Anatomical and respiratory changes in shoot tips of interior and coastal Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco.) seedlings during bud development

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ABSTRACT

Bud development, embryonic shoot respiration and root elongation of coastal and interior seedlots of Douglas fir *(Pseudotsuga menziesii* (Mirb.) Franco.) were compared from September through March after four months growth under nursery production conditions. Apical development was described in terms of morphology, mitotic index, and cell numbers. Embryonic shoot development was described in terms of number of leaf primordia, and cell divisions in leaf primordia. Respiratory rates of shoot tips were measured during primary leaf initiation, bud-scale initiation, preformed leaf initiation and dormancy.

Morphological and anatomical stages of bud development in the interior variety occurred about four weeks in advance of the coastal variety. Respiratory rates of developing embryonic shoots decreased rapidly during preformed leaf initiation in both varieties. The interior variety decreased more rapidly, reflecting the earlier morphological development, and reached a basal rate in November, about four weeks in advance of the coastal variety. The lowest levels of respiration in the embryonic shoot coincided with the end of cell divisions in the apex. Cell divisions resumed in mid-February in both varieties. Cells in all apices were dividing by March 1. Flushing was variable but occurred more rapidly in the interior variety. Respiration rates increased in early-March during shoot elongation in both varieties but the interior variety had lower respiratory rates than the coastal variety.

Root elongation showed two peaks of activity, one in the fall and one in the spring, and decreased to a minimum in December in both varieties. The interior variety ceased root elongation completely whereas, the coastal variety continued at a reduced level during the winter. On March 1 root elongation in the interior variety increased rapidly coinciding with the resumption of rapid cell division in the shoot apex. The relationship was less marked in the coastal variety.

The relationship between these findings and current definitions of dormancy are discussed.

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Chapter I

INTRODUCTION

Douglas fir *(Pseudotsuga menziesii* (Mirb.) Franco) occupies a large proportion of the most productive forest lands of Western North America. Its distribution is extensive with a north south range of 4800 Km from British Columbia (55°N) throughout the Pacific Northwest and along the Rocky Mountains well into Mexico (19°N) (Silen 1978). Douglas fir exists as two varieties (Little 1953), var. *glauca* which is distributed throughout the Rocky Mountain area and var. *menziesii* which grows on the islands and coastal regions. Despite this botanical distinction most traits display a clinal variation along its range (Schober 1963).

Heavy logging of this resource requires a commitment to continuous reforestation. In 1985, 16 million (9 million coastal and 7 million interior) Douglas fir seed was sown in containers in British Columbia. The total sowing in all age classes and stock types of Douglas fir in British Columbia in 1986 will be 40 million (Pelchat 1985 pers. comm.). With such large numbers of seedlings the financial burden of losses due to seedling mortality will become considerable. The causes of mortality are many, however, those due to the poor physiological conditioning of nursery grown crops may be minimised if the appropriate cultural practices are applied at the correct time (Duryea 1985). Many seed sources are collected of each variety, and since the effects of environmental cues on development differ according to ecotype (Heide 1974), each variety may have unique cultural requirements for maximum survival at the desired time and location of planting. These exact requirements are not always known, consequently prediction of the safe handling period, for lifting, storage and planting date may sometimes result in poorly conditioned seedlings.

During development seedlings undergo anatomical and morphological changes due to activity of their apical and subapical meristems. These changes are also reflected in changes in seedling physiology. Many workers have indicated that treatments which adversely effect bud development in conifers will be detrimental to shoot growth and subsequent yield (van den Berg and Lanner 1971; Colombo 1982, Lavender and Stafford 1985). Thus, detailed information is required of both anatomical and physiological characteristics of conifer ecotypes for the best reforestation success (Duryea 1985). Despite the importance of this information few workers (Carlson *et al.* 1980; Macey 1982; Simpson and Macey 1985 have attempted this approach.

This study was undertaken to investigate bud development of an interior and a coastal seed source of Douglas fir seedling nursery production stock. By following anatomical stages, in conjunction with respiratory rates of embryonic shoots, it was possible to make deductions concerning the relationship between ontogeny of the embryonic shoot and its energy requirements. Respiration rates indicate the capacity (Pollock 1953; Bachelard and Wightman 1973) of buds to provide energy for all processes involved in growth (Beevers 1973; Lambers *et al.* 1981). The concept of dormancy and past categorization of the classical dormant period are related to findings of anatomical and respiratory changes during bud development. Both bud anatomy (Bachelard 1980) and respiratory rates (Pollock 1953) were considered of primary importance for the elucidation of the problem of dormancy.

Chapter II

LITERATURE REVIEW

2.1 Section 1: The control of bud development

Two ecotypes of the same species may respond differently to the same set of environmental conditions because environmental factors interact with the plant's genetic predisposition in the control of bud development. These differences in bud development are important to growers wishing to grow a number of ecotypes in the same nursery and assure proper physiological conditioning. The literature presented will be limited to control of bud development imposed by the environment and the differences in response due to ecotypic differences between seed sources. More general reviews of control are by Samish (1954), Wareing (1956), Romberger (1963), Vegis (1966), Noodén and Weber (1978), Bachelard (1980), and Lavender (1981).

2.1.1 Environmental control

Daylength and temperature are important in the control of the dormant period (Wommack 1964; Nienstaedt 1966b; Lavender *et al.* 1968; Campbell and Sugano 1975; van den Driessche 1975; Erez and Lavee 1977; Owens *et al.* 1977; Pollard and Logan 1977; Nelson and Lavender 1979).

In seedlings long photoperiods prolonged the free growth phase in *Picea mariana* (Mill.) BST. (black spruce) (Pollard and Logan 1977) and Douglas fir

(Lavender and Overton 1972). Short photoperiods promoted bud set of Douglas fir (Lavender *et al.* 1968; Lavender and Overton 1972), induction of bud-scale and leaf primordial initiation in *P. glauca.* (Moench). Voss. (white spruce) (Macey 1982) and black spruce (Pollard and Logan 1977) and higher rates of foliar initiation in *P. sitchensis* (Bong.) Carr. (Sitka spruce) (Cannell and Cahalan 1979).

Pollard and Logan (1977) found that once preformed leaf initiation had begun photoperiod was unimportant in subsequent development but that temperature had the main effect. Temperature was found to have its effect on rate of primordia production (Macey 1982). Other reports on the effects of temperature on winter bud development are conflicting. Whereas, low night air or soil temperatures (6-10°C) hastened bud development under 18 hour days in Douglas fir (Lavender *et al.* 1968; Lavender and Overton 1972), Brix (1971) demonstrated no such effect under 16 hour days at constant low temperatures (8°C).

Moisture, nutrient status and light intensity were found to play a role in the onset of bud formation in seedlings (Lavender *et al.* 1968; van den driessche 1969a, 1978; Lavender and Hermann 1970; Cheung 1973; Drew and Ferrell 1977; McCreary *et al.* 1978; Pollard and Logan 1979; Blake *et al.* 1979), but only before the onset of preformed leaf initiation (Pollard and Logan 1977).

The interaction between photoperiod and temperature during chilling has been complicated by the use of a number of chilling temperatures between (0-5°C) which were thought to be equally effective (Lavender 1981). Wommack (1964) found temperatures between 4 and 6°C to be optimum for chilling for Douglas fir seedlings. Approximately 2000 hours at 4.4°C resulted in terminal bud flushing of Douglas fir coastal and interior varieties (Van den Driessche 1975). Campbell

(1979a) showed that photoperiod had its greatest effect in substituting for chilling early in the chilling process, but its effect became insignificant near its fulfillment. Mild temperatures (20°C) prior to chilling under short days (9hrs) was found to enhance the effectiveness of chilling temperatures of coastal Douglas fir seedlings (Lavender and Stafford 1985). However, warm periods during chilling were found to negate the accumulated chilling hours (Erez and Lavee 1971; Van den Driessche 1975). Once chilling had been fulfilled the rate of flushing of Douglas fir seedlings was found to be dependent on soil temperatures (Lavender *et al.* 1973).

Many mature trees begin bud formation when photoperiods are at a maximum, or even irrespective of photoperiod (Perry 1971; Noodén and Weber 1978). However, short photoperiods are considered to promote winter bud development (Heide 1974; Campbell and Sugano 1975). Presumably the control over bud development in mature trees has a stronger endogenous component (Lavender 1981) than in seedlings.

2.1.2 Seed source variation

Genetic variation both between and within populations is great in coastal (Campbell 1979b; Christophe and Birot 1979) and interior Douglas fir (Wright *et al.* 1971; Rehfeldt 1983a). However, populations of coastal and interior varieties are readily differentiated according to their phenology, growth potential and cold hardiness (Rehfeldt 1983a). Griffin and Ching (1977) found these characters varied, with the exception of bud burst, between 18 wind pollinated families of Douglas fir. The most significant contrast was between those from coastal and interior ranges.

Coastal seedlings showed greater epicotyl growth, grew for a longer period before setting bud, showed less capacity to set buds in response to moisture stress and were less cold hardy (Griffin and Ching 1977). The ability of coastal sources to maintain sustained growth in competition for light and space was also confirmed by Irgens-Moller (1968). The trait of early bud break was not selected for in coastal sources (Irgens-Moller 1967, 1968) except in those from high elevations (Ritchie 1984).

Interior Douglas fir sources have been shown to possess rapid responses to geographic, physiographic and climatic variables. Low summer precipitation (Hermann and Lavender 1968; Rehfeldt 1978b; Griffin and Ching 1977; Irgens-Moller 1968; White *et al.* 1979; Sorensen 1983) and a short frost free season (Irgens-Moller 1967, 1968; Griffin and Ching 1977; Emmingham 1977; Sorensen 1983) were correlated with early bud set, low top to root ratios, and reduced height growth compared with the coastal seed sources. Interior sources of Douglas fir were slower growing than coastal sources (Irgens-Moller 1967; Sorensen 1983) but had greater cold hardiness (Munger and Morris 1936; Wright *et al.* 1971; Hattemer and Koning 1975; van den Driessche 1977; Rehfeldt 1983a). Early bud flushing tended to occur in interior sources from areas of low July precipitation (Campbell 1974; White *et al.* 1979), although it has been reported that the variation in intravarietal flushing time is so great that intervarietal differences become insignificant (Campbell and Sugano 1975; Campbell 1979a).

2.2 Section 2: Shoot, root and bud development

Plant growth and development is a correlated pattern of many interrelated growth processes of root and shoot. Meristematic regions initiate and differentiate various tissues and organs, and these processes occur by the coordinated action of cell division, elongation and differentiation. Thus, anatomical observations are important to any study of plant growth and development. Gross morphological characteristics of bud phenology may ignore changes in the rates and direction of cell division and cell elongation in meristematic regions. The literature presented here concerns studies of phenological development of apices of seedlings and mature conifers, and a general account of shoot and root development.

2.2.1 Modes of shoot growth

Northern temperate conifers are capable of exploiting two modes of shoot growth. These have been referred to as fixed, episodic (Lanner 1976; Lavender 1981) or proleptic growth (Tomlinson and Gill 1973), and free (Jablanzcy 1971; Pollard and Logan 1976; Lanner 1976) or sylleptic growth (Tomlinson and Gill 1973).

Proleptic growth occurs by extension of the preformed leaf and stem units of the primordial shoot and by definition is preceded by a period of dormancy (Tomlinson and Gill 1973). This type of growth occurs in most temperate Pinaceae. Sylleptic growth occurs when new stem units are initiated and extended simultaneously (Jablanzcy 1971; Tomlinson and Gill 1973). It is the mode of growth in seedlings from germination to bud set (Pollard and Logan 1976).

2.2.2 Bud development

In the Abietoideae, a vegetative bud consists of an embryonic shoot enclosed by bud scales. The embryonic shoot consists of an unelongated axis, preformed leaf primordia and an apical meristem (apex), which is that portion of the embryonic shoot above the youngest primordium (Parke 1959). The shape and volume of the apex and cytohistological zones change during the annual growth cycle. These changes have been used in phenological and anatomical studies (Cecich and Miksche 1970; Owens and Molder 1973 a,b; 1977, 1979) and in detailed analyses of apical growth and organ formation (Cannell 1976; Cannell 1978; Kremer 1984).

The annual cycle of bud development in mature trees in the Abietoideae has been divided into bud-scale initiation, leaf initiation, and dormancy (no cell divisions or new primordia initiated) (Sterling 1946; Parke 1959; Owens and Molder 1973a, b, 1979). The annual cycle of bud development in seedlings was briefly described by Allen (1947).

> "The seedling apex, beginning as an undifferentiated mass of cells, during the growing season produces foliage leaf primordia simultaneously with slow elongation, forms cataphylls, and finally lays down a second compliment of foliage leaf primordia in the winter bud. This cycle of events was not produced in mature plants which produced only one set of foliage leaf primordia in one season"

Germinants of white spruce, *Picea rubens* Sarg. (red spruce), black spruce, *P. abies* Ch. Karst.(Norway spruce), and *Abies balsamea* Ch. Mill. (Balsam fir), grown in the Maritime Provenances, had similar bud development (Jablanczy 1971). These species underwent primary leaf initiation until August, then bud-scale initiation until the beginning of September, followed by leaf initiation. The time of height growth cessation was not stated, but Cannell and Cahalan (1978) showed that height growth continued during bud-scale initiation in Sitka spruce seedlings.

Gregory and Romberger (1972) and Romberger and Gregory (1977) followed changes in apical diameter and plastochron duration in Norway spruce for 180 days after germination. Apical diameter increased gradually to a maximum after 140 days and plastochron duration decreased from 18.5 to 5.7 hrs. Kremer (1984) reported similar findings in jack pine germinants. Carlson *et al.* (1980) found that apical mitotic indices of Douglas fir seedlings increased during early bud formation. Cannell and Cahalan (1979) showed that, in Sitka spruce, shorter photoperiods (10hr) induced greater numbers of foliar units, greater apical dome diameters, and an earlier cessation of height growth than long (17hr) photoperiods. Cannell (1978) demonstrated the relationship between primordial size, rates of initiation and the amount of apical tissue in this species.

2.2.2.1 Bud-scale initiation

Mature trees: In the spring the leaf primondia of Douglas fir become mitotically active before shoot elongation begins (Owens 1968; Owens *et al.* 1985). Apical activity resumes in the peripheral zone (PZ), then in the rib meristem (RM), and lastly in the apical initial zone (AI) (Owens 1968; Owens and Molder 1973a). The apex divides slowly at first and then bud scales are initiated, elongate and enclose the apex. During bud-scale initiation shoot elongation occurs, first from division of cells present in the dormant bud followed by cell elongation after flushing (Owens *et al.* 1985). Apical meristematic activity is generally low during early shoot elongation (Owens and Molder 1973b, 1977). The rate of bud-scale initiation increases as the rate of shoot elongation increases (Owens and Molder 1973b; Owens *et al.* 1985). Shoot elongation ceases at the end of bud-scale initiation (Owens *et al.* 1977; Bachelard 1980; Owens and Singh 1982; Owens 1984) or during early leaf intiation (Owens and Molder 1973b, 1979).

Seedlings: There is little literature dealing directly with bud-scale initiation after germination in seedlings. Allen (1947) gave a detailed description of apical development of the embryo during germination and briefly described apical development until the formation of the second set of leaf primordia.

2.2.2.2 Initiation of preformed leaves

The point at which bud-scale initiation ceases and leaf initiation begins has often been referred to as bud set in seedlings (Wareing 1954; Hermann 1967; Lavender *et al.* 1968; Lavender *et al.* 1969; Cheung 1973; D'Aoust 1981) and in mature trees (Dormling 1973; Ekberg 1979). Perry (1971) referred to this period as winter-bud formation. The process of initiation of preformed leaves is similar in mature trees and seedlings.

Mature trees: The shift from bud-scale to leaf initiation commenced by early-July in mature coastal Douglas fir (Owens and Molder 1973a) and coincided with the cessation of shoot elongation and an increase in apical growth. Cytohistological zonation became more distinct during early leaf initiation in Douglas fir (Owens and Molder 1973a), and other conifers (Cecich and Miksche 1970; Owens and Molder 1975, 1976; Kremer 1984).

Initiation of preformed leaves has been described in many conifers and is characterised by early rapid rates which gradually diminish as bud formation nears completion (Parke 1959; Owens 1968; Pollard 1973, 1974; Owens and Singh 1982; Owens and Molder 1973a, b, 1975, 1976, 1977; Owens 1984). Owens and Molder (1973a) found that the early stage of leaf initiation produced two thirds of the final number within six weeks, this was followed by a 25 percent reduction in apical elongation and mitotic frequency in all zones. Preformed leaves were initiated until mid-November, by which time the apex was reduced to a small dome, distinct zonation disappeared, and mitotic frequency had declined by 80 percent.

Seedlings: Carlson *et al.* (1980) followed mitotic index throughout preformed leaf initiation and reported a general decline in mitotic index between September and November in coastal Douglas fir but bud development was not studied in detail.

In *Picea* and *Abies* germinants, initiation of preformed leaves began as late as September and continued into November (Jablanczy 1971). Gregory and Romberger (1972), and Romberger and Gregory (1977) spruce, and Cannell and Cahalan (1979), indicated that apical-dome sizes in spruce had reached their maximum by the commencement of leaf initiation and leaf primordia were produced at the expense of apical-dome tissues. Shoot elongation in Sitka spruce ceased at the beginning of preformed leaf initiation (Cannell and Cahalan 1979) Preformed leaf initiation ceased in Sitka spruce about 60 days after the beginning of 10 hour inductive photoperiods (Cannell and Cahalan 1979).

2.2.2.3 Dormancy

Mature trees: There is general agreement in anatomical studies that the dormant period is distinguished by a lack of cell divisions in the apical meristem. The dormant bud of Douglas fir consists of numerous bud scales enclosing the embryonic shoot, which possesses all the leaf primordia for the following season. The apex is a low dome and apical volume is at its smallest. No mitoses occur in the apex during this period (Owens and Molder 1973a). These findings confirmed those of Sterling (1947) for Douglas fir and Parke (1959) for *A. concolor* Lindl. & Gord. (white fir).

Cytohistological zonation was present but not clear in dormant Douglas fir apices (Owens and Molder 1973a), and in other conifers (Owston 1969; Owens and Molder 1975, 1976). Cecich (1977) suggested that the lack of distinct zonation during this stage may be related to the apex assuming a storage function and becoming engorged with lipid material.

Seedlings: Development of the apex of seedlings of Douglas fir after the cessation of primary-leaf initiation is similar to that of mature trees (Allen 1947; Jablanczy 1971).

2.2.3 Root development

The organization of conifer root apicies has been reviewed by Romberger (1963); Torrey and Feldman (1977) and Sutton (1980). The anatomical and morphological development of roots has been described for Douglas fir (Bogar and Smith 1965). The root system is heterorhizic, and adventitious roots may also be produced (Bogar and Smith 1965). Root growth in Douglas fir throughout the annual cycle and its relationship to the development of the shoot was reported by Kreuger and Trappe (1967). Root growth in general has been reviewed by Riedacker (1976) and Sutton (1980).

Following germination the primary root develops into a complex branched structure consisting of long laterals (elongating roots) and short laterals (absorbing, feeding or nutritional roots) (Sutton 1980). Long lateral roots are sparsely branched and relatively fast growing. Short lateral roots are initiated close together, often in pairs, on opposite sides of the parent root and become less than 5mm long (Bogar and Smith 1965). Their apical meristems are round and have a slow rate of cell division.

Roots may have several endogenously controlled growth periods during the annual cycle (Sutton 1980). Each period of growth may last 2 to 4 weeks (Riedacker 1979). The growth pause is accompanied by the formation of a dark brown suberized root tip (Sutton 1980). Periodicity may be controlled by moisture stress (Kreuger and Trappe 1967), temperature, photoperiod (Stahel 1972), and nutrient distribution between the shoot and root (Drew 1982).

2.3 Section 3: Respiration and Metabolism

Respiration and its contribution to growth have been reviewed extensively (Laties 1957; Barnes and Hole 1978; ap Rees 1980; Raison 1980; Wiskich 1980; Lambers *et al.* 1983b). It is known that numerous active processes, including metabolic changes, transpire as buds develop towards the dormant state and emerge from it (Tyurina 1976; Durzan 1976; Noodén and Weber 1978; Wieser 1979). Pollock (1953) emphasised the importance of respiratory activity of buds in the relationship between growth and dormancy phases during the annual cycle, but no study has compared the ontogeny of the embryonic shoot with its respiration in conifers. Selected literature will be presented concerning the respiration and metabolism of buds and bud parts during the annual cycle. No attempt has been made to present the literature according to real or artificially imposed physiological phases owing to the confusion of terms used in the past to describe the physiology of bud development. The terminology concerning the physiological concept of dormancy will be presented in a later section.

The information available for conifers is limited and there are no respiratory studies for developing buds of seedlings. Respiratory rates of vegetative buds of

Douglas fir stem cuttings were measured by Bhella and Roberts (1975) from July through April, and Kozlowski and Gentile (1958) followed respiratory rates of *Pinus strobus* (eastern white pine) (White pine) buds from March through May.

A number of workers have studied respiration of vegetative buds of hard wood species. Chan-Thom (1951) described the change in respiratory rates of a *Pyrus* cultivar (hardy pear) throughout the annual cycle under natural, greenhouse and storage conditions. Peterkova *et al.* (1976) measured respiratory rates of vegetative buds throughout the annual cycle of another *Prunus* cultivar (apricot), and Hatch and Walker (1969) followed respiratory rates of leaf and flower buds of 'Gleason Elberta' peach and 'Chinese' apricot. Pollock (1953) demonstrated that the capacity of buds to respire over a range of oxygen tensions changed in the summer, autumn and winter in *Acer saccharum* Marsh. (Sugar maple) and *A. platanoides* L. (sycamore). He also demonstrated the effect of bud scales and temperature upon respiratory rates and metabolism in these two species. Bachelard and Wightman (1973) described respiratory rates of vegetative buds of *Populus balsamifera* (L.) (poplar) between February and May, and Kato (1981) reported those of *Vitis vinifera* (grape) between September and February.

Respiration in flower buds has also been studied in a number of angiosperms including apricot (Tétényi 1964), *Rhododendron* cultivars (Ballantyne 1963, 1966, 1968), Lilac, Cherry, Mulberry,and Maple (Komarnitski *et al.* 1981) and pear (Cole *et al.* 1982).

Most studies of vegetative buds used intact buds and there was generally little information about the anatomical stage of bud development within the annual growth cycle, and designations of physiological phases were not clearly specified.

Most of these workers associated low levels of bud respiration with a physiological phase equivalent to Romberger's (1963) rest phase.

2.3.1 Respiratory changes during the annual cycle

Despite the lack of precise deliniation of physiological phases there were some common trends in the bud respiratory characteristics in all the above studies. Respiratory rates increased with the resumption of bud growth in the spring (Chan-Thom 1951; Pollock 1953; Tétényi 1964; vanden Born 1960; Hatch and Walker 1969; Bachelard and Wightman 1973; Tyurina 1979). This respiratory increase indicated a dramatic change in physiological activity. The increment was four-fold in poplar (Bachelard and Wightman 1973) and six-fold in hardy pear (Chan-Thom 1951). Past work has shown that certain areas of the embryonic shoot and subterminal regions contribute a larger proportion of the respiratory activity than others. (Ball and Boel 1944; vanden Born 1960; Rzhanova 1973).

Towards the end of shoot elongation in mature trees the developing bud behaves as a sink for numerous metabolites and engages in active syntheses of new compounds involved in growth. Using radio-labelled tracers, Semin and Madis (1964) showed that nutrient substances (phosphorus) actively entered the buds of several woody fruit species during development. As buds develop changes in metabolism have been related to the presence of bud scales limiting oxygen supply (Pollock 1953).

Following this active phase there was a period of reduced respiratory activity commonly referred to as rest. The timing of this phase depended upon the study. Chan-Thom (1951) found rates decreased to a minimum in September in buds under natural conditions, whereas both Kato (1981) and Bhella and Roberts (1975) found the lowest rates occurred in November. Pollock (1953) also noted a decrease in the capacity of buds to respire during autumn and early winter. Hatch and Walker (1969), however, found no relationship between the rest period and respiration rate.

During autumn metabolic changes were reported in buds and other plant tissues associated with cold hardiness (acclimation) Cold hardiness has been broadly reviewed by Parker (1959, 1963), Weiser (1970), Senser *et al.* (1974), Arronson *et al.* (1976), Levitt (1980), and Graham and Patterson (1982). Its acquisition is a multistage process and is a result of many physiological and structural changes in cells of the entire plant (Steponkus 1984). Evidence indicates that carbohydrates are stored and transformed (Parker 1963), lipids synthesised (Siminovitch *et al.* 1968, 1975; Singh *et al.* 1975) and changes occurred in phosphorus (Semin and Madis 1964), amino acid (Durzan 1968, 1969; Kacperska-Palacz *et al.* 1977), RNA (Erikson 1977) and protein metabolism (Durzan 1969; Craker *et al.* 1969; Pomeroy *et al.* 1970; Bixby and Brown 1975). Several physiological studies have dealt with the environmental requirements of conifer seedlings during the stages of cold hardening (Timmis and Worrall 1975; Tinus 1980; Ritchie 1984; Simpson and Macey 1985).

A period of chilling is necessary for the normal resumption of bud growth in Douglas fir seedlings (Lavender *et al.* 1968). Leaf primordia of *Prunus cerasus* L. var. Montmorency (Cherry) (Pollock and Olney 1960), vegetative buds of sugar maple (Pollock 1960), and flower buds of *Rhododendron* (Ballantyne 1963), were found to increase their respiratory rates after exposure to chilling temperatures. The rise in respiratory rate may be related to the removal of some metabolic block under the influence of chilling temperatures (Pollock and Olney 1959; Pollock 1960). Respiratory rates were found to rise with increased chilling time but remained constant or dropped slightly with time in unchilled leaf primordia of maple buds (Pollock 1960). 2,4-dinitrophenol (DNP) increased respiratory rate to a greater degree in unchilled than chilled primordia (Pollock 1960). The uncoupler DNP has been used by a number of workers to indicate the availability of phosphate acceptors (Ballantyne 1968; Trunova 1968; Wiskich *et al.* 1964; Wiskich 1980). The low respiratory rates and low response to DNP during dormancy probably owes its existance to a limiting metabolite, i.e. phosphate acceptor (ADP), responsible for preventing the cells from reaching their maximum respiratory capacity (Romberger 1963; Trunova 1968).

There is a relationship between bud moisture content and dormancy. Low water content of buds is a universal property of buds during dormancy (Wierszyllowski *et al.* 1960; Little 1970; Cottingnes 1983b). Respiratory rates were found to rise when dormant dehydrated buds were allowed to hydrate between February and May (Kozlowski and Gentile 1958). The rise in water content of buds coincides with the end of dormancy and an increase in DNA levels (Cottingnes 1983a). The resumption of DNA synthesis after dormancy is followed by other metabolic events. Chandry *et al.* (1970) suggested that rest was broken and protein synthesis stimulated by a liberation of DNA from a DNA-phosphoprotein complex in the resting state. Protein and amino acid synthesis were found to be rapid at bud break (Durzan 1969; Bachelard and Wightman 1973).

2.3.1.1 Cyanide (CN) resistant respiration

Cyanide resistant (CN-resistant) respiration is widespread in higher plants (Lambers 1980; Henry and Nyns 1975) and micro-organisms (Henry and Nyns 1975; Seidow 1982). CN-resistance may occur in both exised and intact tissues (Henry and Nyns 1975; Solomos 1977; Storey 1980; Day *et al.* 1980; Lambers *et al.* 1980a; Laties 1982) and may vary during the dormant period (Cole *et al.* 1982; Farrar 1980). CN-resistant respiration has been found to have at least two component pathways in plant mitochondria; the alternative pathway terminating in the alternative oxidase, and the residual pathway. The nature of the alternative oxidase remains obscure (Rich *et al.* 1977; Siedow 1982) as does the branch point of the alternative pathway from the cytochrome chain (Storey 1976; Rustin *et al.* 1980).

The flux of electrons through the alternative pathway may be controlled by the arrangement of carriers in the branching region which provides a switch for directing electron transport either through the cytochrome pathway or through an alternative pathway (Storey 1976). Since the alternative pathway is non-phosphorylating (Solomos 1977) this results in a partial uncoupling of phosphorylation from oxidation. This pathway may be inhibited specifically by substituted hydroxamic acids (Schonbaum *et al.* 1971), disulfiram (Grover and Laities 1981) and n-propyl gallate (Siedow and Grover 1980). Hydroxamic acids will also inhibit peroxidase and polyphenol oxidase, although these enzymes are also inhibited by CN (Rich *et al.* 1978).

After inhibition by CN and salicylhydroxamic acid (SHAM) a residual oxygen consuming pathway remains in isolated mitochondria (Theologis and Laties 1978), potato and tomato slices (Day *et al.* 1980) and root, leaf and bud tissues (Lambers *et al.* 1983a; Cole *et al.* 1982). The nature of residual respiration is unknown but monoxygenases may be involved (Day *et al.* 1980).

It has been suggested (Palmer 1976; Theologis and Laties 1978; Lambers 1980) that the alternative pathway handles the overflow of a saturated cytochrome pathway. The changing balance of electron flow through the CN-sensitive and alternative pathways maintains cation flux through the TCA cycle and maintains energy charge.

2.4 Section 4: Definitions of dormancy

There is no satisfactory definition of the term dormancy which satisfies both physiological and anatomical approaches to the phenology of bud development. In this review of the literature on dormancy an attempt will be made to indicate the confusion surrounding this concept, so that a meaningful terminology may be specified, without reiterating past misconceptions.

Classically, dormancy has been defined as any case in which a tissue predisposed to elongate does not do so (Doorenbos 1954). This definition is equivalent to that enunciated by Molish (1922) and has been followed by other reviewers of this phenomenon (Samish 1954; Wareing 1956; Richardson 1958; Romberger 1963; Vegis 1966; Sarvas 1974; Perry 1971; Noodén and Weber 1978; Tyurina 1979; Champagnat 1983). A rich and varied terminology has been used by these reviewers to categorize the apparent change in physiology during the classical dormant period.

The physiological terminology for dormancy in the literature was found to fall into two categories, those describing physiological states or those describing physiological phases. Physiological states are defined here as conditions existing within the bud resulting from internal or external factors that may occur at any time during the annual cycle. Physiological phases describe apparent sequential changes in the physiology of buds during development. Generally, these terms are based on one single criterion, that is, the ability of buds to resume growth under favourable, but unnatural, conditions. This criterion has led 'dormancy' to be equated with rest and other physiological phases during development.

2.4.1 Physiological states

Two physiological states have been recognized in bud dormancy:

1) Dormancy imposed by a direct environmental effect of cold, drought or other stresses; and,

2) Dormancy imposed by controlling factors from within the plant, referred to by some as physiological dormancy (Richardson 1958). Most reviewers have split this second state into two further states;

   i) Bud dormancy imposed by influences from other plant parts, ie. correlative inhibition (plant growth regulators).

   ii) Bud dormancy imposed by conditions within the bud itself, ie. physiological state subject to the effects of chilling and photoperiod.

Numerous reviews of dormancy in woody plants have each assigned a confusing array of new and old terms to describe these states. Dormancy imposed by the direct effect of the environment has been referred to as quiescence (Samish 1954; Romberger 1963; Perry 1971; Noodén and Weber 1978), imposed dormancy (Doorenbos 1954), facultative dormancy (Semin and Madis 1964), environmental dormancy (Bachelard 1980), enforced dormancy (Kato 1981), relative or conditional dormancy (Vegis 1966), ecodormancy (Lang *et al.* (1985) or just dormancy (Pollock 1953).

Dormancy imposed by influences exerted by interactions between the bud and other parts of the plant may result in the growth inhibition of axillary and terminal buds (Champagnat 1983; Romberger 1963), and may be broken by defoliation (Molish 1922). This form of physiological dormancy has been referred to as summer dormancy (Doorenbos 1953; Wareing 1956; Bachelard 1980), correlative inhibition (Samish 1954; Romberger 1963; Sĕbánek 1975; Noodén and Weber 1978; Champagnat 1983), relative/conditional dormancy (imposed but not restrained) Vegis (1966) and ectodormancy (Lang *et al.* 1985).

Dormancy imposed by conditions within the bud has been referred to as rest (Pollock 1953; Samish 1954; Romberger 1963; Bachelard 1980), deep organic dormancy (Semin and madis 1964; Tyurina 1979), dormancy 1 (Sarvas 1974), dormancy (Noodén and Weber 1978; Champagnat 1983), winter dormancy (Wareing 1956), endogenous dormancy (Sĕbánek 1975), endodormancy (Lang *et al.* 1985), deep dormancy (Kato 1981), and true dormancy (Vegis 1966).

2.4.2 Physiological phases

The physiological phases delineated according to the degree of bud growth (regrowth) under favourable conditions (Vegis 1966) have resulted in arbitrary and ill defined limits. The regrowth of buds decreases during predormancy, early-rest (Vegis 1966), or pre-rest (Perry 1971). During this phase, several authors have not distinguished between the physiological states of direct environmental dormancy and dormancy imposed upon the bud from within the plant. Thus the use of spontaneous dormancy (Kato 1981), quiescence (Perry 1971), endogenous dormancy (Sĕbánek 1975), and organic dormancy (Semin and Madis 1964; Tyurina 1979) during this phase has only added to the confusion of terms. Following the early phase, bud regrowth decreases to a minimum during true dormancy/middle-rest (Vegis 1966), winter-rest (Perry 1971), dormancy 1 (Sarvas 1974), and increases during chilling until bud break in the spring. This last phase has been called post dormancy or late-rest (Vegis 1966), after-rest (Perry 1971), and dormancy 2 (Sarvas 1974).

The above physiological phases of dormancy are of little value when applied to a developmental study of seedlings under natural conditions. From a practical point of view, these phases do not indicate when the seedling is most hardy. Coastal Douglas fir seedlings were shown to be most hardy, with respect to environmental stress, from December through February (Lavender 1964; Hermann 1967; Hermann and Lavender 1967; Lavender and Wareing 1972). This led Lavender and Cleary (1974) to propose a new set of physiological phases based upon the resistance of Douglas fir seedlings to environmental stress. They divided the dormant period into four phases.

1) Dormancy induction: From mid to late July until mid to late September during which buds developed and after-ripened, and produced a late flush of growth if put into favorable conditions.

2) Dormancy deepening: From mid to late September until mid to late November during which buds almost lost the ability to grow but still remained susceptible to environmental stress.

3) Dormancy: From mid to late November until mid to late February during which buds had the maximum resistance to stress but still retained some regrowth capability.

4) Post Dormancy: From mid to late February until bud break during which resistance to environmental stress was lost and buds regained the ability to grow.

However, if applied to mature trees this interpretation would suggest that their buds were almost always in a stage of dormancy which reduces the usefulness of the term dormancy and confuses it with the occurrence of active processes within the bud (Owens and Molder 1973a).

More recently a physiological model has been proposed to describe and predict in numerical terms the annual growth cycle of vegetative buds of temperate woody species (Fuchigami *et al.* (1982). This scheme has potential use in predicting the physiological state of nursery stock (Simpson 1984) in that physiological, morphological and anatomical data may be used to estimate a seedlings position in the annual growth cycle represented by a 360° Growth Stage Model (°GS). Thus the seedling's readiness for lifting or planting, based of a broad range of attributes, may be used rather than arbitrary lifting and planting 'windows'.

In summary, an essential weakness of the classification of the dormant period according to bud regrowth lies in the assumption that a particular state is a property of the whole bud without recognizing that several meristems exist within a developing bud. Thus, states and phases overlap, and the different tissues within a bud may be in different physiological states of dormancy at the same time. This situation is exemplified by Lang *et al.* (1985) who advocates the use of 'dormancy' to describe only meristematic areas which have ceased cell division, and then goes on to propose yet another new set of terms, eco-, ecto- and endodormancy, to designate the physiological state of buds during their development. It is difficult to see how this terminology clarifies the situation, but the attempt demonstrates how confusion will certainly arise where the same term is used to describe both the physiological state of specific tissues and complex organs. The criterion of environmental resistance, however, requires all apical and lateral meristems to be accounted for in the overall survivability of the seedling, so that from a whole plant view the plant is dormant. The development of this state is a complex process and the physiological and anatomical developmental stages of bud tissues may or may not all be in phase during all stages of seedling development.

2.4.3 Specification of dormancy

Consistent with the classical concept of dormancy the majority of developmental studies of whole plants have used bud set or the termination of height growth to delimit the beginning, and bud break or flushing (emergence of new needles) to delimit the end of the dormant period (Nienstaedt 1966a, b; Lavender *et al.* 1968; Lavender and Hermann 1970; Campbell and Sugano 1975; van den Driessche 1975, 1969, 1977; Sĕbánek 1975; Wample *et al.* 1975; Kleinschmit and Sauer 1976; McCreary *et al.* 1978). According to this view dormancy infers a lack of visible elongation and may occupy 75 percent of the annual growth cycle from mid-July to the following spring in mature trees (Lavender 1981). However, detailed anatomical studies of buds (Sterling 1946; Allen 1946; Parke 1956; Owens and Molder 1973a; Owens *et al.* (1977) and shoot development (Owens 1968; Owens and Molder 1973a,b; Owens *et al.* 1977,1979; Pollard and Logan 1977; Owens 1984) have shown that after cessation of shoot elongation buds continue to initiate preformed leaves and remain meristematically active until late fall. Furthermore, in the spring, cell divisions are visible in the preformed shoot long before visible elongation begins (Owens *et al.* 1985).

Resistance to stress is also an important criterion in the delimitation of dormancy under natural conditions. Lack of apical mitotic activity was found to correlate well with resistance of Douglas fir seedlings to transplanting shock in the winter and spring (Carlson *et al.* 1980). This evidence was derived from physiological studies on coastal Douglas fir seedlings (Lavender 1964; Lavender and Cleary 1974) in which it was demonstrated that seedlings were susceptible to transplanting shock in the fall. The terminology of Lavender and Cleary (1974) included the period of fall apical growth (Owens 1968) within the dormancy phases, and attempted to categorize dormancy, in a similar manner to former physiological studies (Romberger 1963). Categorical definitions of dormancy, such as pre, true, and post dormancy (Sarvas 1974), are difficult to apply and apparently inadequate (Romberger 1963; Sarvas 1974; Lang *et al.* 1985). The difficulty lies in the concept of a partitioned dormancy. In many species steady states and transitional phases during dormancy (Smith and Kefford 1964) are not present (Campbell 1979a).

In seedlings, under natural conditions, it may be more useful to reserve the term dormancy for the period of apical mitotic inactivity, since the classical dormancy period seems to describe a period of reduced, but continuously changing, developmental rates (Campbell 1979a) rather than an absence of growth.

Romberger (1963) was of the opinion that activity of meristems should be used to define the dormant period because of the complex nature of physiological processes occurring during the annual cycle. More recently Lavender (1981) also indicated that only apical meristems of temperate plants are generally considered to have a dormant period during the annual growth cycle. Perry (1971) included an absence of cell divisions as well as a chilling requirement in his dormancy definition. Owens and Molder (1973 a,b) and Owens *et al.* (1977) have consistently defined the dormant period in mature trees according to the anatomical criterion of absence of apical mitotic activity. Recently Simpson and Macey (1985) found some correlation between apical meristematic activity and resistance to frost damage in white spruce seedlings. This provides further evidence for the relationship between mitotic index, dormancy and resistance to stress.

Chapter III

METHODS AND MATERIALS

Douglas fir seedlings were selected from two provenances on the basis of their varietal differences and availability. They were interior dry (seedlot 3442, Lake Carson, 820m elevation) and coastal (seedlot 1207, Desine, 800m elevation).

In 1982 seedlings were raised from seed in styroblock containers under a polythene shelterhouse at the British Columbia Forest Service Nursery, Duncan, B.C. Three styroblock containers of seedlings from each seedlot were left in the Duncan Nursery shelterhouse from May 1982 to March 1983. All cultural practices, fertilizing and watering were carried out according to the nursery operational schedule.

Seedlings from the same seedlots were used for a 1983/1984 study. They were grown at Duncan nursery under the same conditions as the previous year until mid August, when 2,000 seedlings from each seedlot were transferred to the lath house at the University of Victoria. Growing conditions, watering and fertilizer application were as similar as possible to those prevailing in Duncan in the previous year. During August through November, the seedlings were watered 3 times weekly with a weekly application of either 10-20-10 or 20-20-20 fertilizer.

In both years the interior variety was transferred from 18 hour to natural photoperiods during mid-July. The coastal variety received natural photoperiods for the whole growing season (Cardy 1984 pers. comm.).

3.1 Embryonic shoot development

3.1.1 Anatomical

3.1.1.1 Sampling

Ten seedlings of each variety were collected every fortnight between September 1982 and March 1983. By 0900 on each collection date the seedlings were selected at random from the styroblocks, placed in plastic bags and transported to the University of Victoria.

3.1.1.2 Tissue preparation

The bud-scales were removed from the terminal vegetative buds, and the embryonic shoots were excised just below the crown region and fixed in formalin/acetic acid/alcohol (FAA) (Sass 1958). Shoot tips were fixed by mid morning on each collection date in order to avoid confounding effects due to diurnal fluctuations in mitotic index (Macey 1980, Kawazawa *et al.* 1970). The material was dehydrated in a tertiary butyl-alcohol series (Johansen 1940) and embedded in Tissue Prep. Serial longitudinal sections of embryonic shoots were cut at 6μ and stained with safranin and hematoxylin.

3.1.1.3 Measurement

Median sections were selected for apical measurements (Owens *et al.* 1977). Mitotic index, the percentage of cells in division (Owens and Molder 1973, Carlson *et al.* 1980), was determined from the median section of each apex. Dividing cells can be identified by dark staining with hematoxylin of the condensed chromosomes during metaphase, anaphase and telophase. Identification of these phases served as the criterion for cell division in this study. Apical height, width, stage of development and the number of needle primordia along the flanks of the apex were also determined from the median section. The count of needle primordia was taken as a relative indication of the number of primordia formed on the whole embryonic shoot.

Early and late bud-scale initiation were distinguished by observation of the number of bud scales removed during dissection. Early preformed-leaf initiation was the period during which the first two thirds of the total leaf primordia were initiated. The average number of dividing cells per needle primordium was calculated from counts of cell divisions in all needle primordia along the flanks of the median section of each embryonic shoot. Cell divisions in needles and apices were counted using a compound microscope. Cell numbers in apices were counted from photomicrographs of median sections.

3.1.2 Respiration

Respiration rates[1] of shoot tips were measured in two successive years using a clark type polarographic electrode (see pp. 33-34). In the first year respiration was measured from October 1982 through March 1983, however, these dates did not include the entire period of bud development. Therefore, in the second year respiratory rates were measured from August 1983 through December 1983, and during March 1983. Since growing conditions were identical in the two years, respiration data were comparable on similar dates from early bud-scale initiation in the summer through dormancy to bud swelling and flushing in the following spring.

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[1] The term respiratory rate is used here to indicate the respiratory capacity of embryonic shoots at 25°C (Pollock 1953).

3.1.2.1 Sampling 1982/1983

Seedlings were sampled on the same days from the same styroblocks as in the developmental study. On each collection date 20 seedlings of each variety were randomly selected from each styroblock and transported in plastic bags to the laboratory. In order to minimize variation due to diurnal fluctuations in metabolic activity (Richter 1978), respiration rates of embryonic shoots were always measured from mid-afternoon through to late-evening on each collection date. Numbers of replicates varied from two to four for each variety depending on the time of year. Bud replicates included the terminal bud and all lateral vegetative buds within 2 cm of the terminal bud.

3.1.2.2 Sampling 1983/1984

From August through December 1983, 50 seedlings were randomly selected from each seedlot two or three times per month, and transported in plastic bags to the laboratory. Between three and five replicates of 10 to 20 embryonic shoots were made at each collection date. Respiration rates were measured from early-afternoon through the evening. In August 1983 it was necessary to increase the numbers of embryonic shoots to 20 since apices were very small (vanden Born 1960; Bella and Roberts 1975). In March 1984 rates of respiration were monitored during bud swelling and emergence of the needles. This included a part of embryonic shoot development not studied in March 1983.

3.1.2.3 Tissue preparation

Dissection of embryonic shoots varied depending upon the stage of bud development but in all cases the apex and usually some of the smallest subtending primordia were included. In August embryonic shoots were still undergoing primary leaf initiation, consequently apices were excised above the last formed leaf primordium. During bud-scale initiation excision was made above the last formed bud scale. During preformed leaf initiation excision was made below the most basal leaf primordium.

3.1.2.4 Measurement

Following dissection embryonic shoots were kept up to 15 min. in a small glass vial containing the same medium in which oxygen consumption was to be measured. An osmoticum (0.3 M mannitol, 4 mM $MgCl_2$ and 25 mM TES buffer, pH 7.2) (James and Spencer 1979) was used to avoid osmotic shock to excised tissues when placed into the medium. The medium in the respiration chamber was allowed to equilibrate at 25°C. while the wet fresh weight of the buds was determined. Saturation of the medium was ensured by bubbling air through the medium at a lower temperature before equilibrating at 25°C.

For each sample, between 10 and 20 embryonic shoots were transferred to the respiration chamber and all light was excluded by covering the chambers and water jacket with aluminium foil. The plunger and electrode assembly were slid into the chamber to the exclusion of all air bubbles. The first measurement of total oxygen consumption was made from the chart recorder within 5-10 mins. equilibration time.

3.1.2.5 CN-resistance

It was necessary to measure the importance of the alternative pathway and residual oxygen consumption in order to indicate their relative contribution to the total oxygen utilization throughout bud development and dormancy until resumption of bud activity.

Potassium cyanide (KCN) is a reversible inhibitor of cytochrome oxidase (Estabrook 1969), whereas salicylhydroxamic acid (SHAM) is an inhibitor of the alternative oxidase (Schonbaum *et al.* 1971). Both KCN [2] and SHAM were added serially to the same bud samples. Once a steady trace had been obtained 20µl aqueous solution of KCN was injected through the access port to a final concentration of 2mM in the respiration chamber and the amount of inhibition of the CN sensitive pathway noted. Between eight and 12 min. were allowed for penetration of the inhibitor into the tissues so that a steady rate of oxygen consumption was obtained.

Oxygen consumption due to the alternative oxidase was inhibited by injecting 100µl ethanolic solution of SHAM into the chamber to a final concentration of 3 mM. Higher concentrations of SHAM were used but abandoned as they tended to increase KCN inhibited respiratory rates. The volumes injected did not exceed 100µl and the same volume was not found to have a significant effect on rates when added without SHAM. At least 15 min. were allowed for the system to equilibrate due to the effect of the ethanol on dissolved oxygen levels in the medium.

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[2] The maximum concentration of each inhibitor in the sample vessel required for maximum inhibition of Douglas fir shoot tip respiration was determined by titration on each sample date throughout the sample period (1982/1983) and found to be 2mM and 3mM KCN and SHAM respectively.

The proportion of oxygen consumption due to all oxygen consuming pathways, the CN resistant alternative and residual respiration were calculated from the chart recordings in natoms O/min^{-1}.g^{-1} (Estabrook 1969). Oxygen consumption by the alternative pathway was taken as an estimate of the maximum possible flux of electrons through the alternative pathway after inhibition of the cytochrome chain at a particular stage of bud development.

3.1.2.6 The polarographic electrode

Clark type polarographic oxygen electrodes (YSI Model 5331), respiration chambers (YSI Model 5213) and oxygen monitor (YSI Model 53) were obtained from the Yellow Springs Instrument Company, Yellow Springs, Ohio.

The electrode consisted of a sealed platinum wire (cathode) surrounded by an insulated silver reference electrode (anode). The two were connected by a KCl bridge. A voltage was imposed across the two electrodes in the test medium bringing about an electrolytic reduction of oxygen (Estabrook 1969).

The electrode was housed within an acrylic plunger provided with an air trap and access port. The plunger fitted snugly into a precision milled glass respiration chamber. Teflon clips held the plunger in place, and the whole unit, consisting of four respiration chambers, was surrounded by circulating water at 25°C. The design minimised back diffusion and air bubbles were easily eliminated from the system (Estabrook 1969). A magnetic stirrer ensured constant mixing of the medium in the chambers.

The current generated at the electrode was amplified and displayed on a galvanometer (monitor) reading 0 to 100 percent saturation. The system was zeroed using nitrogen and calibrated so that the scale on the meter represented the percentage oxygen saturation in the medium. The meter was connected to a Perkin Elmer Colman 165 chart recorder set to 10 mV sensitivity at a speed of 5 mm/min.

In the 1983/84 study, seedling embryonic shoots were smaller in August and September than at later dates in either 1982/83 or 1983/84. Consequently, in 1983/84 a smaller version of the respiration chamber (YSI Model 5213) and plunger were fashioned from acrylic in order to reduce the standard 3 ml chamber to a 1 ml capacity. The modified chamber was used throughout the 1983/84 study because its performance proved to be satisfactory for the measurement of oxygen consumption of up to 80 mg fresh weight of tissue and results were consistent with those obtained from similar amounts of tissue collected at comparable times in 1982.

3.2 Root development

Numbers of white roots for each seedling were counted on ten seedlings of each variety on each collection date in 1982. The seedlings were those from which embryonic shoots had been excised. Counts were made immediately after fixation of the embryonic shoots. White roots were counted in each of the following size classes; 0-4.9 mm, 5-9.9 mm, and over 10 mm. Notes were kept describing root morphology throughout the sampling period.

In 1983 root growth was assessed by Burdett's semi quantitative index for measuring root growth capacity (Burdett 1979). White roots from ten seedlings were counted and classed according to the following numerical index:

0 = no new roots

1 = some new roots but none greater than 10mm

2 = 1-3 new roots over 10 mm

3 = 4-10 new roots over 10 mm

4 = 11-13 new roots over 10 mm

3.3 Statistical analysis

Variances between varieties and dates were tested with the Fmax test ($\alpha=0.05$) (Bliss 1970) and were found to be homogeneous in all data with the exception of respiration rates between dates from August to October in 1983 and some of the components of respiration throughout the sample period. Lack of homogeneity of respiratory components data was not consistent for any component or variety between dates.

Parametric tests were employed for homogeneous data to test for significant differences between dates and varieties ($\alpha=0.05$). Duncans Multiple Range Test and Contrasts were used for between dates and varieties, respectively, using the GLM procedure for unbalanced sample sizes (SAS 1982).

Data employing percentages (mitotic index and CN-resistant respiration) were normalised using an arcsine transformation (Zar 1974). Data in which proportions were used (leaf activity) were analysed using the Chi-square test (Ryan *et al.* 1985).

Significant differences between varieties ($\alpha=0.05$) were demonstrated for respiration data between August 1983 and October 1983, and for data between February to mid-March in 1984, using independent t tests (Zar 1974). Between dates analysis was performed on data between October 1982 and March 1983 which were homogeneous ($\alpha=0.05$). These data were presented in Appendix A.

A regression of respiration and mitotic index was performed on a portion of the data. Strictly speaking this test should only be used where variances are homogeneous. Both varieties were heterogeneous for respiration rates between dates and there were few data points, however, this representation of the data was included to illustrate a relationship between respiration of the embryonic shoot that may be confirmed with larger sample sizes and more data points.

Chapter IV

RESULTS AND OBSERVATIONS

The seedlings underwent a three to four month free growth phase after germination (Mathews 1985 pers. comm.). The phenology of the two varieties differed in that morphological events in apices from the interior variety preceded those in apices form the coastal variety by four to six weeks under nursery growing conditions.

4.1 Embryonic shoot development

4.1.1 Bud-scale and preformed leaf initiation

4.1.1.1 Coastal variety

On early collection dates there was considerable variation in stage of bud development (Fig. 9). The change from primary leaf initiation (Figs. 1A, 2A) to bud-scale initiation (Figs. 1B, 2B) occurred during the last two weeks of August (Fig. 9). In early September apices were initiating either primary leaves or bud scales (Fig. 9) and MI was 0.95 (Fig. 3). During the last 2 weeks of September, apices were initiating late bud scales or early preformed leaf primordia (Figs. 1C, 2C, 9, 10) and the MI was more than 2.0 (Fig. 3). Mitotic index was greatest during early preformed leaf initiation when analysed by stage (Fig. 9). By early-October apices of all seedlings were initiating preformed leaf primordia (Fig. 9) and zonation was conspicuous (Fig. 2C). By mid-October apices had reached their maximum number of cells (Fig. 4) and apical size.

The increase in apical size appeared to result from cell divisions in the rib meristem (RM) and peripheral zones (PZ) (Fig. 2C). Cell divisions in the RM were chiefly responsible for the rapid increase in apical width during growth of the shoot tip (Figs. 5, 6). A mammillary apex (Allen 1946) was observed in which the mitotically less active apical initials (AI) and central mother cells (CMC) formed a prominent tip on the apex with a constriction between these zones and the subtending PZ zone (Fig. 2C). Apices having similar form were not found in the interior variety because preformed leaf initiation was well underway when the first collection was made (Fig. 7).

Preformed leaf initiation occurred from the PZ and proceeded rapidly up the flanks of the apex. The rate of preformed leaf initiation was steady from September until it ceased in mid-November (Fig. 7). Apices continued to increase in cell number (Fig. 4) and size until mid-October (Figs. 5, 6) then MI decreased (Fig. 3), the rate of preformed leaf initiation exceeded apical growth and apical size began to decrease as preformed leaf primordia encroached upon the apical dome. Mitotic index (Fig. 3) and preformed leaf initiation continued at a decreasing rate until all preformed leaf primordia were initiated in early December (Fig. 7). Mitotic activity of preformed leaf primordia was about 0.7 during most of the period of leaf initiation (Fig. 8). It increased to 1.1 in November, as preformed leaf primordia enlarged, then rapidly decreased to a low rate by mid-December (Fig. 8)

4.1.1.2 Interior variety

Comparable stages of bud development occurred about one month earlier in the interior variety (Fig. 9). In early September apices were observed in both early and late preformed leaf initiation (Figs. 2C, D, 9) and MI was 2.3 (Fig. 3). Mitotic index was highest during the stage of early preformed leaf initiation in the late summer and autumn (Fig. 9). By the beginning of October, few apices were still in late preformed leaf initiation (Fig. 9) and, MI had decreased to 0.7 (Fig. 3). During September apical width, height and cell numbers decreased to a minimum (Figs. 4-6). Apical zonation was initially distinct but became less during late September and October (Figs. 2D, E). Mitotic activity continued at a low level throughout October, and apical width, height and cell numbers showed no significant change (Figs. 3-6).

4.1.2 Nodal diaphragm development

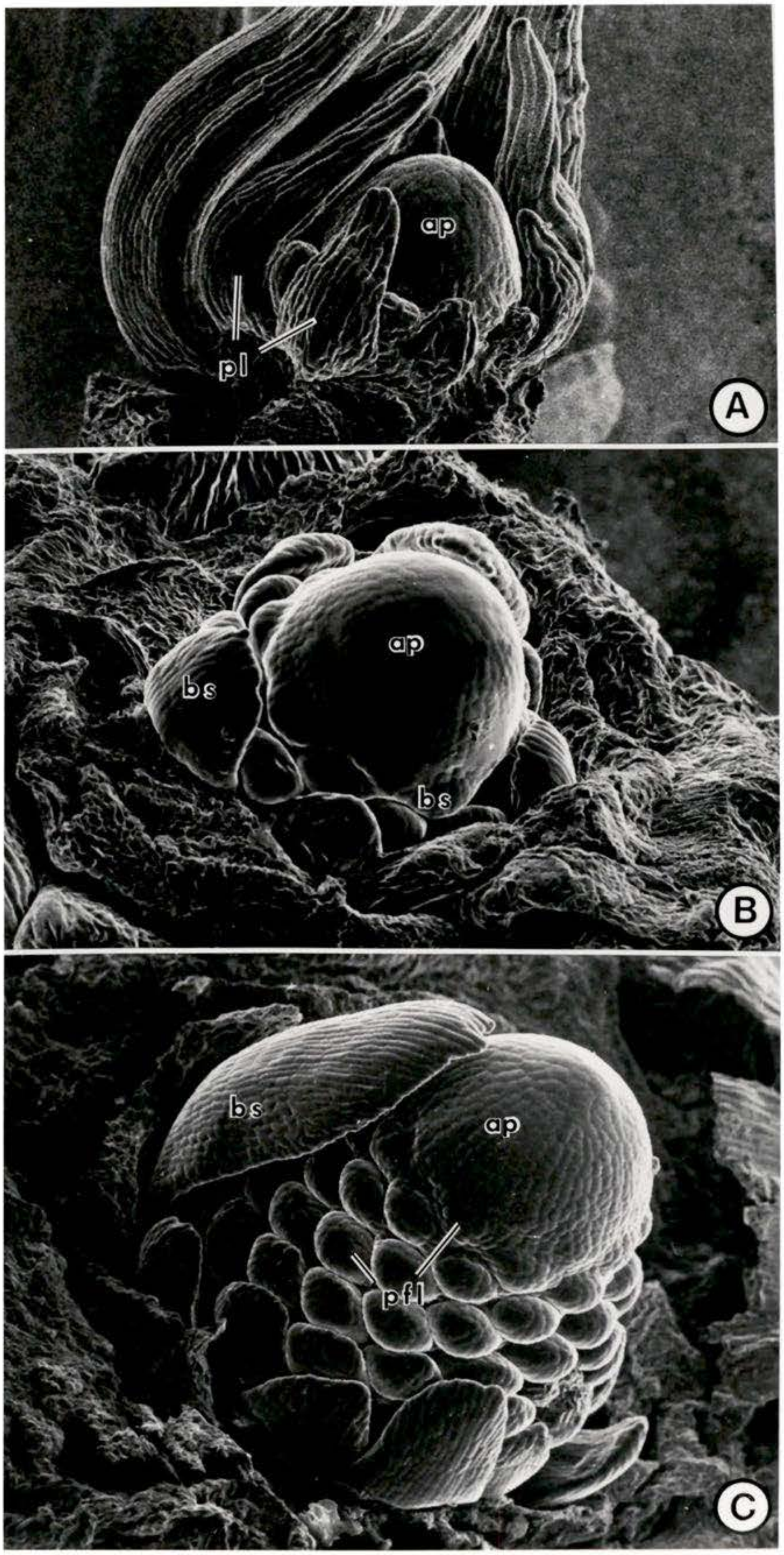
A nodal diaphragm (crown) began to develop at the base of the embryonic shoot during early preformed leaf initiation in both varieties and became clearly visible during late leaf initiation (Fig 2D). Differentiation began late in September and was completed late in October in the coastal variety, whereas the interior variety had already begun crown development in early September and this was completed by October. In both varieties a plate of thick walled cells, five to eight cells thick differentiated at the base of the embryonic shoot and extended to the edge of the cup shaped receptacle bearing the bud scales. It was interrupted by the anastomosing eustele, consisting of procambial strands. By late-November, in both varieties, a space formed immediately below the crown region and remained until bud break in March.

Figure 1: Scanning electon micrographs of living, unfixed, dissected shoot tips of coastal Douglas fir.

A. Apex (ap) during primary leaf (pl) initiation collected in early August. X 95.

B. Apex during bud-scale (bs) initiation collected in early August. X 95

C. Apex during preformed leaf (pf) initiation collected in early September. X 95.


ap
pl
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C

Figure 2: Median longitudinal sections of the developing embryonic shoots of Douglas fir seedlings

A. Apex collected in early-September during primary leaf (pl) initiation showing the apical zone (a) X 60.

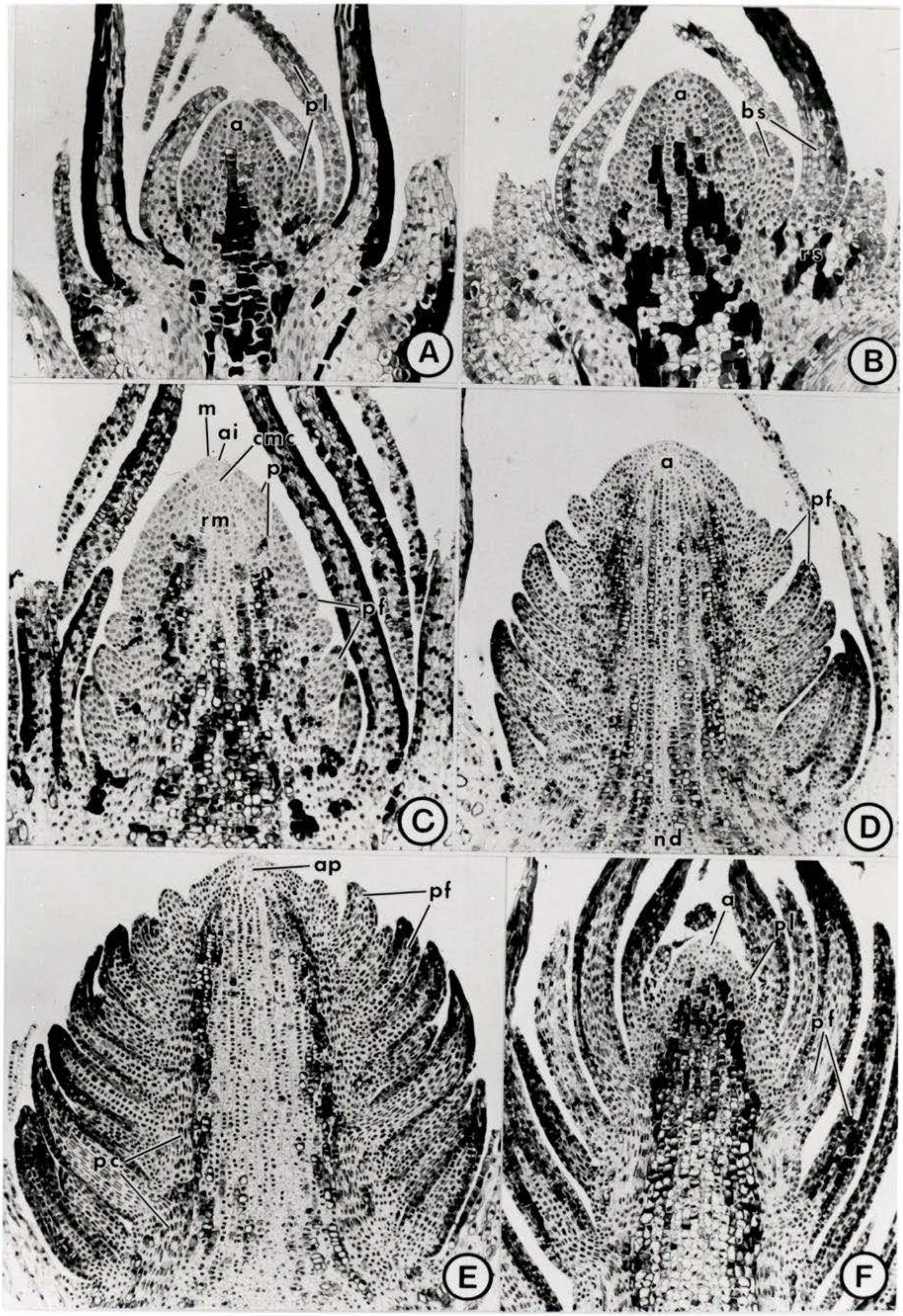
B. Shoot tip during bud-scale (bs) initiation X 60.

C. Embryonic shoot during early preformed leaf (pf) initiation showing the mammillary apex (m) and its zonation into apical initials (ai), central mother cells (cmc), peripheral zone (p) and rib meristem (rm). X 60.

D. Embryonic shoot during late preformed leaf initiation showing the region of formation of the nodal diaphragm (nd). X 50.

E. Dormant embryonic shoot showing apex (ap) and the partially elongated preformed leaf primordia with procambial strands (pc). X 50.

F. The expanding shoot collected in March before emergence from the bud scales showing the formation of current year neoformed leaf primordia (pl) X 50.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4.1.3 Cessation of mitotic activity

No mitotic activity was found in any apices of the coastal variety between mid-December 1982 and March 1983 (Fig. 3), nor was there any change in apical dimensions (Figs. 5, 6). Mitotic activity occurred at a low level in the subtending preformed leaf primordia until mid-January (Figs. 8). Apices of the interior variety ceased mitotic activity in mid-November 1982 and remained inactive until March 1983 (Fig. 3). Apical dimensions remained unchanged from October 1982 to March 1983, and cell divisions ceased in the subtending preformed leaf primordia in mid-November (Figs. 4, 5 and 8).

Considerable variation existed in the timing of the end of mitotic activity within each variety (Fig 9). Some individuals of the interior variety had ceased mitotic activity in late September and those of the coastal variety by mid-November (Fig. 9). Leaf mitotic activity showed a similar variation.

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

4.1.4 Resumption of bud activity following dormancy

Mitotic activity began in the preformed leaf primordia of buds in mid-February in coastal and interior varieties. A sharp increase in mitoses occurred in the apex (Fig. 3) and throughout the embryonic shoot in March when shoot elongation also commenced (Fig. 2F). There was a delay of about two weeks after apical mitosis resumed before an increase in apical dimensions was observed on March 1. Embryonic shoots elongated by internode extension and preformed leaf primordia elongation (Fig. 2F). Apices of elongating shoots were observed to be initiating primordia (Fig. 2F) which had the appearance of neoformed leaves rather than bud scales. Mitotic index exceeded 1.5 in both varieties (Fig. 3) and there was a two to three fold increase in fresh weight (Table 1). Bud fresh and dry weights increased more rapidly in the interior variety than in the coastal variety.

Table 1: Mean[1] fresh and dry weight of coastal and interior shoot tips of Douglas fir

	Fresh Wt.(mg)		Dry wt.(mg)	
Date	Coastal	Interior	Coastal	Interior
Feb 24	0.0313 b[2]	0.0679 a	0.0083 b	0.0112 a
Mar 5	0.0508* b	0.2653* b	0.0116* b	0.0566*[3] b
Mar 13	0.1434* a	0.3753* c	0.0283* a	0.0757* c

[1] Each mean based on 5 replicate weighings

[2] Means followed by the same letter are not significantly different at the $\alpha=0.05$ level within each fresh and dry weight category

[3] Means accompanied by asterisks indicate significant differences between varieties at the $\alpha=0.05$ level using paired contrasts.

Bud scales lightened in colour from their usual dark tan, and enlarged for a time to accommodate the expanding shoot. Flushing began in mid-February in some individuals of both varieties and continued until late-March. By 13 March 1984 about 50 percent of coastal seedlings were undergoing bud swelling, whereas bud swelling was occurring in all interior seedlings. There was a greater difference in onset of bud expansion in individuals within a variety than between varieties, however in both years development in the majority of interior seedlings was about a week in advance of that of the coastal variety.

Figure 3: The average mitotic index (MI) per median section based on 4-8 apices per collection of coastal (□) and interior (●) Douglas fir.

Month abbreviations indicate the first day of the month.

Means on successive dates with the same letter are not significantly different at the $\alpha=0.05$ level using Duncan's multiple range test and asterisks (*) indicate significant differences between varieties at the $\alpha=0.05$ level from paired contrasts.

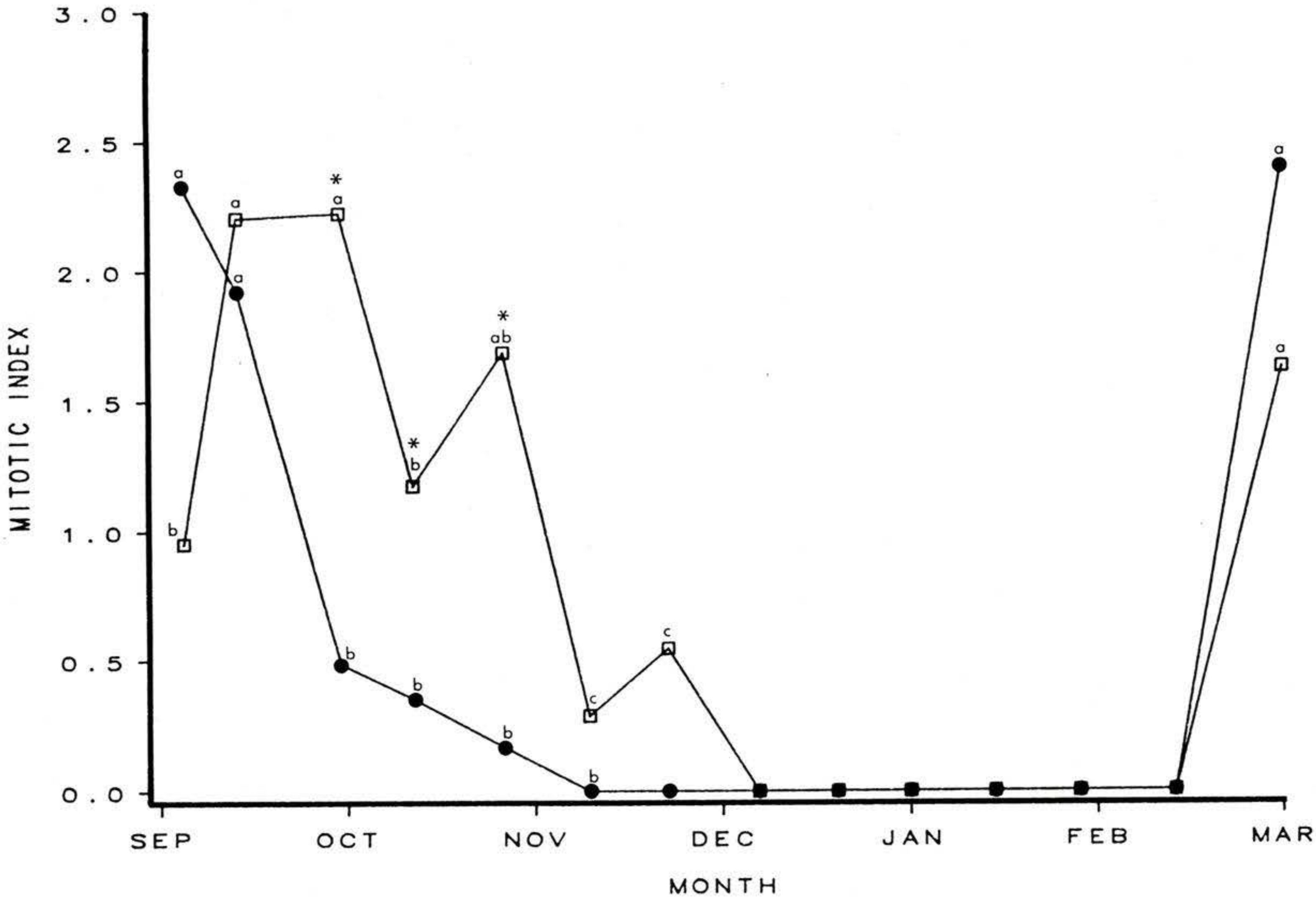
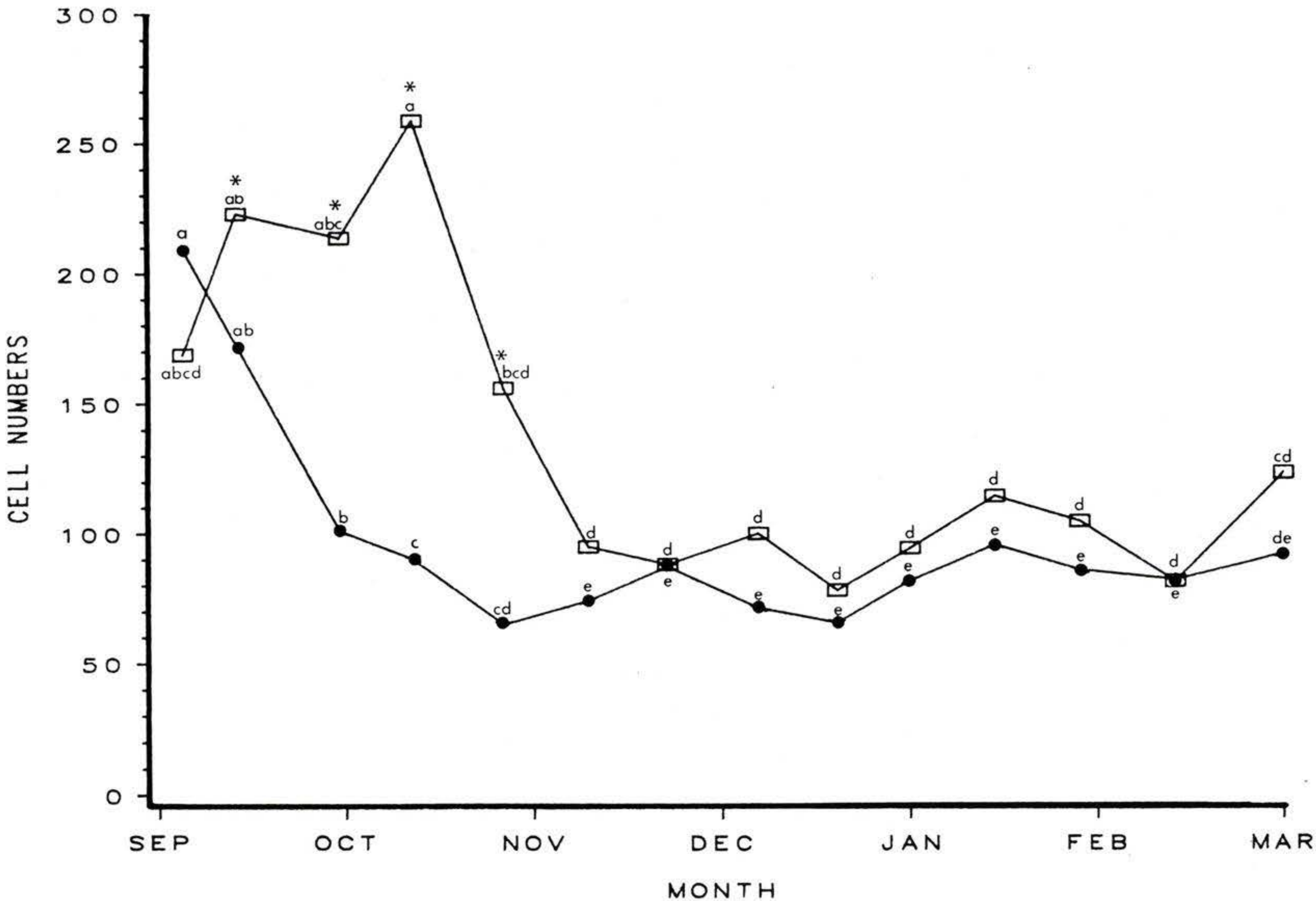

MITOTIC INDEX
3.0
2.5
2.0
1.5
1.0
0.5
0.0
SEP
OCT
NOV
DEC
JAN
FEB
MAR
MONTH

Figure 4: The average number of cells per median section of apices from coastal (□) and interior (●) Douglas fir based on 4-8 apices per collection.

Month abbreviations indicate the first day of the month.

Means on successive dates with the same letter are not significantly different at the $\alpha=0.05$ level using Duncan's multiple range test and asterisks (*) indicate significant differences between varieties at the $\alpha=0.05$ level from paired contrasts.



300
250
200
150
100
50
0
CELL NUMBERS
SEP
OCT
NOV
DEC
JAN
FEB
MAR
MONTH

Figure 5: The average apical height per median section of of coastal (□) and interior (●) Douglas fir based on 4-8 apices per collection.

Month abbreviations indicate the first day of the month.

Means on successive dates with the same letter are not significantly different at the $\alpha=0.05$ level using Duncan's multiple range test and asterisks (*) indicate significant differences between varieties at the $\alpha=0.05$ level from paired contrasts.

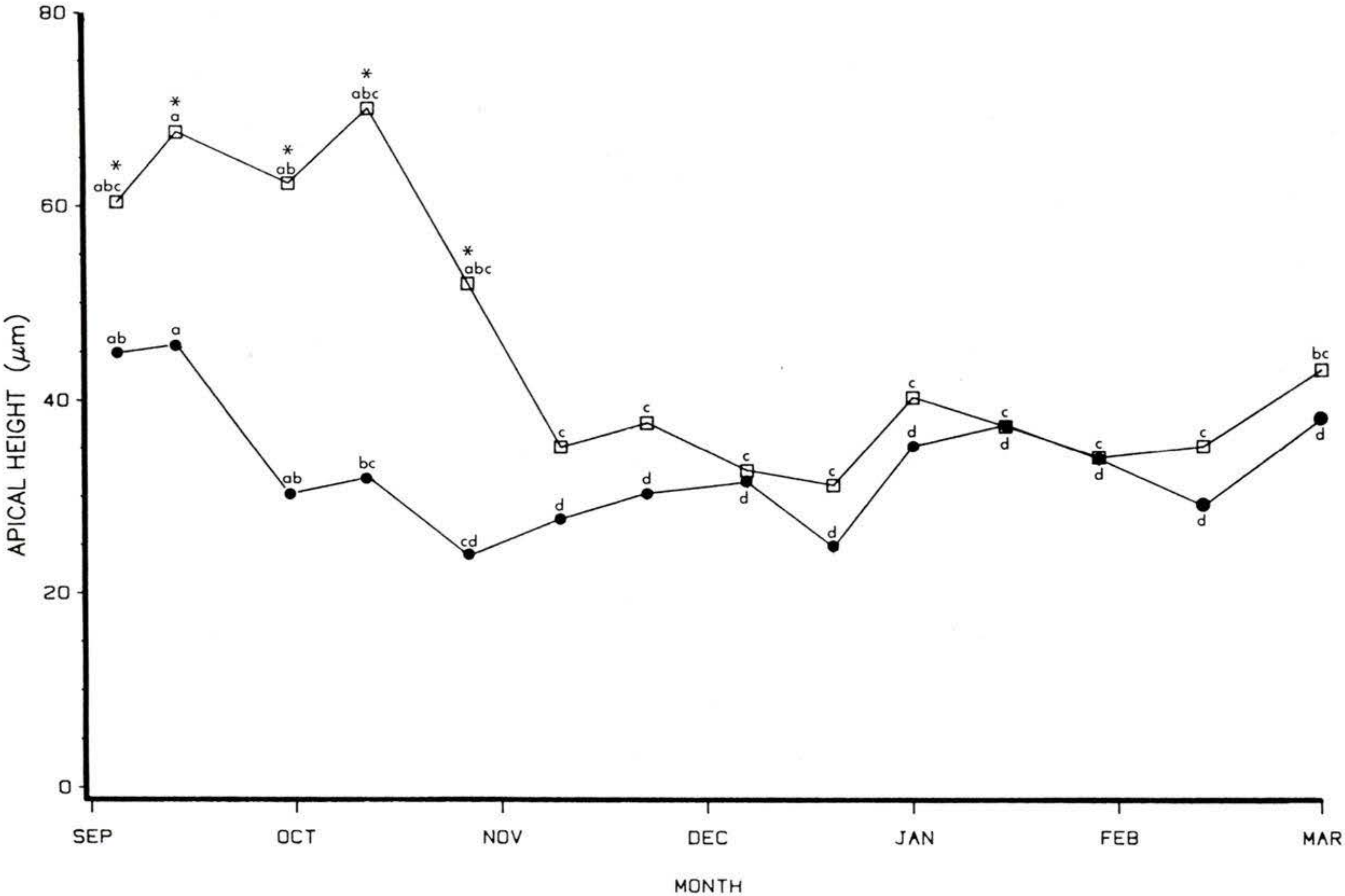
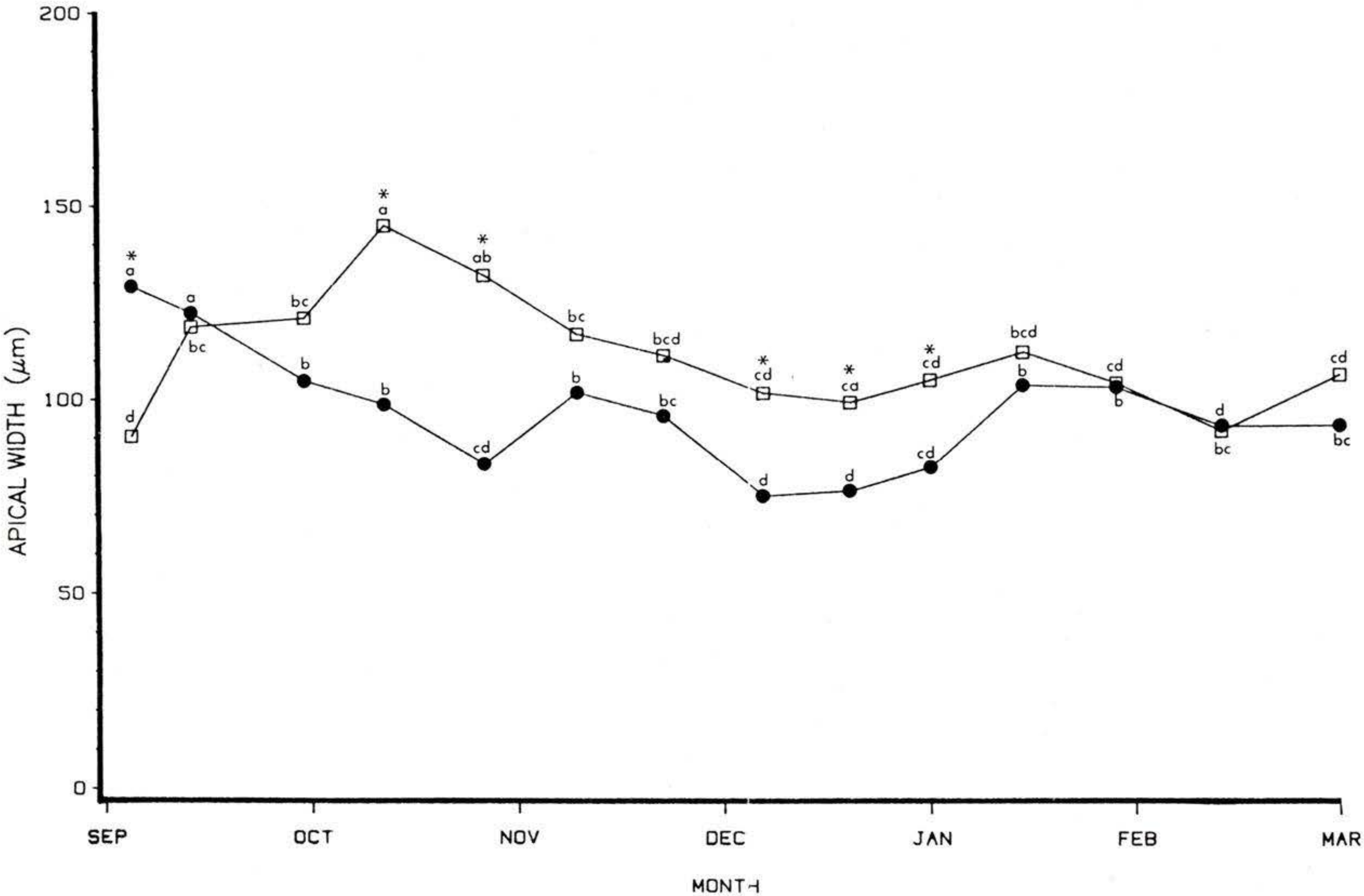

80
60
40
20
0
APICAL HEIGHT (μm)
SEP
OCT
NOV
DEC
JAN
FEB
MAR
MONTH

Figure 6: The average apical width per median section of of coastal (□) and interior (●) Douglas fir based on 4-8 apices per collection.

Month abbreviations indicate the first day of the month.

Means on successive dates with the same letter are not significantly different at the $\alpha=0.05$ level using Duncan's multiple range test and asterisks (*) indicate significant differences between varieties at the $\alpha=0.05$ level from paired contrasts.

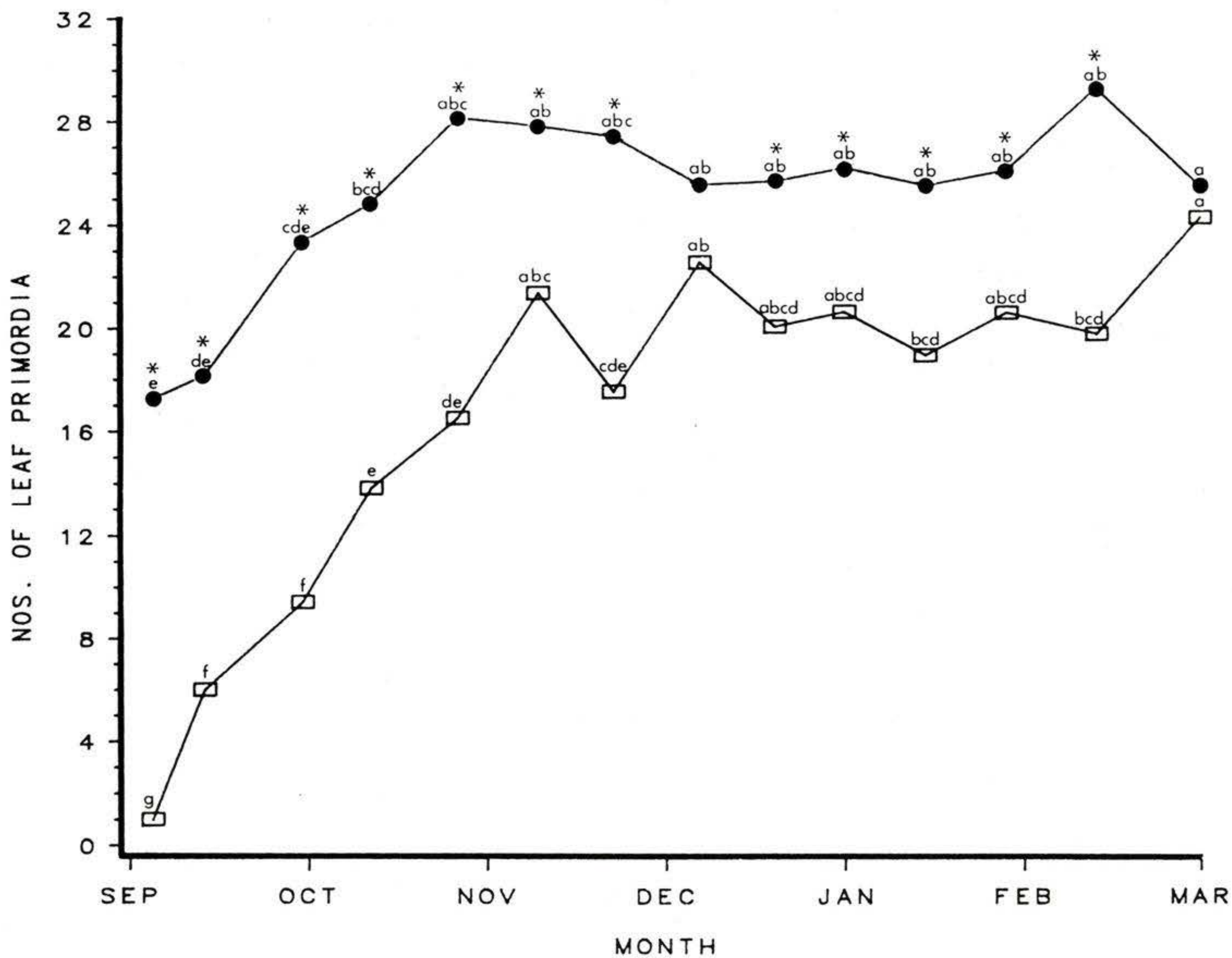


200
150
100
50
0
APICAL WIDTH (μm)
SEP
OCT
NOV
DEC
JAN
FEB
MAR
MONTH

Figure 7: The average number of leaf primordia per median section of embryonic shoots of coastal (□) and interior (●) Douglas fir based on 4-8 apices per collection.

Month abbreviations indicate the first day of the month.

Means on successive dates with the same letter are not significantly different at the $\alpha=0.05$ level using Duncan's multiple range test and asterisks (*) indicate significant differences between varieties at the $\alpha=0.05$ level from paired contrasts.



NOS. OF LEAF PRIMORDIA
0
4
8
12
16
20
24
28
32
SEP
OCT
NOV
DEC
JAN
FEB
MAR
MONTH
* e
* de
* cde
* bcd
* abc
* ab
* abc
ab
* ab
* ab
* ab
* ab
* ab
a
g
f
f
e
de
abc
cde
ab
abcd
abcd
bcd
abcd
bcd
a

Figure 8: Leaf Activity represented by the average number of cell divisions per leaf primordium per median section of embryonic shoots of coastal (□) and interior (●) Douglas fir based on 4-8 apices per collection.

Month abbreviations indicate the first day of the month.

Means on successive dates with the same letter are not significantly different at the $\alpha=0.05$ level. Asterisks (*) indicate significant differences between varieties at the $\alpha=0.05$ level. Chi-square tests (2x2) were employed throughout.

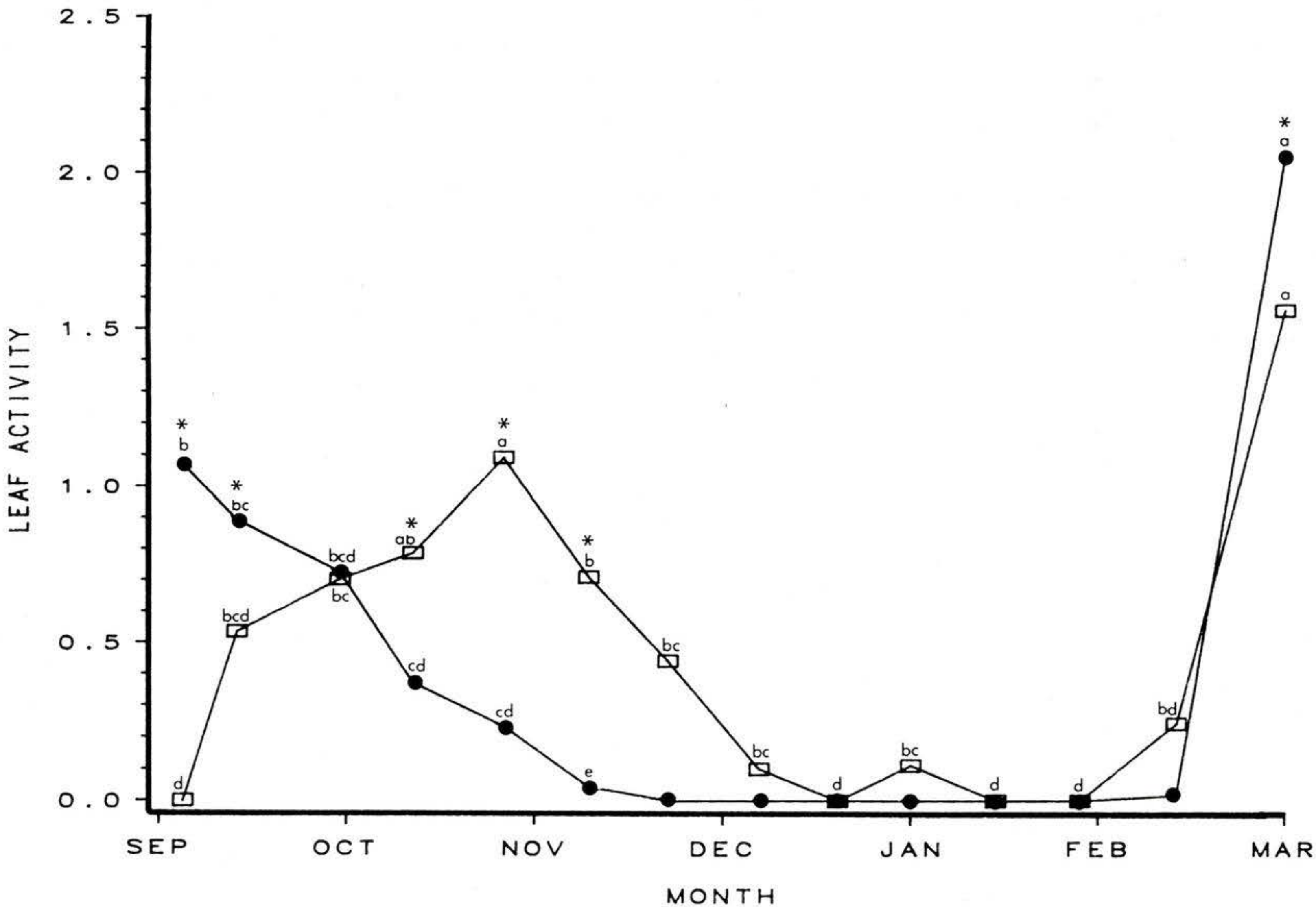
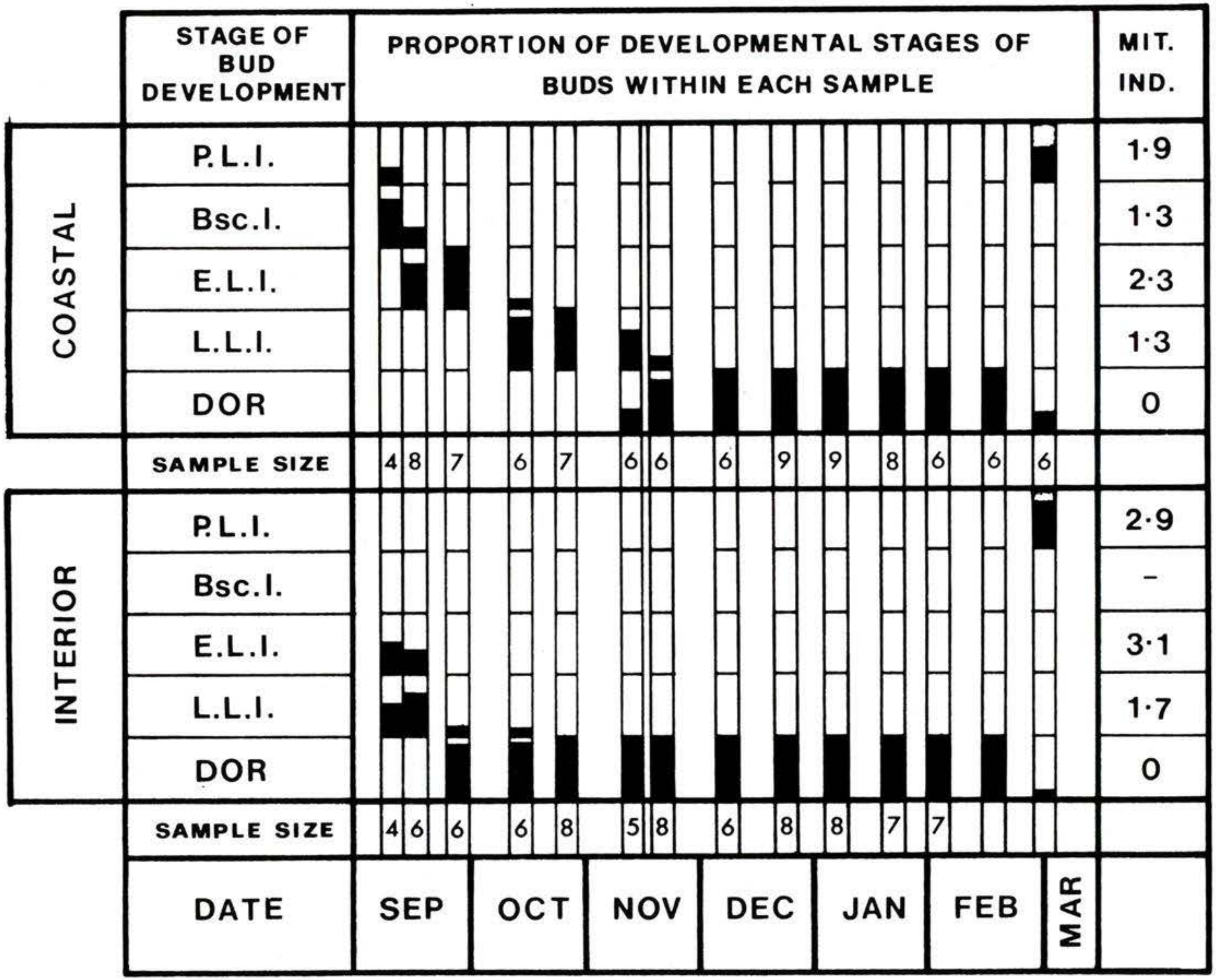

2.5
2.0
1.5
1.0
0.5
0.0
LEAF ACTIVITY
SEP
OCT
NOV
DEC
JAN
FEB
MAR
MONTH
*
b
*
bc
bcd
bc
bcd
d
*
ab
*
a
*
b
cd
cd
e
bc
bc
d
bc
d
d
bd
*
a
a

Figure 9: Percentage of apices at different stages of bud development per collection date and the mean average MI for all apices at each stage of development.

Stages of bud development included primary leaf initiation (P.L.I), bud-scale initiation (Bsc.I.), early leaf initiation (E.L.I), late leaf initiation (L.L.I) and dormant (DOR).


STAGE OF BUD DEVELOPMENT
PROPORTION OF DEVELOPMENTAL STAGES OF BUDS WITHIN EACH SAMPLE
MIT. IND.
COASTAL
P.L.I.
Bsc.I.
E.L.I.
L.L.I.
DOR
1·9
1·3
2·3
1·3
0
SAMPLE SIZE
4 8 7 6 7 6 6 6 9 9 8 6 6 6
INTERIOR
P.L.I.
Bsc.I.
E.L.I.
L.L.I.
DOR
2·9
–
3·1
1·7
0
SAMPLE SIZE
4 6 6 6 8 5 8 6 8 8 7 7
DATE
SEP
OCT
NOV
DEC
JAN
FEB
MAR

4.2 Root development

4.2.1 First year

Root activity had a number of components due to heterorhizic root development. Roots longer than 5mm consisted of only long laterals (elongating roots). Roots shorter than 4.9 mm were composed of short laterals and some elongating roots produced by reactivation of suberised long laterals, The proportion of long laterals in this size class may have been greater during the winter months when rates of elongation were low. Short laterals were produced in large numbers on secondary or tertiary laterals near the junction of root and shoot of the seedling.

Root development of coastal and interior varieties had two active periods, one in the fall and the other in early spring (Figs. 10, 11). Coastal seedlings began root elongation about two weeks later in the fall than interior seedlings, and root elongation was less vigorous in coastal seedlings (Fig. 10). The elongation of newly initiated long and short laterals and existing short laterals was highest in both varieties during September and October. Root elongation of interior seedlings ceased in mid-December, but that of the coastal variety did not stop during the winter although elongation slowed considerably.

Root elongation in all size classes occured in late-January to February in coastal seedlings (Fig. 10) but not until early March in the interior variety (Fig. 11). Elongation of long laterals was at a maximum in both varieties in early March. Both varieties showed a significant decrease in numbers of white roots in late-April indicating a reduction in both elongation and initiation of long and short lateral roots.

4.2.2 Second year

Counts of white roots longer than 10 mm gave an estimate of the amount of elongation of long laterals (Fig. 12, 13). Both varieties showed fluctuations in root elongation between August and mid-October. Root elongation of the coastal variety increased from a low level in late-August to a maximum in September then decreased September through December. In the interior variety, root elongation was high early in September, decreased later in September, increased during mid-October then decreased rapidly from October to early-November. This last decrease was more rapid than in the coastal variety.

Figure 10: Numbers of white roots in three length classes for 10 root systems of coastal Douglas fir seedings per collection date.

Vertical bars on each date indicate the frequency of;

White roots <1-4.9 mm,

White roots 5-9.9 mm,

White roots >10 mm.

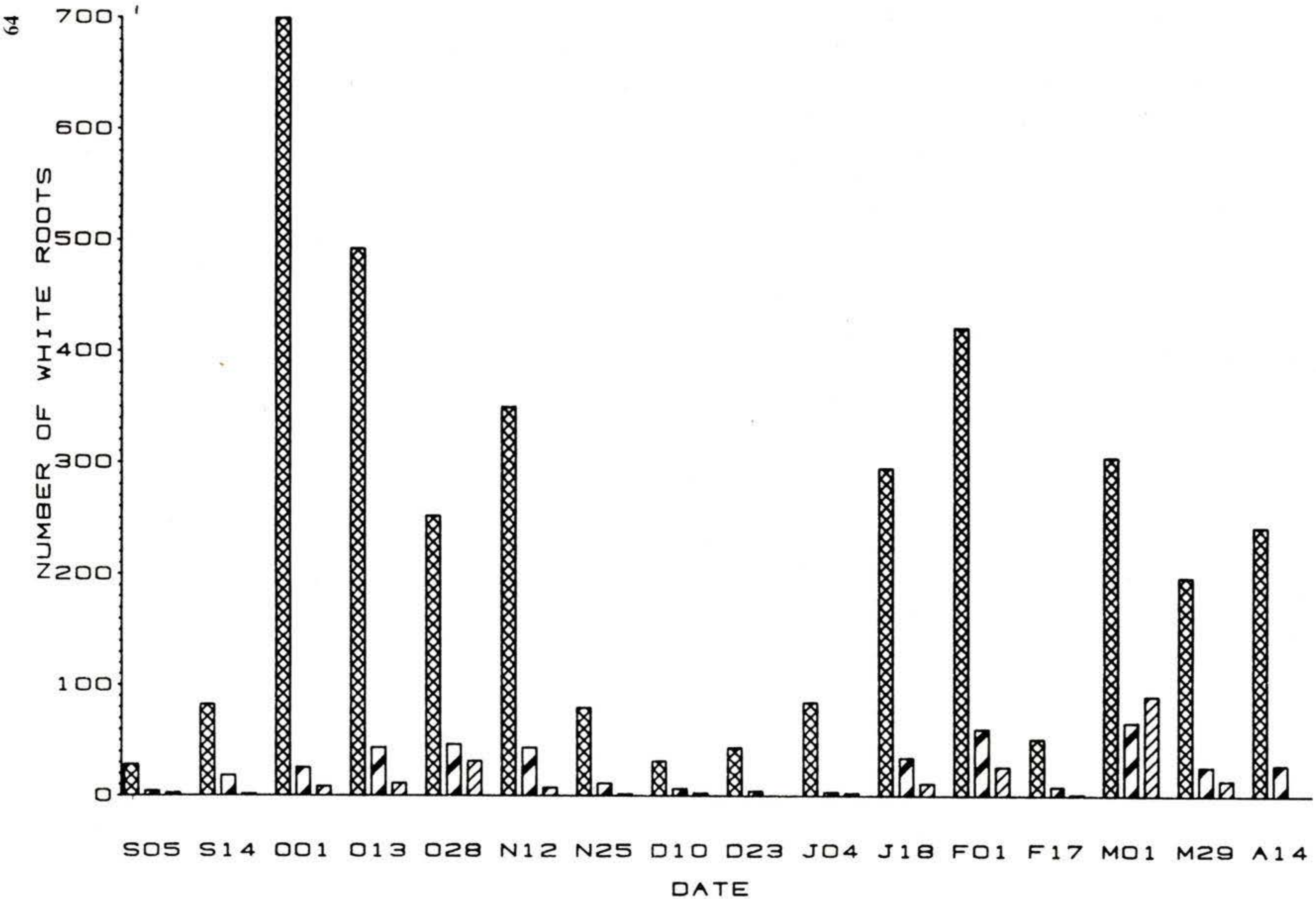

64
NUMBER OF WHITE ROOTS
700
600
500
400
300
200
100
0
S05 S14 O01 O13 O28 N12 N25 D10 D23 J04 J18 F01 F17 M01 M29 A14
DATE

Figure 11: Number of white roots in three length classes for 10 root systems of interior Douglas fir seedlings per collection date.

Vertical bars on each date indicate the frequency of;

White roots <1-4.9 mm,

White roots 5-9.9 mm,

White roots >10 mm.

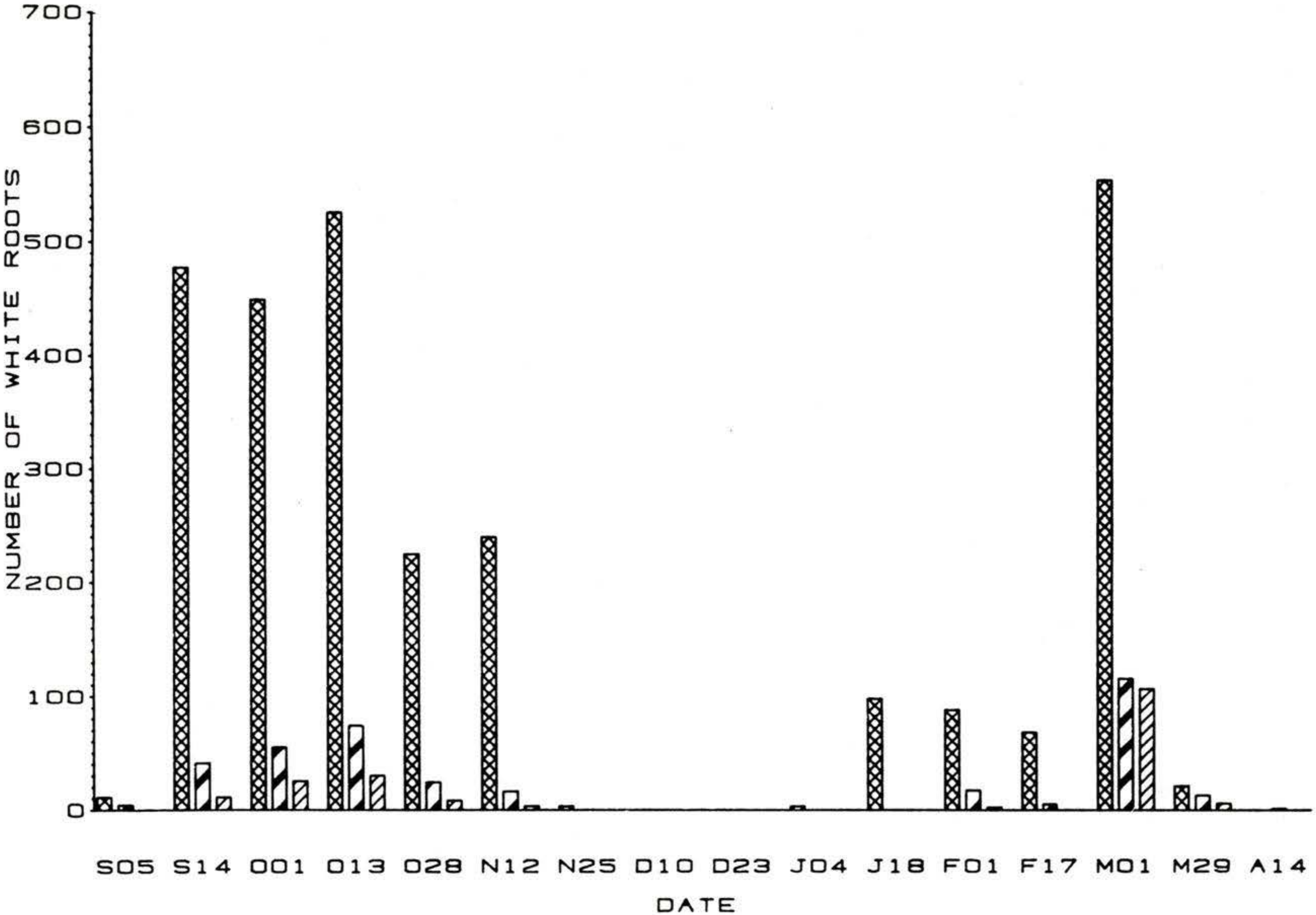

700
600
500
400
300
200
100
0
NUMBER OF WHITE ROOTS
S05 S14 O01 O13 O28 N12 N25 D10 D23 J04 J18 F01 F17 M01 M29 A14
DATE

Figure 12: Frequency of root systems of coastal Douglas fir seedlings in each class of Burdett's semi-quantitative activity index based on 40 seedlings per collection.

Vertical bars on each date indicate the frequency of;

 Root systems with no white roots,

 Presence of white roots <10mm,

 Presence 2-3 white roots >10mm.

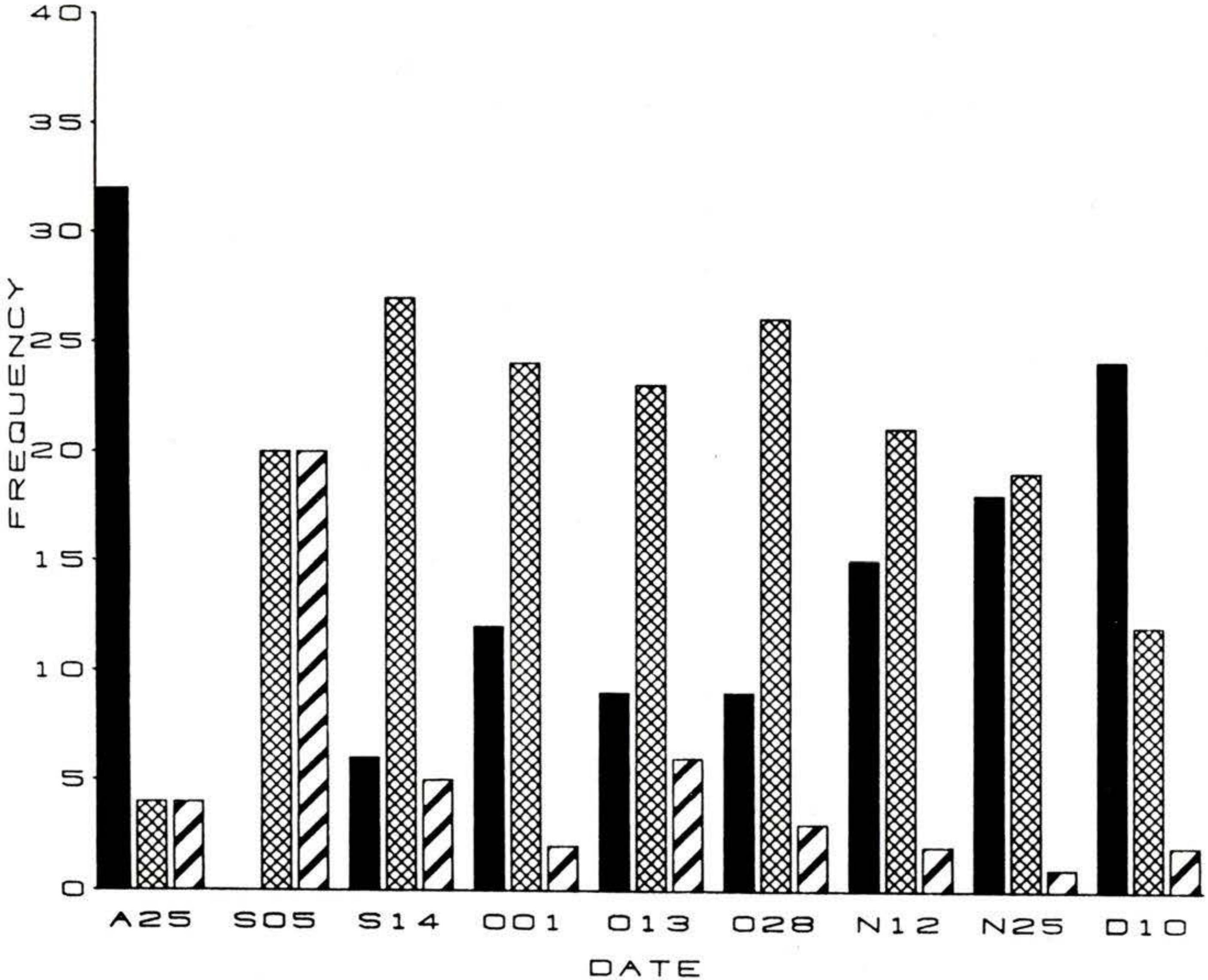
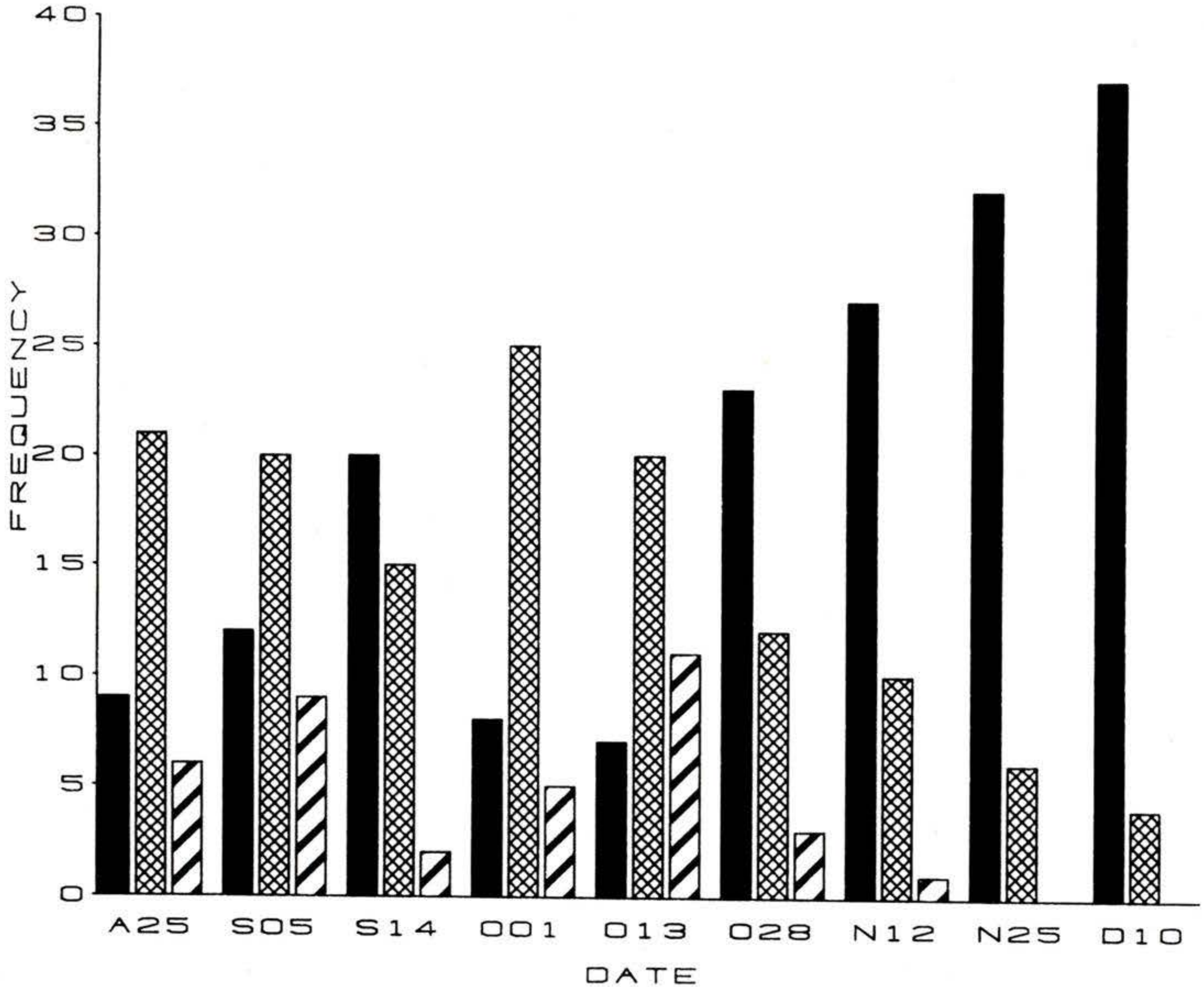

FREQUENCY
0
5
10
15
20
25
30
35
40
A25
S05
S14
O01
O13
O28
N12
N25
D10
DATE

Figure 13: Frequency of root systems of interior Douglas fir seedlings in each class of Burdett's semi-quantitative activity index based on 40 seedlings per collection.

Vertical bars on each date indicate the frequency of;

- Root systems with no white roots,
- Presence of white roots <10mm,
- Presence 2-3 white roots >10mm.


FREQUENCY
0
5
10
15
20
25
30
35
40
A25
S05
S14
O01
O13
O28
N12
N25
D10
DATE

4.3 Respiration of embryonic shoots

4.3.1 Total oxygen consumption

Respiratory rates of embryonic shoots were combined for the two years for each of the coastal and interior varieties (Fig. 14)

In late-August respiratory rates of both varieties were high (900-1200 natoms $O/min^{-1}.g^{-1}$) but decreased steadily to mid-October levels when rates were low (200-400 natoms $O/min^{-1}.g^{-1}$). Respiratory rates remained at this low basal rate between October and February. Respiratory rates remained slightly elevated until late-November in the coastal variety, whereas the interior variety tended to decrease to a basal rate of respiration in late-October. There was a trend for respiratory rate to decrease to a minimum in late-December and January. The higher fall respiratory rates in the coastal variety were shown most clearly in 1983/1984 data but are also indicated in 1982/1983 data (Fig. 14). In late-November there was a significant increase in respiration rate in the interior variety (α=0.05). Respiratory rates increased rapidly with the resumption of shoot elongation in March (Fig. 14). Regression of respiratory rate and MI was found to conform closely to a quadratic model in both varieties (Figs. 15, 16).

Respiratory rates were generally higher in the coastal variety than in the interior variety during August through October and during March. There was no difference from November through February.

Figure 14: Combined respiratory rates (natoms $O/min^{-1}.g^{-1})$ for 1982/1983 and 1983/1984 of developing embryonic shoots of coastal and interior Douglas fir seedlings.

Data from 1982/1983 are shown with a continuous line and data from 1983/1984 are shown with a dotted line for coastal ( ) and interior ( ) Douglas fir seedlings.

Each mean is based upon 2-5 replicates of 10 embryonic shoots during the two years.

Means on successive dates with the same letter are not significantly different at the α=0.05 level using Duncan's multiple range test in 1982/1983 (Appendix A).

Asterisks indicate significant differences between varieties at the α=0.05 level from t tests performed on 1982/1983 (**) and 1983/1984 (*) data.

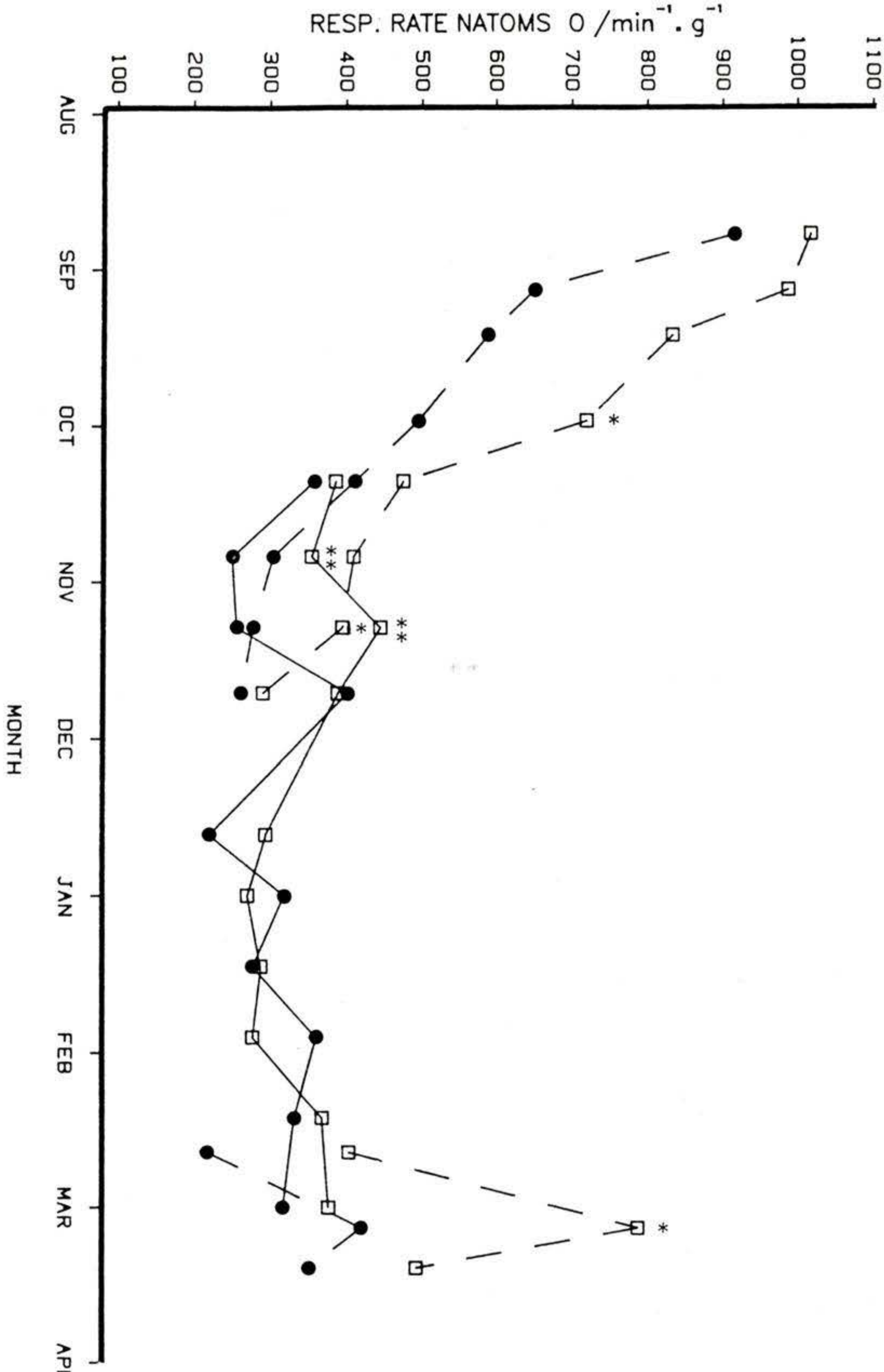
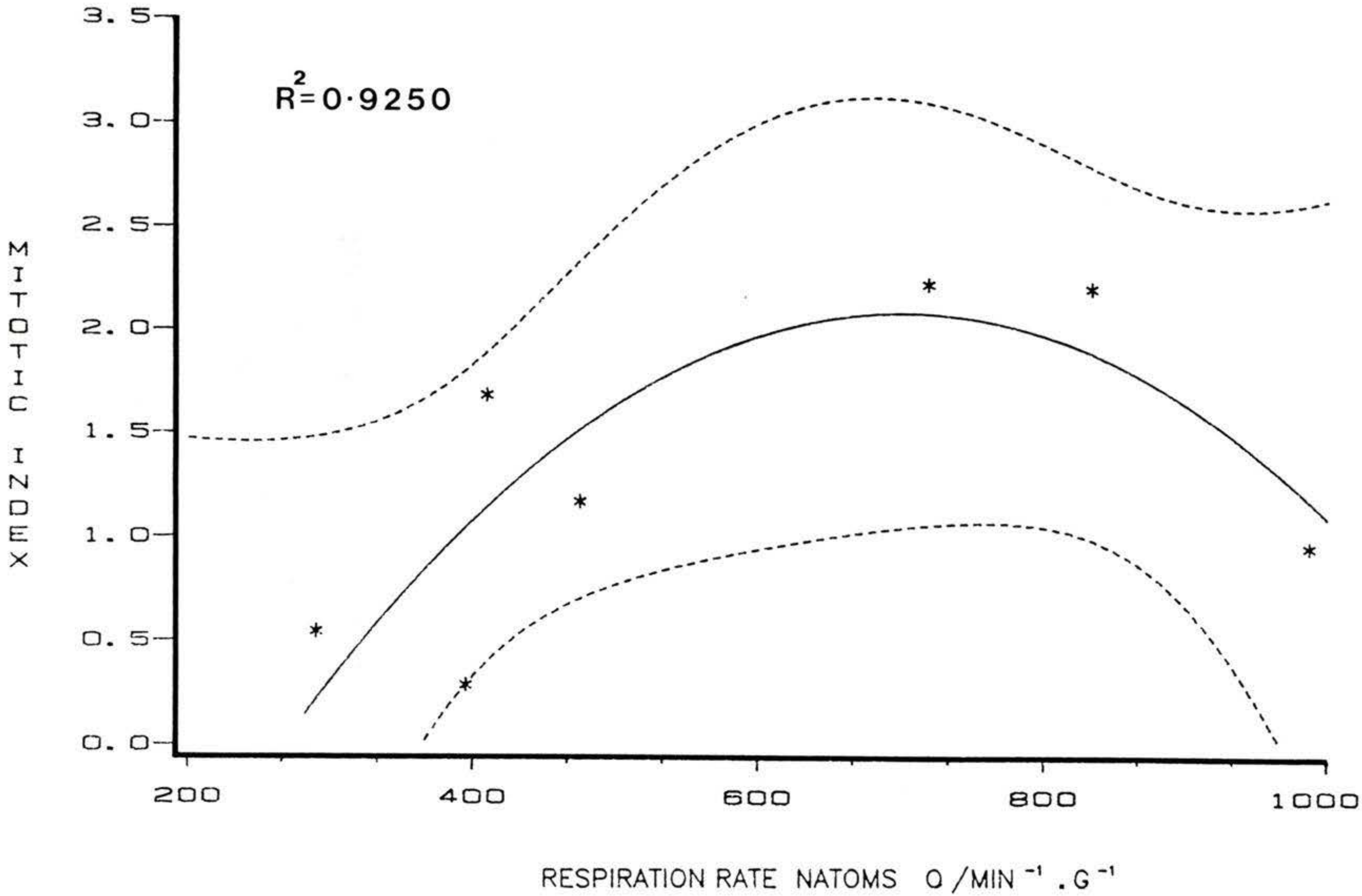

RESP. RATE NATOMS O /min^{-1}. g^{-1}
100
200
300
400
500
600
700
800
900
1000
1100
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MONTH
*
**
*
**
*

Figure 15: Quadratic regression curve of mitotic index (MI) and respiratory rates of embryonic shoots of coastal Douglas fir seedlings.

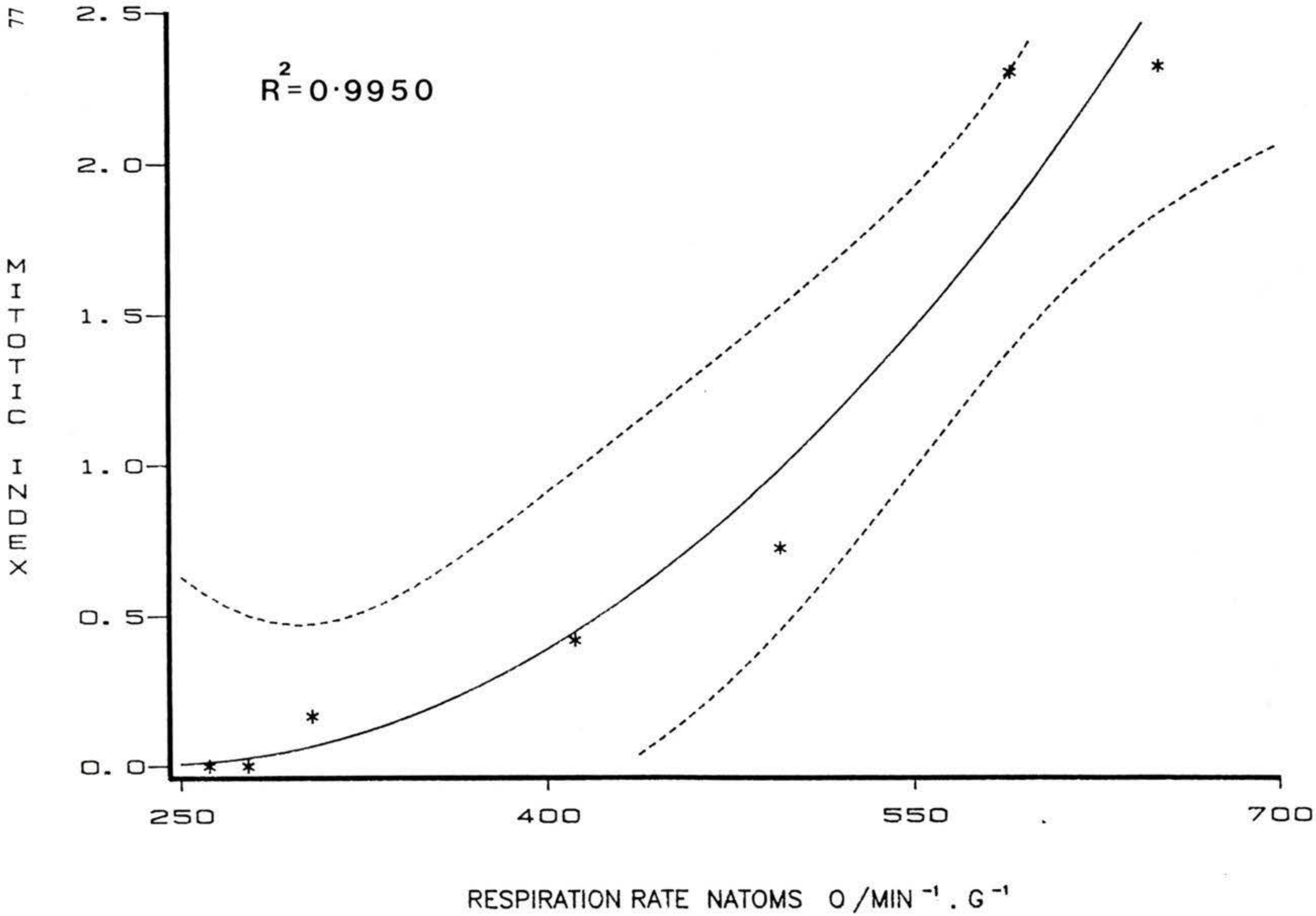
Broken lines indicate 95% confidence intervals about the regression curve.



R² = 0·9250
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
MITOTIC INDEX
200
400
600
800
1000
RESPIRATION RATE NATOMS O /MIN⁻¹ . G⁻¹

Figure 16: Quadratic regression curve of mitotic index (MI) and respiratory rates of embryonic shoots of interior Douglas fir seedlings.

Broken lines indicate 95% confidence intervals about the regression curve.



R² = 0·9950
MITOTIC INDEX
2.5
2.0
1.5
1.0
0.5
0.0
250
400
550
700
RESPIRATION RATE NATOMS O /MIN ⁻¹ . G ⁻¹

4.3.2 CN resistant respiration

Alternative pathway respiration did not vary significantly in either variety from mid-September through March (Tables 2, 3, 4, and 5).

During August and early-September (Tables 4, 5) the alternative and residual pathways in both varieties were very low compared with October levels. From September through February the alternative pathway accounted for between about 2 to 3 (Tables 2, 4) and 3 to 4.5 (Table 3, 5) percent of total respiration in coastal and interior varieties, respectively. Cyanide resistance accounted for between 6 and 27 percent of total respiration, and did not vary significantly from October through March. Resdual respiration was variable both within and between varieties and years and accounted for the majority of variation in CN-resistant respiration. There was an apparent increase in cyanide resistance in both varieties from approximately 11 percent during September through December to about 30 percent in early and mid-March in coastal and interior varieties due to the contribution of both residual and alternative respiration (Tables 4, 5).

Table 2: Percentage[1] of CN-resistant respiration due to alternative and residual respiration after sequential inhibition by CN and SHAM of isolated embryonic shoots of interior Douglas fir seedlings in 1982/1983

Date	Alternative	Residual	Total (CN-resistance)
Oct 13	4.73 a[2]	29.94 a	35.01 a
Oct 28	-1.52 a	28.98 a	27.45 ab
Nov 12	5.10 a	16.27 bcd	21.36 ab
Nov 26	-1.15 a	16.85 bc	15.70 b
Dec 23	-3.00 a	24.16 cde	16.61 b
Jan 4	8.74 a	10.47 cde	10.64 b
Jan 18	3.76 a	11.32 cde	15.08 b *[3]
Feb 1	5.04 a	9.42 cde	15.24 b
Feb 17	7.80 a	6.62 de	14.44 b
Mar 1	6.70 a	5.15 e	11.84 b

1 Each mean based on 3-5 replicates of 10 embryonic shoots.
2 Means with the same letter are not significantly different ($\alpha=0.05$) within the same column (Duncan's multiple range test).
3 Means with asterisks indicate significant differences between varieties on this date at the $\alpha=0.05$ level (Student t test).

Table 3: Percentage[1] of CN-resistant respiration due to alternative and residual respiration after sequential inhibition by CN and SHAM of isolated embryonic shoots of coastal Douglas fir seedlings in 1982/1983

Date	Alternative		Residual		Total (CN-resistant)	
Oct 13	-0.75	ab[2]	22.73	a	21.98	a
Oct 28	0.47	ab	22.22	a	22.69	a
Nov 12	1.22	ab	17.46	a	18.68	ab
Nov 26	5.25	a	14.27	a	20.47	ab
Dec 23	-12.51	b	21.29	a	8.78	bc
Jan 4	1.33	ab	19.50	a	20.83	a
Jan 18	-9.48	b	14.10	a	5.12	c *[3]
Feb 1	11.02	a	6.36	a	17.47	ab
Feb 17[4]					16.10	abc
Mar 1	10.79	a	7.25	a	18.04	ab

1 Each mean based on 2-5 replicates of 10 embryonic shoots.
2 Means with the same letter are not significantly different ($\alpha=0.05$) within the same column (Duncans's multiple range test).
3 Means with asterisks indicate significant differences between varieties on this date at the $\alpha=0.05$ level (Student t test).
4 Data missing for alternative and residual components on this date.

Table 4: Percentage[1] of CN-resistant respiration due to alternative and residual respiration after sequential inhibition by CN and SHAM of isolated embryonic shoots of coastal Douglas fir seedlings in 1983/1984

Date	Alternative	Residual	Total CN-resistant
Aug 25	0.00 c[2]	0.00 e	0.00 f
Sep 5	0.00 c	0.00 e *[3]	0.00 f *
Sep 14	2.68 bc	0.00 e	2.68 ef
Oct 1	4.59 ab	10.84 bc	15.40 b *
Oct 13	2.89 abc	3.28 de *	6.15 de *
Oct 28	0.85 bc	5.88 cd *	6.73 d *
Nov 12	0.91 bc	8.79 bc	9.67 cd
Nov 26	0.27 bc	11.32 b	11.59 bc
Feb 24	4.40 abc	8.96 b *	12.27 bc *
Mar 5	5.39 abc	22.21 a *	27.61 a *
Mar 13	8.07 a	19.81 a	27.88 a

1 Each mean based on 3-5 reps. of 10-20 embryonic shoots.
2 Means with the same letter are not significantly different (α=0.05) within the same column (Duncan's multiple range test).
3 Means with asterisks indicate significant differences between varieties on these dates at the α=0.05 level (Student t test).

Table 5: Percentage[1] of CN-resistant respiration due to alternative and residual respiration after sequential inhibition by CN and SHAM of isolated embryonic shoots of interior Douglas fir seedlings in 1983/1984

Date	Alternative		Residual		Total (CN-resistant)	
Aug 25	0.94	d[2]	0.00	e	0.94	d
Sep 5	0.59	cd	9.86	d *[3]	10.44	c *
Sep 14	6.46	bc	1.11	e	7.57	c
Oct 1	4.94	bcd	11.95	bcd	16.87	bc *
Oct 13	1.88	cd	7.80	d *	9.50	c *
Oct 28	3.92	cd	8.82	d *	12.73	c *
Nov 12	1.22	cd	9.95	d	11.20	c
Nov 26	-0.37	d	11.47	cd	11.37	c
Feb 24	10.04	ab	16.89	ab *	26.92	b *
Mar 5	4.60	bcd	16.03	bc *	20.63	b *
Mar 13	13.23	a	21.14	a *	34.38	a *

[1] Each mean based on 4-5 reps. of 10-20 embryonic shoots.
[2] Means with the same letter do not differ significantly ($\alpha=0.05$) within the same column (Duncan's multiple range test).
[3] Means with asterisks indicate significant differences between varieties on these dates at the $\alpha=0.05$ level (Student t test).

Chapter V

DISCUSSION

The purpose of this study was to relate anatomical development of seedling apices to respiration of the shoot tip and both of these to the many physiological stages and phases of dormancy found in the literature. The interpretation of data from these two artificially separated but realistically inseparable aspects of seedling development is a considerable challenge since, morphological and physiological aspects of development have previously been studied in isolation.

In this discussion morphological and anatomical development of the two varieties are compared in terms of their morphological stages (Owens and Molder 1973a), and the implications of developmental differences are examined. Respiratory characteristics of embryonic shoots are discussed with reference to morphological development. The implications of both anatomical and physiological approaches to the concept of dormancy is presented in a separate section. The desired outcome is to demonstrate the inherent weakness in approaching the problems of seedling development from either a physiological of morphological point of view alone. A more complete picture of seedling dormancy must come from an integration of both approaches.

5.1 The growth cycle of seedlings in relation to that of mature trees

The phenology of bud development in seedlings and mature trees and their relationship to shoot elongation differ and are represented in Fig. 17. Differences occur in the phenology with respect to shoot elongation and the duration of apical developmental stages. In mature conifers the preformed overwintering bud partly determines the capacity for shoot growth in the following year, whereas seedlings undergo a period of free growth before bud development begins (Pollard *et al.* 1975).

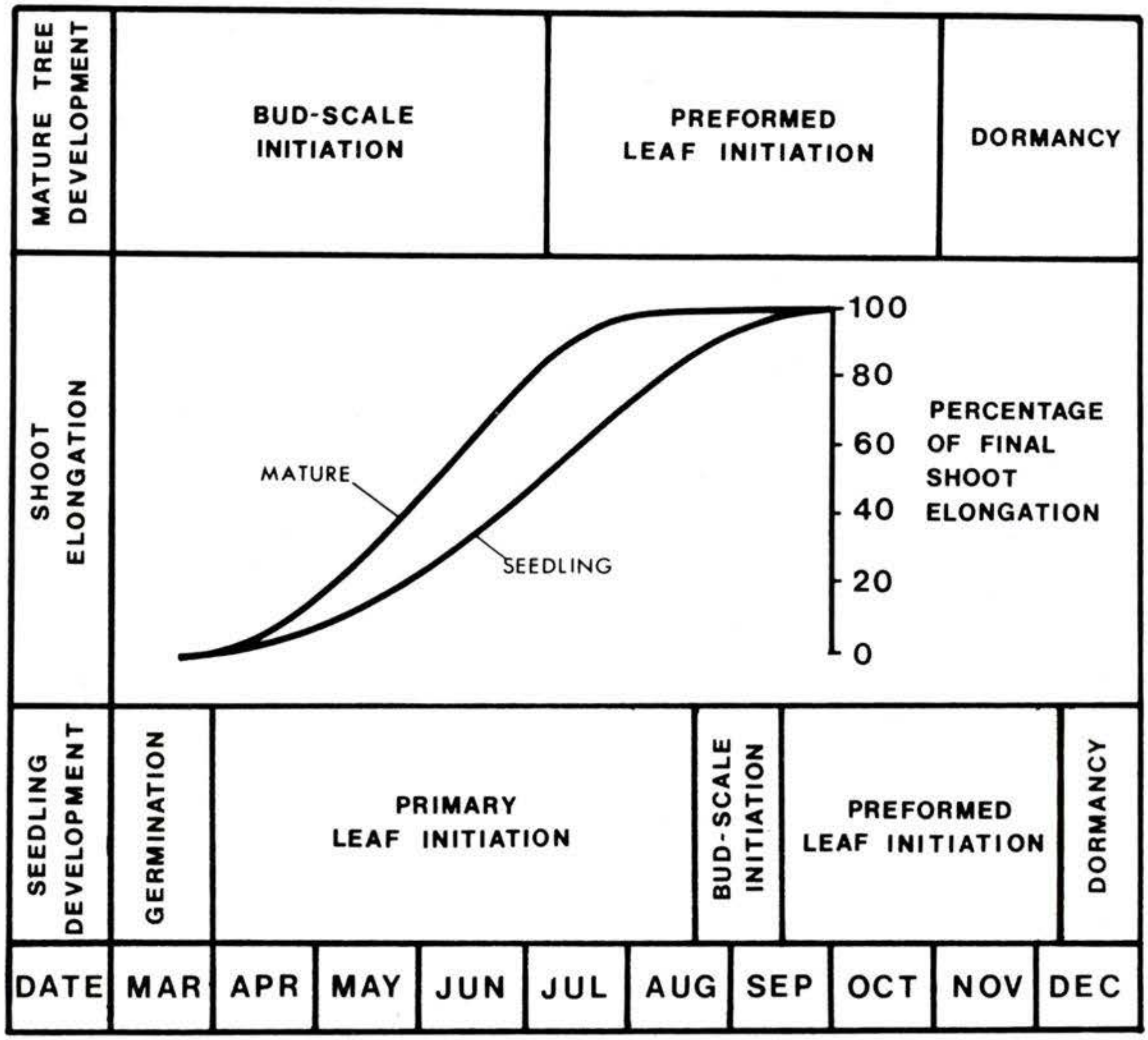
Lateral buds of mature Douglas fir begin bud-scale initiation in the spring soon after apical mitotic activity begins (Owens and Molder 1973a). This is several weeks before the emergence of the new shoots. Bud-scale initiation continues until the end of lateral shoot elongation (Owens *et al.* 1985). This pattern of bud development also occurs in lateral shoots of most other mature members of the Abietoideae (Owens and Molder 1973a, 1976, 1977b; Owens *et al.* 1977).

In terminal shoots of seedlings of the Abietoideae, primary leaves are initiated during shoot elongation and bud-scale initiation does not begin until free growth of the shoot is almost completed (Jablanczy 1971). In the present study morphological events of embryonic shoot formation for Douglas fir seedlings once free growth had ended were similar to those in mature trees except that all events began later in seedlings. In seedlings bud-scale initiation began in late July or August instead of March in mature trees (Owens and Molder 1973a). Owens and Molder (1973a) indicated that bud-scale initiation lasted about three months and preformed leaf initiation four months in mature coastal Douglas fir. In seedlings primary leaf initiation lasted about four months, bud-scale initiation one month, and preformed leaf initiation three months (Figs. 17, 18).

With few exceptions, mature temperate members of the Pinaceae do not undergo free growth during the period of shoot elongation. In most (Lines and Mitchel 1966; Jablanczy 1971) including Douglas fir (Owens and Molder 1973a) the potential for free growth diminishes with age ceasing after 5-10 years (Pollard and Logan 1976). A few, however, retain the capacity for free growth to some extent. Western Hemlock may initiate a few leaf primordia in some lateral buds before bud-scale initiation begins in the spring (Owens and Molder 1984) and long shoots of mature *Larix* undergo some free growth each spring (Remfrey and Powell 1984).

Figure 17: Phenology of lateral bud development and lateral shoot elongation in mature trees and terminal bud development and primary shoot elongation in seedlings.

Data for bud development and shoot elongation for mature coastal Douglas fir was taken from Owens *et al.* (1985). Data for seedling bud development was that of the coastal Douglas fir in this study and shoot elongation was taken from B.C. Ministry of Forests Records (Matthews 1985 pers. comm.)


MATURE TREE DEVELOPMENT
BUD-SCALE INITIATION
PREFORMED LEAF INITIATION
DORMANCY
SHOOT ELONGATION
MATURE
SEEDLING
100
80
60
40
20
0
PERCENTAGE OF FINAL SHOOT ELONGATION
SEEDLING DEVELOPMENT
GERMINATION
PRIMARY LEAF INITIATION
BUD-SCALE INITIATION
PREFORMED LEAF INITIATION
DORMANCY
DATE
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

5.2 Phenological differences in development between coastal and interior varieties of Douglas fir

5.2.1 Primary leaf initiation and bud development

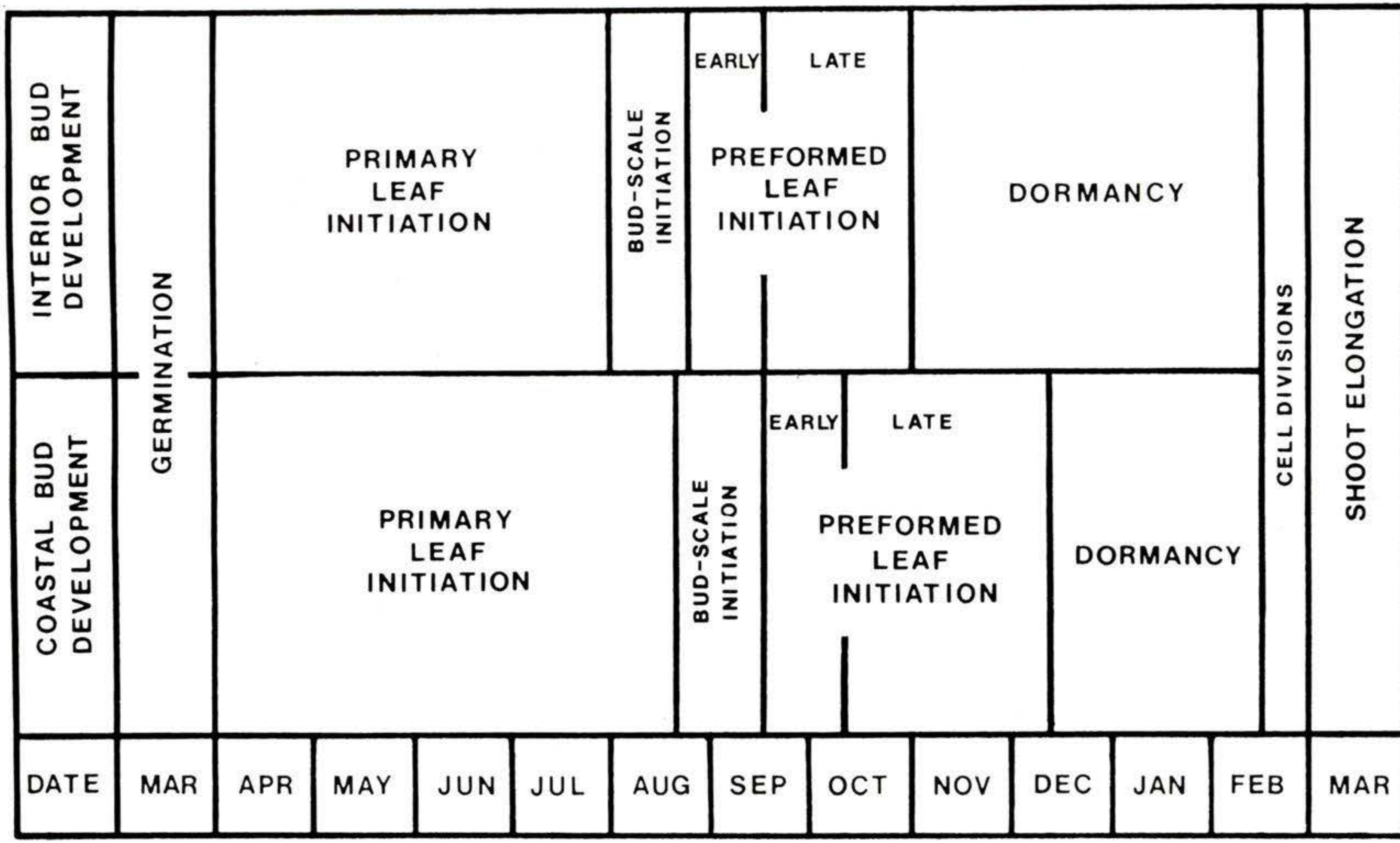
The two varieties of Douglas fir seedlings differed in the timing of their developmental stages. The interior variety was approximately four weeks in advance of comparable stages in the coastal variety. This phenological difference resulted from a longer free growth period and a longer period of bud development following free growth in the coastal variety (Fig. 18).

Free growth had ended in both varieties when sampling began in September. The interior variety was already undergoing preformed leaf initiation whereas the coastal variety had only initiated a few bud scales. However, past evidence suggests that the earlier completion of bud development in the interior variety was due to a shorter free growth period than that of the coastal variety (Irgens-Moller 1968). Inductive 8hr photoperiods applied from germination (Ingens-Moller 1968) and 9hr photoperiods after July (Lavender and Overton 1972) induced the cessation of free growth earlier in the interior than the coastal Douglas fir seedlings. Furthermore, height growth curves for the same seedlots grown under the same nursery conditions showed that the interior variety reached its maximum height approximately three to four weeks before the coastal variety (Matthews 1985 pers. comm.)

Bud development following free growth also contributed towards the differences in phenology between the two varieties. During bud development the MI was higher in apices at comparable morphological stages in the coastal than the interior variety. The decrease in MI was also more rapid in the interior than the coastal variety during comparable stages. These results are similar to those in a study of white spruce, where northern provenances formed the bulk of their preformed leaves within four weeks. After the same four week period, the southern provenances had fewer preformed leaf primordia, but they surpassed the northern provenances in numbers of preformed leaves after eight weeks (Pollard *et al.* 1975). Rapid bud development was suggested to be linked to the avoidance of environmental conditions associated with a continental climate when white spruce seedlings and coastal Sitka spruce seedlings were compared (Pollard *et al.* 1975). The ability in the interior variety of Douglas fir to complete bud development rapidly, even under coastal conditions, indicates that this is a genetically fixed character. Other studies also indicate that the duration of preformed leaf initiation and the number of primordia initiated in seedlings is under genetic control (Pollard and Logan 1973, 1977; Pollard *et al.* 1975). Further investigation would be required to clearly establish this in coastal and interior varieties of Douglas fir seedlings. This would require a study of all aspects of bud morphogenesis, including numbers and rates of production of preformed leaf primordia and the partitioning of growth between the production of leaf primordia and new apical tissue, to determine exactly where the differences in bud morphogenesis lie.

Sarvas (1974) stated that the length of the active height growth period in temperate plants determines the competitiveness of the individual. If there is a cold period, then the active height growth period must be shorter so that its effects do not interrupt the vital functions involved in bud development. This principle was demonstrated in this study by the continuation of free growth of the

Figure 18: A comparison of stages of apical development of coastal and interior Douglas fir seedlings in the first year of growth.


INTERIOR BUD DEVELOPMENT
GERMINATION
PRIMARY LEAF INITIATION
BUD-SCALE INITIATION
EARLY
LATE
PREFORMED LEAF INITIATION
DORMANCY
CELL DIVISIONS
SHOOT ELONGATION
COASTAL BUD DEVELOPMENT
PRIMARY LEAF INITIATION
BUD-SCALE INITIATION
EARLY
LATE
PREFORMED LEAF INITIATION
DORMANCY
DATE
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR

coastal variety into the fall and early cessation of free growth in the interior variety. Prompt response to inductive photoperiods and the lack of sensitivity to late summer and fall temperatures favourable to growth (Lavender and Overton 1972) enable the interior variety to respond to the environmental cues of low summer precipitation (Hermann and Lavender 1968; Irgens-Moller 1968; White *et al.* 1979; Sorensen 1983) and a short frost-free season (Irgens-Moller 1967; Griffin and Ching 1977; Emmington 1977; Sorensen 1983). In contrast, the coastal variety was reported to respond relatively slowly to inductive photoperiods but responded to warm fall temperatures with extended growth (Lavender *et al.* 1968). The coastal genotype is opportunistic by allowing continued growth in the fall. This may constitute a competitive advantage in the intensly competitive lush wet growing conditions in many parts of of the Pacific Northwest and this exploitation of the free growth stage may contribute in a cumulative way to the vigour of the tree in future years (Pollard *et al.* 1975).

The rate of preformed leaf initiation, and the number of primordia formed, are influenced by the conditions prevailing during this developmental stage. The coastal variety had significantly greater apical width but fewer preformed leaf primordia than the interior variety during comparable stages of initiation. This suggestes that the interior variety produced more preformed leaf primordia in the last stages of preformed leaf initiation. Paradoxically this would indicate the interior variety would have a greater potential for elongation in the following year. However, early development of the interior variety under coastal conditions prevailing during August and September may have modified its response from those under continental conditions. The effect of temperature (optimum 25°C) was greater than that of moisture stress, photoperiod, and light intensity on increasing the numbers of preformed leaf primordia produced by apices of Sitka spruce seedlings (Pollard and Logan 1977). Columbo (1982) also found that warm temperatures during preformed leaf initiation resulted in greater numbers of needle primordia in black spruce and jack pine. Therfore, whereas conditions were optimal for bud development in the interior variety during preformed leaf initiation, conditions may have been suboptimal for the coastal variety at the same developmental stage later in the year.

Most seedlings are capable of free growth for some years following germination (Lines and Mitchel 1966) thus it is difficult without further study to ascertain the importance of preformed stem units in relation to free growth in succeeding years. Further investigation to determine the contribution of preformed stem units to seedling height growth and the role of temperature in bud development in the two varieties would be of great use to those wishing to manipulate this stage of morphogenesis under nursery conditions.

5.2.2 Shoot elongation following dormancy

Cell divisions were first observed in several individuals of both varieties on the 14th of February. This was approximately two weeks before cell divisions were seen in all, and the beginning of shoot elongation in some, individuals. Owens *et al.* (1985) reported that cell division preceeded cell elongation in the shoot axis in mature Douglas fir. They showed cell division existed as a distinct stage preceeding cell elongation.

By 1 March there was an apparently greater number of cell divisions in embryonic shoots of the interior variety. This difference was not significant at the

$\alpha=0.05$ level in this study owing to small sample sizes, however, Anon.(1985) showed a difference in MI between the same two seed lots. Had samples been increased in size to overcome the variability of material and performed more frequently during this critical two week period, the basipetal progression of mitotic activity observed by Owens (1968) in mature Douglas fir embryonic shoots might have been seen to progress at different rates in the two varieties resulting in the observed differences.

Intravarietal and intervarietal flushing time was quite variable although a greater proportion of individuals from the interior variety flushed earlier than those of the coastal variety. Owens *et al.* (1985) showed that 10% of final shoot elongation occurred before flushing. Thus, two separate processes may be contributing to the final timing of bud flush: i) rate of cell division following resumption of activity; and, ii) rate of cell elongation following cell division. Since the cell division stage appears to be shorter in the interior variety and Campbell and Sugano (1975) reported that interior Douglas fir sources were able to elongate more rapidly than coastal sources, both processes may be operating in timing of flushing. Such differences would be expected in interior and coastal varieties of Douglas fir since the interior variety has a shorter period for shoot elongation (Sorensen 1983).

Compared to developmental differences in the fall the resumption of cell divisions in the spring was almost synchronous in the two varieties. Sarvas (1974) was of the opinion that the chilling period functioned to zero the developmental clock with respect to the end of dormancy (indicated by date of flushing). Past studies using flushing to indicate the end of dormancy have shown the limitations of this criterion. Lavender *et al.* (1970) used flushing in response to increasing soil temperatures as an argument that soil temperature was increasing the export of postulated gibberellins from the roots. Another interpretation may be that temperature was directly affecting rate of cell division or cell elongation inside the buds. Similarly, the use of flushing in investigations concerning chilling requirements (van den Driessche 1975) gave no information of how different chilling periods affect both cell division and elongation and the importance of these two processes to flushing and subsequent growth and survival.

5.2.3 Shoot-root relations

The elongation of roots and resumption of mitotic activity in shoot apices may be closely correlated in Douglas fir seedlings as has been shown in broad leaved trees (Thelges and Beck 1976). Root elongation in interior Douglas fir increased markedly from a low level on the same sample date (1 March) as shoot apical mitotic activity but decreased during the early stages of shoot elongation. This trend was not as strong in the coastal variety where elongation before 1 March was greater, and more variable, and the increase in March smaller. Lavender and Hermann (1970) and Lavender *et al.* (1969) found chilling necessary for the surge in spring root growth, but bud development (flushing) and root growth were not correlated. They postulated that root activity in the spring was dependant upon a substance exported from the leaves. They attributed the periodicity of low summer, but high fall and spring root activities to soil moisture and not endogenous rhythms. However, there was a poor correlation between root and bud activity (Lavender *et al.* 1969). This may have been due to the fact that they did not determine the onset of bud development, rather, flushing was used to indicate the beginning of shoot growth. It could also have resulted from their choice of material since, in the present study, coincidence of root elongation and apical mitotic activity was less marked in the coastal variety than the interior variety. Other studies have also reported root elongation to preceed visible shoot elongation in Douglas fir (Kreuger and Trappe 1967) and *Cedrus atlantica* (Riedacker 1979). However, these workers also only measured shoot elongation and did not determine when cell division began in buds.

In the fall there was an increase in root elongation and numbers of short roots in both varieties. This occurred slightly later in the coastal variety than the interior variety. During this phase of root development embryonic shoots were undergoing late preformed leaf initiation in both varieties but stages of bud development were somewhat later in the coastal variety. Lavender *et al.* (1968) found root numbers to increase during October in coastal Douglas fir seedlings but attributed their development solely to cold nights. The difference in timing of fall root development between the two varieties suggest that the effect may be linked to development of the bud rather than solely cold nights.

5.3 Shoot apical MI of coastal and interior varieties of Douglas fir seedlings

The MI reflects the rate of apical development at any morphological stage. In September MI was considerably higher in the interior variety than the coastal variety at a similar stage of apical development. The mean generation time (Grif 1963) of apical cells of coastal or interior varieties are not known. If the equivalent mean generation time was the same in each variety, the higher MI in the interior variety would result in the more rapid production of preformed leaf primordia in the interior variety, at least in the later stages of preformed leaf initiation. However, Cannell (1978) suggested that differences in rates of initiation of preformed leaf primordia were due to the amount of new tissue apportioned to primordia. This interpretation does not demand a difference in the rate of production of new tissue, only a modification of the apportionment of tissue between apex and primordia. Since the size and even the true number of preformed leaf primordia cannot be accurately determined, this point remains speculative.

The mitotic activity of the apex may predict physiological changes in seedlings during their development. Mitotic activity decreased to zero following the completion of preformed leaf initiation in both varieties of Douglas fir seedlings in this study. Cessation of mitotic activity has been used to indicate that dormancy had begun in mature trees (Owens 1968; Owens and Molder 1973a). Carlson *et al.* (1980) also correlated the onset of dormancy and hardiness with the end of mitotic activity for coastal Douglas fir seedlings. Frost hardiness and storability in white spruce were also found to be correlated with MI (Simpson and Macey 1985).

Resumption of mitotic activity may also be useful in the prediction safe planting dates in the spring. In this study cell divisions were observed before any cell elongation associated with flushing, therefore buds that appear dormant may be mitotically active and less resistant to stress. These early cell divisions are important in the subsequent development of the embryonic shoot. They add significantly to the numbers of cells in the embryonic shoot and the amount of shoot elongation is partially determined by the number of cells which will elongate

(Owens *et al.* 1985). Experiments in which cell division at this time might be prevented or restricted, possibly through insufficient chilling or environmental stress, could provide valuable information regarding the role of cell division in subsequent seedling development. These findings could be important to the improvement and predictability of seedling stock which must often undergo considerable mistreatment during handling and transportation to the planting site.

5.4 Respiration of coastal and interior varieties of Douglas fir seedlings

The respiration rate and stage of development of the embryonic shoot were closely related. Mitotic index and respiration rate appeared to be correlated at certain times of the year, although this relationship may not have been a direct one. Mitotic index was plotted against respiration rate from September to October and a regression was performed. The best fit was achieved with a quadratic function (R^2=0.9250 and R^2=0.9950 in coastal and interior varieties respectively) although a simple linear regression also fitted the interior variety data very closely (R^2=0.8962). These relationships are presented with some caution since only seven data points were available. More data and larger sample sizes would be needed to confirm the correlation. The quadratic relationship between MI and respiration rate arose owing to low MI during the earliest stages of bud development in the coastal variety. Comparable early stages were not observed in the interior variety. The indication is that MI is not always directly related to respiration of the embryonic shoot. This inconsistency may have arisen from the variable nature of MI during the early stages of bud development (Anon. 1985), which may have been indicated if sampling had been more frequent during bud-scale initiation.

The number of cells undergoing DNA synthesis may contribute more to overall metabolic rates than the process to cell division since the mitotic phase in HeLa human cancer culture cells (Robbins and Morrill 1969) and plant cells (Erikson 1947; Stern and Kirk 1948) were found to have a low requirement for oxidative energy as were G1 and G2 phases of the cell cycle (van't Hof 1968). The S stage of the cell cycle was found to require the most energy (Robbins and Morrill 1969) suggesting that the MI may indicate the rapidity of the mean generation time (Grif 1963) and thus the intensity of cell synthesis. Histological tests for succinic dehydrogenase and peroxidase may have confirmed whether the embryonic shoot in the early stages of bud development, when it consisted largely of apical tissue, was undergoing more intense respiration than later stages when morphological events were progressing more rapidly.

The decrease in respiration of embryonic shoots during bud development in both varieties was partially a consequence of the reduction in the proportion of meristematic compared to differentiating tissues in the embryonic shoot. Respiration rates in the early autumn in both varieties were high as was the proportion of meristematic tissues. vanden Born (1960, 1963) reported high respiratory rates and succinic dehydrogenase activity in the apical region of the embryonic shoots of *Picea glauca* and Riding and Gifford (1971) reported high peroxidase activity in meristematic regions of *Pinus radiata* buds. Further development of the embryonic shoot, during preformed leaf initiation, resulted in an increase in the proportion of differentiating tissues: procambial strands, leaf primordia and pith cells containing ergastic substances. Ergastic substances, commonly tannin (polyhydroxyphenolic) compounds (Hejnowicz 1979) have been extensively reported (Gifford and Wetmore 1957; Owens and Molder 1973a, 1976, 1977; Owens *et al.* 1985). Kefeli and Kadyrova (1971) reported that a reduction in respiration accompanied their accumulation in callus cells of *Pinus elliotii* and that differentiation in embryonic shoots was accompanied by reduced meristematic activity and an increase in inhibitors.

The decrease in respiration in comparable stages in both varieties from the end of bud-scale initiation through preformed leaf initiation may be correlated with important physiological changes in the embryonic shoot. Komarnitskii *et al.* (1981) reported a similar decrease in respiration of flower primordia of several hardwoods following organogenesis. These workers demonstrated an inverse relationship between respiration rate and frost hardiness. Structural changes occur in chloroplasts during the hardening process (Senser *et al.* 1975) and electron transport is reduced (Martin *et al.* 1978 a, b; Ŏquist and Martin 1980).

In the present study the interior variety had a significant (α=0.05) peak (Appendix A) in respiration following the completion of leaf initiation and cell divisions in the apex. This peak coincided with the period of maximum frost hardiness and resistance to stress for samples from the same seed lot (Anon. 1985). The physiological significance of this peak is uncertain and is possibly due to an experimental artifact since a peak at a comparable stage of development was not observed in the coastal variety. However, the interior variety has been demonstrated to have a capacity to develop greater frost hardiness than the coastal variety (Ingens-Moller 1967; van den Driessche 1977). Thus, if this peak was due to some change in metabolism associated with the many active processes occurring during frost hardening; e.g. the accumulation and assimilation of organic solutes (Siminovitch 1967; Siminovitch *et al.* 1968; Levitt 1980), carbohydrate recycling (Senser *et al.* 1971) and, rapid nitrogen metabolism (Erikson 1977), these may be more rapid in the more hardy variety. However, this study made no attempt to determine frost hardiness or the relationship between respiration and the various biochemical processes occurring in embryonic shoots. A more biochemical approach would be required to elucidate such relationships.

In the present study the respiratory characteristics were those of the embryonic shoots rather than the whole buds. Bud scales were removed so that respiration of embryonic shoots could be related to ontogeny without confusion with the respiratory characteristics of the bud scales (Zimmerman *et al.* 1970). Former studies have shown bud scales to exert some kind of effect, either mechanical of metabolic on oxygen utilization by the bud (Pollock 1953, Kozlowski and Gentile 1958; vanden Born 1960). However, it is clear that the increasingly large proportion of mature and scenescent bud scale tissue in the fully-formed bud in the fall would have had a significant effect on the total respiration rate per g. wt. of bud tissue. In turn, in the spring the elongating bud scales would also have modified the respiration rate of the bud compared with that of the embryonic shoot by their mechanical or physiological effects on total bud respiration. Previous respiratory studies using intact buds, which were largely concerned with physiological characteristics within the bud (Chan-Thom 1951; Hatch and Walker 1969; Bhella and Roberts 1975; Peterkova *et al.* 1976), should be compared with caution to the present study due to masking of respiratory changes of the embryonic shoot by other bud parts. Furthermore, all the above studies were on mature trees and none of them gave any indication of the morphological stage of their material during important changes in respiratory activity. It is evident, however, that had respiratory rates of both intact as well as excised buds of coastal and interior Douglas fir seedings been measured some interesting comparisons would have been made and perhaps some areas of doubt in this study cleared up.

The increase in respiration rate in the spring occurred when buds were swollen but had not flushed in both varieties. Former studies on intact buds from mature trees of various species showed much higher respiration rates after bud burst (Chan-Thom 1951; Bachelard and Wightman 1974), however, the influence of bud scales during this process is uncertain. Kozlowski and Gentile (1958) demonstrated that bud scale removal during the spring increased the capacity of unflushed buds to respire to almost the level of those already flushing. Thus, it is possible that in studies using intact buds which were quiescent but capable of increasing their respiratory capacity, that the presence of bud scales limited respiration. Therefore, when flushing occurred a considerable amount of development had already taken place, namely the resumption of cell division and the initial stages of shoot elongation, giving rise to a large initial increase in respiration. In the present study buds were swollen but none had flushed when measurements were made.

Respiration rates of elongating shoots of the interior variety were significantly lower than the coastal variety in March. This result was unusual since the shoots of the interior variety were apparently undergoing more rapid cell division and elongation than the coastal variety. The opposite result would have been expected. One possible interpretation may be that elongating shoots of the coastal variety have a higher capacity to respire at 25°C (the temperature used in this study) than the interior variety. Kreuger and Ferrell (1965) found that in intact Douglas fir seedlings, soon after germination the interior seedlings had higher respiratory rates than the coastal seedlings at 10°C, whereas at 20°C and 35°C the coastal seedlings were higher. It would be interesting to measure respiratory rates, and the alternative pathway component, of elongating shoots at the lower temperatures prevailing during February and March to test this hypothesis.

5.5 Cyanide resistant respiration in coastal and interior Douglas fir seedlings

CN-resistance, that portion of total respiration due to both alternate and residual respiration, did not vary significantly from September through March in both varieties.

During August, in the interior variety, and into early September in the coastal variety, respiratory rates of shoot tips of Douglas fir seedlings were high and variable, and almost completely inhibited by CN. At this time apices were tiny and apical incision caused wounding to a relatively large proportion of the tissue compared with later stages of bud development. Thus, wound respiration may have contributed significantly to the magnitude of respiration at this time. This is consistent with other studies. Respiratory rates of fresh potato slices were three- to five-fold greater than that of the intact tuber, and were inhibited by CN (Laties 1978). Similar findings in other storage organs were reported by Jackobson *et al.* (1970); Laties *et al.* (1972) and Theologis and Laties (1980). The loss of the alternative pathway in wounded tissue slices was reported to be caused by damage and breakdown of membrane lipids (Theologis and Laties 1980). Wound respiration in storage tissues was shown to be temporary (Laties 1978) so had respiratory trials been carried out on wounded apical tissue over extended periods of time the percentage of respiration due to wounding may have been determined. Thus, the magnitude of wound respiration in relation to respiration required for growth of the shoot tip might also have been indicated.

Fall and winter levels of alternative pathway activity were relatively low and constant. Low levels of alternative pathway activity in buds during bud-scale and leaf initiation and dormancy is consistent with the view that when the respiration control rate (RCR) is high (the levels of ADP are high and ATP low) engagement of the alternative pathway is reduced (Storey 1976). ATP levels have been shown to be low during dormancy in several hardwood buds (Tyurina 1978, 1979), although no data are available for conifers. Cole *et al.* (1982) working with pear flower buds found conflicting results. They demonstrated an increase in cyanide resistance during chilling but a decrease during elongation. Furthermore, they found SHAM had no effect on the respiration rate at 25°C but did at 5°C. The engagement of the alternative pathway during chilling has been demonstrated in other tissues (Leopold and Musgrave 1979), and Yoshida and Tagawa (1979) showed that most of the respiration of *Cornus* callus was cyanide resistant below 15°C. Unfortunately, the equipment in my study was not designed to function efficiently at low temperatures. Nevertheless, the results showed that at 25°C the components of respiration did not change significantly during development of the embryonic shoot and entry into dormancy.

Varietal differences in CN-resistant respiration from August to early March were due to differences in residual respiration. The function of residual respiration is not understood (Solomos 1977) and thus its role in these varietal differences is obscure, however, the timing appears to have a developmental basis. In September, the higher residual respiration of the interior variety corresponds to these apices having entered preformed leaf intiation in advance of the coastal variety. The reason for higher residual respiration rates in October is uncertain. In the spring high residual and alternative respiration of the interior variety may be related to the more rapid development following resumption of activity of the embryonic shoot.

The capacity for electron flux through the alternative pathway appears to increase in the spring during shoot elongation (Table 7, 8). Embryonic shoots of both coastal and interior varieties underwent a several-fold increase in the alternative pathway capacity from winter to spring. It has been suggested that the increase in the alternative respiration in the spring of white spruce buds was due to increased substrate mobilisation causing a flux of electrons through the alternative pathway as the capacity of the cytochrome pathway was exceeded (Flannagan-Johnson 1985). Substrate mobilisation was previously proposed as the reason for increased alternative respiration activity in aged potato slices (Theologis and Laties 1978; Laties 1982). Metabolic events associated with the resumption of elongation permit a greater percentage of the total respiration to continue after complete inhibition of the cyanide sensitive pathway. It is not possible to conclude that a greater flux of electron transport was occurring through the alternative pathway in the absence of CN inhibition.

5.6 Dormancy characteristics of coastal and interior Douglas fir seedlings

The results of this study require that the phenomenon of dormancy of Douglas fir seedlings, here defined as the period of mitotic inactivity in the apex, be discussed: morphologically in terms of shoot elongation, anatomically in terms of apical development and MI and, physiologically in terms of the respiratory activity of the embryonic shoot.

The physiological, anatomical and morphological evidence indicated dormancy developed in both varieties as a state of low respiration and MI from approximately the end of October to March. The decrease in respiration began approximately with the end of bud-scale initiation and the basal rate of respiration was reached in both varieties during late preformed leaf initiation. This period of respiratory decrease demonstrated that physiological changes occurred within the embryonic shoot long after 'bud set' and height growth cessation. Both of these have been used as criteria for dormancy in Douglas fir by Lavender *et al.* (1968), van den Driessche (1969), Lavender and Overton (1972) and in other species by Kleinshmit and Sauer (1976). Cessation of lateral shoot elongation and height growth respectively, occurred at the beginning of preformed leaf initiation in both mature conifers (Owens and Molder 1973a; Owens *et al.* 1977) and Sitka spruce seedlings (Cannell and Cahalan 1979). This is when respiratory rates and MI were high in coastal and interior Douglas fir seedlings. Pollard *et al.* (1975) referred to the period of leaf initiation as the 'onset of dormancy' leaving the term vague and undefined but truer to events inside the bud, i.e. the start of development of the dormant bud.

At the end of dormancy, cell division resumed and respiration increased before flushing in the spring. Flushing is a gross morphological criterion for the end of dormancy which has frequently been used in the past (Semin and Madis 1964; Hermann 1967; Worrall and Mergen 1967; Lavender *et al.* 1973; Sĕbánek *et al.* 1975; Campbell and Sugano 1975; van den Driessche 1975, 1977; Nelson and Lavender 1979; Pollard and Ying 1979b). In the present study, the increase in respiration rate in the spring was associated with rapid cell division when some growth of the shoot had occurred but flushing had not. Owens *et al.* (1985) found cell divisions were frequent before flushing in mature Douglas fir embryonic shoots until shoots had reached 10 percent of their final length. After flushing cell divisions decreased and cell elongation increased. The use of gross morphological characteristics has come about through a liberal interpretation of the classical definition of dormancy (Doorenbos 1953). This has led to some confusion over where the dormant period occurs within the annual cycle.

In mature Douglas fir, buds were considered dormant (Owens 1968; Owens and Molder 1973a) when all cell divisions ceased in the shoot apices. If this criterion is applied to seedlings the coastal and interior varieties ceased mitotic activity in December and November, respectively, after a period of embryonic shoot development, and resumed activity at essentially the same time in March prior to shoot elongation. Therefore, the dormancy period began later in the fall in the coastal than the interior variety. Observations of organ initiation, DNA synthesis and cell division in the apex following the end of shoot elongation led Owens and Molder (1973a) to use the term dormancy for buds in mature conifers in which none of these were occurring (Owens *et al.* 1977; Owens 1984). Other workers also concerned with the histochemistry of apices, have associated dormancy with a limited ability to duplicate DNA in potato buds (Tuan and Bonner 1965) and a relatively low DNA level in ash (Cottinges 1983). There is also good evidence that in coastal Douglas fir nursery seedlings the cessation of mitotic activity in the apices coincides with the period of maximum resistance to stress during handling (Carlson *et al.* 1980).

The decrease in respiratory rate of coastal and interior Douglas fir seedlings overlaps with the rest period of Romberger (1966) and dormancy deepening of Lavender and Cleary (1974) in seedlings. Bella and Roberts' (1975) rest period coincided with the time at which mature coastal Douglas fir were undergoing late preformed leaf initiation (Owens and Molder 1973a). The winter basal rate of respiration was reached during late leaf initiation in both varieties of Douglas fir in this study. Other physiological studies of dormancy in intact buds have correlated the decrease in respiration with a loosely defined period of rest in some soft and hardwood species (Chan-Thom 1951; Pollock 1953; Bachelard and Wightman 1973; Peterkova *et al.* 1976; Kato 1981), including stem cuttings of young coastal Douglas fir (Bella and Roberts 1974, 1975). Without morphological data these studies have given a confusing picture of the relationship of rest to dormancy.

The relationship of bud respiration to rest depends largely upon the techniques used to determine the rest period. Respiration rates of Douglas fir seedlings decreased during preformed leaf initiation which is the time corresponding to rest; the inability to flush under favourable conditions of photoperiod and temperature (Romberger 1966). However, no correlation was found between rest and respiration rate in apricot buds (Hatch and Walker 1969) or flower primordia of hard woods (Komarnitski *et al.* 1981), whereas there was in Douglas fir (Bella and Roberts 1975). Hatch and Walker (1969) used the concentration of gibberellic acid required to cause flushing to delineate rest, Bella and Roberts (1974) used the effect of 18 and 9hr photoperiods on respiration rate and succinic dehydrogenase activity of the terminal and lateral buds of Douglas fir, whereas Komarnitskii *et al.* (1981) specified only that the stem cuttings were allowed to flush at room temperature. Others (Chan-Thom 1951; Pollock 1953; Peterkova *et al.* 1976; Kato 1981) used differing values of photoperiod and temperature to establish the minimum response to favourable growth conditions. Thus rather than put into question any correlation of respiration rates with rest the conflicting evidence suggests that rest itself may be too vaguely defined. Since, regrowth trials were not performed in this study, no comparison can be made with the physiologist's definition of rest and the period of reduced respiration in this study.

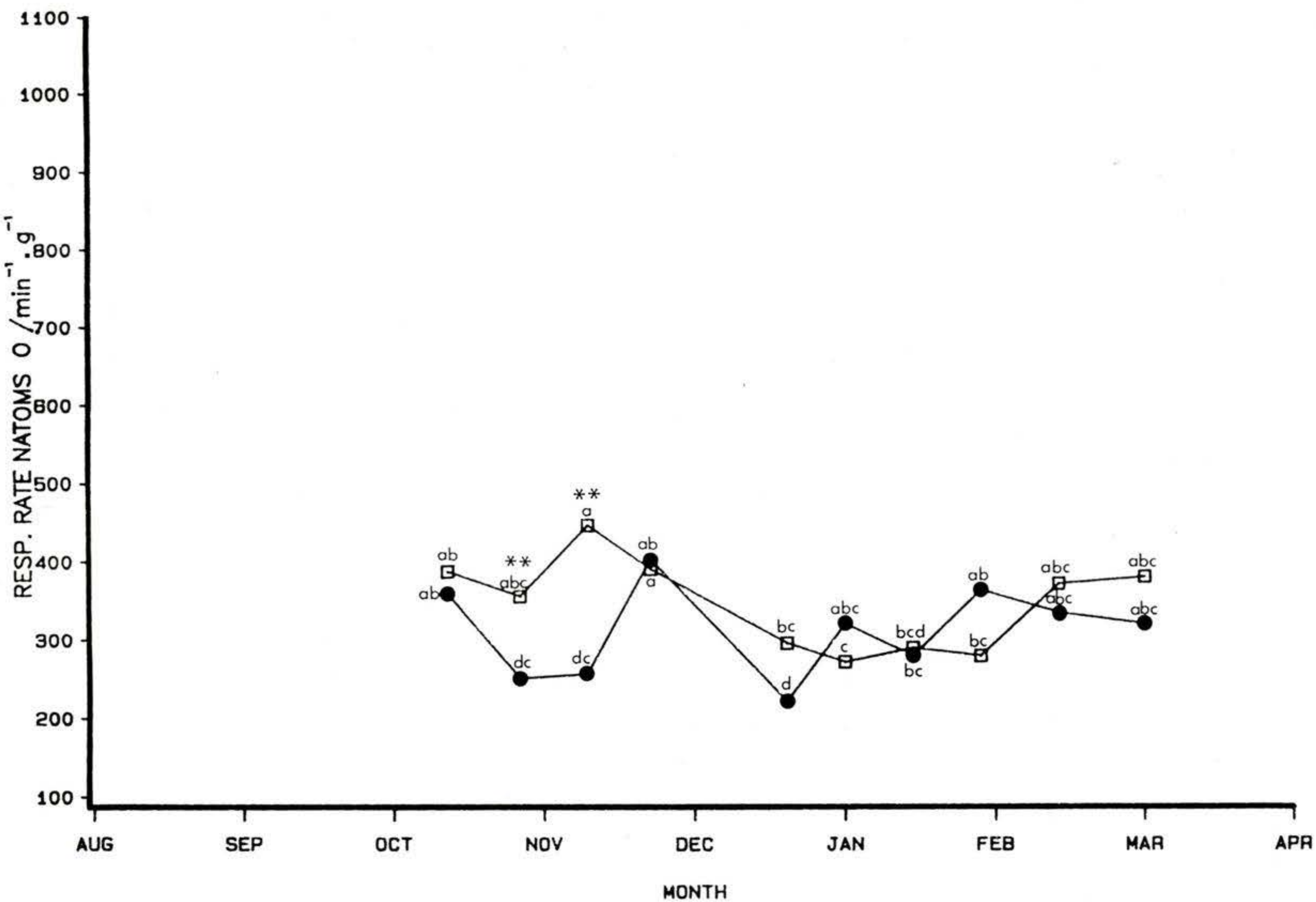
In conclusion the classically defined period of dormancy, when aplied to seedlings, describes a period of development characterised by many energy dependent physiological and morphological changes in buds. Seedlings are not resistant to stress to the same degree at all times during this period. Measurements of MI, respiration rates of shoot tips and other approaches including changes in chlorophyll fluorescence in needles (Hawkins and Lister 1985) are techniques that may be used to indicate the beginning and duration of this hardy period. The terminology describing physiological changes throughout the classical dormant period is of limited value for describing physiological changes revealed by the above techniques since, generally, it is based on a criterion other than that of hardiness. The use of the term dormancy exclusively for the hardy period would have more meaning in terms of seedling survival and would avoid referring to seedlings undergoing rapid bud development as 'dormant'. This terminology would not be entirely new since dormancy, as defined by developmental morphologists (Owens and Molder 1973a), is delimited by an absence of cell divisions in the apical meristem. This period correlates closely with the beginning and end of the hardy period in Douglas fir seedlings.

Work is needed to clarify the dormant period in terms of existing physiological tests of seedling vigour. This approach may be extended, eventually, to formulate a predictive model of the whole annual cycle of seedling development using both morphological stages and physiological tests under standardised physiological conditions. This approach has already been attempted by Fuchigami *et al.* (1982). Such a model would enable decisions concerning lifting, storage and planting out of the many species and ecotypes of economically important conifers to be made with greater confidence and precision.

Appendix A

RESPIRATION RATES 1982/1983

The graph shows respiration rates of embryonic shoots of coastal (□) and interior (●) Douglas fir seedlings. Dates with the same letter were not significantly different ($\alpha=0.05$) using the Duncan's multiple range test. Significant differences between varieties determined from paired Contrasts ($\alpha=0.05$) were indicated with asterisks (**).


RESP. RATE NATOMS O /min⁻¹.g⁻¹
1100
1000
900
800
700
600
500
400
300
200
100
AUG
SEP
OCT
NOV
DEC
JAN
FEB
MAR
APR
MONTH
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Title of Thesis

Anatomical and respiratory changes in shoot tips of interior and coastal Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco.) seedlings during bud development.

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