 2). More apices under HT had completed bud-scale initiation than under LT, and more apices under D had completed bud-scale initiation than under ND. After 4-5 weeks, most apices under GSD had completed bud-scale initiation (Table 2). There was no trend between HT and LT or ND and D and completion of bud-scale initiation.

Type of SD significantly influenced number of bud scales initiated (Table 3). More bud scales were initiated under GSD than under ASD and under D than under ND (Table 4), but the influence of moisture was only weakly significant (Table 3).

Fig. 2-5. Light micrographs of median longitudinal sections of shoot tips of Douglas-fir seedlings.

Fig. 2-3. Recently-initiated neoformed leaves (nl) are expanding as internodes elongate. Darkly staining phenolic substances have accumulated in cuboidal pith (ph) cells. x90.

Fig. 2. Apical meristem (am) initiating neoformed leaves prior to treatment.

Fig. 3. Apical meristem initiating bud scales (bs) prior to treatment.

Fig. 4. Apical meristem initiating bud scales after 1-2 weeks of treatment. x100.

Fig. 5. Apical meristem completed bud-scale initiation and in the transitional phase after 3-5 weeks of treatment. Crown cells (cr, between arrows) were differentiating. Crown cells were radially enlarged and not phenolic-filled. x90.

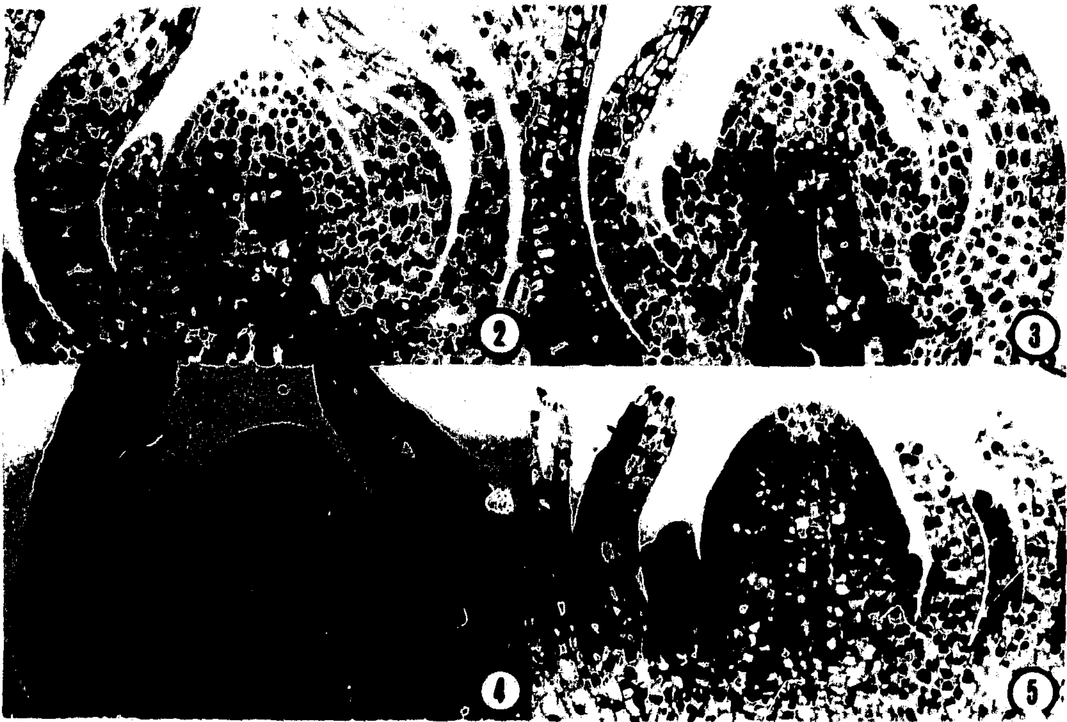

2
3
4
5

Table 1: Percentage of apices initiating bud scales after 1-2 weeks under different dormancy induction treatments in Douglas-fir seedlings.

Apices not initiating bud scales were initiating neoformed leaves. Observations were from dissections and sectioned material. n = 12-15.

Treatments: ASD - abrupt short day, GSD - gradual short day
HT - high temperature, LT - low temperature

TREATMENT	NO. OF WEEKS	PERCENTAGE OF APICES INITIATING BUD SCALES
NO DROUGHT		
ASD-HT	1.0	100.0
ASD-LT	1.0	100.0
GSD-HT	2.0	92.3
GSD-LT	2.0	66.7
DROUGHT		
ASD-HT	1.0	100.0
ASD-LT	1.0	100.0
GSD-HT	2.0	93.3
GSD-LT	2.0	80.0

Table 2: Percentage of apices initiating bud scales, in the transitional phase, and initiating preformed leaves after 3-5 weeks under different dormancy induction treatments in Douglas-fir seedlings.

Transitional phase is the period between the end of bud-scale initiation and the start of preformed-leaf initiation. Observations were from dissections and sectioned material. n = 14-15.

Treatments: ASD - abrupt short day, GSD - gradual short day
HT - high temperature, LT - low temperature

TREATMENT	NO. OF WEEKS	PERCENTAGE OF APICES INITIATING BUD SCALES	PERCENTAGE OF APICES IN TRANSITIONAL PHASE	PERCENTAGE OF APICES INITIATING PREFORMED LEAVES
NO DROUGHT				
ASD-HT	3.0	20.0	0.0	80.0
ASD-LT	3.0	28.6	0.0	71.4
GSD-HT	4.0	7.1	28.6	64.3
GSD-LT	4.5	33.3	20.0	46.7
DROUGHT				
ASD-HT	3.5	13.3	6.7	80.0
ASD-LT	3.0	21.5	7.1	71.4
GSD-HT	4.5	40.0	33.3	26.7
GSD-LT	5.0	20.0	33.3	46.7

Table 3: Analysis of variance of dormancy induction treatment effects on number of bud scales initiated in Douglas-fir seedlings.

*, significant at 0.05 level.

SOURCE	DF	MS	F	P
Type of short day (SD)	1	227.28	5.38	0.0236*
Temperature (T)	1	15.96	0.38	0.5410
Moisture (M)	1	143.88	3.40	0.0696
SD x T	1	1.52	0.04	0.8499
SD x M	1	37.50	0.89	0.3497
T x M	1	21.78	0.52	0.4754
Error	64	42.26		

Table 4: Numbers of bud scales and preformed-leaf primordia initiated under different dormancy induction treatments in Douglas-fir seedlings.

Numbers are presented as means (±SE). For number of bud scales, n = 5 for GSD-HT-ND, n = 6 for ASD-LT-D, and n = 10 for other treatments. For number of preformed-leaf primordia, n = 9 for GSD-HT-ND and n = 20 for other treatments.

Treatments: ASD - abrupt short day, GSD - gradual short day
HT - high temperature, LT - low temperature

TREATMENT	NUMBER OF BUD SCALES	NUMBER OF LEAF PRIMORDIA
NO DROUGHT		
ASD-HT	19.8 (1.6)	113.5 (6.3)
ASD-LT	18.6 (1.6)	101.1 (4.6)
GSD-HT	22.2 (1.9)	66.0 (7.9)
GSD-LT	21.0 (2.9)	70.2 (2.0)
DROUGHT		
ASD-HT	19.4 (2.1)	88.6 (5.3)
ASD-LT	22.2 (2.2)	79.8 (4.5)
GSD-HT	26.7 (2.6)	56.5 (2.1)
GSD-LT	25.0 (1.8)	68.6 (4.1)

3.3.2 Bud-Scale-Complex Composition and Development

Transitional structures, intermediate between neoformed leaves and bud scales (leaf-bud scale), were potentially the outer-most (first-formed) structures of the BSC. They were green needle-shaped structures of varying length with brown, broad scale-like bases. Leaf-bud scales occurred more frequently in BSCs under ASD than on those under GSD. Number of leaf-bud scales per BSC ranged from 1 to 3. There was no trend between number of leaf-bud scales and treatment.

Bud-scale primordia differentiated into one of four morphologically-distinct bud-scale types. Type 1 bud scales had long acuminate apices with rounded, truncate bases that did not overlap. Type 2 bud scales were less elongated, but broader than type 1. They had medium acute apices and truncate bases that did not overlap. Type 3 bud scales were similar in shape to type 2; however, they were longer and broader, and overlapped along half of their length. Type 4 bud scales were larger than type 3, had broadly acute apices and broadly rounded bases, and greatly overlapped along all of their length. Types 1, 2 and 3 bud scales were the outer, brown, coriaceous bud scales. Type 4 bud scales were the inner, white, foliaceous bud scales. There was no trend between number of each bud-scale type and treatment.

Based on proportion of bud-scale types present, a three-stage BSC development scheme was devised. A BSC comprising: type 1 and/or type 2 bud scales occurred in stage 1 of BSC development; type 1, 2, and 3 occurred in stage 2; and all four bud-scale types occurred in stage 3. In this scheme, a bud-scale type was assigned once it was recognizable rather than when fully differentiated.

On seedlings with apices undergoing bud-scale initiation prior to treatment, BSCs were in stage 1 of development. After 1-2 weeks of SD, BSCs were in stage 1 and 2 (Table 5). More BSCs under ASD were in stage 2 than were those under

GSD. After 1 week under ASD, more BSCs under HT were in stage 2 than were those under LT, and more BSCs under ND were in stage 2 than were those under D. After 2 weeks under GSD, there was no trend between HT and LT or ND and D and passage to stage 2.

After 3 weeks under ASD, most BSCs had reached stage 3 of development (Table 5). More BSCs under HT were in stage 3 than were those under LT, and more BSCs under D were in stage 3 than were those under ND. After 4-5 weeks under GSD, most BSCs were in stage 3 (Table 5). There was no trend between HT and LT or ND and D and passage to stage 3.

Table 5: Percentage of bud-scale complexes (BSCs) in various stages of BSC development after 1-2 weeks and after 3-5 weeks under different dormancy induction treatments in Douglas-fir seedlings.

In this developmental scheme, stage 1 was the most rudimentary. Observations were from dissections. *n* = 7-10 for weeks 1-2, and *n* = 9-10 for weeks 3-5.

Treatments: ASD - abrupt short day, GSD - gradual short day
HT - high temperature, LT - low temperature

TREATMENT	NO. OF WEEKS	PERCENTAGE OF BSCs AT VARIOUS STAGES OF BSC DEVELOPMENT 1	2	NO. OF WEEKS	PERCENTAGE OF BSCs AT VARIOUS STAGES OF BSC DEVELOPMENT 1	2	3
NO DROUGHT							
ASD-HT	1.0	42.9	57.1	3.0	20.0	10.0	70.0
ASD-LT	1.0	80.0	20.0	3.0	10.0	50.0	40.0
GSD-HT	2.0	62.5	37.5	4.0	0.0	11.1	88.9
GSD-LT	2.0	100.0	0.0	4.5	20.0	10.0	70.0
DROUGHT							
ASD-HT	1.0	62.5	37.5	3.5	0.0	0.0	100.0
ASD-LT	1.0	88.9	11.1	3.0	11.1	22.2	66.7
GSD-HT	2.0	70.0	30.0	4.5	20.0	10.0	70.0
GSD-LT	2.0	70.0	30.0	5.0	0.0	10.0	90.0

3.3.3 Transitional Phase

After completion of bud-scale initiation, apices entered a transitional phase during which apical volume increased and new primordia were initiated very slowly, if at all (Fig. 5). Between weeks 3-5, fewer apices under ASD were in the transitional phase than were those under GSD (Table 2). After 3 weeks under ASD, all apices under ND had passed through the transitional phase and only a few apices under D were still in this phase. After 4-5 weeks under GSD, there was no trend between HT and LT and percentage of apices in the transitional phase, and fewer apices under ND were in the transitional phase than were those under D.

The transitional phase under ASD was contracted, as evidenced by the common occurrence of primordia intermediate between bud scales and leaves (bud-scale leaf) on preformed shoots under ASD (Figs. 6-9). Upon dissection of ASD buds, bud-scale leaves were clearly evident on preformed shoots. They were the outer-most (first-initiated) and most-elongated primordia, and had cuspidate to short acuminate apices (resembling apices of rudimentary type 1 bud scales) on otherwise leaf-like primordia (Fig. 6). Bud-scale leaves rarely occurred on preformed shoots under GSD (Figs. 10-13).

3.3.4 Preformed-Leaf Initiation

Once the transitional phase was completed, preformed-leaf initiation began (Fig. 14). After 3-5 weeks of SD, more apices under ASD were undergoing early preformed-leaf initiation than were apices under GSD (Table 2). Under ASD, more apices under HT were undergoing preformed-leaf initiation than those under LT. There was no difference between percentage of apices initiating preformed leaves under ND and D. Under GSD, there was no trend between HT and LT and percentage of apices initiating preformed leaves. More apices under ND were

Fig. 6-9. Scanning electron micrographs of fresh preformed shoots of terminal buds of Douglas-fir seedlings from dormancy induction treatments, collected in early November, showing apical meristem (am) and preformed-leaf primordia (pl). Bud scales and bud-scale receptacle were dissected away. Treatments: ASD, abrupt short day; HT, high temperature; LT, low temperature; ND, no drought; D, drought. Note first-initiated bud-scale-leaf structures (bsl) and elongated preformed-leaf primordia with acute to acuminate apices. x45.

Fig. 6. From ASD-HT-ND, showing first-initiated bud-scale leaf structure with cuspidate to short acuminate apex.

Fig. 7. From ASD-HT-D.

Fig. 8. From ASD-LT-ND.

Fig. 9. From ASD-LT-D.

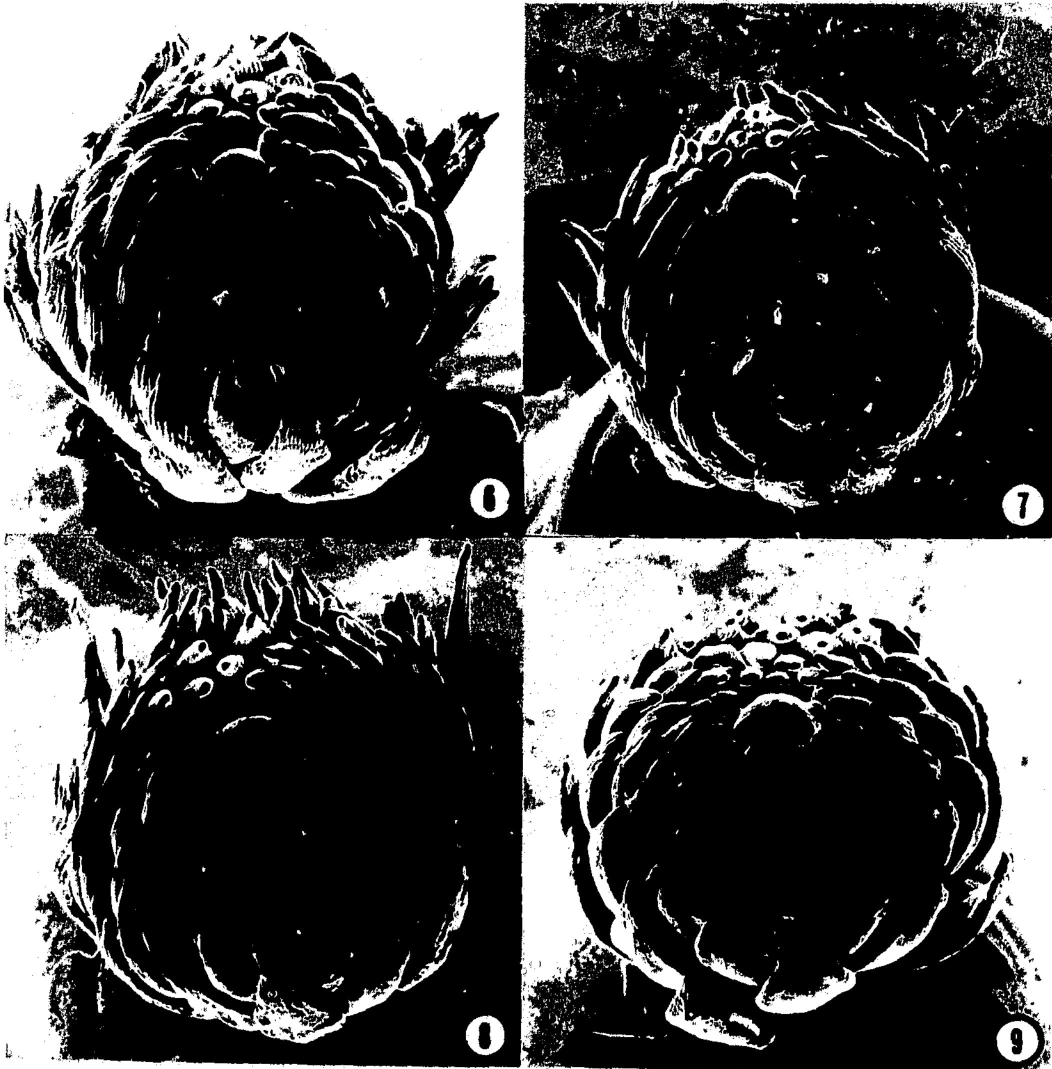


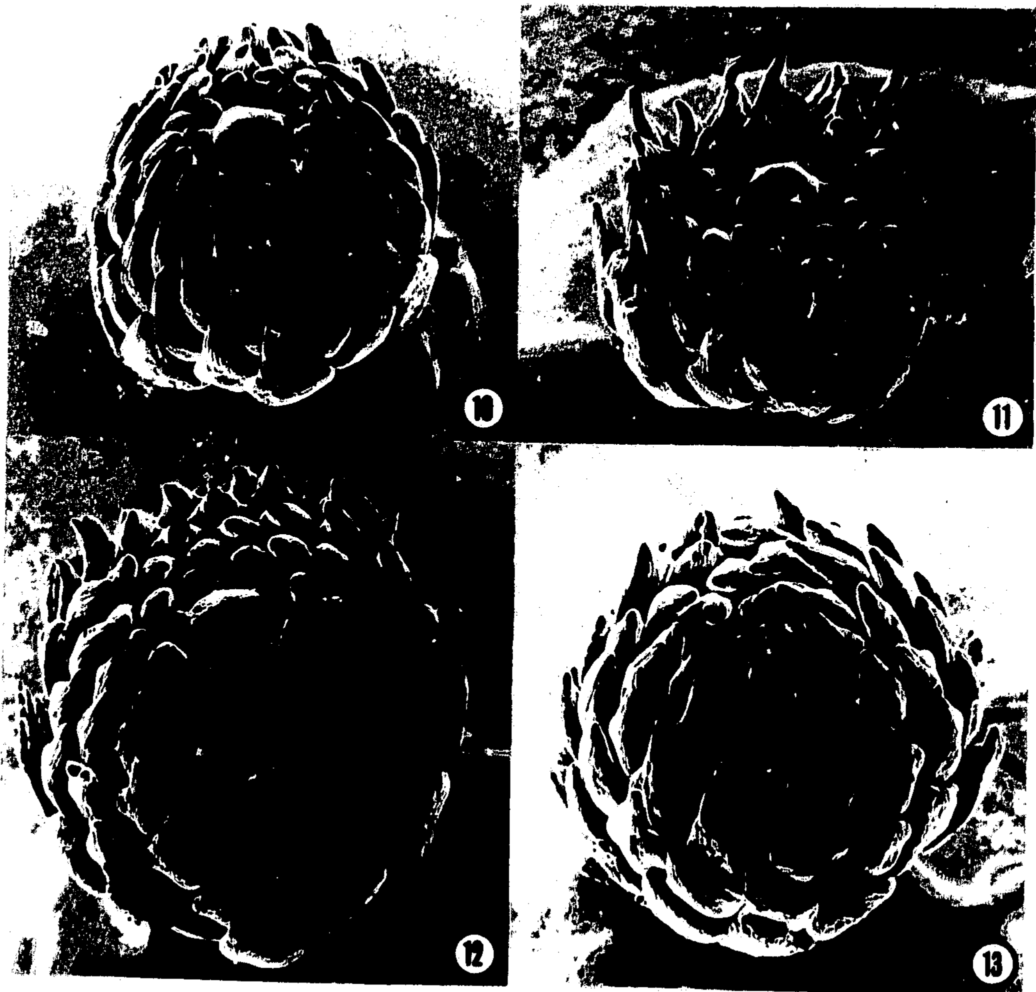
Fig. 10-13. Scanning electron micrographs of fresh preformed shoots of terminal buds of Douglas-fir seedlings from dormancy induction treatments, collected in early November, showing apical meristem (am) and preformed-leaf primordia (pl). Bud scales and bud-scale receptacle were dissected away. Treatments: GSD, gradual short day; HT, high temperature; LT, low temperature; ND, no drought; D, drought. Note apices of preformed-leaf primordia were acute to obtuse. x45.

Fig. 10. From GSD-HT-ND.

Fig. 11. From GSD-HT-D.

Fig. 12. From GSD-LT-ND.

Fig. 13. From GSD-LT-D.


10
11
12
13

undergoing preformed-leaf initiation than those under D.

Type of SD and moisture had a highly significant influence and temperature and type of SD x temperature interaction had a significant influence on number of preformed-leaf primordia initiated (Table 6). More preformed-leaf primordia were initiated under ASD than under GSD and under ND than under D (Table 4). Under ASD, more preformed-leaf primordia were initiated under HT than under LT; whereas, under GSD, more were initiated under LT than under HT (Table 4).

Table 6: Analysis of variance of dormancy induction treatment effects on number of preformed-leaf primordia initiated in Douglas-fir seedlings.

*, significant at 0.05 level; **, significant at 0.001 level.

SOURCE	DF	MS	F	P
Type of short day (SD)	1	33518.26	84.62	0.0001**
Temperature (T)	1	3378.93	8.53	0.0041*
Moisture (M)	1	6891.91	17.40	0.0001**
SD x T	1	25.16	0.06	0.8014
SD x M	1	2960.90	7.48	0.0071*
T x M	1	15.81	0.04	0.8420
Error	142	396.09		

3.3.5 Bud-Scale Receptacle

During bud-scale and early preformed-leaf initiation, cells of the cortex and pith in the shoot beneath the apical meristem and bud scales proliferated forming a flat, broad receptacle. Under ASD, there was greater proliferation of cortical cells relative to pith cells, and the cortical cells enlarged longitudinally resulting in a concave receptacle which surrounded the embryonic preformed shoot (Fig. 15). Under GSD, there was greater proliferation of pith cells, and cortical cells remained

Fig. 14.	Light micrograph of median longitudinal section of apical meristem (am) of a Douglas-fir seedling initiating preformed-leaf primordia (pl), surrounded by inner bud scales (ibs). x100.
Fig. 15-16.	Light micrographs of median longitudinal sections of terminal buds of Douglas-fir seedlings showing embryonic preformed shoot (eps), bud-scale receptacle (r, between arrows), cortical cells (c), vascular tissue (vt), and pith region (ph). x40.
Fig. 15.	From abrupt short day (ASD).
Fig. 16.	From gradual short day (GSD).
Fig. 17.	Light micrograph of median longitudinal section of distal portion of a preformed shoot of a Douglas-fir seedling under abrupt short day (ASD) showing part of apical meristem (am) and last-initiated preformed-leaf primordia (pl) with vacuolate cells (vc) in the apical regions. x140.
Fig. 18-19.	Light micrographs of median longitudinal sections through the crown (cr, between arrows) below preformed shoots of Douglas-fir seedlings in October showing thickness of crown cell walls. x100.
Fig. 18.	From abrupt short day (ASD).
Fig. 19.	From gradual short day (GSD).

14
c
ph
c
15
Sph
16
17
18
19

isodiametric resulting in a convex receptacle which was surmounted by the embryonic preformed shoot (Fig. 16).

3.3.6 Preformed-Leaf Primordium Development

All preformed-leaf primordia elongated, except those initiated last (Fig. 17). By mid-October, preformed-leaf primordia under ASD had elongated more than those under GSD, (cf. Figs. 6-9 with Figs. 10-13) and those under ND had elongated more than those under D (cf. Figs. 6, 8, 10, 12 with Figs. 7, 9, 11, 13). Under ASD, apices of primordia were acute to acuminate; whereas, under GSD, they were acute to obtuse (cf. Figs. 6-9 with Figs. 10-13). Under ASD, apical region cells elongated and became vacuolate (Fig. 17). Consequently, upon bud dissection, apices of preformed-leaf primordia were hyaline.

Maturation of epidermal cells of preformed-leaf primordia occurred basipetally by deposition of ergastic substances along both the adaxial and abaxial surfaces (Fig. 17). The direction of epidermal maturation of preformed-leaf primordia was acropetal along preformed shoots.

3.3.7 Preformed-Shoot Height and Width

Type of SD had a highly significant and moisture had a significant influence on preformed-shoot height (Table 7). In mid-October, preformed-shoot height was greater under ASD than under GSD (Table 8) (cf. Figs. 6-9 with Figs. 10-13) and under ND than D (Table 8) (cf. Figs. 6, 8, 10, 12 with Figs. 7, 9, 11, 13). Treatments and their interactions had no influence on preformed-shoot width (Table 9).

Table 7: Analysis of variance of dormancy induction treatment effects on final preformed-shoot height in Douglas-fir seedlings.

*, significant at 0.05 level; **, significant at 0.001 level.

SOURCE	DF	MS	F	P
Type of short day (SD)	1	1464286.65	36.64	0.0001**
Temperature (T)	1	62954.31	1.58	0.2202
Moisture (M)	1	224638.25	5.62	0.0251*
SD x T	1	2626.18	0.07	0.7996
SD x M	1	49408.80	1.24	0.2760
T x M	1	12857.77	0.32	0.5752
Error	27	39960.69		

Table 8: Final preformed-shoot height and width in mid-October under different dormancy induction treatments in Douglas-fir seedlings.

Height and width (μm) are means (±SE). n = 3-5.

Treatments: ASD - abrupt short day, GSD - gradual short day
HT - high temperature, LT - low temperature

TREATMENT	PREFORMED-SHOOT HEIGHT	PREFORMED-SHOOT WIDTH
NO DROUGHT		
ASD-HT	1144.3 (156.8)	660.8 (23.3)
ASD-LT	1166.2 (118.0)	666.4 (17.7)
GSD-HT	613.8 (47.6)	672.0 (13.8)
GSD-LT	705.6 (37.0)	625.0 (60.4)
DROUGHT		
ASD-HT	1041.6 (199.0)	761.6 (98.1)
ASD-LT	807.8 (126.5)	578.2 (52.8)
GSD-HT	547.7 (79.5)	620.5 (76.0)
GSD-LT	595.8 (67.1)	602.5 (34.8)

Table 9: Analysis of variance of dormancy induction treatment effects on final preformed-shoot width in Douglas-fir seedlings.

SOURCE	DF	MS	F	P
Type of short day (SD)	1	10987.63	0.90	0.3500
Temperature (T)	1	6474.34	0.53	0.4716
Moisture (M)	1	2870.82	0.24	0.6308
SD x T	1	29965.64	2.47	0.1279
SD x M	1	2727.08	0.22	0.6394
T x M	1	18680.54	1.54	0.2256
Error	27	12144.92		

3.3.8 Crown Differentiation and Dimensions

During neoformed-leaf and bud-scale initiation, cells within the pith of the shoot apex were cuboidal, phenolic-filled and thin-walled (Fig. 2). When apices started to initiate preformed-leaf primordia, crown differentiation began in the pith at the base of the embryonic preformed shoot. Cells enlarged radially, walls thickened and phenolics disappeared (Fig. 5). After 3-5 weeks of SD, more cells were differentiating as crown under ASD than under GSD. There was no trend between HT and LT or ND and D and number of crown cells differentiating. By mid-October under both ASD and GSD, the crown tissue formed a diaphragm separating the pith of the preformed shoot from that of the subtending shoot. Crown cell walls which differentiated under ASD were thicker (Fig. 18) than those under GSD (Fig. 19).

Type of SD had a highly significant influence and type of SD x temperature interaction had a significant influence on final crown height in mid-October (Table 10). Crown height was greater under ASD than under GSD (Table 11) (cf. Figs. 18 and 19). Under ASD, crown height was similar under HT and LT; whereas, under

Table 10: Analysis of variance of dormancy induction treatment effects on final crown height in Douglas-fir seedlings.

*, significant at 0.05 level; **, significant at 0.001.

SOURCE	DF	MS	F	P
Type of short day (SD)	1	7502.84	13.07	0.0012**
Temperature (T)	1	469.75	0.82	0.3734
Moisture (M)	1	915.14	1.59	0.2171
SD x T	1	2860.43	4.98	0.0338*
SD x M	1	80.30	0.14	0.7112
T x M	1	129.88	0.23	0.6380
Error	28	574.06		

Table 11: Final crown height and width in mid-October under different dormancy induction treatments in Douglas-fir seedlings.

Height and width (μm) are means (±SE). *n* = 3-5.

Treatments: ASD - abrupt short day, GSD - gradual short day
HT - high temperature, LT - low temperature

TREATMENT	CROWN HEIGHT	CROWN WIDTH
NO DROUGHT		
ASD-HT	113.9 (8.1)	444.2 (80.5)
ASD-LT	131.6 (17.2)	604.8 (58.4)
GSD-HT	78.4 (11.6)	450.2 (43.4)
GSD-LT	115.4 (10.7)	508.5 (31.0)
DROUGHT		
ASD-HT	113.4 (6.2)	421.4 (40.6)
ASD-LT	119.0 (15.6)	375.2 (31.1)
GSD-HT	76.2 (10.8)	353.9 (66.6)
GSD-LT	90.7 (6.2)	436.8 (27.8)

Table 12: Analysis of variance of dormancy induction treatment effects on final crown width in Douglas-fir seedlings.

*, significant at 0.05 level.

SOURCE	DF	MS	F	P
Type of short day (SD)	1	5785.73	0.56	0.4611
Temperature (T)	1	645.92	0.06	0.8047
Moisture (M)	1	97695.38	9.43	0.0047*
SD x T	1	32561.89	3.14	0.0872
SD x M	1	4554.40	0.44	0.5128
T x M	1	22603.98	2.18	0.1508
Error	28	10361.83		

GSD, crown height was greater under LT than under HT (Table 11). Moisture had a significant effect on final crown width (Table 12). Under ASD and GSD, crown width was greater under ND than under D (Table 11).

3.4 Discussion

3.4.1 Bud Development

Shoots of first-year seedlings arise by neoformation, that is leaves and internodes are initiated and mature immediately (*sensu* Halle *et al.* 1978). When a dormancy induction signal is perceived by the seedling, shoot neoformation ceases and bud development begins. The apical meristem initiates first bud scales, and then preformed leaves. Thus, bud development involves the initiation and differentiation of a bud-scale-complex (BSC), and of a preformed shoot within that complex. In the past, seedling bud development was thought to occur quickly, and the presence of bud scales was thought to signify a developed bud (see Heide 1974). Indeed, the term "bud set" suggests that bud development is nearly completed,

rather than just beginning. The present study demonstrates that bud development - having two stages of primordial initiation with a transitional phase in between - is a long, slow process in 1-0 coastal Douglas-fir seedlings which starts in early August and ends in mid-October. The developmental stages were similar, but the phenology was different from that of coastal Douglas-fir seedlings given an early August, moderate moisture stress treatment under natural photoperiod (Fielder and Owens 1989). In that study, bud development started in mid-August and continued until early December.

3.4.2 Effect of Type of SD, Temperature and Moisture on Bud Development

Type of SD affected the rapidity of transition from neoformed-leaf to bud-scale initiation in the present study. Apices under ASD started and completed bud-scale initiation earlier than did apices under GSD. Earlier studies of conifer seedlings have demonstrated a similar SD effect. In *Picea sitchensis* (Bong.) Carr., bud-scale initiation started after only 2 SDs (Cannell and Cahalan 1979). In *P. mariana* (Mill.) B.S.P. and *P. glauca* (Moench) Voss, all bud scales were initiated within 2 weeks in SD (Pollard and Logan 1977). Short days resulted in the appearance of buds within 1-2 weeks in *P. abies* (L.) Karst. (Dormling *et al.* 1968, Heide 1974). Type of SD also affected subsequent stages of bud development in the present study. Development of the BSC occurred faster, the transitional phase was shorter, and preformed-leaf initiation began earlier under ASD than under GSD.

In the present study, temperature affected the speed of transition from neoformed-leaf to bud-scale initiation. Temperature effects were not discernible under ASD because all apices were initiating bud scales after 1 week of SD. Under GSD, the transition from neoformed-leaf to bud-scale initiation occurred more rapidly, and thus buds became visible earlier, under HT than under LT. These

results are similar to those of other studies of Douglas-fir, *Abies procera* Redh., *P. sitchensis* (Owston and Kozlowski 1981), and *P. abies* (Dormling *et al.* 1968, Heide 1974) in which buds became visible earlier under high than under low temperatures in seedlings placed under SD. In contrast, in Douglas-fir seedlings under both SD and LD, buds appeared earlier under low than under high temperatures (Lavender *et al.* 1968). In that study, unlike the other studies, dormancy induction began in June when rapid seedling shoot elongation was just starting. The different developmental stage of the seedlings may account for the contrasting results.

Moisture also affected the rapidity of transition from neoformed-leaf to bud-scale initiation in the present study. Drought hastened the start and completion of bud-scale initiation. An earlier start and completion of bud-scale initiation under D has been shown in other studies. After one week of treatment, more *P. glauca* seedlings under D were initiating bud scales than were those under ND (Macey and Arnott 1986). In Douglas-fir seedlings under both SD and LD, buds appeared earlier under D than under ND (Lavender *et al.* 1968). In second-year *A. magnifica* A. Murr. and *A. concolor* (Gord. and Glend.) Lindl. seedlings, bud-scale initiation was completed earlier under D than under ND (Hallgren and Helms 1988).

In the present study, D delayed the transitional phase and the start of preformed-leaf initiation. In Douglas-fir trees, mitotic frequencies in apices of shoots with low shoot water potentials were lower than those in apices of shoots with high shoot water potentials (Owens *et al.* 1985). In the present study, the longer transitional phase in apices under D may have been due to delayed apical enlargement because of reduced mitotic activity.

3.4.3 Effect of Type of SD, Temperature and Moisture on Numbers of Bud Scales and Preformed-Leaf Primordia Initiated

Type of SD had a significant influence on number of bud scales initiated in the present study. Fewer bud scales were initiated under ASD than under GSD. This appears to occur because duration of bud-scale initiation was shorter under ASD and thereby resulted in fewer primordia becoming bud scales even though rate of bud-scale initiation, which was not determined in the present study, appeared to be faster under ASD.

Moisture also influenced number of bud scales initiated. More bud scales were initiated and bud-scale initiation was completed earlier under D than under ND; therefore, rate of bud-scale initiation was faster under D. In second-year *A. magnifica* and *A. concolor* seedlings, fewer bud scales were initiated under D than under ND (Hallgren and Helms 1988). These contrasting results suggest different morphological adaptations to drought due to age-related differences in organogenic activity of the apical meristem and in moisture stress within the apical meristem. Under natural conditions, first-year seedlings begin bud-scale initiation in response to mid-summer moisture deficits (Lavender *et al.* 1968), and more bud scales could offer the apical meristem greater protection against desiccation. In contrast, second-year seedlings begin bud-scale initiation prior to bud break in the spring, the apical meristem is always surrounded by bud scales, and the need for desiccation protection is reduced. Thus, in second-year *Abies* seedlings under continuous D, rate of bud-scale initiation was slowed and fewer bud scales were initiated (Hallgren and Helms 1988) without risk of desiccation to the apical meristem.

Type of SD had a significant influence on number of preformed-leaf primordia initiated in the present study. More preformed-leaf primordia were initiated under ASD than under GSD, in part because rate of preformed-leaf

initiation, which was not determined in the present study, appeared to be faster under ASD. Gregory and Romberger (1972) demonstrated that an increased rate of primordium initiation was accompanied by an increase in apical volume in *P. abies* seedlings. An increase in apical volume, which was not determined in the present study, could also partially account for the larger number of preformed-leaf primordia initiated, since apical domes provide the sites for primordium initiation (Romberger 1963).

In the present study, temperature had a significant influence on number of preformed-leaf primordia initiated. Apices under ASD initiated more preformed-leaf primordia under HT than under LT. This agrees with an earlier study of *P. glauca* seedlings placed under SD, in which more preformed-leaf primordia were initiated under the high temperature (Pollard and Logan 1977). Also, in *Pinus radiata* D. Don cuttings under simulated natural photoperiod, more foliar primordia were initiated under warm than under cool temperatures (Bollmann *et al.* 1986).

Moisture had a significant influence on number of preformed-leaf primordia initiated in Douglas-fir seedlings. Drought reduced the number of preformed-leaf primordia initiated. Other studies of the effect of D during foliar initiation have reported similar results. In *A. magnifica*, *A. concolor* (Hallgren and Helms 1988), and *P. resinosa* Ait. (Clements 1970), fewer primordia were initiated under D than under ND. Even an inductive 2-week D in *Picea glauca* seedlings reduced the number of preformed-leaf primordia relative to ND (Macey and Arnott 1986). Owens *et al.* (1985) demonstrated that apices of Douglas-fir shoots with low shoot water potentials were smaller than apices of shoots with high shoot water potentials. It is likely that D reduced apical growth in Douglas-fir seedlings in the present study, although apical size was not measured. On smaller apices, the number of preformed-leaf primordia initiated would be reduced because the smaller apical

dome would provide fewer sites for primordium initiation (Romberger 1963).

3.4.4 Functional Terminology

"Bud set" is the term commonly used by growers and physiologists to indicate successful dormancy induction. The present study illustrates that environmental conditions impact not only on bud-scale initiation (the developmental equivalent of "bud set"), but more importantly on the longer process of shoot preformation. This evidence supports the adoption of bud development rather than "bud set" in describing the morphogenetic processes of apical dormancy induction, that precede and/or coincide with the physiological processes, leading to apical dormancy. While dormancy induction marks the end of first-year seedling shoot elongation, it marks the preparatory stage for second-year seedling shoot elongation. This preparatory stage of bud development is a long, slow process.

Chapter 4
COMMERCIAL GREENHOUSE STUDY

4.1 Introduction

Cultural regimes for containerized seedlings are under continual refinement. A critical stage of seedling culture is dormancy induction during which seedling height growth ceases and bud development begins. In British Columbia, most nurseries use moderate moisture stress to induce dormancy (Matthews 1982); a few use short days (SD). Early dormancy is essential in fall-planting stock, and SD show promise for the earlier development of dormancy (see Chapter 3).

Although McCreary *et al.* (1978) prescribed 8 weeks in SD for Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco), growers currently use a 3- or 4-week duration. After 2-3 weeks in SD, height growth has ceased and buds become visible; this is thought to indicate successful dormancy induction. However, when seedlings are returned to natural long photoperiods after 3 weeks in SD, flushing of buds frequently occurs; this suggests that bud development has not been completed. Appearance of buds is an imprecise indicator of stage of bud and dormancy development. In coastal Douglas-fir (var. *menziesii*) seedlings, bud development began in mid-August but was not completed until mid-December; then, apical meristems became dormant, as indicated by a lack of mitoses (Fielder and Owens 1989). Duration in SD is very important to bud and dormancy development of seedlings; if too short, buds will flush after return to long days, and if too long, it may impact on bud and dormancy development because of reduced photosynthesis.

Once height growth ceases, dry matter production in shoots and roots increases (Matthews 1982, Tinus 1982). Although photosynthates are utilized both in bud and dormancy development and in dry matter production, the morphological

characteristics used for grading and culling seedlings in British Columbia are height, shoot diameter at the root collar, and root dry weight (Anon. 1986); consequently, duration in SD is critical because of the impact of reduced photosynthesis on dry matter production.

The objectives of this study were: (1) to determine the timing of bud and dormancy development during and following different SD durations with moderate moisture stress, different SD durations without moderate moisture stress, and moderate moisture stress under natural long days (LD); and, (2) to determine the effects of these treatments on shoot diameter at the root collar and root dry weight in 1-0 coastal Douglas-fir seedlings. This information will be of value in deciding upon the shortest duration in SD, the advantage/disadvantage of combining moisture stress with SD, and the advantage of SD over moisture stress under LD for inducing early dormancy without seriously reducing dry matter production in fall-planting stock.

4.2 Materials and Methods

4.2.1 Study 1

4.2.1.1 Seedling Culture

Coastal Douglas-fir seeds from Vancouver Island (B.C. Ministry of Forests, Registered Seedlot No. 4505; 48°49' N, 123°56' W, elevation 610 m) were stratified at 2°C for 4 weeks before sowing. Seeds were sown on April 9, 1986 in BC/CFS PSB 313A Styroblocks (Beaver Plastics Ltd., Edmonton, Alta.) at the MacMillan Bloedel, Angus P. MacBean Nursery, Yellow Point, B.C. (123°55' long. and 49°4' lat.). Styroblocks were placed in 2 production polyethylene greenhouses, identical

structurally and in orientation, maintained at 20°C/20°C (day/night) at 90 % relative humidity.

Growing medium was a 2:1 peat:vermiculite mix. Nutricote Type 360 (16-10-10) (Chisso-Asahi, Tokyo, Japan) was incorporated into the mix at a rate of 1.3 kg/m^3. Styroblocks were misted up to ten times daily (depending on brightness of weather) during germination. Thereafter, styroblocks containing seedlings were watered on loss of 3 kg below saturated weight. After germination, Plant-Prod Forestry Seedling Starter (11-41-8) (Plant Products Co. Ltd., Bramalea, Ont.) was applied, at a rate of 500-750 g/kl, with each watering during April and May. Subsequently, Plant-Prod Forestry Seedling Special (20-8-20) was applied, at a rate of 750 g/kl, with each watering during June. Throughout seedling culture, Plant-Green iron chelate (13% Fe) (Westcan Horticultural Specialists Ltd., Calgary, Alta.) and Peters Soluble Trace Element Mix (W.R. Grace and Co., Fogelsville, PA) were applied when needed as indicated by foliar analysis. Peters Calcium Nitrate (15.5% N, 20% Ca) was applied as necessary to adjust the pH of the growing medium.

In one greenhouse, photoperiod was controlled by black-out curtains (VRE Greenhouse Systems, Stoney Creek, Ont.). The system required that the polyethylene roof and sidewalls remain intact. Consequently, the greenhouse was cooled by fans when temperatures reached 25°C. The second greenhouse did not have photoperiod control and its polyethylene sidewalls and roof were removed prior to dormancy induction.

4.2.1.2 Dormancy Induction

There were 3 dormancy induction treatments: SD without moderate moisture stress (SD-MS), SD with moderate moisture stress (SD+MS), and long day with

moderate moisture stress (LD+MS). Dormancy induction treatments began on July 4, 1986. Natural photoperiod on July 3 was 16 hours (h) and 6 minutes.

On July 4, photoperiod was reduced to 8 h for the SD treatments. The photoperiod occurred from 08:00 h to 16:00 h. Styroblocks were removed after 3, 4, 5, and 6 weeks in SD (WK SD) and placed under natural photoperiod in the second greenhouse.

Prior to SD-MS, SD+MS and LD+MS, fertilizer application ceased and the growing medium was leached of mineral salts. Seedlings were not fertilized during the first 2 weeks of all treatments. For SD+MS and LD+MS, MS occurred as a cycle over a 2-week period, during which styroblocks were watered to saturation and then re-watered upon loss of 3 kg below saturated weight and onset of seedling wilting. Pre-dawn equilibrium water potentials of seedlings, determined by the pressure chamber technique (Ritchie and Hinckley 1975), reached -1 to -1.5 MPa before re-watering (A. Maher, pers. comm.). For SD+MS, the cycle occurred during the first 2 weeks of SD. For LD+MS, the MS cycle was the induction treatment. For SD+MS and LD+MS, watering of styroblocks based upon loss of 2 kg of saturated weight resumed in week 3.

4.2.1.3 Post-Induction and Over-Wintering Culture

In week 3, application of Forestry Seedling Special resumed with each watering and continued through July for all treatments. Starting in August, Plant-Prod Forestry Seedling Finisher (8-20-30) was applied, at a rate of 750 g/kl, with each watering. Greenhouse sidewalls and roof were put back into place in October. Greenhouse temperatures were maintained between 0-5°C by fans and heating during the fall and winter.

4.2.1.4 Experimental Design

The experimental design was a modified split-plot. For the SD treatments, there were 2 plots of 36 styroblocks each for SD-MS and SD+MS. Each plot was divided across the greenhouse into 4 blocks of 12 styroblocks each. To prevent accidental watering, the plots were separated by a buffer 2 styroblocks wide. Duration in SD was randomized in each block. One styroblock was removed from each of the 4 blocks for each SD duration. Styroblocks were moved to a second greenhouse under natural LD where they were placed in the same block location as in the greenhouse of origin. Each block was surrounded by a buffer of seedlings one styroblock wide.

For the LD+MS treatment, there was one plot of 12 styroblocks. The plot was divided across the greenhouse into 4 blocks of 4 styroblocks each. One styroblock in each block was sampled. After MS ended, these styroblocks were moved within the greenhouse to the area containing the SD-treated seedlings. The styroblocks were placed in the same block location as earlier, within the blocks containing the SD-treated seedlings.

4.2.1.5 Sampling and Transport

On the day before treatments began, 16 seedlings were sampled throughout the 2 greenhouses. Thereafter, 24 seedlings were randomly sampled per collection date from each treatment (6 seedlings from each styroblock). To avoid studying bud development on seedlings that would not meet height standards during grading and thus would be culled, only seedlings greater than 16 cm in height were sampled. As the season progressed, seedlings with acceptable height, which had small shoot diameters and/or poorly developed root systems, were not sampled. Pollard (1974b) reported that seedling size in *Picea glauca* (Moench) Voss influenced bud

morphogenesis. Similarly, in an earlier study of Douglas-fir seedlings, bud development was affected by seedling size (unpublished observations).

Seedlings were bundled in groups of 6 with the roots wrapped in plastic so as to allow aeration and packed upright into cartons. Seedlings were transported by car to the University of Victoria, Victoria, B.C. where they were kept in a lath-house until shoot tips were sampled for dissection and processing.

4.2.1.6 Processing of Samples

Neoformed leaves, and subsequently bud scales (once ergastic substances had accumulated), surrounding shoot tips were cut away. Type of foliar organ being initiated, stage of bud-scale differentiation, and presence of ergastic substances in bud scales were noted. For ease of description, bud scales were grouped into one of 4 bud-scale types (see Chapter 3, Section 3.3.2 for definitions). Dissected shoot tips were sliced along both sides, fixed in formalin-acetic acid-alcohol (FAA), dehydrated in tertiary-butyl alcohol series (Johansen 1940), and embedded in TissuePrep. Serial longitudinal sections were microtomed at 8 μm, and stained with safranin and hematoxylin.

4.2.1.7 Morphological and Anatomical Observations

Eight median longitudinal sections per treatment (2 from each styroblock) per collection date were selected, and traced using a microprojector. Type of foliar organ, numbers of preformed-leaf primordia per flank of the apical meristem/preformed shoot, and crown differentiation were noted. Receptacle width, bud-scale height, apical height and width, and preformed-shoot-axis height and width were determined. Cell counts in the apical meristem - delineated as that part of the apical dome above the last-initiated primordia - were made upon the

microprojection of the section. Numbers of cells in mitosis in the median section of the apical meristem were counted using a compound microscope. Mitotic index was calculated as the percentage of dividing cells in the median section of the apical meristem.

Receptacle width was the transverse distance between the bases of the outer-most bud scales. Bud-scale height was the vertical distance from the base to the apex of the longest outer-most bud scale. Apical height was the vertical distance between the maximum height of the apical dome and a transverse line between the axils of the last-initiated primordia. Apical width was the transverse distance between the axils of the last-initiated primordia. Preformed-shoot-axis height was the vertical distance between a transverse line between the axils of the last-initiated primordia and a transverse line between the bases of the first-initiated preformed-leaf primordia. Preformed-shoot-axis width was the transverse distance between the bases of the first-initiated preformed-leaf primordia.

Prior to treatments and during weeks 1-4 of treatments, neoformed leaves and/or bud scales were dissected away from shoot tips to reveal the apical meristem and its most-recent products. Samples representative of each treatment were fixed in Zirkles fixative, dehydrated in an ethyl alcohol series, processed through an ethyl alcohol:amyl acetate series to pure amyl acetate, dried to critical point, and gold-coated. Gold-coated specimens were observed and photographed using a scanning electron microscope (JSM-35U, JEOL, Tokyo, Japan) operating at 10 kV.

4.2.1.8 Numbers of Bud Scales and Preformed Leaves

After overwintering in the greenhouse, 20 seedlings from each of the 5 WK SD-MS, 5 WK SD+MS, and LD+MS treatments (5 from each styroblock) were randomly sampled in mid-March 1987. Buds were dissected and numbers of bud

scales were counted. In late March, 24 seedlings per treatment (6 from each styroblock) were randomly sampled and planted in a common garden. In late October, seedling shoots were clipped from roots at the root collar, bagged and air-dried. Numbers of preformed leaves on second-year shoots were counted.

4.2.1.9 Speed of Bud Break

In mid-March 1987, 20 seedlings per treatment (5 from each styroblock) were randomly sampled for a dormancy release test. Growing medium was a 2:2:1 peat:vermiculite:sand mix. Pots (3-liter capacity) were loosely filled with growing medium, holes were dibbled into the medium and seedlings were planted in the holes. There were 5 pots per treatment; each pot contained 4 seedlings (1 from each styroblock). Pots were watered to saturation prior to placement in a controlled environment chamber (Conviron E15, Controlled Environment Ltd., Winnipeg, Man.). Pot placement in the chamber was randomized within each of 5 replicates. Conditions for forcing bud flush were 20°C day/night and a 16 h photoperiod, with the growing medium maintained near saturation (Ritchie 1984b).

Every 3 days, terminal buds of seedlings were examined. As soon as the preformed shoot pushed through the bud scales, the bud was said to have flushed (Ritchie 1984b).

4.2.1.10 Root Collar Diameter and Root Dry Weight

In early October 1986, after shoot tips were sampled from 24 seedlings (6 from each styroblock) for the anatomical study, shoot diameter at the root collar was measured. Then, roots were washed to remove the growing medium, roots were clipped from shoots, bagged and air-dried. Samples were dried at 80°C in a forced-air oven for 12 h prior to weighing.

4.2.1.11 Presentation of Results and Statistical Analysis

Although all treatments were sampled and processed for anatomical/morphological study, observations were not obtained for all treatments. At the end of the study, the SD+MS treatment was judged to be an unsatisfactory cultural treatment. Because observations were required only for comparison purposes with the SD-MS treatment, only one SD+MS treatment was selected for observation. The treatment selected was the 4 WK SD+MS treatment because the 3, 5, and 6 WK SD+MS treatments were judged to be unsatisfactory treatments on the basis of physiological data. Anatomical/morphological observations are presented for 3, 4, 5, and 6 WK SD-MS and 4 WK SD+MS. Numbers of preformed leaves and speed of bud break are presented for the 3, 4, 5, and 6 WK SD-MS treatments and the 3, 4, 5, and 6 WK SD+MS treatments.

Because of small sample sizes for anatomical observations, differences between means for the SD-MS and SD+MS treatments ($p < 0.05$) were tested using Student's t-test (SAS 1982). For the SD-MS and SD+MS treatments, an analysis of variance (ANOVA) (SAS 1982) was used to test for treatment effects and interactions on numbers of preformed leaves, days to bud break, shoot diameter at the root collar and root dry weight. Subsequently, Student's t-test was used to test for differences between means.

Anatomical observations from the SD treatments and the LD+MS treatment were not compared statistically because of different stages of bud development. For numbers of preformed leaves, days to bud break, shoot diameter at the root collar and root dry weight, an ANOVA was not used because the experimental design was not balanced; it is impossible to have different durations under natural LD. However, differences between means were tested using Student's t-test.

4.2.2 Study 2

In study 1, seedlings in the 2 greenhouses varied in their developmental stage prior to dormancy induction treatments. Although, sowing dates and growth curves (until desired heights were approached) were the same in both greenhouses, it is likely that after the polyethylene roof was removed from one greenhouse, seedlings received a mild MS. To validate duration of bud-scale initiation and hence phenology of subsequent stages of bud development for the LD+MS treatment in study 1, a second dormancy induction study was conducted in 1987.

Seedlings were from a different seedlot than those used in study 1 (B.C. Ministry of Forests, Registered Seedlot No. 9976; 50°10' N, 125°25' W, elevation 710 m). To confirm that seedling response did not vary with seedlot and thus invalidate this second study, both LD+MS and SD-MS were selected as treatments for seedlings from the same greenhouse.

Ten seedlings from one styroblock per treatment were randomly sampled the day before induction began and immediately examined to determine that apical meristems were initiating neoformed leaves. Dormancy induction began on July 2, 1987 and seedlings were randomly sampled from the same styroblocks each week during the first four weeks of treatment. Neoformed leaves and subsequently bud scales were dissected away to reveal the apical meristem and its most-recent products. Stage of bud development was noted. Fresh specimens representing each treatment were observed and photographed using a scanning electron microscope operating at 10 kV.

4.3 Results

4.3.1 Bud Development During and Following the SD-MS and SD+MS Treatments

4.3.1.1 Bud-Scale Initiation and Differentiation

Prior to the SD treatments in studies 1 and 2, apices of all seedlings were initiating neoformed leaves (Fig. 20). During neoformed-leaf initiation, zonation of the apical meristem was evident. The apical zone was broad and deep, and the rib meristem was narrow and shallow (Fig. 21). Within the first week in SD in both studies, all apices ended neoformed-leaf initiation, then began and completed bud-scale initiation (Figs. 22-23). Mean number (±SE) of bud scales initiated for SD-MS and SD+MS was 20.2 (1.4) and 19.5 (0.9), respectively. Means were not significantly different.

During week 1 in SD, bud-scale differentiation began and structures destined to become type 1 bud scales (Fig. 24) of the bud-scale-complex (BSC) (see Chapter 3, Section 3.3.2 for definitions) were morphologically distinct from undifferentiated bud-scale primordia (Fig. 23). After 3 weeks in SD, terminal buds were evident. Under SD-MS, types 1, 2, and 3 bud scales were visible in BSCs, and ergastic substances were deposited throughout the abaxial cells of type 1 and 2 bud scales and in the abaxial cells of the apical regions of type 3 bud scales. Because of ergastic substance deposition, bud scales appeared brown. Under SD+MS, type 1 and 2 bud scales were visible in BSCs, ergastic substance deposition was not evident and bud scales were pink. After 4 weeks in SD, all three bud scale types were visible in BSCs under SD+MS. All bud scales under SD-MS and SD+MS were brown.

During week 1 in SD, elongation of type 2 bud scales began, and it continued

Fig. 20-25. Scanning electron and light micrographs of shoot tips of Douglas-fir seedlings during and following the short day (SD) treatments.

Fig. 20. Fresh shoot tip before the SD treatment with older neoformed leaves cut away to reveal the apical meristem (am) and recently-initiated neoformed leaves (nl). x75.

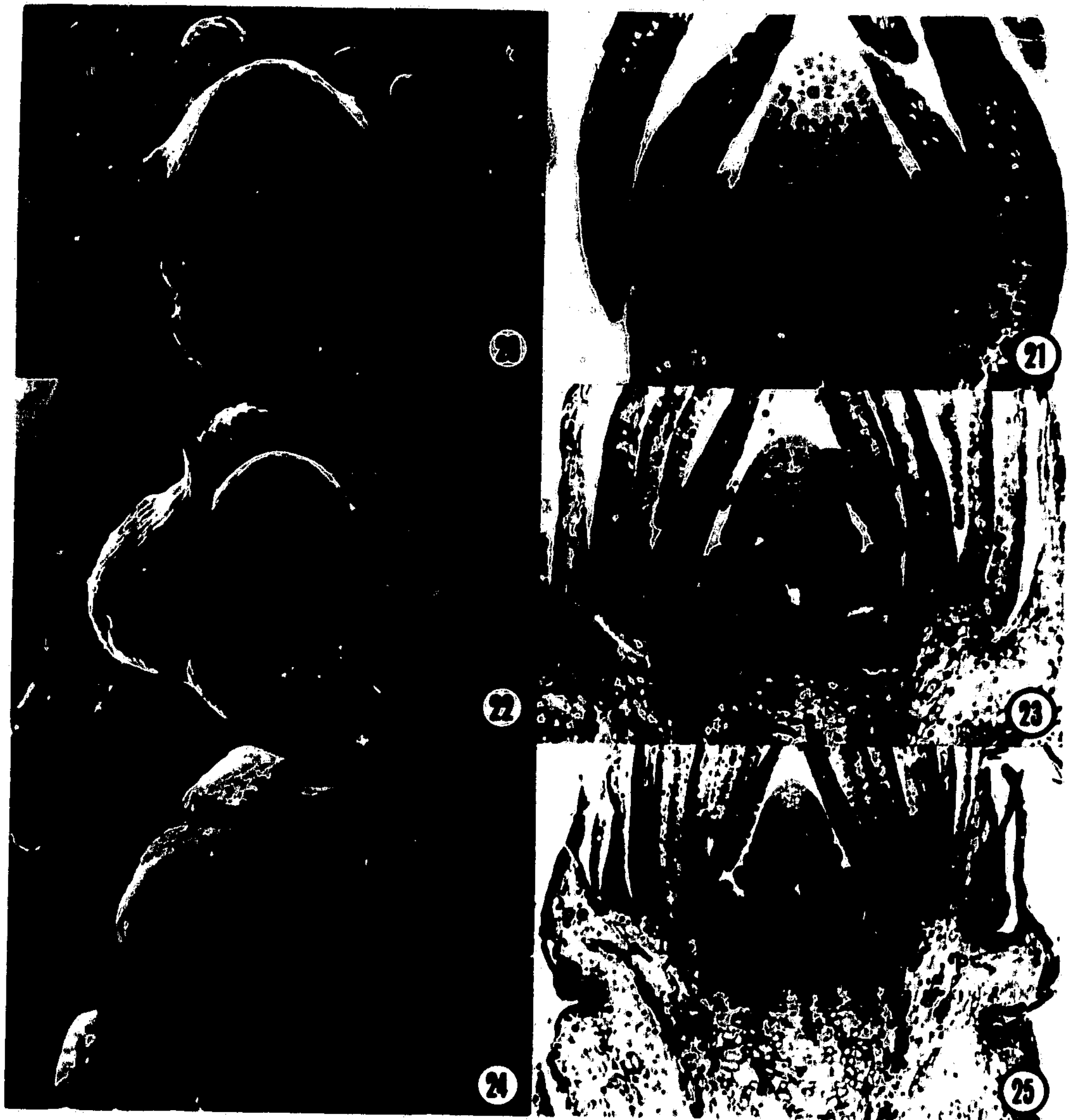
Fig. 21. Median longitudinal section of apical meristem initiating neoformed leaves before the SD treatment, showing broad, deep apical zone (az); narrow, shallow rib meristem (rm); and narrow peripheral zone (pz). Note width of shoot, and number and sizes of pith (ph) and cortical (c) cells in the shoot below the apical meristem. x75.

Fig. 22. Fresh shoot tip after one week in SD with older bud scales cut away to reveal apical meristem, surrounded by recently-initiated, undifferentiated bud scales (bs), initiating preformed leaves (pl). x75.

Fig. 23. Median longitudinal section of shoot tip after one week in SD showing apical meristem, with distinct apical zone, rib meristem, and peripheral zone, starting preformed-leaf initiation. The shoot below the apical meristem widened as the bud-scale receptacle (r), between arrows) developed due to an increase in number and size of pith and cortical cells. Note the base of the embryonic preformed shoot (eps) was flush with the receptacle tissue. x75.

Fig. 24. Gold-coated shoot tip after one week in SD with neoformed leaves cut away to reveal rudimentary type 1 bud scales of the bud-scale complex. x30.

Fig. 25. Median longitudinal section of bud after 3 weeks in SD, showing large parenchyma (pa) cells in reduced petioles (pe) of type 1 bud scales that further widened receptacle. Elongation of cortical cells below bases of bud scales elevated receptacle tissue around the embryonic preformed shoot (eps). x30.



for 4 weeks. Mean final height (±SE) of the longest type 2 bud scale for SD-MS and SD+MS was 3.3 (0.2) mm and 3.2 (0.2) mm, respectively.

4.3.1.2 Bud-Scale-Receptacle Development

Prior to the SD treatments, mean width (±SE) of the shoot below the apical meristem was 0.5 (0.02) mm. After 1 week in SD, shoot width below the apical meristem increased as the bud-scale receptacle developed. Receptacle width after 1 week under SD-MS and SD+MS was 1.1 (0.09) mm and 1.0 (0.07) mm, respectively. During week 2 in SD, there was only a slight increase in receptacle width. However, during week 3 in SD, receptacle development was completed and final receptacle width (± SE) for SD-MS and SD+MS was 2.2 (0.11) mm and 1.9 (0.05) mm, respectively.

In the shoot below the apical meristem, there was a great increase in number of cortical cells and a slight increase in number of pith cells during week 1 in SD (Table 13). The increases were greater under SD+MS than under SD-MS (Table 13). Size of cortical and pith cells increased markedly during week 1 (Table 13). Pith cells were wider than cortical cells (24.8 μm and 17.8 μm, respectively for SD-MS; 23.7 μm and 16.2 μm, respectively for SD+MS). During week 2 and 3 in SD, there was a larger increase in number of pith cells than in number of cortical cells (Table 13). The increases were greater under SD-MS than under SD+MS. Under SD-MS, cell size increased slightly during week 2 and modestly during week 3 (Table 13). Under SD+MS, cell size increased modestly during week 2, but did not increase during week 3 (Table 13). After 3 weeks, pith cells remained wider than cortical cells (30.3 μm and 24.8 μm, respectively for SD-MS; 27.9 μm and 18.7 μm, respectively for SD+MS). These increases in number and size of cortical and pith cells during the initial 3 weeks in SD accounted for most of the increase in

receptacle width.

Table 13: Percentage increases in number and size of pith and cortical cells by week during bud-scale-receptacle development under different dormancy induction treatments in Douglas-fir seedlings.

Treatments: SD-MS - short day without moisture stress
SD+MS - short day with moisture stress
LD+MS - long day with moisture stress

Percentages based on observations from 6 seedlings per treatment.

TREATMENT	NO. OF WEEKS	PERCENTAGE INCREASE IN NUMBER OF CELLS		PERCENTAGE INCREASE IN SIZE OF CELLS	
		PITH	CORTICAL	PITH	CORTICAL
SD-MS	1	22	170	58	84
SD-MS	2	56	14	4	9
SD-MS	3	30	16	17	28
SD+MS	1	53	192	51	67
SD+MS	2	9	2	17	18
SD+MS	3	21	6	<1	<1
LD+MS	1	0	0	0	0
LD+MS	2	10	125	41	74
LD+MS	3	33	21	<1	3
LD+MS	4	<1	11	34	23
LD+MS	5	33	11	<1	<1

In addition to receptacle development from the proliferation and enlargement of pith and cortical cells, receptacle width increased slightly due to an increase in size of parenchyma cells in the reduced petioles of distal-most neoformed leaves and type 1 bud scales (Fig. 25). Expanded petioles were evident after 3 weeks under SD-MS and after 4 weeks under SD+MS.

During week 1 and 2 under SD, the base of the apical meristem was flush with the receptacle tissue (Fig. 23). However, during week 3, cortical cells directly below the bases of bud scales elongated longitudinally elevating the receptacle tissue

around the embryonic preformed shoot (Fig. 25).

4.3.1.3 Preformed-Leaf Initiation

For the SD-MS treatments, preformed-leaf initiation began during week 1 (Figs. 22, 23, 26A). Concomitantly, apical height increased to a maximum value (Fig. 27A). Rate of preformed-leaf initiation steadily increased in week 1 and 2 (Fig. 26A). During this time, zonation of the apical meristem became more distinct than during neoformed-leaf initiation. Both the apical zone and rib meristem were broad and deep.

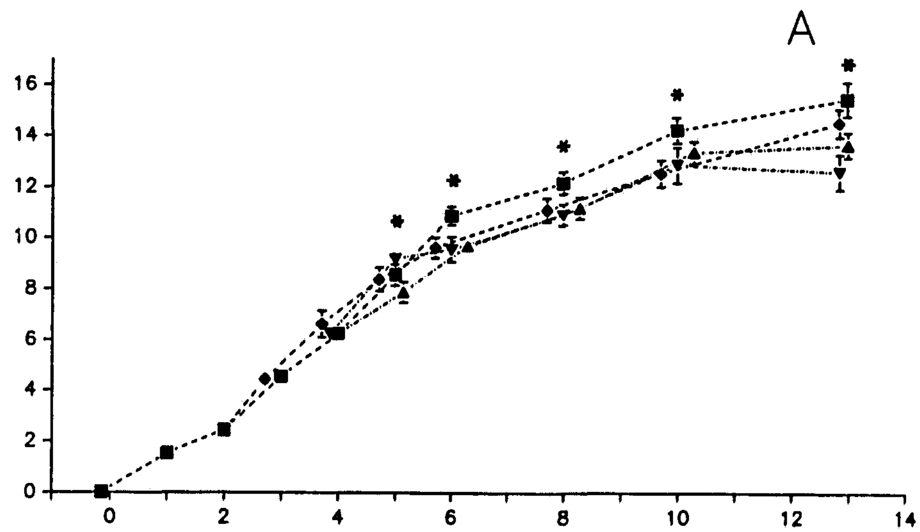
Rate of preformed-leaf initiation then increased rapidly until week 4 for 3 WK SD-MS, week 5 for 6 WK SD-MS, and week 6 for 4 and 5 WK SD-MS (Fig. 26A). Apical height of 3 WK SD-MS was significantly larger than that of 4 WK SD-MS in week 4 and 6 WK SD-MS in week 6 (Fig. 27A). Apical width of 3 WK SD-MS was significantly larger than that of 4 WK SD-MS during weeks 4-6, 5 WK SD-MS during weeks 6-8, and 6 WK SD-MS in week 4 and 6 (Fig. 28A). Number of apical cells increased until week 3 and then decreased as preformed-leaf initiation exceeded apical growth (Fig. 29A). The peripheral zones widened during rapid preformed-leaf initiation (Fig. 30), at times constricting the apical zone. By week 6, 66%, 70%, 71%, and 76% of the total number of preformed-leaf primordia per flank had been initiated under 3, 4, 5, and 6 WK SD-MS, respectively.

Apical height decreased only slightly during slow preformed-leaf initiation (Fig. 27A). During this time, significantly more primordia were initiated each week under 4 WK SD-MS than under 3, 5, or 6 WK SD-MS (Fig. 26A). As preformed-leaf initiation neared completion, all zones of the apical meristem were prominent, but had decreased in size. The apical zone and rib meristem were broader and shallower, and the peripheral zones were narrower. Preformed-leaf initiation was

Fig. 26. Number of preformed-leaf primordia per flank in median longitudinal sections through buds of Douglas-fir seedlings versus date for (A) 3, 4, 5, and 6 WK SD-MS, (B) 4 WK SD-MS, 4 WK SD+MS, and LD+MS. n = 8.

Abbreviations: WK, weeks in; SD, short day; LD, long day; MS, moisture stress
Dashed lines: 3 WK SD-MS (◆), 4 WK SD-MS (■)
Dashed-dotted lines: 5 WK SD-MS (▲), 6 WK SD-MS (▼)
Solid lines: 4 WK SD+MS (□), LD+MS (○)

*, t-test for difference between means of SD treatments was significant at $p < 0.05$.


A
16
14
12
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8
6
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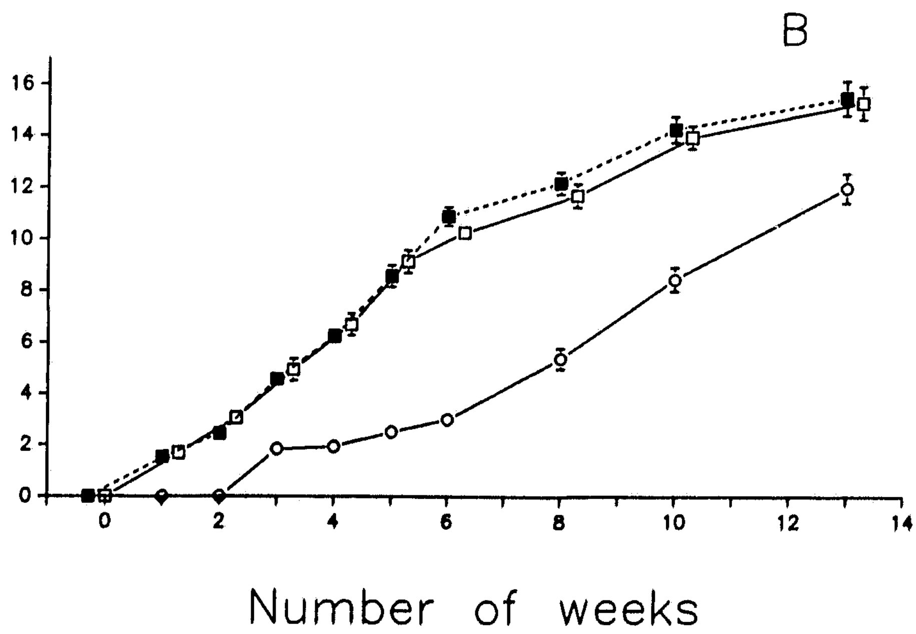
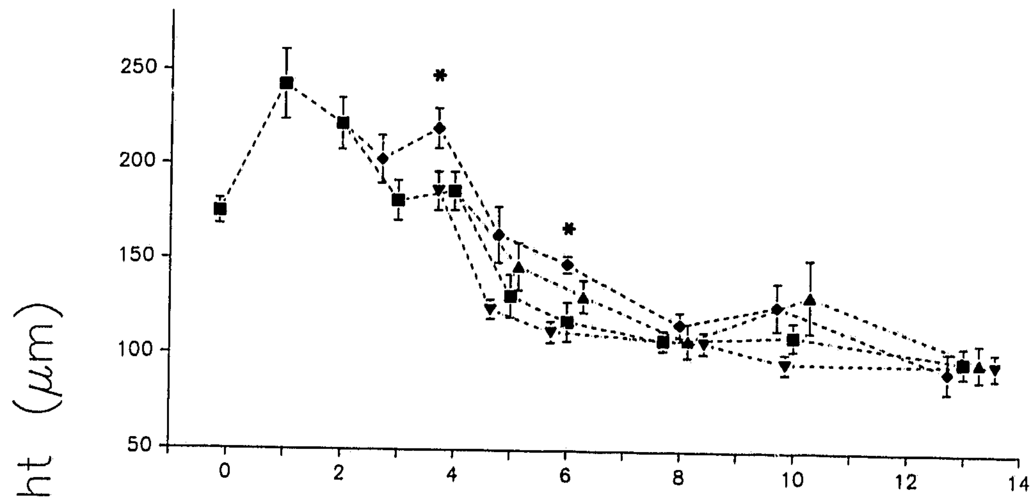

B
16
14
12
10
8
6
4
2
0
0
2
4
6
8
10
12
14
Number of weeks
Number of preformed-leaf primordia per flank

Fig. 27. Height of apical meristem (μm) in median longitudinal sections through buds of Douglas-fir seedlings versus date for (A) 3, 4, 5, and 6 WK SD-MS, (B) 4 WK SD-MS, 4 WK SD+MS, and LD+MS. $n = 8$.

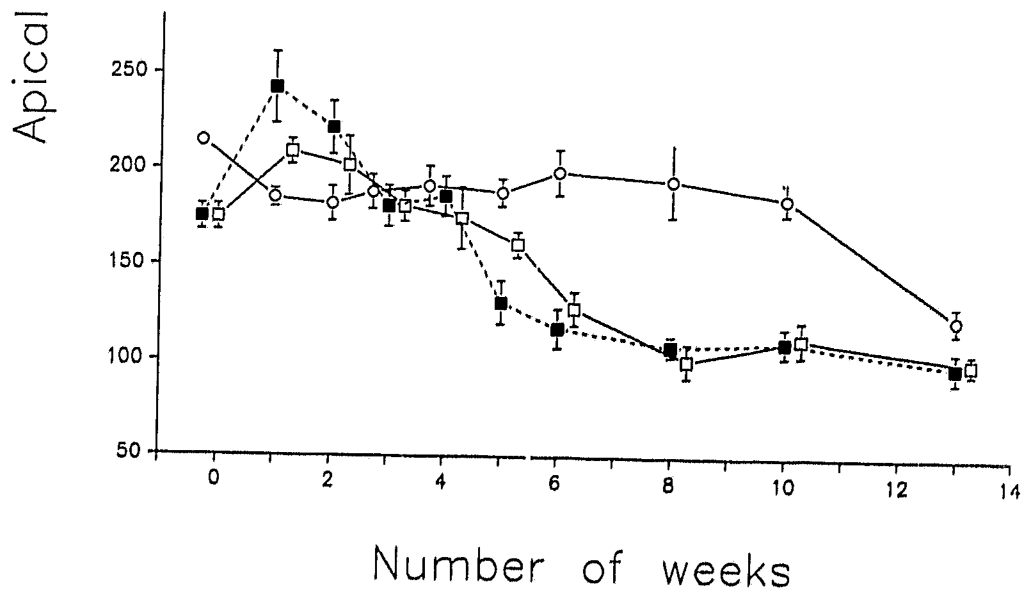
Abbreviations: WK, weeks in; SD, short day; LD, long day; MS, moisture stress
Dashed lines: 3 WK SD-MS (◆), 4 WK SD-MS (■)
Dashed-dotted lines: 5 WK SD-MS (▲), 6 WK SD-MS (▼)
Solid lines: 4 WK SD+MS (□), LD+MS (○)

*, t-test for difference between means of SD treatments was significant at $p < 0.05$.

A



B



Apical height (μm)

Number of weeks

Fig. 28. Width of apical meristem (μm) in median longitudinal sections through buds of Douglas-fir seedlings versus date for (A) 3, 4, 5, and 6 WK SD-MS, (B) 4 WK SD-MS, 4 WK SD+MS, and LD+MS. $n = 8$.

Abbreviations: WK, weeks in; SD, short day; LD, long day; MS, moisture stress
Dashed lines: 3 WK SD-MS (◆), 4 WK SD-MS (■)
Dashed-dotted lines: 5 WK SD-MS (▲), 6 WK SD-MS (▼)
Solid lines: 4 WK SD+MS (□), LD+MS (○)

*, t-test for difference between means of SD treatments was significant at $p < 0.05$.

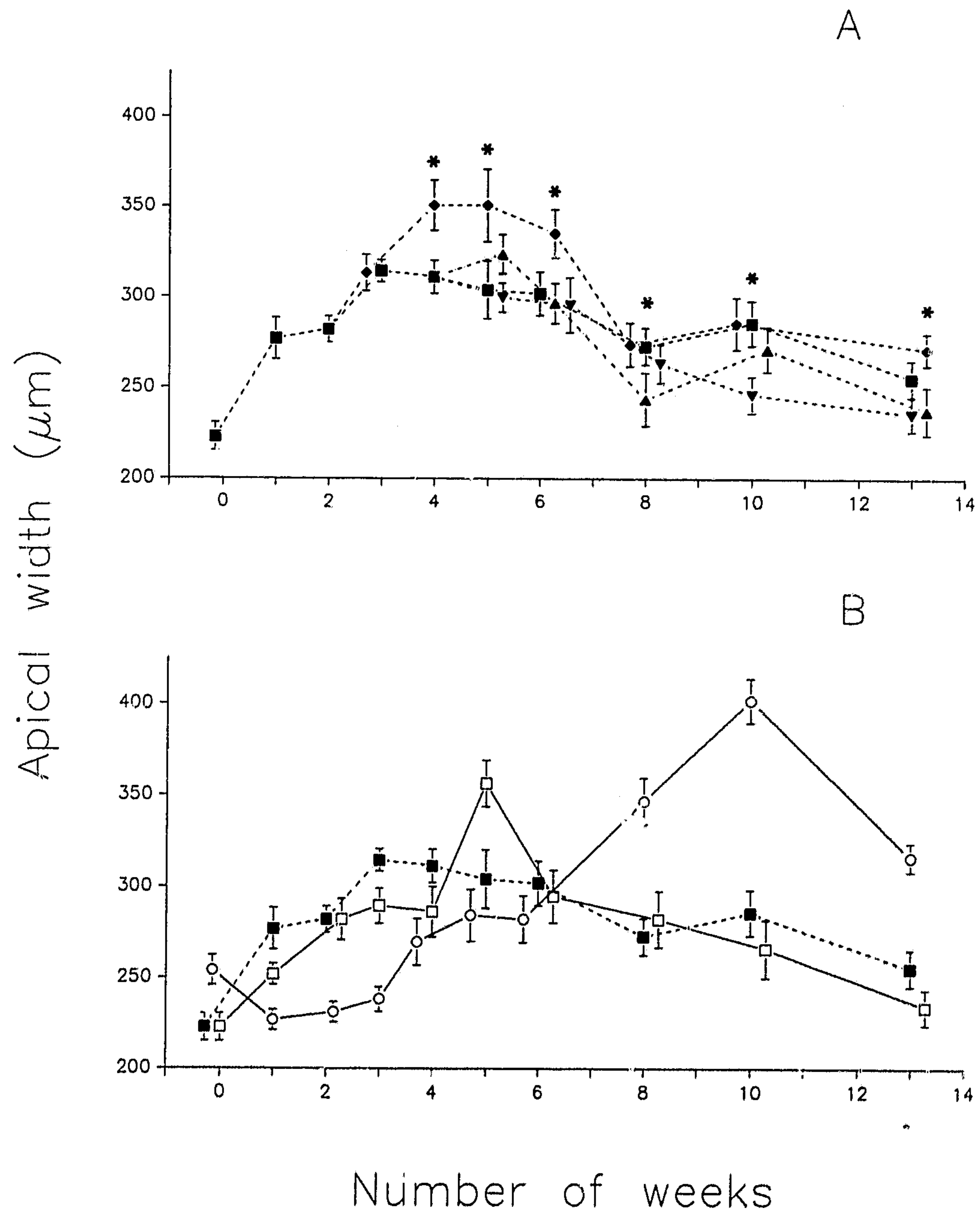
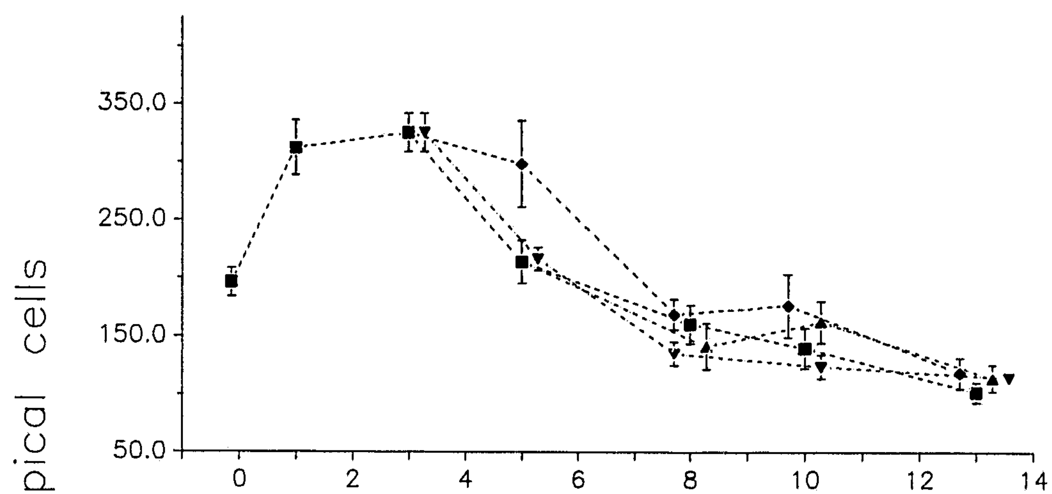
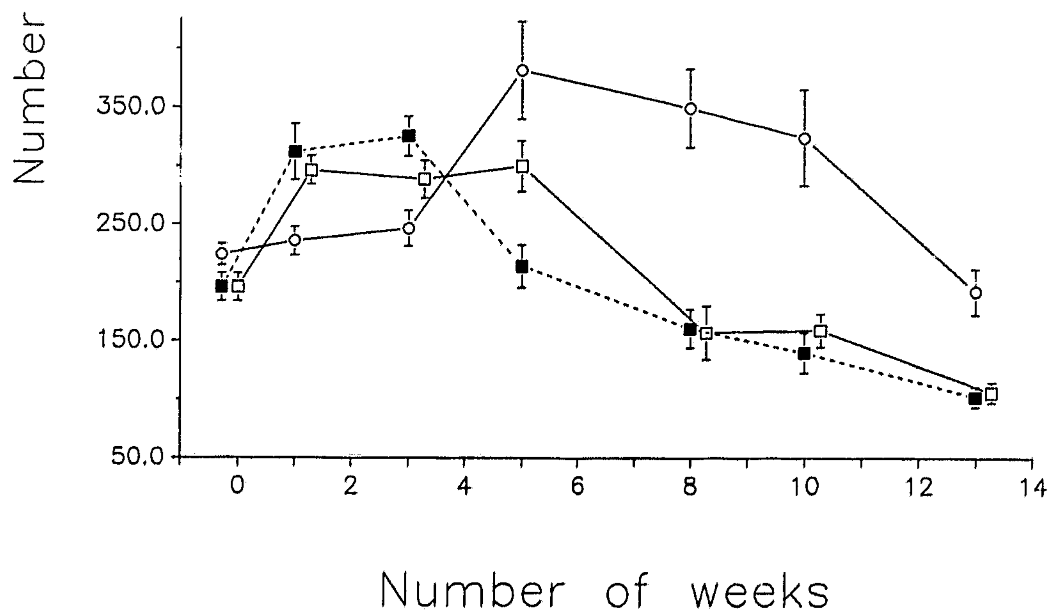

A
B
400
350
300
250
200
0
2
4
6
8
10
12
14
*
Apical width (μm)
Number of weeks

Fig. 29 Number of apical cells in median longitudinal sections through buds of Douglas-fir seedlings versus date for (A) 3, 4, 5, and 6 WK SD-MS, (B) 4 WK SD-MS, 4 WK SD+MS, and LD+MS. $n = 8$.

Abbreviations: WK, weeks in; SD, short day; LD, long day; MS, moisture stress
Dashed lines: 3 WK SD-MS (◆), 4 WK SD-MS (■)
Dashed-dotted lines: 5 WK SD-MS (▲), 6 WK SD-MS (▼)
Solid lines: 4 WK SD+MS (□), LD+MS (○)





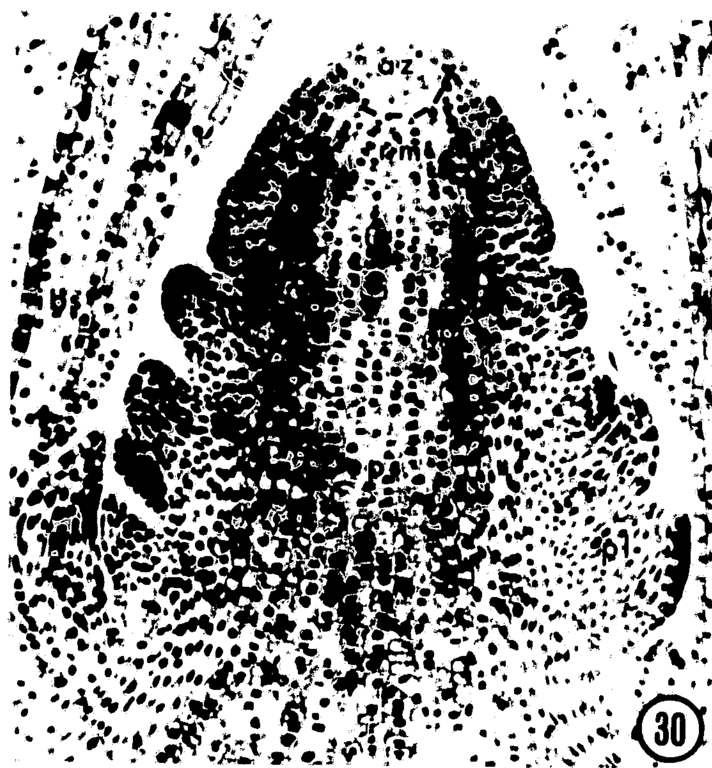
Number of apical cells

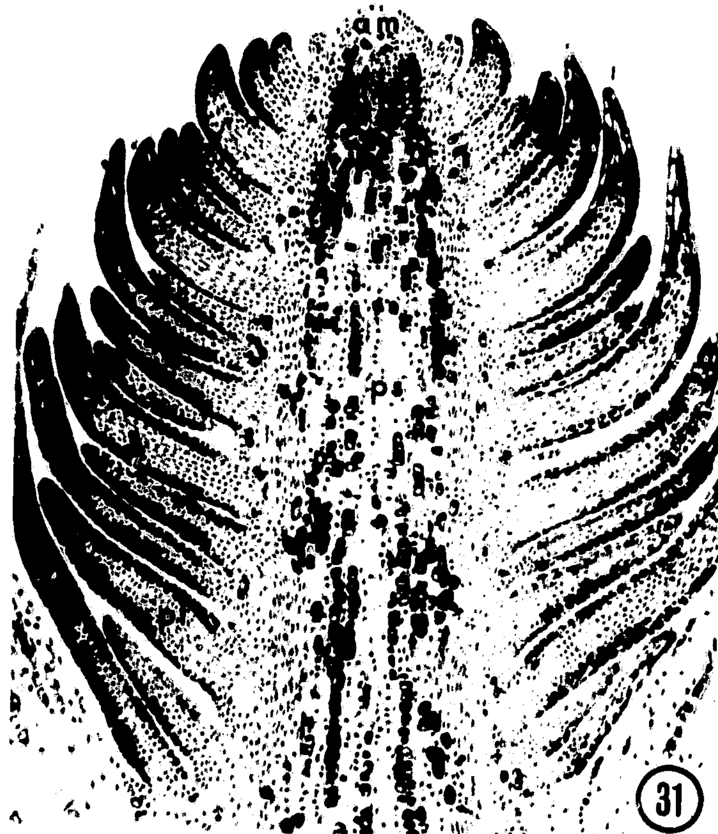
Number of weeks

Fig. 30-31. Light micrographs of median longitudinal sections through buds of Douglas-fir seedlings during and following the short day (SD) treatments.

Fig. 30. Embryonic preformed shoot (eps) surrounded by inner bud scales (ibs) during rapid preformed-leaf (pl) initiation after 5 weeks, showing apical zone (az), rib meristem (rm), and peripheral zone (pz). Note widened peripheral zone. x105.

Fig. 31. Preformed shoot (ps) after 13 weeks. Apical meristem had completed preformed-leaf initiation. Note minimum height of apical meristem. x45.


az
am
bs
p
pl
30


am
p
31

completed and minimum apical heights were reached by week 10 for 6 WK SD-MS (Figs. 26A, 27A, 31). In week 10, apical width of 6 WK SD-MS was significantly smaller than that of 3, 4, and 5 WK SD-MS (Fig. 28A). Preformed-leaf initiation was completed and minimum apical heights were reached by week 13 for 3, 4, and 5 WK SD-MS (Fig. 26A, 27A).

There were no significant differences in number of preformed-leaf primordia initiated between 4 WK SD-MS and 4 WK SD+MS (Fig. 26B). By week 6, 67% and 70% of preformed-leaf primordia had been initiated under 4 WK SD+MS and 4 WK SD-MS, respectively. Apical heights of 4 WK SD-MS and 4 WK SD+MS were not significantly different (Fig. 27B). With two exceptions, apical widths and number of apical cells of 4 WK SD-MS and 4 WK SD+MS were not significantly different (Figs. 28B, 29B).

For the SD-MS treatments, mean number of preformed leaves ranged from 140.0 to 175.3 (Table 14). Duration in SD had a highly significant effect on number of preformed leaves initiated (Table 15). Significantly more preformed leaves were initiated under 3 WK SD-MS than under 4, 5, and 6 WK SD-MS. Significantly more preformed leaves were initiated under 4 and 5 WK SD-MS than under 6 WK SD-MS. For SD+MS treatments, mean number of preformed leaves ranged from 134.9 to 164.3 (Table 14). Significantly more preformed leaves were initiated under 3, 4, and 5 WK SD+MS than under 6 WK SD+MS.

4.3.1.4 Mitotic Activity During Bud Development

During organogenesis, the majority of mitoses were in the peripheral zone of the apical meristem; it is this zone that gives rise to primordia. As preformed-leaf initiation began in week 1 in SD-MS, mitotic index (MI) was the same as during neoformed-leaf initiation (Fig. 32A). The period of increase in MI during weeks 3-5

Table 14: Numbers of preformed leaves initiated in buds of Douglas-fir seedlings from different dormancy induction treatments.

Treatments: WK SD-MS - weeks in short day without moisture stress
WK SD+MS - weeks in short day with moisture stress
LD+MS - long day with moisture stress

Numbers (±SE) are means from 24 seedlings per treatment.

TREATMENT	NUMBER OF PREFORMED LEAVES
3 WK SD-MS	175.3 (5.6)
4 WK SD-MS	165.7 (5.9)
5 WK SD-MS	162.1 (5.1)
6 WK SD-MS	140.0 (5.3)
3 WK SD+MS	164.1 (5.2)
4 WK SD+MS	163.4 (7.0)
5 WK SD+MS	164.3 (5.5)
6 WK SD+MS	134.9 (5.4)
LD+MS	159.4 (6.4)

Table 15: Analysis of variance of dormancy induction treatment and location in greenhouse effects on number of preformed leaves initiated in buds of Douglas-fir seedlings.

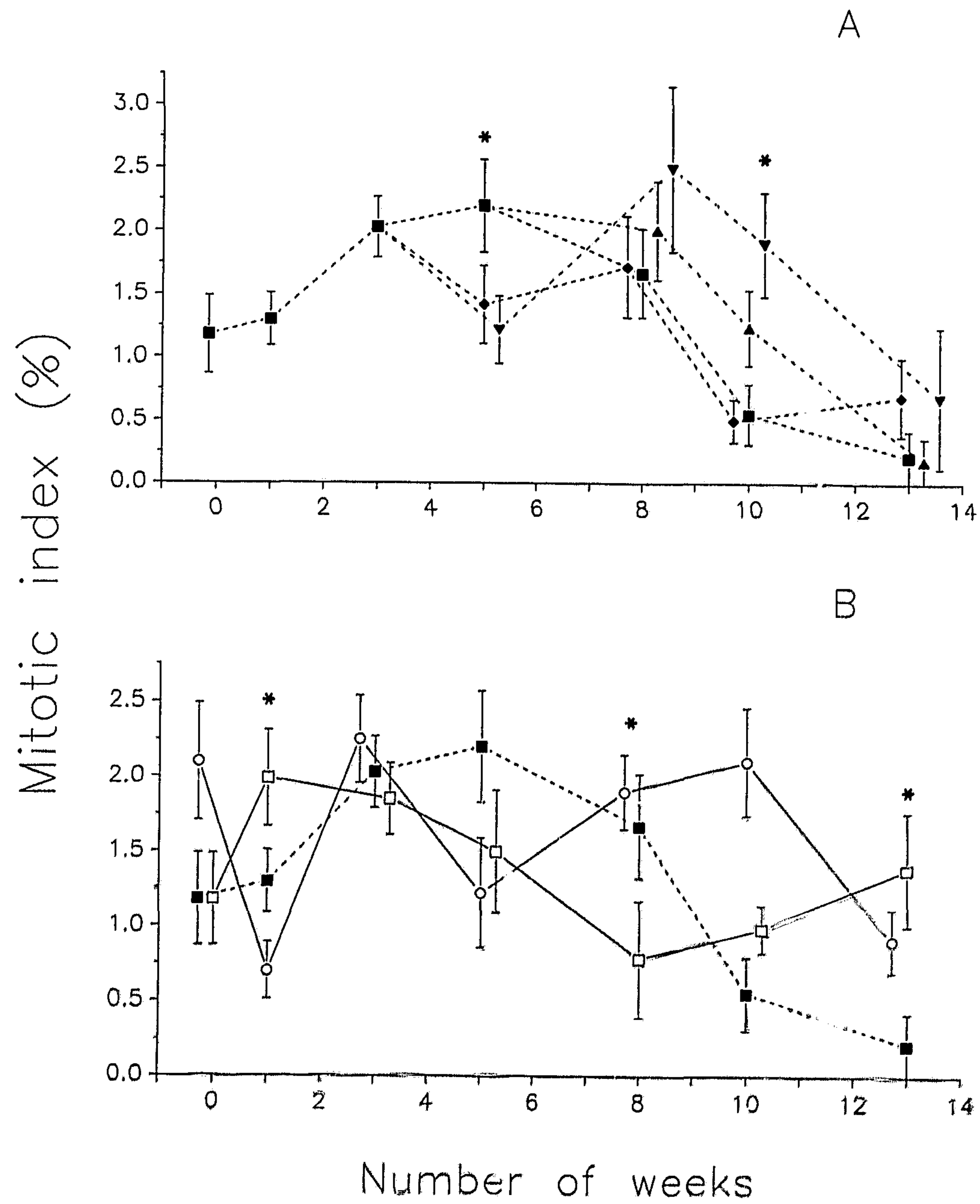
**, significant at $p < 0.001$.

SOURCE	DF	MS	F	P
Moisture regime (MR)	1	812.63	1.04	0.3087
Duration in short day (DSD)	3	10054.81	12.89	0.0001**
Location in greenhouse	3	250.00	0.32	0.8105
MR x DSD	3	376.48	0.48	0.6947
Error	181	779.84		

Fig. 32. Mitotic index of apical meristem (μm) in median longitudinal sections through buds of Douglas-fir seedlings versus date for (A) 3, 4, 5, and 6 WK SD-MS, (B) 4 WK SD-MS, 4 WK SD+MS, and LD+MS. $n = 8$.

Abbreviations: WK, weeks in; SD, short day; LD, long day; MS, moisture stress
Dashed lines: 3 WK SD-MS (◆), 4 WK SD-MS (■)
Dashed-dotted lines: 5 WK SD-MS (▲), 6 WK SD-MS (▼)
Solid lines: 4 WK SD+MS (□), LD+MS (○)

*, t-test for difference between means of SD treatments was significant at $p < 0.05$.


A
3.0
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
12
14
*
*
Mitotic index (%)
B
2.5
2.0
1.5
1.0
0.5
0.0
0
2
4
6
8
10
12
14
*
*
*
Number of weeks

coincided with rapid preformed-leaf initiation (Fig. 26A, 32A). Between weeks 6-10, MI decreased as preformed-leaf initiation slowed (Fig. 26A, 32A). In week 10, MI of 5 and 6 WK SD-MS was significantly greater than that of 4 WK SD-MS, and MI of 6 WK SD-MS was significantly greater than that of 3 WK SD-MS (Fig. 32A). As MI approached zero in week 13, preformed-leaf initiation was completed (Figs. 26A, 32A). By week 13, mitoses were absent in 50%, 88%, 88%, and 75% of the apices in 3, 4, 5, and 6 WK SD-MS. Although not significantly different, MI of 4 and 5 WK SD-MS were less than those of 3 and 6 WK SD-MS (Fig. 32A).

During week 1, MI of 4 WK SD+MS was significantly greater than that of 4 WK SD-MS, despite MS during week 1 (Fig. 32B). However, MI of 4 WK SD+MS decreased after week 1; whereas, MI of 4 WK SD-MS increased until week 5. In week 8, even though MI of 4 WK SD-MS was decreasing, it was significantly greater than MI of 4 WK SD+MS (Fig. 32B). Mitotic index of 4 WK SD+MS increased in weeks 10 and 13 and was significantly greater than that of 4 WK SD-MS in week 13 (Fig. 32B). By week 13, mitoses were absent in 88% and 25% of apices in 4 WK SD-MS and 4 WK SD+MS, respectively.

4.3.1.5 Crown Differentiation

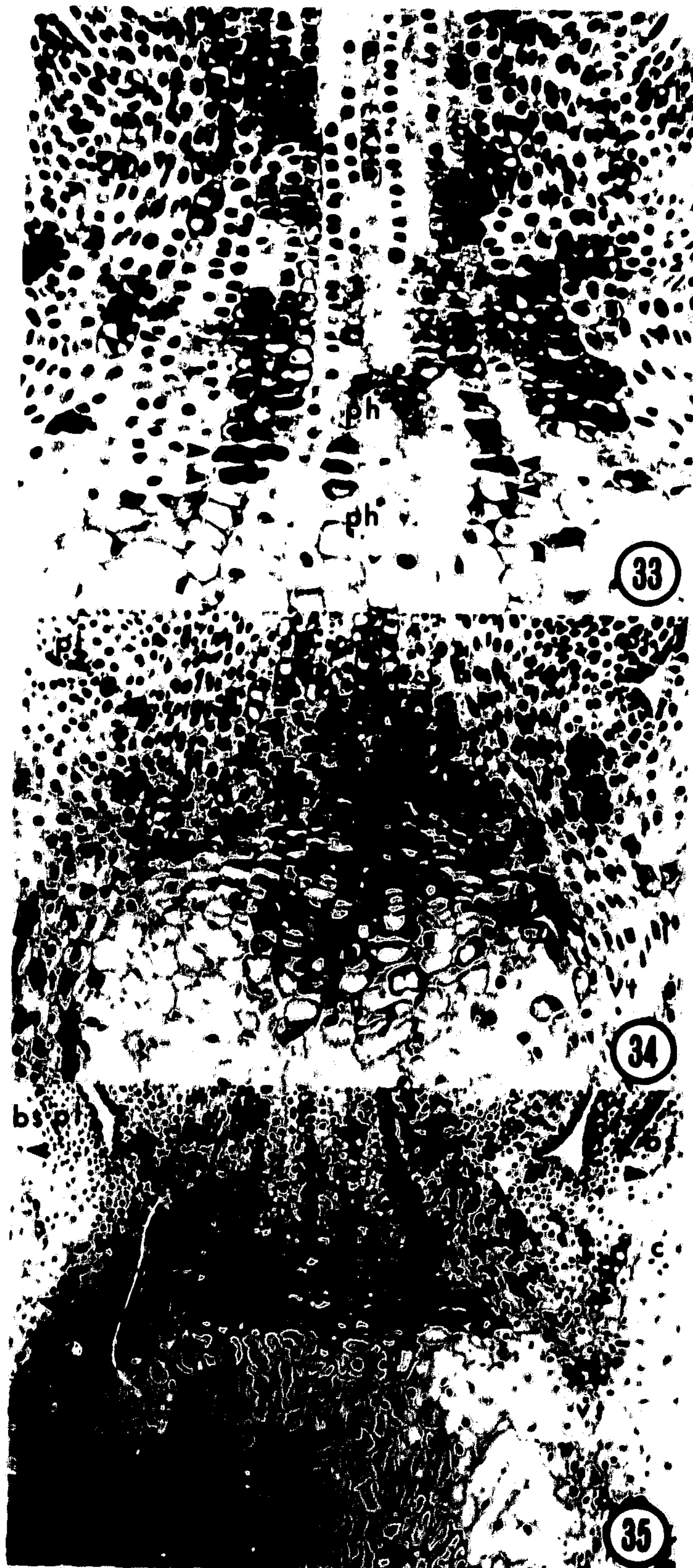
Crown differentiation in the SD-MS and SD+MS treatments was first apparent during rapid preformed-leaf initiation in week 3. A band of radially elongated cells partially extended across the pith at the base of the embryonic preformed shoot (Fig. 33). By week 5 for SD+MS and week 6 for SD-MS, as rapid preformed-leaf initiation ended, the band of cells extended entirely across the pith (Fig. 34). Thickening of crown cell walls within the pith occurred during weeks 8-13 (Fig. 35). Between weeks 10-13, cells with thickened walls became apparent within the vascular strands and cortex, and the crown extended through the cortex to the

Fig. 33-35. Light micrographs of median longitudinal sections through the bases of preformed shoots (see preformed-leaf primordia (pl)) during crown differentiation during and following the short day (SD) treatments.

Fig. 33. A band of radially elongated cells (between arrows) partially extending across the pith (ph) after 3 weeks in SD. x160.

Fig. 34. Band of cells (between arrows) extended entirely across the pith between vascular tissue (vt) after 5-6 weeks. x100.

Fig. 35. Crown (cr) cells with thickened walls in pith after 13 weeks. Crown cells extending through the cortex (c) (between arrows) to the bases of inner bud scales (ibs) were apparent. x50.


ph
ph
33
p
34
bs
p
b
c
35

bases of the inner bud scales (Fig. 35).

In early October, for the SD-MS treatments, crown height and width ranged from 139.2 to 166.0 μm and 626.5 to 962.1 μm, respectively (Table 16). There were no significant differences in crown height among the SD-MS treatments, but crown width of 3 and 5 WK SD-MS was significantly wider than that of 6 WK SD-MS.

For 4 WK SD-MS and 4 WK SD+MS, respectively, crown height was 155.0 μm and 154.7 μm, and crown width was 848.6 μm and 861.6 μm (Table 16). There were no significant differences in crown height and width between 4 WK SD-MS and 4 WK SD+MS.

Table 16: Crown height and width in buds of Douglas-fir seedlings from different dormancy induction treatments by early October.

Treatments: WK SD-MS - weeks in short day without moisture stress
WK SD+MS - weeks in short day with moisture stress
LD+MS - long day with moisture stress

Heights and widths are means (±SE) from 8 sections per treatment.

TREATMENT	CROWN HEIGHT (μm)	CROWN WIDTH (μm)
3 WK SD-MS	166.0 (6.0)	962.1 (35.7)
4 WK SD-MS	155.0 (9.6)	848.6 (83.6)
5 WK SD-MS	148.1 (9.7)	847.9 (51.1)
6 WK SD-MS	139.2 (12.3)	626.5 (34.7)
4 WK SD-MS	155.0 (9.6)	848.6 (83.6)
4 WK SD+MS	154.7 (11.3)	861.6 (40.7)
LD+MS	141.0 (6.3)	774.7 (29.3)

4.3.1.6 Preformed-Shoot-Axis Height and Width

For the SD-MS treatments, preformed-shoot-axis height and width in early October ranged from 1258.4 to 1640.4 μm and 596.2 to 783.6 μm, respectively

(Table 17). Preformed-shoot-axis height and width of 3 and 4 WK SD-MS were significantly greater than those for 6 WK SD-MS. Preformed-shoot-axis width of 3 and 5 WK SD-MS were significantly greater than those of 5 and 6 WK SD-MS, respectively.

Table 17: Numbers of preformed-leaf primordia per preformed-shoot-axis flank and preformed-shoot-axis height and width in buds of Douglas-fir seedlings from different dormancy induction treatments by early October.

Treatments: WK SD-MS - weeks in short day without moisture stress
WK SD+MS - weeks in short day with moisture stress
LD+MS - long day with moisture stress

Numbers, heights and widths are means (± SE) from 8 sections per treatment.

TREATMENT	NO. OF PREFORMED-LEAF PRIMORDIA	PREFORMED-SHOOT-AXIS HEIGHT (µm)	PREFORMED-SHOOT-AXIS WIDTH (µm)
3 WK SD-MS	14.6 (0.6)	1551.2 (80.6)	783.6 (17.4)
4 WK SD-MS	15.5 (0.7)	1640.4 (87.3)	737.2 (29.8)
5 WK SD-MS	13.7 (0.5)	1479.8 (36.1)	739.0 (12.9)
6 WK SD-MS	12.6 (0.7)	1258.4 (70.6)	596.2 (15.7)
4 WK SD-M	15.5 (0.7)	1640.4 (87.3)	737.2 (29.8)
4 WK SD+MS	15.3 (0.6)	1520.8 (88.5)	696.1 (33.7)
LD+MS	12.0 (0.6)	1101.3 (52.9)	664.0 (31.9)

For 4 WK SD-MS and 4 WK SD+MS respectively, preformed-shoot-axis height was 1640.4 µm and 1520.8 µm, and preformed-shoot-axis width was 737.2 µm and 696.1 µm (Table 17). Preformed-shoot-axis height and width of 4 WK SD-MS and 4 WK SD+MS were not significantly different.

The number of leaf primordia per preformed-shoot-axis flank was not always proportional to shoot-axis height. Individual preformed-leaf primordia for 4 WK

SD-MS occupied more axis tissue than those for 4 WK SD+MS (Table 17). The amount of axis tissue occupied per primordium for 4 WK SD-MS and 4 WK SD+MS was 106 μm and 99 μm, respectively.

4.3.2 Speed of Bud Break After the SD-MS and SD+MS Treatments

After natural chilling, buds of seedlings from SD-MS treatments flushed 4.8 to 6.9 days after being placed under forcing conditions in March (Fig. 36). Duration in SD had a highly significant effect on days to bud break (Table 18). Buds from 4 WK SD-MS flushed significantly faster (4.8 days) than those of seedlings from other SD durations (5.4 to 6.9 days). Buds from 6 WK SD-MS flushed significantly slower (6.9 days) than buds from 3 and 5 WK SD-MS (5.7 and 5.4 days, respectively). After natural chilling, buds of seedlings from SD+MS treatments flushed 4.6 to 6.3 days after being placed under forcing conditions in March (Fig. 36). Buds from 4 WK SD+MS flushed significantly faster (4.6 days) than those from 3 WK SD+MS (5.7 days).

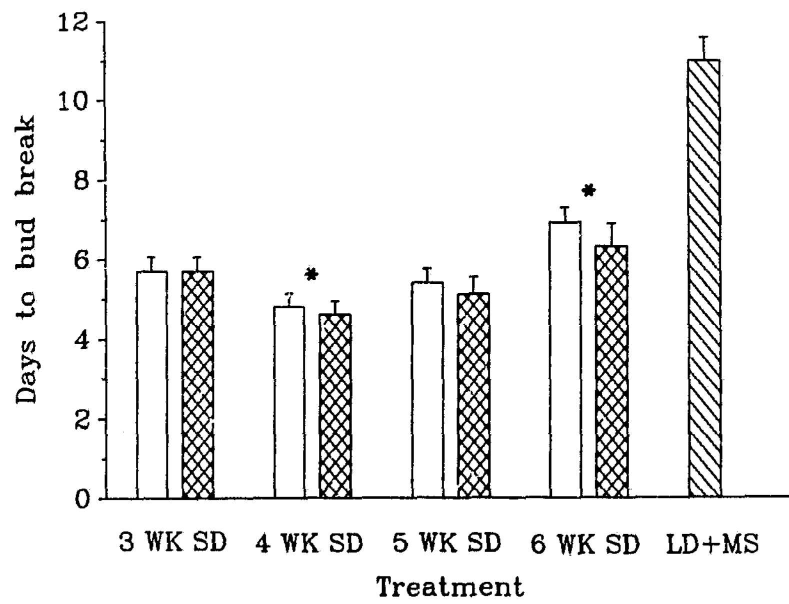
Table 18: Analysis of variance of dormancy induction treatment, location in greenhouse, and location in controlled environment chamber effects on days to bud break in Douglas-fir seedlings. **, significant at $p < 0.001$.

SOURCE	DF	MS	F	P
Moisture regime (MR)	1	2.76	0.90	0.3444
Duration in short day (DSD)	3	25.25	8.25	0.0001**
Location in greenhouse	3	10.11	3.30	0.0222
Location in controlled environment chamber	4	4.58	1.50	0.8864
MR x DSD	3	0.66	0.21	0.2062
Error	145	3.06		

Fig. 36. Days to bud break in March of terminal buds of overwintered Douglas-fir seedlings from different dormancy induction treatments. $n = 20$.

Abbreviations:	WK, weeks in; SD, short day; LD, long day
Unshaded bars:	no moisture stress
Shaded bars:	moisture stress

*, t-test for difference between means of SD treatments was significant at $p < 0.05$.



12
10
8
6
4
2
0
Days to bud break
*
*
3 WK SD
4 WK SD
5 WK SD
6 WK SD
LD+MS
Treatment

4.3.3 Root Collar Diameter and Root Dry Weight for the SD-MS and SD+MS Treatments

For SD treatments, shoot diameter at the root collar ranged from 3.1 to 3.5 mm (Table 19). Duration in SD had a highly significant effect on root collar diameter (Table 20). Root collar diameter of 3 and 4 WK SD-MS was significantly larger than that of 5 and 6 WK SD-MS, and root collar diameter of 3 and 4 WK SD+MS was significantly larger than that of 5 WK SD+MS.

Table 19: Shoot diameter at the root collar and root dry weight in early October of Douglas-fir seedlings given different dormancy induction treatments.

Treatments: WK SD-MS - weeks in short day without moisture stress
WK SD+MS - weeks in short day with moisture stress
LD+MS - long day with moisture stress

Diameters and weights (±SE) are means for 24 seedlings.

TREATMENT	ROOT COLLAR DIAMETER (mm)	ROOT DRY WEIGHT (g)
3 WK SD-MS	3.5 (.10)	0.73 (.04)
4 WK SD-MS	3.5 (.10)	0.73 (.03)
5 WK SD-MS	3.2 (.09)	0.60 (.04)
6 WK SD-MS	3.1 (.09)	0.55 (.04)
3 WK SD+MS	3.5 (.07)	0.64 (.03)
4 WK SD+MS	3.3 (.09)	0.59 (.04)
5 WK SD+MS	3.1 (.08)	0.62 (.03)
6 WK SD+MS	3.2 (.06)	0.70 (.03)
LD+MS	3.2 (.07)	0.62 (.03)

For SD treatments, root dry weight ranged from 0.55 to 0.73 g (Table 19). An ANOVA indicated a highly significant moisture regime x duration in SD interaction on root dry weight (Table 21). Root dry weight of 3 and 4 WK SD-MS was significantly greater than that of 5 and 6 WK SD-MS; whereas, root dry weight

of 6 WK SD+MS was significantly greater than that of 4 and 5 WK SD+MS. For 3 and 4 WK SD, root dry weight of the SD-MS treatment was significantly greater than that of the SD+MS treatment; whereas, for 6 WK SD, root dry weight of the SD-MS treatment was significantly less that of the SD+MS treatment.

Table 20: Analysis of variance of dormancy induction treatment and location in greenhouse effects on shoot diameter at the root collar in Douglas-fir seedlings.

**, significant at $p < 0.001$.

SOURCE	DF	MS	F	P
Moisture regime (MR)	1	0.0469	0.26	0.6088
Duration in short day (DSD)	3	1.3078	7.33	0.0001**
Location in greenhouse	3	0.2440	1.37	0.2541
MR x DSD	3	0.1712	0.96	0.4129
Error	181	0.1783		

Table 21: Analysis of variance of dormancy induction treatment and location in greenhouse effects on root dry weight in Douglas-fir seedlings.

**, significant at $p < 0.001$.

SOURCE	DF	MS	F	P
Moisture regime (MR)	1	0.0122	0.44	0.5084
Duration in short day (DSD)	3	0.0626	2.24	0.0850
Location in greenhouse	3	0.0240	0.86	0.4627
MR x DSD	3	0.1907	6.83	0.0002**
Error	181	0.0279		

4.3.4 Bud Development During and Following the LD+MS Treatment

4.3.4.1 Bud-Scale Initiation and Differentiation

Prior to the LD+MS treatment in study 1, no bud scales were visible with the unaided eye. However, upon dissection, it was evident that apices were undergoing bud-scale initiation (Fig. 37). From sectioned material, it was apparent that the transition from neoformed-leaf to bud-scale initiation was a recent one (Fig. 38). Under LD+MS, bud-scale initiation was completed after 3 weeks in 37.5% of seedlings and after 4 weeks in the remaining seedlings.

In study 2, prior to the LD+MS treatment, apices of all seedlings were initiating neoformed leaves. Within the first week in LD+MS in study 2, all apices ended neoformed-leaf initiation and began bud-scale initiation (Fig. 39). Bud-scale initiation was completed after 4 weeks. Despite the start of bud-scale initiation prior to the LD+MS treatment in study 1, the observation that duration of bud-scale initiation in study 2 was the same as that in study 1, validates the presentation of observations of subsequent stages of bud development observed in study 1 as typical of those following a LD+MS treatment.

Zonation of the apical meristem during bud-scale initiation was less distinct than that during subsequent preformed-leaf initiation; the apical zone was broad and deep, and the rib meristem was narrow and deep (Fig. 38). During week 1 in LD+MS, apical height and apical width decreased (Figs. 27B, 28B), while number of apical cells remained unchanged (Fig. 29B). Apical width remained constant during weeks 1-3, but increased in week 4 (Fig. 28B). Apical height and number of apical cells remained unchanged during bud-scale initiation (Figs. 27B, 29B). Mean number (±SE) of bud scales initiated for LD+MS was 33.4 (1.7).

Fig. 37-42.	Scanning electron and light micrographs of shoot tips of Douglas-fir seedlings during and following the long day with moisture stress (LD+MS) treatment.
Fig. 37.	Gold-coated shoot tip before the LD+MS treatment with neoformed leaves cut away to reveal the apical meristem (am) initiating bud scales (bs). x75.
Fig. 38.	Median longitudinal section of apical meristem initiating bud scales before the LD+MS treatment, showing broad, deep apical zone (az); narrow, deep rib meristem (rm); and narrow peripheral zone (pz). Recently-initiated neoformed leaves (nl) are expanding as internodes elongate. Note width of shoot, and number and sizes of pith (ph) and cortical (c) cells in the shoot below the apical meristem. x60.
Fig. 39.	Fresh shoot tip after one week of LD+MS with neoformed leaves cut away to reveal the apical meristem initiating bud scales. x75.
Fig. 40.	Median longitudinal section of bud after 4 weeks showing widening of the shoot below the apical meristem as the bud-scale receptacle (r, between arrows developed due to an increase in number and size of pith and cortical cells. Note the apical meristem surmounted a convex receptacle. x40.
Fig. 41.	Median longitudinal section through bud after 5 weeks. Elongation of cortical cells below bases of bud scales elevated the receptacle until it was flush with the base of the embryonic preformed shoot (eps). x35.
Fig. 42.	Fresh shoot tip after 4 weeks, surrounded by last-initiated bud scales, starting to initiate preformed leaves (pl). x75.

38
40
41
42

After 1 week in LD+MS, only rudimentary type 1 bud scales were visible. After 4 weeks, terminal buds were evident, types 1 and 2 bud scales were visible, and ergastic substances were deposited in abaxial cells of the apical regions in type 1 bud scales. After 5 weeks, all 3 bud scale types were visible and ergastic substances were deposited throughout the abaxial cells of all types.

In LD+MS, elongation of type 2 bud scales began during week 2 and continued for 5 weeks. Mean final height (±SE) of the longest type 2 bud scale was 2.3 (0.2) mm.

4.3.4.2 Bud-Scale-Receptacle Development

Prior to LD+MS, mean width (±SE) of the shoot below the apical meristem was 0.6 (0.02) mm. Shoot width remained unchanged during week 1 in LD+MS, but during week 2 it increased to 1.0 (0.05) mm as the bud-scale receptacle developed. Receptacle width remained unchanged during week 3 and increased slightly to 1.4 (0.08) mm during week 4. After 5 weeks, receptacle development was completed, and final receptacle width was 1.8 (0.11) mm.

During week 1 of LD+MS, there were no changes in number and size of pith and cortical cells in the shoot below the apical meristem (Table 13). During week 2, there was a great increase in number of cortical cells and a slight increase in number of pith cells (Table 13); size of cortical and pith cells increased markedly (Table 13). Pith cells were wider than cortical cells (24.8 μm and 17.4 μm, respectively). During weeks 3 and 5, there was a larger increase in number of pith cells than in number of cortical cells. Cell size increased modestly during week 4, but did not increase in week 5. After 4 weeks, pith cells were still wider than cortical cells (29.9 μm and 22.1 μm, respectively). These increases in number and size of cortical and pith cells accounted for most of the widening of the receptacle.

In addition to the proliferation of pith and cortical tissue, receptacle width under LD+MS increased slightly due to an increase in size of parenchymal cells in the reduced petioles of distal-most neoformed leaves and type 1 bud scales. For the LD+MS treatment, expanded petioles were first evident after 5 weeks.

During weeks 2-4 of the LD+MS treatment, the apical meristem surmounted a convex receptacle (Fig. 40). During week 5, cortical cells directly below the bases of bud scales elongated longitudinally elevating the receptacle tissue until the base of the embryonic preformed shoot became flush with the top of the receptacle (Fig. 41).

4.3.4.3 Preformed-Leaf Initiation

In the LD+MS treatment, preformed-leaf initiation began during week 3 in 37.5% of seedlings and during week 4 in the remaining seedlings (Fig. 42). Zonation of the apical meristem during preformed-leaf initiation under LD+MS was the same as that described earlier for SD treatments. Rate of preformed-leaf initiation was slow during weeks 3-6 (Fig. 26B). During this time, apical height remained unchanged from that during bud-scale initiation (Fig. 27B). Apical width increased between weeks 3-4, then remained constant until week 6 (Fig. 28B), and number of apical cells increased (Fig. 29B). Rate of preformed-leaf initiation was rapid during weeks 6-10 (Fig. 26B). During rapid preformed-leaf initiation, apical height remained unchanged (Fig. 27B), apical width increased markedly (Fig. 28B), and number of apical cells decreased (Fig. 29B). There was a slight decrease in rate of preformed-leaf initiation between weeks 10-13 (Fig. 26B). During this time apical height, apical width, and number of apical cells decreased (Figs. 27B, 28B, 29B). By week 13, preformed-leaf initiation was not completed (Fig. 26B, 43), nor were minimum apical height, apical width or number of apical cells reached (Fig.

27B, 28B, 29B, 43). Mean number of preformed leaves initiated was 159.4 (Table 14).

4.3.4.4 Mitotic Activity During Bud Development

Bud-scale initiation in the LD+MS treatment occurred until week 3 or 4. During week 1 of MS, MI decreased from its pre-treatment value of 2.1% (Fig. 32B). By week 3, one week after MS ended, MI had increased to a value of 2.2% (Fig. 32B). Mitotic index again decreased, coinciding with slow preformed-leaf initiation, during weeks 3-6 and then increased during rapid preformed-leaf initiation between weeks 6-10 (Fig. 26B, 32B). It decreased during weeks 10-13 as rate of preformed-leaf initiation slowed (Fig. 26B, 32B), but had not approached zero by week 13 (Fig. 32B).

4.3.4.5 Crown Differentiation

Crown differentiation in the LD+MS treatment was first apparent during week 5. A group of radially elongated cells was centrally located within the pith at the base of the embryonic preformed shoot (Fig. 44). These cells changed little during weeks 5-10. Between weeks 10-13, the crown region extended entirely across the pith and cell walls had thickened (Fig. 45). By week 13, cells with thickened walls became apparent within the vascular strands and cortex, and the crown extended through the cortex to the bases of the inner bud scales. Crown height and width were 141.0 μm and 774.7 μm, respectively (Table 15).

4.3.4.6 Preformed-Shoot-Axis Height and Width

Preformed-shoot-axis height and width by early October for the LD+MS treatment were 1101.3 μm and 664.0 μm, respectively (Table 16). Although preformed-leaf initiation was not completed in early October, it was slowing; any

Fig. 43-45.	Light micrographs of buds of Douglas-fir seedlings following the long day with moisture stress (LD+MS) treatment.
Fig. 43.	Preformed shoot (ps) after 13 weeks. Apical meristem (am) had not completed preformed-leaf (pl) initiation. Note that height of apical meristem was not minimal. x40.
Fig. 44-45.	Light micrographs of median longitudinal sections through the bases of preformed shoots (see preformed-leaf primordia (pl)) during crown differentiation following the LD+MS treatment.
Fig. 44.	A group of radially elongated cells (between arrows) centrally located within the pith (ph) after 5 weeks. x140.
Fig. 45.	Crown (cr) cells with thickened walls extended entirely across the pith after 13 weeks. x70.

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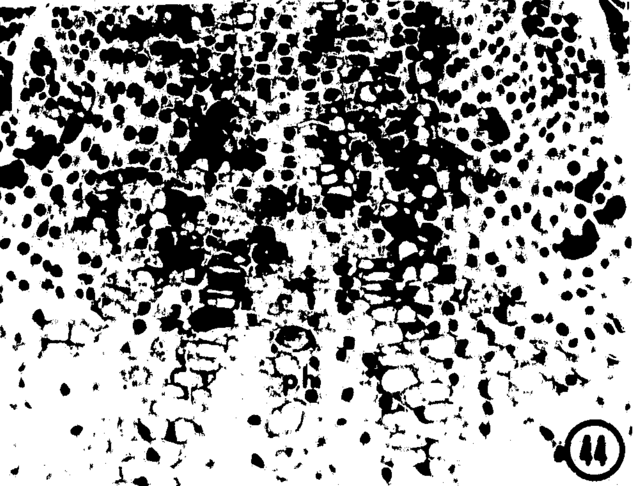
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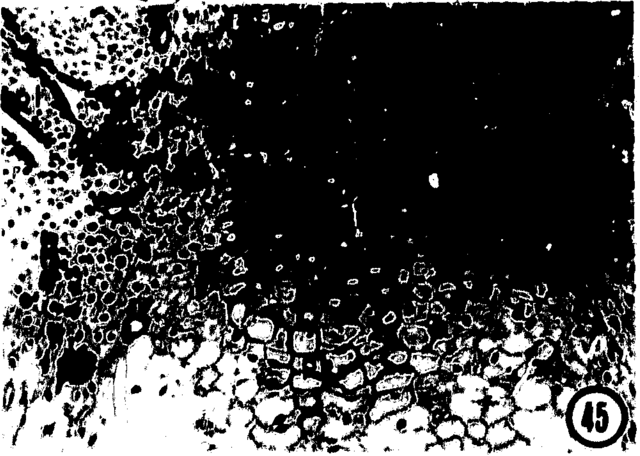
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subsequent increase in shoot-axis height was small.

4.3.5 Speed of Bud Break After the LD+MS Treatment

After natural chilling, buds of seedlings from the LD+MS treatment flushed 11.0 days after being placed under forcing conditions in March (Fig. 36).

4.3.6 Root Collar Diameter and Root Dry Weight for the LD+MS Treatment

Shoot diameter at the root collar and root dry weight were 3.2 mm and 0.62 g, respectively (Table 17).

4.3.7 Comparison of Bud Development Under the SD and LD+MS Treatments

Fully-formed buds of the SD and LD+MS treatments were similar in appearance; however, the number of bud scales in the BSC of these treatments was different. Mean number of bud scales initiated for SD-MS, SD+MS, and LD+MS was 20.2, 19.5, and 33.4, respectively; means for SD-MS and SD+MS were significantly different than the mean for LD+MS. Mean number of preformed leaves initiated under the SD treatments ranged from 134.9 to 175.3 (Table 14); under the LD+MS treatment, 159.4 preformed leaves were initiated (Table 14). Significantly more preformed leaves were initiated under 3 WK SD-MS than under LD+MS. Significantly fewer preformed leaves were initiated under 6 WK SD-MS and 6 WK SD+MS than under LD+MS.

Preformed-shoot-axis heights for 3, 4, 5, and 6 WK SD-MS and 4 WK SD+MS were significantly greater than preformed-shoot-axis height for LD+MS (Table 17). However, only preformed-shoot-axis width for 4 WK SD-MS was significantly greater than that for LD+MS.

4.3.8 Comparison of Speed of Bud Break After the SD and the LD+MS Treatments

After natural chilling, buds of seedlings from the SD treatments flushed 4.6 to 6.9 days after being placed under forcing conditions in March (Fig. 36); whereas, buds of seedlings from the LD+MS treatment flushed after 11.0 days (Fig. 36). Buds from the SD treatments flushed significantly faster than buds from the LD+MS treatment.

4.3.9 Comparison of Root Collar Diameter and Root Dry Weight for the SD and LD+MS Treatments

Shoot diameter at the root collar for the SD treatments ranged from 3.1 to 3.5 mm (Table 19); under the LD+MS treatment, shoot diameter at the root collar was 3.2 mm (Table 19). Root collar diameter of 3 WK SD-MS and 3 WK SD+MS was significantly larger than that of LD+MS; whereas, root collar diameter of LD+MS was significantly larger than that of 5 WK SD+MS. Root dry weight for the SD treatments ranged from 0.55 to 0.73 g (Table 19); under the LD+MS treatment, root dry weight was 0.62 g (Table 19). Root dry weights of 3 WK SD-MS, 4 WK SD-MS and 6 WK SD+MS were significantly larger than those of LD+MS.

4.4 Discussion

4.4.1 Bud Development During and Following the SD-MS and SD+MS Treatments

There was a uniform response to SD by seedlings in the present study; all apices ended neoformed-leaf initiation and began bud-scale initiation within the first week in SD. In contrast, Lavender *et al.* (1968) reported that "bud-set" in Douglas-fir seedlings was not strongly photoperiodic. In that study, rapid shoot elongation was just starting when seedlings were placed in SD; whereas, shoot

elongation was slowing in the present study. This may have reduced the response they obtained from SD. Uniform "bud-set" in *Picea abies* seedlings under SD was thought to indicate a lack of genetic variance in photoperiodic response (Dormling *et al.* 1968). Similarly, the present study indicates a fairly strong genetic control of bud-scale initiation in response to SD in Douglas-fir seedlings.

Within the first week in SD, apices of Douglas-fir seedlings began and completed bud-scale initiation, enlarged but initiated no or few new primordia (transitional phase), and then began rapid preformed-leaf initiation. In contrast, when *P. sitchensis* seedlings were placed in SD, apices began and completed bud-scale initiation during week 1, entered the transitional phase during week 2, and began rapid preformed-leaf initiation during week 3 (Cannell and Cahalan 1979). The rapid passage through these stages in Douglas-fir seedlings may result from the initial warm temperature of the nyctoperiod (immediately after the black-out curtains were closed), the high light intensity in the greenhouse during the photoperiod, and/or the optimal mineral nutrition of commercially cultured seedlings.

Significantly more preformed leaves were initiated under the 3 WK SD-MS treatment than under the 4, 5, or 6 WK SD-MS treatment. After removal from SD, apical widths for the 3 WK SD-MS treatment were significantly larger than those for the 4, 5, or 6 WK SD-MS treatment. This may have provided more sites for primordium initiation if primordium size was equal. The earlier return to natural LD for the 3 WK SD-MS treatment seedlings may have provided the photosynthates for increased apical growth. It has been suggested that buds (and new leaves) have the highest priority for carbon allocation within a tree (Waring and Pitman 1985). An additional cause of the difference in number of preformed leaves between the 3 and 6 WK SD-MS treatments is discussed below.

Significantly more preformed leaves were initiated under the 4 and 5 WK SD-MS treatments than under the 6 WK SD-MS treatment. This was not due to differences in apical size since after return to natural LD, apical widths for the 4 and 5 WK SD-MS treatments were not significantly different than those for the 6 WK SD-MS treatment. However, apices of the 6 WK SD-MS treatment seedlings ended preformed-leaf initiation earlier than apices of the 3, 4 and 5 WK SD-MS treatments seedlings, and thus initiated fewer preformed leaves. The earlier completion of preformed-leaf initiation may have been due to reduced photosynthesis because of the longer duration in SD.

Although not statistically significant, differences in MI in early October among the 3, 4, 5, and 6 WK SD-MS treatments appear to be biologically significant. Mitotic index in apices from the 4 and 5 WK SD-MS treatments was less than MI in apices from the 3 and 6 WK SD-MS treatments. Mitotic activity may have stopped earlier in apices from the 4 and 5 WK SD-MS treatments, and consequently, these apices may have become dormant earlier. This hypothesis was supported by results of the days to bud break test. Buds from the 4 WK SD-MS treatment flushed faster than buds from the 5 WK SD-MS treatment, and buds from both 4 and 5 WK SD-MS treatments flushed faster than buds from the 3 and 6 WK SD-MS treatments. This suggests that apices from the 4 WK SD-MS treatment entered dormancy earlier, satisfied the chilling requirement earlier, and responded earlier to natural environmental factors involved in bud break (Campbell and Sugano 1975). Buds from the 6 WK SD-MS treatment, flushed significantly slower than buds from the 3 and 5 WK SD-MS treatment, even though preformed-leaf initiation was completed earlier in the former. The reduction of photosynthesis caused by the longer duration in SD may have impacted on the physiology and/or biochemistry of dormancy development, dormancy breaking, and/or bud break.

4.4.2 Bud Development During and Following the LD+MS Treatment

Neoformed-leaf initiation ended and bud-scale initiation began within the first week of the LD+MS treatment in the present study. In contrast, in an earlier study of coastal Douglas-fir under LD conditions, 30% of seedlings continued to initiate neoformed leaves for several weeks after MS ended (Fielder and Owens 1989). The immediate, uniform response in the present study may reflect the sampling design in which only seedlings that exceeded a certain height were sampled; whereas, the variation in the earlier study may reflect completely random sampling. In nursery practice, it is recognized that all seedlings in a greenhouse do not receive MS uniformly (A. Maher, pers. comm.). Large seedlings develop plant moisture stress before small seedlings. Thus, by sampling only larger seedlings in the present study, there was a greater chance that these seedlings had received the MS cue.

One function of bud scales is to reduce water loss from the apical meristem (Wareing and Phillips 1978). In the present study, the rapid change from neoformed-leaf to bud-scale initiation in response to MS would accomplish this. Furthermore, it was not uncommon to find regions of scales interspersed with leaves along seedling shoots (unpublished observations); such scales may have been initiated in response to brief MS events during seedling culture. Lavender (1985) proposed that in areas with dry summers, seedlings begin quiescence (i.e. initiate bud scales) in response to MS because the moisture deficit is the annual environmental condition that precedes winter. He contrasted this pattern with that in areas with wet summers in which shortening photoperiod is the constant environmental cue that precedes winter (Wareing 1969). However, the rapid photoperiodic response of bud development demonstrated in the present study suggests that MS results in bud-scale initiation primarily as desiccation protection and shortening photoperiods further cues bud (and, hence dormancy) development.

Preformed-leaf initiation was initially slow in the LD+MS treatment in the present study. In contrast, in an earlier study of Douglas-fir seedlings induced by MS, preformed-leaf initiation was initially rapid (Fielder and Owens 1989). Initial rapid preformed-leaf initiation was also observed during bud development in mature Douglas-fir trees (Owens 1968). Owens *et al.* (1985) demonstrated a 2 to 4 week retardation of the change from bud-scale to preformed-leaf initiation in buds on shoots of mature trees with low shoot water potentials. Seedlings in the present study may have received greater MS than seedlings in the earlier study (Fielder and Owens 1989), and thus had a longer physiological recovery period during which preformed-leaf initiation by the apex was slow.

4.4.3 Bud Development During and Following the SD and the LD+MS Treatments

Bud development in first-year seedlings involves the initiation and differentiation of a bud-scale complex, and of a preformed shoot within that complex. The present study demonstrates that the sequence of developmental stages is constant, but the phenology and characteristics of the stages vary with type of dormancy induction treatment (Fig. 46). The SD and LD+MS treatments caused an immediate change from neoformed-leaf to bud-scale initiation (Fig. 46). However, rate of bud-scale initiation was faster, duration of bud-scale initiation was shorter, and fewer bud scales were initiated under the SD treatments than under the LD+MS treatment. Preformed-leaf initiation was initially rapid and then slow for the SD treatments; whereas, it was initially slow, then rapid and finally slow for the LD+MS treatment (Fig. 46). Rate of preformed-leaf initiation was faster and duration of preformed-leaf initiation was shorter for the SD treatments than for the LD+MS treatment. However, apices from the SD treatments initiated the same number of preformed leaves as apices from the LD+MS treatment, with the

Fig. 46. Comparison of stages of apical activity/dormancy and of days to bud break in Douglas-fir seedlings after the short day (SD) and long day with moisture stress (LD+MS) treatments.

Period	Moisture stress under long day	Short day
MID-APRIL TO EARLY JULY	NEOFORMED-LEAF INITIATION	
EARLY JULY	DORMANCY INDUCTION TREATMENTS BEGAN	
	MOISTURE STRESS UNDER LONG DAY	SHORT DAY
NUMBER OF WEEKS SINCE INDUCTION TREATMENT BEGAN IN EARLY JULY (1–13)	BUD-SCALE INITIATION	BUD-SCALE INITIATION
	PREFORMED-LEAF INITIATION: SLOW	PREFORMED-LEAF INITIATION: RAPID
	PREFORMED-LEAF INITIATION: RAPID	PREFORMED-LEAF INITIATION: SLOW
DURATION OF EVENTS NOT DETERMINED	PREFORMED-LEAF INITIATION: SLOW	MITOTIC ACTIVITY IN APICAL MERISTEM
	MITOTIC ACTIVITY IN APICAL MERISTEM	DORMANCY
	DORMANCY	
MID-MARCH	SEEDLINGS PLACED UNDER CONDITIONS TO FORCE BUD BREAK AFTER NATURAL CHILLING	
DAYS TO BUD BREAK (2–12)	BUD BREAK	BUD BREAK

exception of apices from the longest duration in SD.

Rudimentary terminal buds were visible after 3 weeks in the SD treatments and after 4 weeks in the LD+MS treatment. Despite the same external appearance of these rudimentary buds, the stages of bud development were different. For the SD treatments, bud-scale initiation was completed 2 weeks earlier and preformed-leaf primordia were being rapidly initiated; whereas, bud-scale initiation was completed and preformed-leaf initiation was just starting for the LD+MS treatment (Fig. 46).

In nurseries using LD+MS as a dormancy induction treatment, lammas growth often occurs several weeks after MS ended and fertilization has resumed (A. Maher, pers. comm.). It appears there is a point after which bud development cannot be interrupted given sufficient mineral nutrition. Under a LD+MS treatment, bud development on some seedlings is slow enough that this developmental point is not reached when fertilization resumes.

In Douglas-fir trees, low mitotic frequencies in apices of shoots with low shoot water potentials have been reported (Owens *et al.* 1985). Similarly, in the present study, MI decreased dramatically during the first week of the LD+MS treatment. In contrast, MI increased during week 1 of the SD+MS treatment. Van den Driessche (1989) demonstrated a decrease in osmotic potential at full turgor of Douglas-fir seedlings placed in SD. If MS levels were similar under LD+MS and SD+MS in the present study, SD may have resulted in an osmotic adjustment. Seedlings under the SD+MS treatment may have experienced some degree of MS in week 1 to account for smaller apical height and width, slower differentiation of bud scales, and smaller pith and cortical cells in the bud-scale receptacle as compared to seedlings under the SD-MS treatment. Diurnal MI under the SD+MS treatment, not determined in the present study, may have shown a decrease in MI

before osmotic adjustment occurred.

During preformed-leaf initiation, MI for the SD treatments and the LD+MS treatment followed the same trends. High MI corresponded with rapid rates of preformed-leaf initiation, while decreasing MI coincided with the slowing and completion of preformed-leaf initiation. The pattern was similar to those reported earlier for Douglas-fir seedlings (Carlson *et al.* 1980, Fielder and Owens 1989).

Apical meristems from the SD treatments entered dormancy - delineated by MI - earlier than apices from the LD+MS treatment (Fig. 46). Consequently, after over-wintering, buds from the SD treatment flushed more rapidly under forcing conditions than did buds from LD+MS (Fig. 46). Similarly, in *P. mariana* (Mill.) B.S.P. seedlings, buds from the SD treatment flushed more rapidly than did those from the LD treatment (Columbo 1986). In the present study, the difference in timing of dormancy development was related to the speed of bud development. Preformed-leaf initiation was completed and MI approached zero in the SD-treated seedlings at the time that preformed-leaf initiation began slowing and MI began decreasing for the LD+MS treatment.

In the present study, individual preformed-leaf primordia for the SD+MS and LD+MS treatments occupie less preformed-shoot-axis tissue than primordia from the SD-MS treatment. In *P. sitchensis* seedlings, Cannell (1978) demonstrated that preformed-leaf primordia were smaller when apical dome area was reduced. During preformed-leaf initiation in the present study, apical heights of the SD+MS and LD+MS treatments were smaller than those of the SD-MS treatment. Thus, for the SD+MS and LD+MS treatments, less "vertical" apical tissue was available to give rise to each primordium.

The crown formed a barrier of thick-walled cells separating the preformed-shoot from the subtending shoot; it was primarily a tissue within the pith region,

with extensions through the cortex to the bases of the inner bud scales. In the present study of Douglas-fir seedlings, cavity formation within the pith below the crown was not observed, but it was observed in earlier studies (Fielder and Owens 1989, Krasowski and Owens 1989). Because the pith portion differentiated before the cortical portion for the SD and LD+MS treatments, the primary function of the crown may relate to the pith barrier. This supports an earlier suggestion that the crown functions in freezing avoidance of the preformed shoot (Sakai 1979, 1982). The large apoplast of the crown permits rapid water movement from the preformed shoot to the cavity. Instead of ice forming in the preformed shoot, ice forms in the cavity below the crown.

Although completion of crown differentiation in the present study coincided with completion of shoot preformation and hence subsequent apical meristem dormancy, it is not clear that the crown is part of dormancy development. Crown differentiation may be a cold-hardiness mechanism, and like other cold-hardiness mechanisms, may be cued by shortening photoperiods. There is support for this hypothesis. In the present study, crown differentiation started in late July for the SD treatments and in mid-August for the LD+MS treatment. In high elevation coastal Douglas-fir seedlings, crown differentiation began earlier in September than in low elevation seedlings (Krasowski and Owens 1989). Likewise, crown differentiation began in early September in interior Douglas-fir and in late September in coastal Douglas-fir (Fielder and Owens 1989). These SD-LD, elevational and varietal differences in starting dates of crown differentiation suggest a genetic response to photoperiod that ensures crown differentiation prior to the first freezing event.

4.4.4 Root Collar Diameter and Root Dry Weight

Despite the reduction in photosynthesis due to SD treatments in the present

study, root collar diameter and root dry weight exceeded minimum grading standards. This may be explained by the timing of the SD induction. Because treatments were finished by mid-August, seedlings were returned to natural LD when rates of photosynthesis may have been maximum (Bourdeau 1959, McGregor and Kramer 1963), and maximum dry-matter production may have compensated for earlier losses. Furthermore, Waring and Pitman (1985) suggested that buds have a higher priority for photosynthate allocation than wood production. Thus, once rapid preformed-leaf initiation was completed within 6 weeks under the SD treatments, there may have been a shift of photosynthates away from buds towards shoots and roots.

In the present study, reduction of photosynthesis under the longer durations in SD apparently caused a decrease in dry-matter production. Root collar diameter and root dry weight were significantly larger for the shorter durations than for the longer durations. Similarly, reduced photosynthesis, due to decreased light intensity, affected dry-matter production in *Larix occidentalis* Nutt. seedlings; root collar diameter and root dry weight under high light intensity were significantly larger than those under lower light intensities (Vance and Running 1985).

There was no significant difference in root collar diameter between the SD-MS and SD+MS treatments in the present study. Root dry weight of the 3 and 4 WK SD+MS treatment was significantly smaller than that of the 3 and 4 WK SD-MS treatment. Similarly, in *L. occidentalis* under high light intensity, moisture stress reduced root dry weight (Vance and Running 1985). In Douglas-fir under LD, root dry weight also decreased with increasing MS (Carlson 1978). This suggests that MS affected photosynthate allocation to roots more than to shoots in the present study, and agrees with an earlier observation in *L. occidentalis* that reduction of light intensity affected photosynthate allocation to roots more than to shoots (Vance and

Running 1985).

4.4.5 Dormancy Development as a Growth Process

Dormancy induction is commonly viewed as an end-point - shoot growth ceases and buds become visible. The present study demonstrates that dormancy induction and development are a growth process, the duration of which is dependent upon type of induction treatment. Until dormancy development is recognized as an active process, the dormancy status of seedlings will continue to be misinterpreted.

Chapter 5

SUMMARY AND CONCLUSIONS

Both studies clearly demonstrated that dormancy induction and dormancy development are ongoing developmental processes and not developmental end-points. The major summary points are as follows:

1. Bud development involves the initiation and differentiation of a bud-scale complex, and of a preformed-shoot within that complex.

2. The sequence of stages of bud development is constant, but the phenology and characteristics of the stages and final bud anatomy vary with type of dormancy induction treatment.

3. Speed of bud break after over-wintering is related to the timing of bud development and dormancy development as delimited by mitotic activity.

The study under controlled environment conditions demonstrated that:

1. An abrupt change from natural photoperiod to SD (ASD) resulted in faster transition from neoformed-leaf to bud-scale initiation, faster completion of bud-scale initiation, and faster passage through the transitional phase to preformed-leaf initiation than did acceleration of the natural shortening of the photoperiod (GSD). Fewer bud scales, but more preformed leaves were initiated under ASD than under GSD.

2. Drought hastened the start and completion of bud-scale initiation, but delayed passage through the transitional phase and hence start of preformed-leaf initiation relative to ND conditions. More bud scales, but fewer preformed-leaf primordia were initiated under D than under ND.

The study under commercial greenhouse conditions demonstrated that:

1. Both the SD and LD+MS treatments were effective in immediately cueing the change from neoformed-leaf to bud-scale initiation. The uniform response under the LD+MS treatment was apparently due to sampling only larger seedlings; they developed plant moisture stress earlier than smaller seedlings. Under the SD treatments, bud-scale initiation was completed within the first week; whereas, under the LD+MS treatment, bud-scale initiation continued for 3-4 weeks.

2. Relative to the SD-MS treatment, SD+MS delayed differentiation of bud-scale types and deposition of ergastic substances within the bud scales. However, rate of preformed-leaf initiation and number of preformed leaves initiated were not significantly different between the SD-MS and SD+MS treatments. The pattern of mitotic index (MI) during bud development for the SD+MS treatment suggested that there was a recovery period after the 2-week MS. These observations together with the reduction of root dry weight for the SD+MS treatment, the possibility of severe rather than moderate MS, and the time and anxiety required to monitor/apply MS suggest that the use of SD+MS as a dormancy induction treatment is not needed and indeed is somewhat detrimental.

3. A comparison of MI between the SD+MS and LD+MS treatments indicated that the effect of MS under SD was not as great as that under LD. This suggested that SD resulted in osmotic adjustment.

4. Duration in SD-MS affected rate of preformed-leaf initiation, MI, days to bud break, shoot diameter at the root collar, and root dry weight. Of the durations investigated, the 3 and 4 WK SD-MS treatments had the greatest root collar diameters and root dry weights. Mitotic index and the speed of bud break test indicated that the 4 WK-SD treatment developed dormancy earlier than the 3 WK-SD treatment. This suggests the use of a 4-week duration in SD for early dormancy induction.

5. The advantages of the SD treatments over the LD+MS treatment for early induction of dormancy was clearly demonstrated by the phenology of bud development, MI and speed of bud break test. Furthermore, dry matter production for the shorter durations in SD was greater than that for the LD+MS treatment.

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