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Title: THE PHYSIOLOGY OF DORMANCY OF WESTERN HEMLOCK (TSUGA HETEROPHYLLA (RAF.) SARG.) SEEDLINGS

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The physiology of dormancy of western hemlock (Tsuga heterophylla (Raf.) Sarg.) seedlings was examined. Many areas proved to exhibit similarities to those elucidated for Douglas-fir, although significant differences were also found and necessitate the use of caution in extrapolating data from one species to the other.

Dormancy of western hemlock seedlings was initiated most rapidly under a regime consisting of an 8 hour photoperiod, a warm temperature regime (25°/20°C), and moderate moisture stress (12-15 bars, PMS). A regime of warm temperature and moderate moisture stress was able to induce dormancy under a 16 hour photoperiod, although not as rapidly as with the short photoperiod. The photoperiod experienced by the seedling in the fall was found to have a slight but significant effect on the date of spring bud break after natural overwintering, with a long photoperiod during the fall delaying the date of bud break. The chilling requirement of western hemlock was found to be considerably less than that of Douglas-fir. If seedlings were preconditioned with six weeks of mild, short days the requirement was met by four weeks of

a constant 5°C temperature. Seedlings not receiving this beneficial pretreatment generally had their chilling requirement fulfilled by six to eight weeks of constant chilling.

Cold storage of western hemlock seedlings for four weeks during October resulted in delayed bud break if they were then placed in a growth room or very high mortality if they were planted into a cold frame. A daily photoperiod during cold storage reduced mortality although it was still high. Cold storage during December or February was found to have no adverse effect on the seedlings. A 16 hour daily photoperiod during February storage provided seedlings which resumed growth most rapidly in the growth room and earliest in the cold frame.

Western hemlock was found to have four distinct phases of dormancy similar to those which occur in Douglas-fir. The third phase during which the chilling requirement is being fulfilled is shorter for western hemlock and results in the transition into the fourth phase earlier than Douglas-fir. The phase of dormancy which the seedling is in appears to have a controlling influence on the response of the seedling to a variety of treatments and cultural manipulations.

Indole-3-acetic acid and abscisic acid were identified as endogenous hormones in western hemlock and the seasonal variation of these hormones was determined. The ratio of IAA to ABA correlated well with the phases of dormancy, although no causal relationship was established. Abscisic acid level was found to increase with increasing moisture stress experienced by the seedling, while the photoperiod experienced by the seedling had little effect on the abscisic acid level. A threshold moisture stress of about 12 bars was found to be the point

where ABA levels began to increase rapidly in the needles of western hemlock seedlings, with the most rapid increase occurring between 16 and 22 bars.

The Physiology of Dormancy of Western Hemlock (*Tsuga heterophylla* (Raf.) Sarg.) Seedlings

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INTRODUCTION

With the growing demand being placed on our timber resource, the necessity of good management becomes increasingly important. Regeneration is one stage of the timber cycle where proper planning will become more vital. By reducing the lag time between harvest and the establishment of a vigorous, fully stocked new stand the forest land will be able to produce a maximum volume of wood in a minimum amount of time. This, of course, assumes proper management of the new stand from the time it becomes established until it reaches harvest age.

In the Pacific Northwest, the use of nursery seedlings has become the most common method of regeneration. By using seedlings, the forester is attempting to speed up the regeneration process and to gain several years in comparison to using direct or natural seeding to produce the new stand. Although the advantages of using seedlings are readily apparent, there are also disadvantages which must be recognized and dealt with. Foremost among the disadvantages are the various stresses which the seedlings will be exposed to in the process of getting them from the nursery into the ground at the site. They must then become rapidly established and produce vigorous growth.

Although western hemlock (Tsuga heterophylla (Raf.) Sarg.) has long been recognized as one of the most productive and valuable trees in the Pacific Northwest forest, it has traditionally been neglected in reforestation in favor of Douglas-fir. But with the increased

demand for wood products, and especially fiber, western hemlock will have to play a much more important role in future regeneration. Total productivity of western hemlock exceeds that of Douglas-fir on equal sites because hemlock tolerates a much higher stocking level. Thus, because of the increasing value of hemlock fiber, hundreds of thousands of acres in Oregon and Washington which are capable of supporting hemlock forests will be more valuable in terms of net worth and productivity if they are regenerated with hemlock.

Unfortunately, regeneration with hemlock seedlings has not been consistently successful in the past. In many cases this was due to the poor quality of the planting stock. With the adaptation of containerized nursery production to western hemlock, many of the problems of poor planting stock have been reduced or eliminated. Although the relatively recent implementation of container systems prohibits accurate estimation of their ultimate place in producing planting stock, such techniques do offer some advantages to the nurseryman and forester. Containerization has allowed the production of species that are slow or difficult to grow and has generally shortened the production time necessary to grow seedlings for reforestation. The efficiency of production is increased as the result of a container system and the planting season can be extended in comparison to bare-root planting. In many situations, container seedlings are thought to have a greater chance of survival and growth than do bare-root seedlings because container seedlings will be planted with an undisturbed root system which is not possible with bare-root stock. This potential advantage will depend on the individual site, and the regeneration

method and seedling type will have to be matched to the particular problems of the site in question.

There are disadvantages to the production of containerized seedlings. First, the cost of production is higher than for bare-root seedlings. However, for western hemlock this additional expense will in many cases be offset by the improved quality of the planting stock and therefore the increased chance for establishment on the regeneration site. Second, a higher level of technical knowledge is needed to produce seedlings with a good potential for survival and subsequent growth. The long term effects of environmental manipulations must be understood and utilized to produce high quality planting stock. The research effort which is being put into the area of container seedling production is minimizing this disadvantage.

One area of physiology which has been shown to affect the potential survival of the seedling is that of dormancy. Many species of forest trees have been found to go through several distinct physiological stages during their dormant periods. The stage of dormancy has a great effect on the response of the seedling to its environment. By understanding these stages, the detrimental effects of many nursery practices, such as lifting and cold storage, can be reduced and a healthy seedling can be produced which will have the greatest possible chance of survival when it is planted in the field. As an example, it is known that cold storage of Douglas-fir lifted in the fall during the early stages of dormancy drastically reduces their survival, whereas cold storage has little effect on the survival of seedlings lifted in the winter. Since containerization increases the potential for

manipulating the growing season and dormancy inducing regime, a more complete understanding of the dormant physiology of the seedlings becomes increasingly important.

Research in this area of physiology has been conducted primarily with Douglas-fir and little information is available on western hemlock and other forest species. Therefore, this work is intended to fill the gap in information on western hemlock's physiology during dormancy. A number of areas pertaining to western hemlock were examined and included dormancy induction, chilling requirements, and cold storage effects. The presence of several distinct physiological phases during the dormant period was examined and the endogenous levels of indole-3-acetic acid and abscisic acid were determined. Information on this subject is essential for efficient production of western hemlock seedlings for regeneration. With the use of container technology and the understanding of the physiology of dormancy, regeneration with western hemlock will become a viable alternative to meet the increasing demand placed on our timber resources.

LITERATURE REVIEW

In the area of tree physiology, the majority of work has dealt with species of major economic importance and western hemlock has been largely overlooked as research material in the Northwest. With the current emphasis on the place of western hemlock in high-yield forestry, as evidenced by the recent Western Hemlock Management Conference, knowledge of the physiology of this species is becoming a high priority for research.

Western hemlock grows in the wet, mild climate of the Pacific Northwest and coastal western Canada and its typical habitat is limited to the moister northern slopes and creek bottoms (Fowells, 1965). It occurs over a wide variety of soils but grows best on deep, well drained soils with deep organic layers and adequate moisture. Soil pH varies throughout its range but is generally between 3.5 and 5.5. Hemlock grows best at elevations between sea level and 2,000 feet, although it is found at altitudes of up to 6,000 feet in portions of its range. Over most of its range, the pure western hemlock forest is the natural climax type.

The morphology and development of vegetative and sexual buds of western hemlock have been studied by Owens and Molder (1973, 1974). Eis (1974) compared the root system morphology of western hemlock and Douglas-fir. Although Douglas-fir had a larger root spread and roots of larger diameter, hemlock had a higher density of thin, rope-like roots and absorbing roots. He also found that hemlock roots followed old decaying roots more frequently than Douglas-fir.

Due to its productivity, growth rates of western hemlock have been studied for many years, both in plantations (Walters, 1964) and in natural stands (Godman, 1953; Buckland, 1956; Gregory, 1957; Griffith, 1960). Although geographic sources will vary in their rate of growth and total growth (Bengtson, McGregor, and Squillace, 1967), western hemlock generally will produce its maximal growth during June and in many cases continues growing into late August or early September. This gives western hemlock a longer growing season than many species, including Douglas-fir.

Western hemlock has also been included in controlled environment and nursery studies. Brix (1971a) found that maximum dry weight, stem diameter, and stem length of western hemlock occurred at a constant temperature of 18°C. This is in contrast to the fluctuating optimum thermoperiod for most forest species. In a nursery study, van den Driessche (1968) showed that western hemlock had a net assimilation rate similar to that of Douglas-fir but had a higher annual pattern of relative growth rate and stem extension growth. In their study of photosynthesis of conifer species under natural conditions, Hodges and Scott (1968) found western hemlock to have a high net photosynthetic rate in comparison to the other species they studied. In addition, they found hemlock to have a low light saturation point which explains in part why it is able to regenerate so prolifically under natural stands and tolerate dense or partial shade. Keller (1969) showed that much of hemlocks' response to light intensity was due to the prior environment. Seedlings grown under low intensity light did not photosynthesize as well when placed under high intensity light and vice versa.

While its growth rate has been studied, work on other physiological responses of hemlock as they relate to nursery production of seedlings is not readily available. Optimum and natural germination conditions and early growth have been studied (Ching, 1958; Minore, 1972). Long and Winjum (1974) summarized many aspects of nursery production and regeneration with western hemlock. In their workshop they provided a review of the current knowledge and spelled out desired research needs to implement a successful regeneration program for western hemlock. In addition, Piesch (1974) has set forth a program of tree improvement for western hemlock. Research on silvicultural practices in western hemlock stands are also beginning to appear in the literature (Debell, 1975; Williamson and Ruth, 1976).

Containerized Seedlings

Nursery practices are of great important to the production of quality stock for regeneration. General practices have been reviewed by Aldhous (1972) and Abbott and Eliason (1968). Areas which have received the most study have been directly related to production and include early mortality of seedlings (Soos and Walters, 1963; Gashwiler, 1971), nutrition (Calvert and Armson, 1975; van den Driessche and Webber, 1975), soils and seedbeds (Minore, Smith, and Woollard, 1969; Wilson and Campbell, 1972), and other biotic factors such as weeds, fungi, and insects. Various root treatments such as pruning or wrenching have received attention as methods to increase survival potential of seedlings (Trappe, 1971; Eis and Long, 1972; Larson, 1975; Edgren, 1975; Tanaka, Walstad, and Borrecco, 1976). Work has also been done

to assess the effect of root exposure on survival of Douglas-fir seedlings (Hermann, 1962, 1964a). Studies by Lopushinsky and Beebe (1976) and Hermann (1964b) have shown that Douglas-fir and ponderosa pine seedlings with larger root systems, as expressed by the shoot-root ratio, had a better chance of survival, especially on harsh sites.

Water relations play an important part in seedling production and will be discussed later. Research has shown that the date of lifting and cold storage of stock prior to planting can have a large influence on survival of the seedlings, and these areas will also be discussed in some detail later.

The introduction of the containerized forest seedling has been hailed as one of the most promising new developments in forestry and nursery production of these seedlings has generally increased (TerBush, 1975, 1977). The interest in containerization is evidenced by the recent symposium on the subject (Tinus, Stein, and Balmer, 1974). That symposium covered such broad areas as the objectives of containerization, container and handling systems, container nursery practices, seedling production, field performance of container seedlings, and economic aspects. Prior to that symposium, the use of container seedlings for regeneration in the Northwest was reviewed by Walters (1969) and by Kinghorn (1970). Both favored the continued usage of containers due to the potential advantages which they possess.

Stein, Edwards, and Tinus (1975) and Stein and Owston (1975) have outlined the advantages and outlook for container seedlings. Some of the advantages they listed for container seedling production are accelerated production to meet demand, greater and more efficient

production, extended planting seasons, improved survival and growth after outplanting, and reduced production problems of some species. Container seedlings are planted with their root system intact and with a root system that has been better protected than the typical bare-root seedling. This gives the container seedling the advantage of not losing close contact with growing medium when moved from the nursery to the field. Container seedling production has given a boost to species which have typically been difficult to produce by standard nursery practices because of the increased control over environmental variables which the system offers. Species in this group include western hemlock, true firs, and spruces. The container system is also applicable to species which have in the past been produced as bare-root seedlings, such as Douglas-fir and the pines.

There are many types of containers and methods of production currently employed and new ones are constantly being developed. Cayford (1972) and White and Schneider (1972) described most of the types of containers available. Probably the most widely used container in the Northwest is the "styroblock-2," which has a rooting volume of about 2.5 cubic inches and contains 98 cavities per square foot. The range of sizes and spacings of the other systems is rather large and gives the nurseryman a wide choice. Matthews (1971) has outlined a production manual for container seedling production in Canada, and Owston (1972a, 1974) has outlined suggested cultural techniques for use in the Northwest. Arnott (1975a) has outlined the technique used to produce containerized western hemlock seedlings in British Columbia. All these methods recognize the importance of such factors as potting mediums,

watering and fertilizing regimes, temperature and light controls, and hardening procedures.

The influence of the type of rooting medium on root development has received considerable attention (Harris, 1967; Edgren, 1973; Karlsson and Koyats, 1974; Hockings and Mitchell, 1975). Composition of the rooting medium has been found to have a significant effect on root growth and distribution within the container. The most commonly used media today are mixtures of peat moss and vermiculite in various proportions. This medium has generally produced good results, but there are data (Edgren, 1973) which suggest pure peat moss is a superior medium to this mixture.

In terms of survival and growth, field performance of containerized seedlings is the most important measure of success. Due to the relative newness of container seedling usage, data on field trials are still relatively limited but new data are constantly becoming available. Owston and Stein (1974) have published a method for making uniform field comparisons of containerized and bare-root nursery stock. Only by making the uniform comparisons can the true value of container seedlings be assessed and a valid guideline for their use in regeneration be formed.

Arnott (1971, 1975b) compared bare-root and container seedlings of Douglas-fir and western hemlock on Vancouver Island. The type of stock produced little difference in survival of Douglas-fir, but survival of western hemlock plugs was 65% whereas survival of bare-root stock was only 29%. Maximal survival occurred with fall or early spring planting dates and the major cause of mortality was drought. Planting trials

for containerized lodgepole pine (Endean and Hockings, 1973) and white spruce (Hockings and Endean, 1974) have been made but without bare-root comparisons. In both studies, survival was high and the largest seedlings prior to planting generally had the best performance.

Planting trials have been conducted in Western Oregon using containerized and bare-root Douglas-fir and noble fir (Owston and Stein, 1972; Owston, 1972b). Survival of all stock was relatively good but significantly higher for those grown in containers and survival was better after spring planting than after fall planting. First year height growth of containerized trees averaged about 2-1/2 times greater than bare-root stock. This study utilized seedlings grown in 7 to 10 inch long containers. While these large containers may not be feasible on a large operational scale, they may have a use in reforestation of difficult sites, particularly droughty ones.

Containerized nursery seedlings in general, and western hemlock in particular, are proving themselves to be viable alternatives to bare-root stock. As research continues, many of the problems of production and field performance will be overcome or lessened. Work on physiological responses of containerized seedlings to environmental stimuli is incomplete and deserves continued study.

Dormancy

Tree growth has been a subject of study for many years and has been covered by many comprehensive texts (Kramer and Kozlowski, 1960; Kozlowski, 1964, 1971; Zimmermann and Brown, 1971). The growth response to the various site factors will determine the productivity of

forest stands and therefore is of interest to foresters. However, the responses of trees, and especially seedlings to environmental factors during the dormant period are often more significant.

Dormancy is a response to a variety of unfavorable growth environments, and in perennial temperate plants, it is an adaptation to withstand the cold winter period without damage. A number of major reviews have been published on the subject of dormancy (Doorenbos, 1953; Samish, 1954; Romberger, 1963; Vegis, 1964). In addition, there are several general and less comprehensive articles on the subject of dormancy (Vegis, 1963; Wareing, 1965; Perry, 1971). There is general agreement that the dormant period is composed of more than one distinct physiological state. All plant physiologists recognize at least two states, summer or imposed dormancy and winter dormancy or rest.

Summer dormancy is a result of external conditions unfavorable for growth, such as high temperatures or drought. The dormancy of summer buds is readily broken by returning the plants to favorable growth conditions. If the unfavorable environment is maintained, summer dormancy will gradually deepen until the plant will not respond with resumed growth when placed in an environment favorable to growth.

Plants whose growth is thus limited by internal rather than external conditions are said to be in a state of winter dormancy. Samish (1954) further divides this period into pre-rest, rest, and post-rest. Under natural conditions, the transition from summer to winter dormancy may be triggered by the lengthening nights of the fall.

In turn, winter dormancy is normally broken by the period of low temperatures common to most temperate zone winters.

Smith and Kefford (1964) suggest a series of steady-state and transitional phases which are chemically regulated. By defining the phases of dormancy in this way, the different responses to environmental stimuli between quiescent buds during a summer drought and post-dormant buds in April awaiting flushing can be explained. Weiser (1966) has presented a similar series of stages which are based on the aquisition and loss of cold acclimation.

A series of four physiological phases has been postulated for Douglas-fir which successfully explains the response to environmental stimuli (Lavender and Cleary, 1974). These phases are dormancy induction, dormancy deepening, dormancy, and post dormancy. The classifications are based on the ability of seedlings to resume growth under favorable environmental conditions as well as their ability to withstand stresses.

Regardless of the classification system you chose to follow, the appreciation of the physiological phases of dormancy and their consequences on survival must be taken into consideration within the scheduling of nursery practices. Only if a seedling is in the proper phase when it is utilized for regeneration can its potential be realized (Lavender and Hermann, 1976).

Research on dormancy has been primarily concerned with buds, since there is little evidence that entire root systems (Romberger, 1963) or the cambium (Lavender, Hermann, and Zaerr, 1970; Worrall, 1971) have a true winter dormancy.

The biochemistry of plants during dormancy has received some attention, although few significant correlations with the states of dormancy have been found. Durzan (1968) studied the amino acid changes of Picea glauca at the onset of dormancy and found that the changes were related to the metabolism of arginine. Bachelard and Wightman (1973) studied the biochemistry of dormant vegetative buds of Populus balsamifera. They found an increased level of catabolic metabolism of carbohydrates and proteins during late March when the dormancy status was decreasing. They also found an increased anabolic metabolism about two weeks prior to bud burst which resulted in a net synthesis of cellular constituents. von Rudloff (1975) has studied the seasonal variation of terpenes in Picea pungens and the only large changes he found in the terpenes he studied were in the buds and young leaves immediately after bud break. Pollock (1953) studied the respiration rate of Acer buds and found that limitation of oxygen uptake was severe in the late summer, was lessened in the fall, and was removed during the late winter. He postulated that one or more substances was produced during the anaerobic metabolism that had growth inhibiting activity on the bud. Simmonds and Simpson (1972) have proposed a model which shows the regulation of dormancy by the activity of the Krebs cycle and pentose phosphate pathway.

Factors Affecting Growth and Dormancy

There are a great many environmental variables that affect plant growth. The most commonly studied ones are light, temperature, water and nutrients. Their effects on tree growth have been reviewed by

Geartner (1964). Each of these factors can initiate dormancy in plants when they become unfavorable for growth.

Since the early work of Garner and Allard (1920, 1923, 1930) much has been learned about the effect of photoperiod on growth, reproduction, and dormancy, and a number of reviews have been published (Wareing, 1948, 1956; Olmsted, 1951; Downs and Borthwick, 1956; Nitsch, 1957a; Borthwick and Hendricks, 1960; Hendricks and Borthwick, 1963). Although most studies of photoperiod have been directed at finding the photoperiod of maximum growth, many have also determined the length of day which causes a cessation of growth and the induction of dormancy. Some of the genera studied in which a critical photoperiod have been found are Abies (Vaartaja, 1960; Arnott, 1974), Pinus (Wareing, 1950a, 1950b, 1951); Zahner, 1955; Watt, 1961; Fowler, 1961), Tsuga (Nienstaedt and Olson, 1961), and several hardwood species (Kramer, 1936; Jester and Kramer, 1939; van der Veen, 1951; Ashby, 1961). Several of these studies also used the technique of interrupting the dark period with a short period of light. From studies of this type, it has been found that the length of continuous darkness is responsible for the photoperiodic effect. Dinus (1968) found that as the interruption was moved closer to the center of the dark period, the effect was increased.

Studies have shown that a long photoperiod is necessary for bud break (Phillips, 1941; Wareing, 1953; Hellmers, 1959). These studies contended that bud break was a photoperiodically mediated event. Today it is known that many woody species will break bud in response to favorable temperature regardless of the photoperiod if the chilling requirement has been fulfilled (Wareing, 1969). Conversely, Lavender

(1977) has shown that a 16 hour photoperiod will stimulate bud break of Douglas-fir seedlings maintained under unfavorable (3°C) temperature. Most work has failed to show a relationship between photoperiod and root growth, although one study (Stahel, 1972) did show that an 18 hour photoperiod did maintain a constant rate of root growth in Sitka spruce.

In a recent study, McGee (1976) found that the light intensity during the previous growing season had a greater influence on bud break than did the light intensity at the time of bud break. Differences of up to 17 days in bud break were found due to prior conditions before outplanting oak seedlings. While some studies have been done to assess the effect of light intensity on growth and survival of evergreen species (Brix, 1970; Ronco, 1970; Fairbairn and Neustein, 1970), none have been done to assess the effect on date of bud break. This information could prove to be a valuable tool for regenerating sites where early growth and establishment of seedlings is required.

The way in which plants use photoperiod as a time measurement has never been clearly understood, although phytochrome has been implicated for quite a while. In a recent article, Heide (1977) has postulated a new model for this phenomena. He hypothesizes that the diurnal change in responsiveness to the phytochrome pigment system is dependent upon a circadian rhythm in membrane functioning and configuration, which is reflected in a parallel rhythm in membrane binding capacity for phytochrome P_{fr}. In his model, phytochrome and the circadian clock are thus integrated into one structural and functional unit. He also showed how his model could account for long and short day plants.

While the ability of short photoperiods to induce dormancy in woody plants is well documented, it is in most instances not the primary factor of dormancy induction in the Pacific Northwest. Most species native to the Northwest generally have set resting buds in midsummer when photoperiods should produce continued growth. A more important factor in inducing dormancy in much of this region is the water stress seedlings experience during the dry summers.

Many of the responses of plants to water stress have been reviewed by Hsiao (1973). Effects of water stress on individual physiological processes have also been investigated. One common response in conifers is stomatal closure. In examining several conifers, Lopushinsky (1969) found that water stress required to cause stomatal closure varied with species. Species which tolerate high leaf moisture stress before closure were less able to survive a drought. Stomatal response is also affected by light intensity, and significant interactions of intensity and water stress have been shown (Strothmann, 1967; Davies and Kozlowski, 1974, 1975a; Pereira and Kozlowski, 1977). As would be expected, transpiration also decreases in trees and seedlings as the moisture stress on them increases (Hellkvist, 1970; McColl, 1973). Unterscheutz, Ruetz, Geppert, and Ferrell (1974) found that Douglas-fir seedlings which had experienced prior soil moisture stress decreased transpiration more in response to low plant water potentials than did plants which had experienced no soil moisture stress, thus showing a preconditioning mechanism for water conservation in seedlings.

Water stress has been shown to affect photosynthesis and respiration (Brix, 1962; Zavitkovski and Ferrell, 1968, 1970; Puritch, 1973;

Lopushinsky, 1975). Photosynthesis was found to have a three phase decrease as water stress increased. Initially there was a rapid linear rate of decline, followed by a second more gradual reduction, and, finally, a steady rate of zero net photosynthesis. This reduction scheme generally followed the increase in carbon dioxide diffusion resistance (Brix, 1972). Decline in respiration was similar to photosynthesis, but was only reduced to between 40 and 75% of the maximum rate, although this varies with species.

Heth and Kramer (1975) have shown that the ability of various species of pine to withstand water stress is also related to other climatic conditions. Survival was most likely if seedlings were in a moist, cool environment when experiencing water stress and least likely in a warm, dry environment. Keller and Tregunna (1976) showed that the prior environment had an effect on the water relations of western hemlock. Seedlings raised under shaded conditions were least able to survive water stress under exposed conditions. Cleary (1970) demonstrated that Douglas-fir in western Oregon set bud in late June prior to the more severe water stress in July and August. In the dormant condition, seedlings were better able to survive the water stress placed upon them. Recognizing the problem of drought in successful regeneration, Heiner and Lavender (1972) found that Douglas-fir seedlings with the capacity for early, vigorous growth of roots and shoots had better survival potential on sites which experienced water stress conditions during the early summer. They suggested collection of seed from early flushing trees to produce seedlings which could adapt and would have increased survival potential for regeneration on droughty sites.

Water stress is often accompanied by temperatures that are unfavorable for growth. Temperature is another factor which can have an effect on growth and the initiation of dormancy in seedlings.

The effect of temperature and fluctuating temperatures on growth has been investigated for several tree species (Larson, 1967; Hellmers, 1963a, 1966a, 1966b; Lavender and Overton, 1972; Good and Good, 1976). In addition, the interaction of temperature and light has also been studied (Hellmers, 1963a; Brix, 1967; Kozlowski and Borger, 1971). In general, each species has a different optimum temperature regime for early development and maximum growth. In some cases, the optimum temperature has been shown to change throughout the growth season (Kramer, 1967) and with the level of soil fertility (Cochran, 1972). Most species require a 5 to 15°C fluctuation between day and night temperatures for their best growth, although western hemlock appears to grow best at a constant temperature (Brix, 1971a).

Studies on the effect of temperature on photosynthesis and respiration (Krueger and Ferrell, 1965; Sorensen, 1964; Sorensen and Ferrell, 1973) have shown that there is an optimum temperature for photosynthesis, with a reduction at temperatures above or below the optimum range. Respiration rates were generally found to increase with increasing temperature. Other studies have shown that the net assimilation rate is influenced by temperature but temperature was found to be less important than the light factor (Wilson, 1966; Farmer, 1975).

Although root activity seems to be uncorrelated with many environmental factors, there is a strong correlation with temperature. Soil temperature has been shown to affect the distribution of shoot and root

growth (Barney, 1951; Larson, 1970; Heninger and White, 1974). There is an optimum soil temperature for root growth that is generally higher than the optimum for shoot growth. In a water culture study with slash pine, Shoulders and Ralston (1975) showed that nutrient uptake by the roots was influenced by temperature. Uptake of most nutrients was increased as temperature increased in the range of 16°C to 28°C.

Fewer studies have been done on the effect of temperature on dormancy, and those which have been done present a confusing picture. In most cases, other variables within the studies can account for much of the seemingly contradictory findings. Variations among ecotypes of the same species have been shown to exist (Irgens-Moller, 1957; Pringle, Elliott, and Degenhardt, 1975).

Hellmers, Genthe, and Ronco (1970) reported that terminal bud formation of Engelmann spruce was controlled by the day temperature and that high day temperatures inhibited bud formation. Working with two varieties of Douglas-fir, Lavender and Overton (1972) found that low soil temperatures hastened dormancy of seedlings grown under a long photoperiod but had little effect on seedlings grown under a short photoperiod. Under a short photoperiod, dormancy in var. menziesii was postponed by low air temperatures and dormancy in var. glauca was postponed by warm days and cool nights. Paton and Willing (1968) reported that high temperatures were able to inhibit the normal short day control of the onset of dormancy in Populus. They postulated that the effect was due to temperature having a direct effect on processes controlling the onset of dormancy. Magnesen (1971) reported that warm temperatures stimulated bud set in Norway spruce and that low

temperatures delayed bud formation. He also reported that low night temperatures promoted the rapid development of buds if the photoperiod was of the proper length.

The interaction of temperature and photoperiod has been the subject of several studies. Several papers have discussed the effect of temperature on the critical photoperiod (Vegis, 1953; Slee and Shepherd, 1972; Heide, 1974a, 1974b). These studies showed that as the temperature was increased, the critical photoperiod to maintain growth was also increased; and that if the photoperiod remained the same as temperature was increased, the incidence of bud formation was increased. The effectiveness of temperature increased in the area of the critical photoperiod and was lessened by very long or very short photoperiods. Magnesen (1969) also found that warm temperatures increased bud set in Norway spruce. However, he considered photoperiod to be the primary trigger for dormancy initiation in that species and considered temperature as the factor promoting bud maturation and frost hardiness. Dormling, Gustafsson, and von Wettstein (1968) found that temperature and photoperiod during bud maturation of Picea abies had a great influence on the time of flushing and the amount of growth during the flush. Mild temperatures and short days resulted in earlier flushing and more growth. A similar phenomenon has been found in Douglas-fir and will be discussed later.

Plant response to nutrition has been studied primarily as a method to get increased growth from seedlings and established trees (Brix and Ebell, 1969; Krajina, Madoc-Jones, and Mellor, 1973; Cochran, 1973), although it is known to affect other physiological processes like

photosynthesis and respiration in Douglas-fir (Brix, 1971b). Nutrient concentrations in trees in established forests and in nursery situations have been determined for many species (Beaton et al., 1965; Beaton et al., 1965; van den Driessche, 1969a; Benzian and Smith, 1973). For western hemlock seedlings, normal whole plant concentrations on a percent dry weight basis have been listed as: Nitrogen - 1.3 to 1.8%; Phosphorus - 0.11 to 0.19%; Potassium - 0.3 to 0.9%. Western hemlock usually has a lower concentration of nutrients than Douglas-fir when grown under similar conditions. Care must be taken when comparing mineral concentrations since age of the tree, the height from which the sample was taken, and the season have very large effects on the concentration (Lavender and Carmichael, 1966; Lavender, 1970). In a recent study, van den Driessche and Webber (1975) found that soluble nitrogen concentration was increased in Douglas-fir by high temperature or increased moisture stress, but total nitrogen concentration was not significantly affected.

The use of a nutrient deficiency to induce dormancy has rarely been studied, but Cheung (1972, 1975) found that dormancy could be induced in containerized western hemlock by withholding nitrogen. This treatment, however, resulted in lowered nitrogen content and pale green foliage. He also found that the individual factors of short photoperiod, water stress, cold temperatures, and spraying ethrel or maleic hydrazide on the seedlings also resulted in dormancy of the seedlings.

Very few studies have looked at the interactions of more than two of these environmental factors on the initiation of dormancy. One which has been done considered the interactions of photoperiod,

temperature, and moisture stress on the induction of dormancy in Douglas-fir (Lavender, Ching, and Hermann, 1968). In the three year study, their results were somewhat erratic from year to year but they were able to show significance for several of the factors studied as well as significant interactions. They generally found that dormancy was induced by a short photoperiod, low night temperatures, and water stress, although the temperature effect was not consistent. They found a significant interaction of temperature and photoperiod on dormancy induction throughout the three years studied. Seed source was also significant throughout the study. Because of the importance of dormancy of nursery seedlings to successful regeneration, studies of this type must be performed for all important species. Understanding of the interactions will give the nursery manager increased flexibility, especially with container seedlings.

Chilling Requirement

Once plants have entered the state of winter dormancy, their growth is limited by internal conditions. In many species this limitation is normally overcome by the period of low temperatures common to most temperate zone winters. The necessity of cold temperatures during the winter for early, vigorous growth in the spring has been recognized for many years (Coville, 1920). Coville also showed that the effect of cold temperatures was limited to parts of the plant which actually experienced them. The length of exposure to low temperatures necessary to break dormancy is referred to as the chilling requirement. The length of the chilling requirement varies between species and has

also been found to vary among ecotypes or provenances of the same species (Calvin, 1957; Perry and Wang, 1960; Kriebel and Wang, 1962). Variations in chilling requirement within a single tree have been shown. Eggert (1951) showed that terminal buds of apple required less chilling than lateral buds and that flower buds required less chilling than leaf buds. Nienstaedt (1966) found that young white spruce required more chilling than older ones.

There is also a variation within species as to the date of bud break after chilling has been fulfilled (Morris, Silen, and Irgens-Moller, 1957; Irgens-Moller, 1967; Nienstaedt, 1972; Sharik and Barnes, 1976). This variation is genetic in origin and differences of 15 to 18 days have been observed within a range of 20 to 40 miles. The response appears to be a multi-factorial response of the plant to environmental factors at the time of bud break. This genetic control of the chilling requirement and date of bud break must be recognized and taken into consideration when seed or seedlings are moved from their place of origin to alternative planting sites in reforestation (Campbell, 1974) or horticulture (Weinberger, 1950).

A period of mild temperatures and short photoperiods prior to chilling has been found to be beneficial to Douglas-fir (Sugano, 1971; Lavender and Wareing, 1972). During this period primordium maturation is taking place within the bud and the process is slowed by low temperatures. Shoot elongation in the spring was found to be increased because of this period of mild temperatures. The maturation process probably occurs in most species and should be recognized in the

production of nursery stock. If seedlings are chilled too soon after bud set, reduced spring growth may result.

The chilling requirement for many forest species have been studied (Olson and Nienstaedt, 1957; Winton, 1964; Berry, 1965; Jensen and Gatherum, 1967; Lyr, Hoffmann, and Richter, 1970; Steinhoff and Hoff, 1972). A wide range has been found, but most fall within the range of 260 to 2000 hours of temperatures below 5°C. Wommack (1964) showed that 12 weeks at a constant 40°F was required for maximum response to a subsequent favorable environment in Douglas-fir. However, its response after 8 weeks of chilling was almost as great. Therefore, in nursery operations geared to produce seedlings in a minimum period of time, the shortest duration of chilling that will produce near optimum bud break will be used. Data on the minimum chilling requirement as well as the optimum chilling period are therefore important from an operational standpoint.

Naturally fluctuating temperatures are less effective in satisfying the chilling requirement (Lavender and Cleary, 1974). During warm winter days, which are not uncommon in the Northwest, there is actually a reversal of some of the chilling which has taken place before. Repeated freezing and thawing periods are also less effective than a constant 3-5°C temperature. As a consequence, it is probable that the natural chilling received during a typical winter is equivalent to only 8 to 10 weeks of constant 3 to 5°C temperatures.

Several papers have demonstrated that long photoperiods may, at least in part, substitute for the chilling requirement for several species (Olmsted, 1951; Olson, Stearns, and Nienstaedt, 1959;

Nienstaedt, 1966, 1967; Worrall and Mergen, 1967; Farmer, 1968). Data have recently been interpreted to show that long photoperiods could substitute for the entire chilling period of Douglas-fir (Roberts, Tomasovic, and Fuchigami, 1974). The data, however, show that in some cases less than 25% of the seedlings were able to achieve bud break.

Once the chilling requirement has been naturally fulfilled, growth is not immediately resumed. Instead, the proper environmental stimuli must be received by the tree to initiate bud break. Must studies have shown that warmer temperatures in the spring are the primary factor responsible for growth resumption (Hermann and Lavender, 1965; Nagata, 1967; Lavender and Hermann, 1970; Campbell and Sugano, 1975; van den Driessche, 1975). The rate of bud break is influenced by the temperature, with warmer temperatures causing more rapid bud break. Photoperiod will influence bud break in the spring if the chilling requirement has not been fully met. Plants in this category will generally have reduced growth and, in most cases, abnormal or deformed growth.

Cold Hardiness

A phenomenon which is closely related to dormancy, although not synonymous with it, is cold hardiness or frost resistance. This ability of plants to tolerate low temperatures without injury has been the subject of several general reviews (Parker, 1963; Weiser, 1970; Alden and Hermann, 1971). Cold hardiness, or the lack of it, is of concern to foresters and nurserymen since frost injury in the nursery will reduce the survival potential and field performance of seedlings (Hermann and Zaerr, 1973; Hermann, 1974; Tanaka, 1974).

The ability of plants to develop cold hardiness increases when they become dormant and little hardiness can be developed before this time (Glerum, Farran, and McLure, 1966; Tumanov, Kuzina, and Karnikova, 1964, 1973a; Alden, 1971; Tumanov, Kuzina, Karnikova, and Khvalin, 1972). There are, of course, differences between species as to the degree of hardiness they possess and this is a function of their native climate (Sakai and Okada, 1971). The degree of frost hardiness which can be developed is also related to provenances within a species (Campbell and Sorensen, 1973; Maronek and Flint, 1974) with northern and high elevation provenances developing more hardiness than their southern and low elevation counterparts. There are also seasonal changes within individual trees as hardiness is gradually acquired and lost (Parker, 1961). Several methods for determination of cold hardiness exist and four of them have been compared by van de Driessche (1969c).

The study of the biochemistry of acquisition and loss of frost hardiness has been directed mainly at carbohydrate metabolism. In an early study, Dexter (1933) found that hardening was favored by an accumulation of carbohydrates due to increased photosynthesis and reduced respiration. More recently, Aronsson, Ingestad, and Lööf (1976) have been able to correlate hardiness with changes in carbohydrate content of Pinus silvestris and Picea abies. They found that the sucrose content increased significantly as hardiness developed under short day conditions, although they recognized this as only one factor of the many which may be involved. A similar conversion of starch to sugar was found to occur in willow and poplar as hardiness increased (Sakai,

1966). Chen and Li (1977) were able to show a decreased starch level and increased sugar, protein, and RNA levels as hardiness was developed under stress conditions in Cornus stolonifera. These changes were similar to those in seedlings which were hardened under short day conditions.

The uptake and incorporation of minerals has been studied during the process of hardening. Potassium and calcium levels were found to have no effect on frost hardiness of Pinus silvestris by Christersson (1975). Phosphorus, on the other hand, has been found to have an influence, although the mechanism is unclear. Biglov (1964) suggests that the effect of increased uptake is due to increasing and maintaining the level of high-energy phosphorus compounds involved in the process of hardening. Grenier and Willemont (1975), however, feel that the effect is due to the incorporation of phosphorus into lipids by de novo synthesis during frost hardening.

The actual process of gaining cold hardiness appears to occur in two stages (Weiser, 1970; Howell and Weiser, 1970). Under natural conditions, the first phase is induced by short days and the second phase is induced by low temperatures. Research into the effects of light and temperature on the development and loss of cold hardiness have provided some insight into how these environmental variables can be utilized to assure frost hardiness of nursery seedlings.

Light intensity during the first phase of hardening has been shown to influence the rate of hardening of Douglas-fir seedlings (van den Driessche, 1970; Timmis and Worrall, 1975). This was true for both long and short photoperiods. As light intensity decreased, the

photoperiod necessary for inducing hardiness was increased. This indicates that the amount of photosynthesis is an important factor during the first stage. If the intensity was sufficiently high, hardiness developed more rapidly under short photoperiods than under long ones.

Low temperatures have been found to promote hardiness more rapidly than warm ones (Tumanov and Krasavtsev, 1959; McGurie and Flint, 1962; Irving and Lanphear, 1967a, 1967b; Horiuchi and Sakai, 1973). Hardiness was increased by low temperatures even under long day conditions, although it was not as great as under short day conditions. Andrews and Pomeroy (1974) have found that diurnal freezing induced a higher degree of hardiness than continuous low temperatures.

While these studies have shown that hardiness can be increased by varying only one environmental factor, the degree of hardiness is not as great as that which is accomplished by the natural two phase hardening. Therefore, light and temperature must be used in combination to get the maximum benefit in terms of hardiness.

Studies which have looked at combinations of both factors provide support for the two phase nature of hardening (Zehnder and Lanphear, 1966; van Huystee, Weisner, and Li, 1967; Fuchigami, Weiser, and Evert, 1967; van den Driessche, 1969b; Sigeru and Sakai, 1972). Maximum hardiness in the species studied was found to develop if a period of short days preceded a gradual lowering of the temperature. The photoperiod during the period of low temperature was found to have little effect during that phase of hardening. Sub-freezing temperatures appear to be necessary for the development of maximum hardiness.

Other factors may be involved in the acquisition of cold hardiness, although they are probably less important than temperature and light. Chen, Li, and Burke (1977) showed that water stress increased cold hardiness of Cornus stolonifera under both long and short photoperiods. The increase reached a maximum after seven days of stress after which there was no further increase and the increase was small in comparison to the natural hardiness which the species develops. Timmis and Tanaka (1976) have shown that container density has an effect on cold hardiness of Douglas-fir with seedlings grown at lower densities having a greater degree of cold hardiness than those grown at higher densities. They attributed much of this effect to the better light and temperature conditions which occurred in the lower density treatments. They also found that mild water stress increased the capability of the seedlings to harden although it had no direct effect on the hardiness.

Dehardening typically occurs in the spring after the chilling requirement is met, although the two do not appear to be correlated (van Huystee, Weiser, and Li, 1967; Sigeru and Sakai, 1972; Murray and Byrnes, 1975). Loss of hardiness is brought about primarily by the consistently warm temperatures which the plants experience in the spring, and warm night temperatures are more effective than day temperatures in reducing hardiness.

Date of Lifting and Cold Storage

That the date of lifting influences the subsequent survival of the seedlings has been observed for several species, including Douglas-fir.

During some periods of lifting, the effect is expressed to a greater degree when the seedlings are cold stored prior to outplanting. This effect is closely tied to the physiological condition of the seedlings as expressed by the phases of dormancy which were discussed earlier.

Very little work has been done with container-grown seedlings in this area and most of the literature has dealt with bare-root stock. The influence of the date of lifting and planting on Douglas-fir has been realized for quite a few years (Lavender and Wright, 1960; Walters and Soos, 1961; Lavender, 1964; Smith and Walters, 1965; Krueger and Trappe, 1967). These studies showed that survival and vigor of fall-lifted stock were lower than that of seedlings lifted in the winter or spring. The magnitude of this effect increased as the stresses, such as water stress, at the planting site increased. This reduced survival was thought to be due to the disruption of the physiology of the seedlings at a time when they were susceptible to stresses encountered due to lifting. In general, lifting dates prior to December, when seedlings are in a deepening dormancy phase, result in poorer survival and reduced vigor. The date before which lifting is detrimental varies with location and varies at the same location between years. Since the general morphology of the dormant seedlings is the same throughout the dormancy cycle, the time of changes from one phase to another cannot be determined by simply looking at the seedlings.

One method of assessing the physiological condition of planting stock which has received a considerable amount of attention is the root regenerating capacity of the seedling at the time of lifting. The general method is to plant lifted seedlings into a container and place

them in an environment favorable for growth. After a specified length of time, seedlings are harvested and the incidence of new root growth observed.

Numerous studies of this sort have yielded a useful measure of the ability of seedlings to survive lifting and planting stresses (Stone, 1955; Stone and Schubert, 1959a, 1969b; Stone, Jenkinson, and Krugman, 1962; Stone, Schubert, Bensler, Baron, and Krugman, 1962; Zaerr, 1967; Lavender, Hermann, and Zaerr, 1970). In general, the root regenerating capacity of most coniferous species is low during the summer and rises during the fall to a peak during the winter and early spring. Brown (1976) has shown that this pattern is also present in western hemlock.

It has been found that short days followed by chilling of Douglas-fir seedlings prior to root regenerating tests increases the number of active roots (Zaerr and Lavender, 1974). Krugman and Stone (1966) found a similar effect of cold nights on the root regenerating capacity of ponderosa pine. This effect of cold temperatures may be an indication that seedlings in the deep dormancy stage, when the chilling requirement is being fulfilled, have an increased root regenerating capacity.

Although the root regenerating capacity cycle is not the same as the normal root growth periodicity, it does provide a valuable tool to assess the potential for survival. In order for a seedling to survive at its new site, it must have the capability to initiate new root growth when conditions are favorable. Thus a low root regenerating capacity would indicate a seedling which would not be able to establish itself and therefore die. Lifting seedlings during periods when root

regenerating capacities are high produce the maximum chance for successful regeneration.

Another method to determine the effect of lifting date on survival is to see if the seedlings will break bud in response to a favorable environment, including a long photoperiod (Lavender, Hermann, and Zaerr, 1970). This method will determine if seedlings are in the dormant stage and therefore less susceptible to the stresses experienced during lifting and planting. Seedlings in the dormancy deepening stage will not break bud under these favorable conditions and the transition between the two stages can thus be measured.

Both these methods take several weeks of work before the results are available. Because of this time factor, research is constantly going on to find a rapid method to determine the stage of dormancy a seedling is in and therefore its ability to survive lifting and planting.

Cold storage of seedlings after lifting is a common nursery practice and is reviewed in several papers (Hockings and Nyland, 1971; Brown, 1971, 1973). The objective of cold storage is to provide seedlings at the time they are needed for regeneration. High elevation sites which cannot be planted until after the natural date of bud break in the nursery will require seedlings that have been held in cold storage to maintain dormancy. This will, in essence, lengthen the planting season available for regeneration. In instances where nursery seedlings cannot be lifted from the nursery due to frozen ground during the winter, cold storage offers the possibility of providing stock which is lifted in the fall and stored until needed. Cold storage can

also be used as a method to hold over surplus stock.

Many aspects of the process of cold storage have been studied, such as storing seedlings in open bales vs. polyethylene bags (Duffield and Eide, 1959; Slayton, 1970; Mullin, Bunting, and Rogers, 1974) and using different temperatures during storage (Deffenbacher and Wright, 1954; Aldhous, 1964; Aldhous and Atterson, 1973). While storage in closed bags maintains more moisture in seedlings, open bale storage does not seem to adversely affect survival. Storage temperature does have an effect on the future survival and growth of seedlings. About 2°C has been shown to be the best temperature for storing many conifers. Storage at temperatures below 0°C usually has detrimental effects on survival after planting. In many instances, storage of seedlings at temperatures above 2°C has resulted in problems of molding, and this problem has been reviewed by Hopkins (1975).

Hellmers (1962) has noted that a disappearance of starch occurs in Jeffrey pine seedlings during storage and he concluded that this was directly related to decreased field survival. This being the case, he suggested using a rapid starch determination as a check of stock prior to shipment. Ronco (1973) noted a similar reduction in carbohydrates in Engelmann spruce during storage. Although he was unable to show a correlation between food reserves and survival, he did suggest that survival may be adversely affected by carbohydrate levels below a certain threshold level at the time of planting.

Moisture content of seedlings during storage has also been studied as a method of determining the potential survival after planting (Eliason, 1962; Tarrant, 1964; Långström, 1971). While

relationships vary depending on the species, there does seem to be a general correlation between water content or weight loss of seedlings and mortality. This provides another possible rapid check of stock after cold storage to assess their quality.

Field survival, of course, will be the measure by which the success of cold storage must ultimately be measured. Studies of survival of cold stored seedlings have yielded a somewhat cloudy picture (Ruth, 1953; Simon, 1961; Jorgensen and Stanek, 1962; Dick, 1963; Tregunna and Crown, 1974). In some studies survival and growth was unaffected after cold storage, whereas in others, growth and survival were markedly reduced by storage. In many of the studies, especially the early ones, the date seedlings were lifted and placed into cold storage was not mentioned or considered inconsequential. As with stock planted immediately after lifting, the date of lifting has a large effect on the ability of seedlings to withstand cold storage.

Cold storage of seedlings at temperatures below freezing has been shown to delay spring bud break of several species (Nyland, 1974); however, the seedlings were better able to withstand spring frosts than freshly dug seedlings. Cold storage for 12 weeks at 2°C has been shown to satisfy the chilling requirement of Douglas-fir and stored seedlings broke bud as rapidly as seedlings which had received a natural chilling (van den Driessche, 1977).

As mentioned, the date of lifting has a major influence on the ability of seedlings to withstand cold storage. Fall lifting followed by cold storage further reduces the survival of fall lifted seedlings in many species. The date after which seedlings can be safely stored

varies with the species and will vary with the fall conditions experienced by the seedlings. Kahler and Gilmore (1961) found that lifting and storage of loblolly pine prior to late December resulted in increased mortality. Working with Norway spruce, western hemlock, Douglas-fir, and western red cedar, Aldhous (1967) found that Norway spruce could be stored if lifted after November and the remainder of the species if lifted after December. He also found that all the species studied stored better at 2°C than at -5°C. The commonly used date in the Northwest before which cold storage will be detrimental to Douglas-fir is mid-November (Winjum, 1963; Hermann, Lavender, and Zaerr, 1972). If Douglas-fir must be lifted and cold stored prior to this time, a daily photoperiod during the storage will reduce to some degree the amount of harm done. Seedlings stored during the safe period also seem to benefit from a daily photoperiod during storage.

The adverse effect of fall cold storage on Douglas-fir appears to be exerted through the roots. Hermann (1967) has shown that the sensitivity of roots to exposure has a seasonal variation and that sensitivity is high during the early fall. With bare-root seedlings, this may be a reason for reduced survival. Lavender and Wareing (1972) have shown that the root temperature also has an effect on survival after storage. They found that much of the adverse effect of storage was reduced if the roots were maintained at 15°C while the shoots were maintained at 2°C. Thus, with bare-root stock, the exposure and cold temperature experienced by the roots seems to be responsible for the harm to seedlings. With containerized seedlings, the root exposure

would not be a problem during cold storage but low temperature could cause reduced survival.

Growth Regulators

Since the original discovery of auxin by Went, a great deal of research has been done in the areas of plant hormones and growth regulators, and several reviews have been published (Giertych, 1964; van Overbeek, 1966; Glaston and Davies, 1969; Wolter, 1973). Several broad categories of hormones have been defined and include auxins, gibberellins, cytokinins, inhibitors, and ethylene. Other postulated hormones are thought to exist, such as the flowering hormone and the wound hormone. As the research on hormones became more sophisticated, it became evident that the actions of hormones in plants were interrelated. Thus, the absolute concentration of a hormone may not be as important as the balance between it and other hormones. Although the exact mechanism of hormonal action has not been elucidated, many hormones have been found to affect protein synthesis in some way.

In many instances, changes in hormone levels are brought about by changes in the environment in which the plant is growing (Nitsch, 1957b, 1963; Villiers, 1966; Tillberg, 1974; Naqvi and Engvild, 1974). The most influential factors are photoperiod, moisture relations, and temperature. This relationship between the environment and hormone level can be used to explain much of the effect of environment on growth and development of plants, although the specific mechanism is not yet clear.

The theory that dormancy is regulated by hormonal control has been reviewed by Smith and Kefford (1964) and Wareing and Saunders (1971). Work in this area has concentrated primarily on the interaction of inhibitors, such as abscisic acid (ABA), and promoters, such as auxins and gibberellins. Work on the effects of the individual hormones also has appeared, although the ability to attribute an effect to a single hormone is difficult.

The application of exogenous hormones and regulators as a method to control dormancy induction and release has been attempted for several species (Lockhart and Bonner, 1957; Larson, 1960; Ashby, 1963; Farmer, 1966; Pharis, Ruddat, and Phillips, 1967; Hassig and King, 1970; Altman and Goren, 1974). In general, these studies have primarily concentrated on the use of ABA and other retardants as methods to induce dormancy and on gibberellic acid (GA) as a method to break dormancy. A wide variety of responses to hormone application is evident from these studies. In some instances, the response of a single species varied with the time of application. Responses ranged from successful initiation or breaking of dormancy to no detectable response. Because of the erratic and unpredictable response, Lavender and Zaerr (1972) suggested that the most effective and practical way to control dormancy in nursery stock is to regulate the seedlings' environment.

Determination of the levels of endogenous hormones throughout the year have shown some general trends which may have some implications as to the hormonal regulation of dormancy. The seasonal changes in ABA and indole-3-acetic acid (IAA) will be discussed in more detail later.

Early work postulated that the balance between ABA and IAA was responsible for the control of dormancy (Allen, 1960; Giertych and Forward, 1966). ABA was found to increase during the initiation of dormancy and decrease at the time of bud break. IAA followed a trend opposite that of ABA.

As the specificity of bioassays has been improved and instrumentation developed for quantitative analysis of hormones, the emphasis has shifted to the possibility of the balance between ABA and GA as the controlling mechanism of dormancy (Eagles and Wareing, 1964; Thomas, Wareing, and Robinson, 1965; Bachelard and Wightman, 1974). GA content in the bud normally increases in the spring prior to bud break and is accompanied by a decrease in ABA. It is interesting to note that in Douglas-fir much of the GA increase is thought to be due to movement of GA from the roots into the shoots and that the root synthesis is dependent on soil temperature (Lavender, Sweet, Zaerr, and Hermann, 1973).

While these hormone trends correlate very well with dormancy periods, the question is yet to be answered if they are, in fact, regulating the dormancy of plants or rather are just a parallel physiological process. An answer to this question may be found when the mechanisms of actions are found for the hormones and lies within the realm of molecular biology. At the present time, however, there appears to be good evidence to implicate hormones, at least to some degree, in the regulation of dormancy.

Indole-3-Acetic Acid

Indole-3-acetic acid is the major naturally occurring auxin in most plant species. Other naturally occurring auxins have been

reviewed by Bentley (1959) and the biochemistry of IAA has been reviewed by Schneider and Wightman (1974). IAA is generally considered as a promoter of growth, although in some instances and at high concentrations it can act as a growth inhibitor. It has been shown to influence many processes within the plant and these can be found in most plant physiology texts.

Various bioassays have been the standard method of detection of IAA since its discovery. However, due to the lack of specificity of some of the bioassays and the inability to definitively identify the compound, several new methods have been presented to get quantitative and qualitative information about IAA levels. Among these methods are spectrophotofluorometry (Powell, 1964; Stoessl and Venis, 1970) and gas-liquid chromatography (Dedio and Zalik, 1966; DeYoe and Zaerr, 1976a, 1976b).

Identification of IAA in confiers has been complicated by the large number of compounds which interfere with isolation and detection of the hormone. However, with new isolation techniques, IAA has been identified as an endogenous substance in several genera, including Pinus (Aldén and Eliasson, 1970), Picea (Steen, 1972), and Pseudotsuga (DeYoe, 1974).

In studies which have investigated the seasonal variation of IAA content in conifers, a similar trend has been found for several species (Aldén, 1971; DeYoe, 1974; Dunberg, 1976). IAA concentration begins to increase immediately prior to bud break in the spring and reaches a maximum during late spring or early summer. During the late summer the concentration decreases and is generally so small during the winter

that it is not detectable. In Douglas-fir, the maximum concentration during the spring has been found to fall in the range of 0.7 to 2.5 micrograms of IAA per kilogram of fresh weight.

The effect of IAA on root growth has been known for some time; however, little correlation between IAA concentration and root regeneration potential has been found (Zaerr, 1967). Brown (1976) has recently shown that the ratio of ABA and IAA can be correlated with root regenerating capacity of western hemlock. IAA may thus have an effect on the phenomena of root regenerating potential, although it is not yet clearly understood. Roberts and Fuchigami (1973) have shown that exogenous auxin can increase rooting of Douglas-fir seedlings as did cold treatments. They advanced the hypothesis that auxin is not the limiting factor of rooting during bud dormancy and that the cold treatment is responsible for bringing levels of promoters and inhibitors into proper balance for rooting of cuttings.

Eliasson (1975) has shown that in excised stem segments exogenously applied IAA has an influence on maintaining ABA levels. Under control conditions both IAA and ABA levels decreased. This indicated that auxin may be an important factor for maintaining high ABA levels in stems of intact plants. He viewed his results as support for the hypothesis that high auxin levels maintain high ABA levels and that it is the high ABA level that prevents the growth of lateral buds.

Abscisic Acid

Since its initial isolation as a component of the β-inhibitor complex, ABA has been thought to be intimately related to dormancy. The

general topic of ABA has been reviewed by Addicott and Lyon (1969) and Milborrow (1974) and aspects of the biochemistry and physiology of ABA have been discussed in several smaller reviews (Wareing, Good, and Manuel, 1968; Addicott, 1970; Milborrow, 1973). There is general agreement that ABA functions as an endogenous growth regulator in many plant species. It is generally considered to be inhibitory to growth and a wide variety of processes have been thought to be under its control as stated in most plant physiology texts. ABA may antagonize the effects of growth promoters such as auxins, gibberellins, and cytokinins, but in most cases it does so at noncompetitive sites.

There is good evidence that many of the effects of ABA are manifest through a reduction or alteration of RNA synthesis (Kahn, 1970; Sankhla and Huber, 1974; Walbot, Clutter, and Sussex, 1975). Exogenous ABA has been shown to alter the qualitative enzyme synthesis as well as a quantitative reduction of synthesis. The reduced synthesis appears to be due to the inhibition of phosphorus incorporation into RNA.

As with most hormones, bioassay was the original method for determining inhibitor content of extracts. However, in addition to the normal limitations of bioassays, this type of detection of ABA also suffers from the need to provide a growth promoter in many of the systems in order to magnify the effect of ABA. Several other methods for qualitative and quantitative determinations of ABA have been identified, with the most commonly used methods being spectropolarimetry (Cornforth, Milborrow, and Ryback, 1966; Milborrow, 1967, 1968; Feucht, Khan, and Daniels, 1974) and gas-liquid chromatography (Lenton, Bowen, and Saunders, 1968; Davis, Heinz, and Addicott, 1968; Lenton, Perry,

and Saunders, 1971). Both methods have been shown to provide reproducible results and can be used to detect submicrogram levels of ABA.

Changes in the ABA level in plants throughout the year, and especially during the dormant period, has led many authors to speculate on the role of ABA or the β-inhibitor complex in controlling dormancy in many species (Hemberg, 1949, 1958; Phillips and Wareing, 1958a; Bowen and Hoad, 1968; Little and Eidt, 1968; Tumanov, Kuzina, and Karnikova, 1973b; Düring and Bachmann, 1975). These studies have shown an increase in ABA level prior to initiation of dormancy in the fall and a maintenance of that level into early winter. At some point, the level begins to fall and is low by the time of spring bud break. Webber (1974) found such a trend in Douglas-fir. Maximum levels in the buds occurred in October and were 1.2 micrograms of ABA per gram of fresh weight. While he found that ABA level followed the dormancy cycle, he thought it had a secondary role and might not have a causal relationship.

This relationship between ABA and dormancy is further supported by the observations that application of exogenous ABA can cause dormancy to be induced in several species (Eagles and Wareing, 1963; El-Antably, Wareing, and Hillman, 1967). The ability to induce dormancy with exogenous ABA has, however, been rather erratic and some studies have been unable to induce dormancy in this manner (Hillman, Hockings, and McWha, 1973).

The possibility of an interaction of ABA and the gibberellins in controlling dormancy was discussed earlier. Alvin, Hewett, and Saunders (1976) followed the levels of ABA and cytokinins in willow

throughout the year. They found that dormancy was associated with a high ABA level and a low cytokinin level. Spring growth was preceded by a decreased level of ABA and an increased level of cytokinins. Others who have followed ABA and cytokinin levels (Taylor and Dumbroff, 1975; Dumbroff and Brown, 1976) have found only slight variations of ABA level throughout the dormant period of Acer saccharum. Cytokinin levels did increase prior to bud break but were not considered to be the controlling factor of bud break.

In order for ABA to regulate dormancy, it must have a relationship to environmental stimuli to control its level in the plant. Many early studies showed photoperiod to have a direct influence over the inhibitor level in several species (Wareing, 1954; Phillips and Wareing, 1958b, 1959; Nitsch and Nitsch, 1959; Kawase, 1961). Under short day conditions, the inhibitor level was found to increase and the length of the dark period rather than the light period was actually responsible for the increases. This build up of inhibitor was rapid and could be detected after two short day cycles. Because of the strong influence of photoperiod on induction of dormancy, they considered the build up of inhibitor to be a causal factor. Much of this work was done prior to the actual isolation of ABA and with bioassays which were not specific for ABA. Therefore, the actual nature of the compound or compounds causing the inhibition was not clear at the time.

Recent studies (Zeevaart, 1971, 1974; Lenton, Perry, and Saunders, 1972) using optical rotatory dispersion or gas-liquid chromatography have been unable to confirm the increase of ABA under short photoperiods. In fact, in some instances ABA level was found to be higher

under long photoperiods than under short ones. This evidence has caused a re-evaluation of the role of ABA in regulating dormancy. It may be that another inhibitory substance with an R_f value similar to ABA may be influenced by photoperiod and thus caused the increased inhibition in the early bioassays, although this is not yet clear. Short photoperiods, while not changing ABA concentrations, may be causing reduced levels of a promoter and thus be altering the balance between inhibitors and promoters. Whatever the case may be, there are ample indications that hormones are in some way, either directly or indirectly, involved in dormancy and this will be an exciting area of future research.

A number of interesting articles have been published dealing with ABA and the water relations of plants. Reports that the application of exogenous ABA to plants could cause stomatal closure and reduce transpiration (Mittelheuser and Van Steveninck, 1969; Jones and Mansfield, 1970) led to a surge in research on the possible use of ABA as an antitranspirant. A number of papers have investigated this area and provided some understanding of its mechanism (Horton, 1971; Kriedemann, Loveys, Fuller, and Leopold, 1972; Mizrahi, Scherings, Arad, and Richmond, 1974; Davies and Kozlowski, 1975b; Nordin, 1976). The exogenous ABA appeared to be acting directly on the guard cell of the stomata to result in closure and was effective at very small concentrations with as little as 0.02 micrograms per cm^2 causing a response in some species. The response was rapid and occurred in one to thirty minutes after application, depending on the species. The response time was well correlated with stomatal sensitivity of the species. The

reduction of transpiration from one application was also a long lasting response. This reduction lasted up to 21 days and, again, was dependent on the species and the environmental conditions under which they were maintained. These studies were carried out in growth rooms since ultra-violet radiation will cause the isomerization of ABA from its natural active form to an inactive form. This fact, of course, makes the field use of ABA as an anti-transpirant unfeasible.

This work with exogenous ABA has led several of the authors to speculate on the role of endogenous ABA in controlling the water relations of plants. Work on the levels of endogenous ABA in relation to various forms of water stress seems to support this idea. ABA levels have been shown to increase in detached wheat leaves as they wilt (Wright and Hiron, 1969) as well as in intact seedlings under water stress (Hoad, 1973; Dörffling, Šonka, and Tietz, 1974). Up to 20 fold increases were reported. This increase has been shown to be due to a rapid increase in de novo synthesis rather than the release of a bound form (Milborrow and Robinson, 1973; Harrison and Walton, 1975). Rasmussen (1976) showed that while ABA levels increased with rapid stress, the same stress built up over an 18 day period did not significantly raise ABA levels in tomato leaves. Apparently the increase in some species occurs only in response to a rapid stress build up.

A wide variety of conditions can cause a water imbalance in plants and include a stream of warm air being blown over the plants (Hiron and Wright, 1973), low relative humidity (Mizrahi, Blumenfeld, and Richmond, 1970; Hoad, 1975), and waterlogging of the root system (Wright and Hiron, 1970). Under all conditions, an increase in ABA

concentration was found. In plants which experienced a water deficit at the roots or an increased transpiration rate, stomatal closure generally occurred and allowed the regaining of leaf turgor within two hours. In these cases, however, ABA levels did not return to the pre-stress level for several days.

Recent work seems to indicate a threshold value of water stress above which ABA concentrations begin to rapidly increase. Zabadal (1974) found that ABA levels began to increase at a plant water potential of -10 to -12 atmospheres for two species of Ambrosia. Beardsell and Cohen (1975) found a similar threshold effect at -8 to -10 bars in maize and sorghum. They also found that ABA increases coincided with a threshold value of stomatal resistance. Stomatal closure occurred at similar values and the level of ABA continued to increase after stomatal closure. Working with Douglas-fir, Blake and Ferrell (1977) showed an ABA increase at a water potential of -10 to -12 bars. They also found that soil water potential was more closely related to ABA level than plant water potential. This methodology was more closely related to the natural environment experienced by Douglas-fir. They suggested that roots are the drought sensing organ and that the message is then acted upon in the needles by stomatal closure and ABA increases. It has been suggested that high ABA levels induced by water stress may contribute to the cessation of shoot growth during the summer (Saunders, Harrison, and Alvin, 1973). Because of the importance of water stress in inducing dormancy under natural conditions in the Pacific Northwest, there is the possibility that ABA is in some way

regulating the process. However, there is only circumstantial evidence for this at the present time.

ABA levels in drought tolerant varieties of corn have been found to be up to four times higher than in the normal varieties under the same conditions (Larque-Saavedra and Wain, 1974). This may be part of the reason for the increased tolerance. With the current emphasis to find drought tolerant varieties of Douglas-fir, this relationship may provide a new tool for the assessment of potential tolerance.

MATERIALS AND METHODS

The western hemlock seedlings used throughout this study were obtained from the Crown Zellerbach Wood Nursery in Aurora, Oregon and were in their first season of growth. The seedlings were actively growing in molded units of black polyethylene when delivered and were then maintained in greenhouses at the Forest Research Laboratory until they were needed for studies. The individual tubes were approximately six inches long and three quarters of an inch in diameter. Rooting volume was about three cubic inches and rooting medium was a mixture of ground Douglas-fir bark and peat moss. Seedlings were grown at a density of about 90 seedlings per square foot. Seeding of the containers was in late February or early March with a low elevation (500') Western Oregon seed source (tree seed zone 051) and were grown in temperature controlled greenhouses at the nursery. Original height of the seedlings when obtained varied from year to year in the range of four to eight inches but was quite uniform within the individual years.

Most of the studies were carried out in controlled environment rooms at the Forest Research Laboratory. The rooms were ten feet square and seven feet high with lighting provided by 42 eight foot high-output, cool white fluorescent tubes and six 100 watt incandescent bulbs. Light intensity at the seedling top varied but was generally around 400 foot candles (4000 lux) as measured by a Weston illumination meter, model number 756. The growth rooms provided control of photoperiod, thermoperiod, and day and night temperatures.

Temperature and relative humidity were constantly recorded by a hygrothermograph.

Dormancy Induction

The ability to initiate dormancy is an essential tool in the production of containerized seedlings. In this way, the nurseryman is able to provide seedlings in the proper condition at the time that they are desired for regeneration. The effects of individual environmental factors on initiating dormancy in western hemlock have been studied (Cheung, 1973), but the most effective system combining several of these factors has not been determined for the species and was the goal of this study.

Three pairs of treatments, long and short photoperiods, warm and cool temperature regimes, and moderate and low moisture stresses, were used in a 2x2x2 design and the conditions are shown in Table 1.

Table 1. Treatments used to test the initiation of dormancy.

Treatment	Photoperiod	Temperature[1]	Moisture Stress[2]
1	16 hrs	cool	low
2	16 hrs	cool	moderate
3	16 hrs	warm	low
4	16 hrs	warm	moderate
5	8 hrs	cool	low
6	8 hrs	cool	moderate
7	8 hrs	warm	low
8	8 hrs	warm	moderate

[1]Cool = 18°/12°C; Warm = 25°/20°C

[2]Low = <5 bars stress; Moderate = 12-15 bars stress

Originally, the effect of nutrient level was also to be included, but the presence of a slow release fertilizer in the planting medium necessitated studying this factor at a subsequent time. The effect of low soil temperatures was assessed using cold water baths under a short photoperiod. In these treatments both temperatures and both moisture stress levels were tested. The treatments were applied to two seed sources, the low elevation source (500') and a high elevation source (1000'), and each treatment was composed of 25 seedlings of each source.

Warm and cool temperature regimes were maintained by using two growth rooms and the day temperature was maintained for 16 hours. Each growth room was set for a 16 hour day length coinciding with the day temperature and short day treatments were maintained by using blackout boxes to maintain darkness during the last 8 hours of light. The blackout boxes were constructed to allow free air circulation to minimize temperature differences and were used whenever short photoperiods were required. Moisture stress was monitored by weighing the plant blocks to measure the water content of the blocks. Low moisture stress treatments were watered to field capacity every other day and never experienced pre-dawn plant moisture stress of more than 5 bars, as measured by a pressure chamber apparatus (Scholander, Hammel, Bradstreet and Hemmingsen, 1965).[1] Moderate moisture stress treatments

[1]Throughout this thesis, the water status of the seedling will be expressed as plant moisture stress rather than as xylem pressure potential (Waring and Cleary, 1967). The plant moisture stress will be defined as the absolute value of the xylem pressure potential and the units of the moisture stress will therefore be positive. A larger positive value for moisture stress will thus indicate the same change in water status as would a more negative value for xylem pressure potential.

were watered when the blocks had lost two pounds of water and corresponded to a pre-dawn moisture stress of 12 to 15 bars. Water baths were maintained at 10°C and blocks in these treatments were enclosed in water-tight metal boxes to prevent water from getting to the root system.

Prior to beginning the treatments, all seedlings were maintained in a growth room set for a 16 hour photoperiod at a constant temperature of 20°C and were well watered for a period of six weeks to be sure they were all vigorously growing at the time the study started. On September 15, 1975 treatments began and original shoot lengths were measured to the nearest millimeter. Shoot length was measured weekly until the conclusion of the study on December 1, 1975 when the seedlings were harvested. The major criterion for determining dormancy was the cessation of shoot elongation since terminal bud formation of western hemlock is difficult to observe. At the time of harvest, each seedling was examined and the incidence of a visible terminal bud was recorded. Additional harvest data included stem diameter and length, root length and activity, root and shoot dry weight after oven drying for three days at 70°C, and shoot-root ratios.

In the following year, seedlings of the low elevation source were obtained which did not include the slow release fertilizer in the planting mix so the effect of nutrient level could be assessed. The effect of nutrient level was studied on selected combinations of the other three factors in the original study and were: a) a short photoperiod, moderate water stress, and a warm or cool temperature; b) a long photoperiod, low moisture stress, and a warm temperature; and c) a

natural photoperiod, moderate moisture stress, and a warm temperature (Table 2). The natural photoperiod varied from slightly over 13 hours at the start of the treatment to about 9-1/2 hours at the time of harvest.

Table 2. Treatments used to test the initiation of dormancy (second study).

Treatment	Photoperiod	Moisture Stress[1]	Temperature[1]	Nutrient Level
1	8 hrs	moderate	warm	high
2	8 hrs	moderate	warm	low
3	8 hrs	moderate	cool	high
4	8 hrs	moderate	cool	low
5	16 hrs	low	warm	high
6	16 hrs	low	warm	low
7	natural	moderate	warm	high
8	natural	moderate	warm	low

[1]See Table 1

Manipulation of the nutrient level was done by varing the amount of a modified Hoagland's solution which the seedlings received. The solution used contained 1400 ppm of nitrogen, 357 ppm of phosphorus as phosphate, and 1655 ppm of potassium plus other macro- and micronutrients that had been successfully used on Douglas-fir. For a high nutrient level, 3 ml of solution was applied once a week to each seedling. The low nutrient level treatment utilized 0.3 ml of the solution weekly.

Methodology of the other environmental variables was the same as in the initial study and each treatment consisted of 50 seedlings. All seedlings were again pre-conditioned with favorable conditions for growth prior to the treatments which began on September 6, 1976. Shoot length was measured weekly throughout the study and the cessation of growth was again used as an indicator of dormancy. Foliage samples were collected at two-week intervals and analyzed for nitrogen by the micro-Kjeldahl method (A.O.A.C., 1950). These analyses indicated that the low nutrient level was having no effect on the foliage nutrient level. Therefore, the low nutrient level treatments received no nutrient solution after the fifth week of the study.

On November 15, 1976, 10 weeks after treatments started, 25 seedlings of each treatment were harvested and the same data recorded as in the initial study. The other 25 seedlings were placed outside under partial shade with the blocks surrounded by sawdust to determine the effect of the dormancy inducing regime on the subsequent spring growth.

Chilling Requirement

Most studies dealing with the chilling requirement of temperate plants indicate that a constant temperature of around 5°C is most effective in fulfilling the requirement. However, plant species do vary in the duration of such treatments required to stimulate resumed growth. Work with Douglas-fir has shown that a period of mild, short days after bud set is necessary in order to receive the beneficial effects of chilling. Therefore, this study was undertaken to ascertain

the chilling requirement of western hemlock and the effect of conditions prior to chilling on the effectiveness of the chilling received.

Seedlings used in this study were transplanted into Crown Zellerbach Dee-Pots. These containers are 10 inches long and 2-1/3 inches in diameter and contain 40 cubic inches of rooting volume. The additional planting mix was identical to that in which the seedlings were growing. In addition, some seedlings were transplanted into clear plastic cylinders measuring 15 inches long and 4 inches in diameter using the same planting mix. The cylinders were covered with removable black plastic so that the root system remained in darkness. These cylinders are designed to observe root growth and allow a calculation of the rate of growth without disturbing the root system. All transplanting was done at the end of July 1974 and, after transplanting, the seedlings were maintained in a growth room set for an 8 hour photoperiod and a 27°/21°C temperature regime until buds developed and the treatments began.

Seedlings were chilled for either 4, 6, 8, or 12 weeks at a constant 5°C temperature. This chilling took place in a room which was equipped with fluorescent tubes to provide an 8 hour daily light period at an intensity of about 100 foot candles. To test the effect of the pre-chilling environment, the seedlings were exposed to each of the periods of chilling after one of the following pre-treatments: 1) immediately after bud set; 2) bud set followed by 3 weeks of mild, short days; 3) bud set followed by 6 weeks of mild, short days; 4) bud set followed by 6 weeks of mild, long days; and 5) bud set followed by 6 weeks of natural days (Table 3).

Table 3. Treatments used to assess the chilling requirement.

Pretreatments were:	Chilling treatments (2°C) were:
1) None	1) 4 weeks
2) 3 weeks of short day	2) 6 weeks
3) 6 weeks of short day	3) 8 weeks
4) 6 weeks of long day	4) 12 weeks
5) 6 weeks of natural day	After chilling, seedlings were placed in a growth chamber.

Each of the treatments was made up of 30 seedlings and consisted of 25 in the Dee-Pots and 5 in the clear plastic cylinders. The study was begun on October 1, 1974.

The effectiveness of each of the pretreatment-treatment sequences was measured by moving the seedlings into a growth room set for a 12 hour photoperiod and a 22°/18°C temperature regime immediately after the end of the chilling period. The rapidity with which terminal and lateral buds resumed growth under these conditions was the primary criterion for assessing treatment efficiency, and the buds were examined twice a week. Observations of root growth in the clear plastic cylinders were made periodically throughout the study.

After 6 weeks in the growth room, seedlings were harvested in a manner similar to the first study. Data recorded for each seedling included stem diameter and length, number of new shoots, root length and activity, and oven dry weight of the shoot and the root.

Effect of Fall Photoperiod Upon Spring Bud Break

As mentioned in the chilling requirement study, some species benefit from a period of short days prior to chilling. This study was conducted to establish whether the effects of the photoperiod during the fall would be expressed in the vigor of spring growth after natural over-wintering. If long photoperiods during the fall after bud set have no effect on the spring growth, the necessity for careful scheduling of greenhouse photoperiodic conditions is reduced during this period.

Western hemlock seedlings were exposed to short, natural, or long photoperiods under mild conditions from October 1, 1974 until November 19, 1974. Each treatment consisted of 30 seedlings identical in previous potting and history as those used in the study of the chilling requirement. Once again, each treatment was composed of 25 seedlings in Dee-Pots and 5 seedlings in clear plastic cylinders.

After the photoperiodic treatments were ended, all seedlings were placed outside under partial shade to receive natural over-wintering. Each container was surrounded with sawdust to minimize the amount of freeze damage to the roots. In the spring of 1975 each seedling was examined every other day and the incidence of terminal and lateral bud break was recorded. This was the primary measure of the extent to which the fall photoperiod affected the seedlings in the spring.

Seedlings were watered to field capacity twice a week until July 14, 1975 at which time watering was discontinued. The date at which terminal bud first appeared was then recorded. On September 30 and October 1, 1975 all seedlings were harvested and data recorded for

each seedling included shoot elongation, stem diameter and length, root length and activity, and shoot and root oven-dry weights.

Effect of Cold Storage Upon Growth Response

Cold storage of seedlings is a common operational process in many nurseries and can provide good planting stock. Unfortunately, cold storage can also have a detrimental effect on the planting stocks survival potential if it is not timed correctly. Cold storage of Douglas-fir in the wrong phase of dormancy has generally resulted in high mortality rates after planting of the seedlings (Lavender, 1964). Very little work has been done in the area of cold storage of containerized seedlings and the purpose of this study was to determine if the same storage-dormancy interaction shown for bare-root Douglas-fir is also present in containerized western hemlock.

Seedlings obtained from the nursery were kept in their original containers and placed in a greenhouse at the Forest Research Laboratory and allowed to go dormant under moderate water stress. Buds began appearing on the seedlings in early September.

On October 1, 1975 the first cold storage treatments began. Two groups of 50 seedlings in their original containers and two groups of 50 seedlings which had been transplanted into Dee-Pots the day before were moved into a room which was maintained at about 5°C. During this period, one group of each pair received an 8 hour daily photoperiod while the remaining seedlings were stored in darkness. The photoperiod was provided by fluorescent tubes and had an intensity of about 100 foot candles. All seedlings were watered once a week to maintain soil

moisture during the storage. Following one month of cold storage, half of each group of 50 seedlings were moved into a growth room and the other half were planted in a cold frame to be observed the following spring.

Seedlings in the growth room received a 16 hour photoperiod and an 18°/12°C temperature regime and were well watered in order to assess the growth response immediately following the cold storage. Seedlings were examined twice a week and the incidence of broken terminal and lateral buds was recorded. After six weeks in the growth room, the seedlings were harvested and the data recorded included terminal elongation, number of new shoots, stem diameter and length, root length and activity, and shoot and root oven dry weights.

Seedlings transplanted into the cold frame were maintained under partial shade and received natural over-wintering in order to assess the growth response the following spring. In the spring, seedlings were examined once a week to determine the date of terminal and lateral bud break and any mortality which occurred. Seedlings were harvested on October 4, 1976 and data recorded included stem length and diameter, root length and activity, and shoot and root oven dry weight.

Seedlings which were not placed into cold storage in October were maintained in an unheated greenhouse with the natural photoperiod until either December 1, 1975 or February 1, 1976. On these dates the cold storage procedures similar to those for the October group were repeated with the single modification that an extra group of seedlings in the February treatment received a 16 hour photoperiod during the storage. The entire study was duplicated using seedlings of the same

seed source which were obtained from the nursery immediately prior to the storage dates. This procedure gave an indication of the results which could be expected for seedlings under an operational situation.

Results from this study in relation to the seedlings which were transplanted into Dee-Pots prior to cold storage stimulated an additional study of various types of root disturbance before storage. In addition to using seedlings grown in the polyethylene blocks, seedlings grown in styro-2 containers were also used to see if the container type had any effect on the results. The styro-2 containers were molded units of styrofoam. Individual cavities were approximately four and one-half inches deep and one inch in diameter. Rooting volume was about two and one-half cubic inches and the planting medium was a mixture of peat moss and vermiculite. Seedlings were growing at a density of about 90 seedlings per square foot. All seedlings were allowed to go dormant in a greenhouse under the natural photoperiod and with moderate moisture stress.

This follow-up study examined the effect of storage for one month during December 1976 followed by six weeks in a growth room. A variety of treatments were applied to the seedlings the day before cold storage and included leaving them in their original containers, pulling them out and replacing them into their original containers, transplanting them into Dee-Pots using the same type of planting mix or soil, and removing them from their containers with storage in a closed, clear polyethylene bag. Seedlings stored out of containers were planted into Dee-Pots using the planting mix after the storage period. The container seedlings were watered once a week and received an 8 hour

photoperiod during storage. In addition, seedlings which had not been disturbed prior to storage and ones transplanted into bark were also maintained in dark storage as a check of the influence of the photoperiod. Each treatment in this study was composed of 20 seedlings and the procedures used after cold storage were the same as those in the initial study of seedlings moved into the growth room.

Depth of Dormancy Stages

An understanding of the physiological stages of dormancy is essential to proper planning of nursery operations to provide quality planting stock. Application of any stress associated with nursery operations, such as lifting or cold storage, during an improper phase usually results in seedlings with a greatly reduced survival potential. The phases of dormancy and their implications on permissible nursery practices are reasonably well understood for Douglas-fir. The purpose of this study was to determine if a similar set of stages of dormancy existed in western hemlock seedlings. If they did, they would probably require the additional nursery planning which is necessary for several other species in order to provide quality planting stock.

Seedlings were allowed to go dormant in a greenhouse under moderate moisture stress. On October 15, 1975 three groups of 25 seedlings were brought into a growth room to test their growth response. The growth room was set for a 16 hour photoperiod and an 18°/12°C temperature regime coinciding with the photoperiod and the seedlings were well watered. One group was maintained with an 8

hour photoperiod, a second with a 16 hour photoperiod, and the third with a 16 hour photoperiod made up of 8 hours of high intensity light (400 foot-candles) and 8 hours of low intensity (20 foot-candles) light. The low intensity light was produced by layering cheese cloth over a frame until the proper intensity was achieved. The effects of the treatments were evaluated by examining the seedlings twice a week and recording the dates of terminal and lateral bud break for each seedling.

The seedlings were harvested after they had been in the growth room for six weeks. Data recorded at this time included terminal elongation and number of new shoots, stem diameter and length, root activity and length, and shoot and root oven dry weights.

Seedlings which were not used in the October test were moved outside under partial shade and were packed in sawdust until December 15, 1975 or February 15, 1976. On these dates, seedlings were placed into the same growth room and the same procedure as that used in October was repeated.

Data from this study also prompted a further study the following year in order to obtain a more precise understanding of the stages and their relative lengths. The procedures used in this study were identical to those used in the initial study except that test dates were more frequent. Tests began on October 1 and 15, November 1 and 15, December 1 and 15, 1976, and January 1, 1977.

Hormone Extraction and Purification

A wide variety of plant processes are known to be under hormonal control. With the improved techniques of isolation and identification now available to tree physiologists, many of these hormones are being reported as endogenous in several coniferous species. This study was primarily undertaken to determine the endogenous shoot levels of ABA and IAA in western hemlock growing under natural conditions as a possible explanation of part of the physiology of the species during dormancy.

Seedlings used in this study were collected from Green Mountain (T9S, R8W, Sec. 36) in the Oregon Coast Range and were part of the dense natural regeneration of western hemlock common in that area. Seedlings were pulled from the ground and transported to the Forest Research Laboratory in plastic bags under moist conditions. Seedling height was generally between 1.0 and 1.5 m and collections were made on June 26, September 24, December 4, 1975, February 3, April 6, and May 6, 1976. A collection was also made on July 30, 1975 and was used to determine the percent recovery of the hormones during the isolation and measurement process.

Upon arrival at the Forest Research Laboratory, the seedlings were placed in a cold room where terminal and lateral shoots were cut into segments approximately 5 cm long. The material was then placed in a preweighed 2 liter erlenmeyer flask and the weight of the material placed in the flask recorded. The material was then covered with 95% methanol and maintained at -15°C for six weeks. During this period, the methanol was changed at two-week intervals and the methanol

extracts were combined into a single extract for each date. At this point, known quantities of authentic IAA and ABA were added to the extract of July 30, 1975 for determination of the recovery after purification.

Each extract was then evaporated in vacuo at 40°C. The evaporation process was halted when all traces of methanol were removed from the extracts. Each extract was then taken up in 30 ml of 95% methanol and stored at -15°C until further purification could be performed.

Solvent Partitioning

The general fractionation procedure was similar to that used by DeYoe (1974) with minor modifications (Figure I). The extracts were evaporated to dryness on the rotary evaporator, taken up in 50 ml of diethyl ether, and partitioned against 50 ml of 0.1 N sodium bicarbonate. The aqueous phase was partitioned in the same manner twice more with 50 ml of fresh ether each time. The three ether fractions were combined and partitioned with 50 ml of fresh sodium bicarbonate. The aqueous phases were then combined and acidified to pH 2.5 to 3.0 by titrating with 10% HCl. The resulting acidic aqueous fraction was then partitioned twice with 40 ml volumes of ether and the ether fractions combined. The ether, which contained the IAA and ABA as well as a number of other substances, was then reduced to dryness in vacuo at 15° C, taken up in 30 ml of methanol and stored at -15°C.

Column Chromatography

Column chromatography using Sephadex LH-20 has been shown to be an effective method of general purification and separation of IAA and

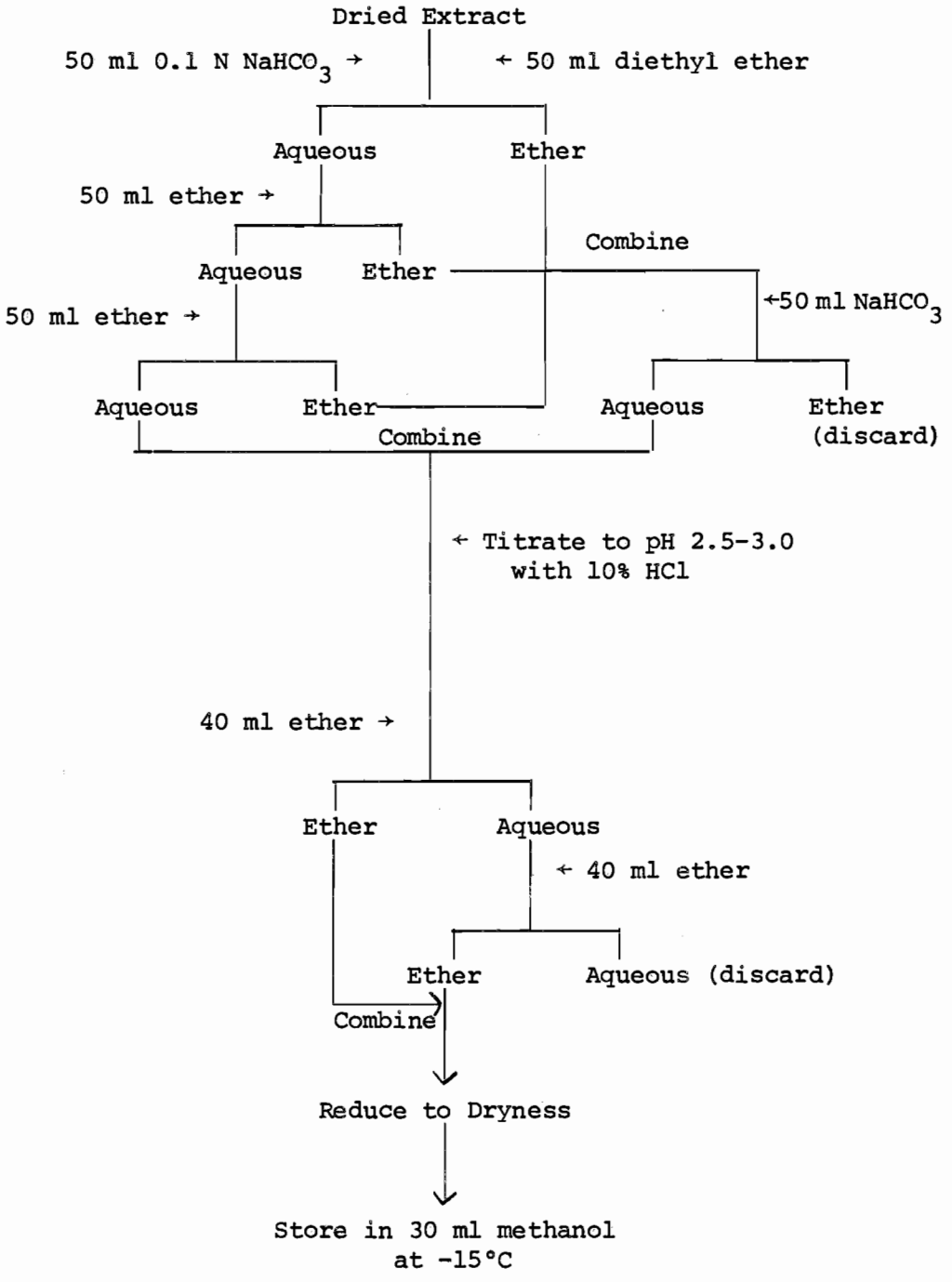

Figure I. Solvent partitioning chart.

ABA (Steen and Eliasson, 1969) and was used in this study. Two columns were made using 250 ml burets which had an inside diameter of 3 cm. Each buret was prepared for use by placing a glass wool plug above the stopcock over which a layer of sterilized sand was poured to provide a horizontal surface for the Sephadex. The Sephadex was activated by mixing 55 grams of Sephadex LH-20 with 200 ml of acidified ethanol (2 ml of 0.001 N HCl per 200 ml of 95% ethanol) for each column and allowing the mixture to swell for 38 hours. The mixture was then reslurried and carefully poured into the columns being sure not to get any trapped air pockets. The beds were allowed to settle by opening the stopcock and allowing a constant flow of acidified ethanol from a reservoir through the columns. Final bed lengths of the columns were 25 and 27 cm.

The columns were calibrated by running standards which contained authentic IAA and ABA in acidified ethanol through the columns and collecting 5 ml fractions. The optical density between 200 and 350 nm of each fraction was then measured using a Beckman ACTA III double beam spectrophotometer to determine fractions which contained ABA and which contained IAA by their absorption pattern in this wavelength range.

Each extract was evaporated to dryness, then redissolved in 2 ml of acidified ethanol and carefully applied to the surface of the column by pipet and drifted several centimeters into the bed with 5 successive 2 ml applications of acidified ethanol. During this process, care was taken not to disturb the upper bed surface of the column. At this point, a 1 liter reservoir of acidified ethanol was connected to the upper portion of the column and the system was closed with a rubber

stopper. The flow rate was adjusted to 40 drops per minute and the respective ABA and IAA fractions were collected. The fractions were evaporated to dryness and redissolved in 15 ml of methanol for storage at -15°C.

After each sample was eluted, the column was cleaned by washing with 250 ml of pyridine:95% ethanol (1:1, v/v) followed by sufficient 95% ethanol to remove all traces of the pyridine.

Preparation of Derivatives

Due to the low volatility of the free acids of IAA and ABA, esters, which are more volatile, were prepared. The methyl ester of both hormones was produced using diazomethane gas as described by Schlenk and Gellerman (1960) and termed micromethylation. Prior to methylation, the extracts were reduced to dryness and then dissolved in a 3 ml of acetone:methanol (9:1, v/v) and placed in small vials. The system used consisted of two test tubes and the vial containing the sample connected in series. In tube 1 was placed approximately 7 ml of diethyl ether and in tube 2 was placed 0.7 ml of diethyl ether, 0.7 ml of 2-(2-ethoxy-ethoxy) ethanol, and 1.0 ml of 60% potassium hydroxide. Immediately prior to derivatization, approximately 3 grams of Diazald (N-methyl-N-nitroso-p-toluenesulfonamide) was added to tube 2 and the system closed. A steady stream of nitrogen was then bubbled into tube 1 and the resulting diazomethane gas produced in tube 2 was bubbled through the sample vial for approximately 30 minutes. The production of diazomethane was stopped by adding glacial acetic acid:ether (1:1, v/v) to tube 2. The entire procedure was carried out in a ventilated

hood. The derivatives thus formed were then evaporated to dryness under a stream of nitrogen, redissolved in 1 ml of methanol, and stored at -10°C. This procedure was used for all ABA and IAA samples as well as on standards of authentic ABA and IAA.

When IAA samples were analyzed by GLC, a number of interferring substances were evident. Therefore, the additional purification step of thin-layer chromatography was used on the IAA extracts. This procedure was not used on the ABA extracts since no further purification was needed to obtain good analyses. One hundred µl of the methylated IAA extract was applied to 20x20 cm plates coated with silica gel "G." A spot of authentic methylated IAA was applied on a separate portion of the plates. Air-tight tanks were used to develop the plates and the atmosphere inside the tank was allowed to become saturated with the 2-butanone:N-hexane (1:3, v/v) solvent prior to inserting the plates for development. The solvent was allowed to ascend 10 cm before the plates were removed and allowed to air dry. The R_f value of IAA in this system, as detected by the fluorescence of the authentic methylated IAA under ultra-violet light, was 3.5 to 4.0 and a band of approximately 1 cm encompassing this R_f value was removed from each plate. The gel thus collected was mixed with 2 ml of methanol to elute the IAA and poured into a Buchner funnel which drained into a test tube inside a suction flask. The gel was then washed with an additional 2 ml of methanol after which suction was applied until the silica gel G was dry. The methanol extract containing the methylated IAA was then reduced to dryness in a micro-vial under a stream of nitrogen, after which the sample was redissolved in 100 µl of methanol and stored at -10°C.

A second derivative of the IAA samples was produced by making the trimethylsilyl derivative following the procedure of Grunwald, Mendez, and Stowe (1969). The derivatization was accomplished by placing 100 µl of the previously methylated IAA extract into a micro-vial and reducing it to dryness under a stream of nitrogen. The sample was then dissolved in 60 µl of acetonitrile and 40 µl of bis(trimethylsilyl)-acetamide (BSA) and the vial was closed and allowed to remain at room temperature for 1 hour prior to analysis. Due to the high reactivity of this derivative, analysis by gas-liquid chromatography (GLC) was performed as soon after formation as possible.

Supplemental Extractions of ABA

A series of extractions were made of actively growing containerized nursery seedlings to ascertain the effect of photoperiod and moisture stress on ABA levels in the shoots of western hemlock. Conditions tested included a long photoperiod and low moisture stress (control), a long photoperiod and moderate moisture stress, and a short photoperiod and low moisture stress. Extractions were made prior to beginning treatments and again after 2 and 4 weeks of each treatment. Duplicate samples were made for each extraction and the extraction process was the same as that previously described except that the fresh weight of material used in each sample was around 100 grams. Each sample was solvent partitioned and the methyl derivative of ABA produced using the same procedure as before. Due to the sensitivity and specificity of the analytic system used for ABA, additional purification using the Sephadex column was not needed.

A final series of extracts were made to determine the effect of various levels of plant moisture stress on the ABA level of the seedlings. The pre-dawn moisture stress was measured using a pressure chamber apparatus after which approximately one gram of mature current year needles were used as the extraction material. Moisture stresses ranged from 3 to 27 bars at the time of extraction. Extraction, solvent partitioning, and derivatization were by the same procedures as outlined earlier except that volumes used in solvent partitioning were reduced due to the smaller extraction volumes.

Hormonal Analysis

A Hewlett-Packard 5750-B Research Chromatograph equipped with flame ionization and electron capture detectors was used to analyze all extracts for IAA and ABA, except for the final series for ABA. All columns used in the analysis were stainless steel with a 1.6 mm inside diameter and were 1.9 m in length. Gas-Chrom Q 80/100 mesh was used as the solid support for all stationary liquid phases tested. The solid support was coated by dissolving a known weight of the stationary liquid phase in a measured volume of an appropriate solvent for the liquid phase and slowly adding a known weight of Gas-Chrom Q with constant stirring. The mixture was allowed to sit for 5 minutes, was restirred into a slurry and poured into a Buchner funnel. Suction was applied until the excess solvent was collected in a vacuum flask and its volume measured. The coated solid support was placed in a heated fluidizer and dried under a constant stream of nitrogen until all the solvent was evaporated. From the initial and final volumes of the

solvent, the weight of the liquid phase, and the weight of the solid support, the percent coating of the solid support was determined.

One end of the column was plugged with a small piece of glass wool to prevent loss of the packing. The coated packing was then slowly poured into the open end of the column using a paper funnel. The column was in a vertical position and constantly vibrated during this process to ensure the even packing necessary for an effective column. When the column was fully packed, the open end was also plugged with glass wool and appropriate fittings for connection to the machine were placed on both ends of the column. The columns were conditioned by attaching them to the gas chromatograph and maintaining the oven temperature at the maximum for the stationary liquid phase for 48 hours.

A number of stationary liquid phases and coating percentages were initially tried and included DC-11 (2.9%), QF-1 (1.7%), SE-30 (2.3%, 3.5% and 4.7%), and XE-60 (3.5%), all of which were obtained from Applied Science Laboratories, Inc., State College, Pennsylvania. After preliminary testing of the columns with samples of authentic IAA and ABA derivatives, the 4.7% SE-30 (methyl) and 3.5% silicone GE XE-60 (25% cyanoethyl, methyl) columns were chosen for analysis of the extracts due to their appropriate retention times and peak shapes. The conditions under which the analyses were performed are listed in Table 4.

Injection of all samples into the columns was done with a 10 µl syringe which was thoroughly cleaned between injections. The area of the appropriate peak from each injection was measured using a polar planimeter.

Table 4. Conditions used for GLC analysis of extracts on Hewlett-Packard chromatograph.

Column	Derivative	Temperature °C			Retention Time (min)
		Injector	Oven	Detector	
SE-30 (4.7%)	Me-IAA[1]	235	160	250	6.1
SE-30 (4.7%)	TMS-IAA[2]	240	160	260	7.4
XE-60 (3.5%)	Me-IAA	235	180	250	8.1
XE-60 (3.5%)	TMS-IAA	240	160	260	7.2
XE-60 (3.5%)	Me-ABA	235	200	270	12.3

[1]Methyl ester (Me) derivative

[2]Trimethylsilyl (TMS) derivative

Analysis of IAA

The XE-60 and SE-30 columns used in the qualitative and quantitative analysis of the IAA extracts by GLC were attached to the gas chromatograph and allowed to come to equilibrium overnight under the conditions shown in Table 4. Helium was used as the carrier gas for both columns and a flow rate of 20 ml/min was used during the IAA analyses. Flame ionization detectors were used for the detection of IAA.

The retention times (R_t) of the two derivatives of IAA on each of the columns was determined by injecting the derivatives of authentic IAA into the columns. These R_t's were then used to identify IAA peaks produced by the extracts. The analysis of either of the derivatives on either of the columns was completed within a single day. A 5 µl injection of each extract derivative was used for analysis and represented 1/200 of the total extract. Range and attenuation were adjusted to

produce the most sensitive detection possible for each extract without going off scale. Following the analysis, a period of approximately 30 minutes elapsed prior to the injection of the next sample to allow compounds of longer R_t's to elute from the column. Random extracts were periodically re-analyzed to be sure the results obtained were reproducible. As further evidence of the qualitative identification of IAA, a small quantity of the appropriate derivative of authentic IAA was added to each extract and the peak enhancement noted. Thus, by utilizing two derivatives on two columns for a total of four determinations on each extract, the qualitative identification of IAA in western hemlock shoots was obtained.

Due to the linearity of detector response to concentration of IAA at lower levels, the quantity of each of the derivatives of IAA for each of the extracts could also be determined. Standard curves for each derivative on each column were made by injecting known quantities of authentic IAA derivatives. The graph of these results made it possible to relate the peak areas obtained from the extracts to a concentration of IAA in each injection.

The quantity of IAA present in the extract used to obtain the rate of recovery was determined by using the standard curves of peak area vs. IAA quantity. This value was compared with the quantity of IAA originally added to the extract to determine the recovery rate. The recovery rate of IAA after all purification procedures was found to be 32%. Knowing the fresh weight of material extracted, the percent recovery, and the portion of the sample injected and its concentration of IAA, it was possible to express quantitatively the extracted IAA in

terms of µg/kg of fresh weight of the shoot material. Since the extracts analyzed also covered a period of one year it was possible to show a seasonal variation in the level of IAA in seedlings growing under natural conditions.

Analysis of ABA

The XE-60 (3.5%) column used in all ABA analyses was attached to the gas chromatograph and allowed to come to equilibrium with the operating conditions listed in Table 4. The column was connected to a Ni^{63} electron capture detector which was set for a pulse interval of 15 µsec. Helium was used as the carrier gas and a mixture of 10% methane in argon was used as the purge gas. Flow rates were adjusted to provide a ratio of carrier gas to purge gas of 1:4. The R_t of the methyl ester of ABA in this system was determined by injection of the authentic derivative and this R_t was used to identify the ABA peak produced by the extracts.

Because of the great sensitivity of the detector for ABA and because the detector was easily overloaded, all extracts were diluted 100 fold with methanol prior to analysis. Injections into the system consisted of 1 µl of the extract being analyzed and 2 µl of methanol. Approximately 1 hour elapsed between injections to allow all compounds to elute from the column and to allow the detector to recover its sensitivity. All extracts were analyzed the same day and random extracts were re-analyzed to test for reproducibility. Due to slight changes in the sensitivity of the detector during the day, samples of known quantities of authentic ABA were periodically injected and were used as

standards for quantification of the amount of ABA present in the extracts.

As further qualitative evidence of the presence of ABA in western hemlock shoots, small quantities of the authentic ABA derivative were added to the extracts and the peak enhancement noted. In addition, random extracts were placed under ultraviolet light for 2 hours to cause conversion of the cis-ABA to the physiologically inactive trans-isomer. Upon analysis of these extracts a peak corresponding to the trans-isomer and a reduced peak of the cis-isomer were noted.

This system proved to be quite sensitive to ABA and sub-nanogram quantities were easily detected. The extract used to determine recovery of the extracted ABA was used in the same manner as that of IAA and showed a recovery of 37%. As with IAA, it was possible to determine the quantity of extracted ABA on a fresh weight of the tissue basis and determine the seasonal trend of endogenous levels of ABA in seedlings growing under natural conditions.

Analysis of the extracts to determine the effect of photoperiod and moisture stress on ABA levels in seedlings was done in the same manner. In this case, however, ABA content of the treatments was related to the control extracts rather than just quantified. Because relative differences were being investigated, the rate of recovery for the purification procedure was not determined and was assumed to be the same for all extracts.

Analysis of the final series of extracts of ABA was carried out on a Varian Model 3700 Gas Chromatograph equipped with a Ni^{63} electron capture detector. The injection, oven, and detector temperatures were

249°C, 200°C, and 299°C respectively. A flow rate of 26 ml/min of purified N_2 was passed through the XE-60 (3.5%) column and produced a R_t of 7.3 minutes. All other procedures of analysis and identification were the same as those used for other ABA extractions.

RESULTS AND DISCUSSION

At the onset of this study of the physiology of dormancy, it was thought that western hemlock would exhibit responses very similar to those of Douglas-fir throughout this period. In some instances this assumption proved to be correct. However, in other instances it was shown that western hemlock and Douglas-fir differed in their responses. These dissimilarities must be recognized to insure production of quality western hemlock planting stock and justify the use of caution in extrapolating data on Douglas-fir to cover western hemlock. Much of this study was carried out in growth chambers and greenhouses and caution must also be used in extrapolating this data to field conditions (Evans, 1963). Because of the continuous changes in the environmental factors under natural conditions some responses may be altered from those observed under controlled environment conditions. The studies were conducted using seedlings which were in their first year of growth or in the beginning of their second year. These are the time periods when cultural practices at the nursery are the most intensive and have the greatest effect on the survival potential of the seedlings after they are planted. No studies were conducted using seedlings which had had two growing seasons. The physiology of dormancy of older seedlings or mature trees may be somewhat different than that of one year old seedlings, although this was not examined.

Throughout this study western hemlock seedlings proved to be excellent research material. Responses of the population proved to be very consistent within any individual treatment. This was especially

true of bud break data, i.e., in most cases the time span from first to last seedling to break bud within a treatment was less than 10 days. This is in sharp contrast to Douglas-fir seedlings which will in many instances take up to six weeks to cover this interval.

Dormancy Induction

The two methods of determining the success of the treatments on initiating dormancy were the total elongation of the seedlings during the 10 week study and the number of weeks until they were within 1 mm of their final length. These two criteria gave an indication of when seedlings stopped elongation as well as the amount of elongation which took place prior to this time. These methods were used as the criteria due to the difficulty of visually determining the date on which western hemlock buds began to develop.

The effect of individual factors is shown in Tables 5 and 6. Photoperiod was clearly the most influential factor in inducing dormancy in seedlings from both elevations, with a short photoperiod hastening dormancy. The temperature and level of moisture stress did not have a significant effect on the number of weeks until elongation ceased but the elongation prior to this time was significantly different for these two factors with a warm temperature or moderate moisture stress reducing elongation. For both low and high elevation stock, the individual factor of moisture stress reduce elongation to a greater extent than did warm temperatures. The three factors exert their effect by producing conditions unfavorable for growth and thereby leading to a reduced growth rate followed by formation of a resting bud.

Table 5. Effect of individual factors on elongation (cm) during study.[1,2]

Factor	Level	Low Elevation Seedlings	High Elevation Seedlings
Photoperiod	Long (16 hrs)	4.67a	5.16a
	Short (8 hrs)	1.99b	1.96b
Temperature	Cool (18°/12°C)	3.94a	4.41a
	Warm (25°/20°C)	2.73b	2.70b
Moisture Stress	Low (<5 bars)	4.28a	4.17a
	Moderate (12-15 bars)	2.38b	2.94b

[1]Values within each factor followed by the same letter do not differ significantly (p = 0.05) by Student's t test.

[2]Each observation is the average of 100 seedlings.

Table 6. Effect of individual factors on weeks until seedlings within 1 mm of final length.[1,2]

Factor	Level	Low Elevation Seedlings	High Elevation Seedlings
Photoperiod	Long	9.3a	9.5a
	Short	3.6b	3.5b
Temperature	Cool	6.7a	6.8a
	Warm	6.2a	6.2a
Moisture Stress	Low	6.8a	6.8a
	Moderate	6.1a	6.2a

[1,2]See Table 5.

The effectiveness of a short photoperiod in inducing dormancy in western hemlock is in agreement with the bulk of the literature reporting this phenomenon for woody plants. In the Pacific Northwest, however, photoperiod is often an insignificant factor in the natural initiation of dormancy for many species, since dormancy is generally induced in mid-summer when photoperiods are still favorable for growth. Moisture stress and temperature are the factors which appear to control dormancy initiation in species native to this region of the country. Because of the large effect of photoperiod, the combining of treatments to look at individual factors reduced the effect of moisture stress and temperature although both factors had a significant effect on elongation.

The interactions of pairs of factors begin to show the effectiveness of warm temperature and moderate moisture stress on initiating dormancy in western hemlock (Tables 7, 8, 9, and 10). All interactions which contained photoperiod as one of the factors was dominated by this

Table 7. Effect of pairs of factors on elongation (cm) of low elevation seedlings during study.[1,2]

		Photoperiod		Temperature	
		Long	Short	Cool	Warm
Moisture Stress	Low	6.22a	2.35c	5.61a	3.40c
	Moderate	3.13b	1.64d	2.71b	2.06d
Temperature	Cool	5.59a	2.28c		
	Warm	3.75b	1.70d		

[1]Values within each interaction followed by the same letter do not differ significantly (p = 0.05) by Duncan's Test.

[2]Each observation is the average of 50 seedlings.

Table 8. Effect of pairs of factors on elongation (cm) of high elevation seedlings during study.[1,2]

		Photoperiod		Temperature	
		Long	Short	Cool	Warm
Moisture Stress	Low	6.33a	2.01c	5.22a	3.13b
	Moderate	3.98b	1.90c	3.61b	2.26c
Temperature	Cool	6.57a	2.25c		
	Warm	3.74b	1.66d		

[1,2]See Table 7.

Table 9. Effect of pairs of factors on weeks until low elevation seedlings within 1 mm of final length.[1,2]

		Photoperiod		Temperature	
		Long	Short	Cool	Warm
Moisture Stress	Low	9.9a	3.7c	6.8ab	6.8a
	Moderate	8.6b	3.6c	6.6ab	5.6b
Temperature	Cool	9.9a	3.4c		
	Warm	8.6b	3.8d		

[1,2]See Table 7.

Table 10. Effect of pairs of factors on weeks until high elevation seedlings within 1 mm of final length.[1,2]

		Photoperiod		Temperature	
		Long	Short	Cool	Warm
Moisture Stress	Low	10.0a	3.6c	6.8a	6.7ab
	Moderate	8.9b	3.4c	6.7ab	5.6b
Temperature	Cool	10.0a	3.6c		
	Warm	8.9b	3.4c		

[1,2]See Table 7.

factor. However, under a long photoperiod, moderate moisture stress or a warm temperature initiated dormancy significantly more rapidly than their counterparts and this was true of both elevational sources. Using the criterion of elongation prior to dormancy initiation, these two factors were in most cases also significant under a short photoperiod. Therefore, although photoperiod was the predominant factor, moisture stress and temperature affected the rapidity of dormancy initiation.

From the practical standpoint of nursery management, the interaction of temperature and moisture stress is the most important interaction. On an operational scale of seedling production, alteration of the natural photoperiod is usually not physically or economically possible and other factors must be used to initiate dormancy. This interaction shows that the combination of moderate moisture stress and a warm temperature can effectively be used to initiate dormancy. In the low elevation seedlings, moderate moisture stress had a greater effect in initiating dormancy than the warm temperature and the combination of the two was more effective than either of them individually. In the high elevation seedlings, moderate moisture stress and a warm temperature had statistically equivalent effects and the combination of the two was again more effective than either of them individually. The high elevation seedlings were shown to be more sensitive to temperature than were the low elevation seedlings and this may be a natural adaptation to their environment.

Tables 11, 12, 13 and 14 show the effect on dormancy initiation when all three factors are used. Here again, photoperiod was the predominant element although the effect of moderate moisture stress and

Table 11. Effect of three factors on elongation (cm) of low elevation seedlings during study.[1,2]

Photoperiod	Moisture Stress	Temperature	
		Cool	Warm
Long	Low	7.53a	4.90e
Long	Moderate	3.65b	2.61c
Short	Low	2.80c	1.90d
Short	Moderate	1.77d	1.50d

[1]Values followed by the same letter do not differ significantly (p = 0.05) by Duncan's Test.

[2]Each observation is the average of 25 seedlings.

Table 12. Effect of three factors on elongation (cm) of high elevation seedlings during study.[1,2]

Photoperiod	Moisture Stress	Temperature	
		Cool	Warm
Long	Low	8.13a	4.54b
Long	Moderate	5.02b	2.94d
Short	Low	2.30c	1.72e
Short	Moderate	2.20c	1.52e

[1,2]See Table 11.

Table 13. Effect of three factors on weeks until low elevation seedlings within 1 mm of final length.[1,2]

Photoperiod	Moisture Stress	Temperature	
		Cool	Warm
Long	Low	10.0a	9.8a
Long	Moderate	9.8a	7.4b
Short	Low	3.6cd	3.8c
Short	Moderate	3.3d	3.9c

[1,2]See Table 11.

Table 14. Effect of three factors on weeks until high elevation seedlings within 1 mm of final length.[1,2]

Photoperiod	Moisture Stress	Temperature	
		Cool	Warm
Long	Low	9.9a	10.0a
Long	Moderate	10.0a	7.9b
Short	Low	3.7c	3.5c
Short	Moderate	3.5c	3.3c

[1,2]See Table 11.

a warm temperature are also evident, especially in treatments with a long photoperiod. The high elevation seedlings again showed greater sensitivity to temperature than the low elevation seedlings. Under favorable conditions for growth of the high elevation seedlings, such as a long photoperiod and a cool temperature, they produced greater elongation than the low elevation seedlings but this effect was reduced or eliminated with a warm temperature.

The effect of cool root temperatures on promoting dormancy is not clearly evident for western hemlock seedlings. Treatments which received this additional factor did not differ significantly from the same treatments without the cool root temperature and the results are shown in Tables 15 and 16. The temperature experienced by the shoots in this set of treatments had a greater effect than when the treatments did not experience cool root temperatures. A warm shoot temperature tended to prolong dormancy induction under this situation and a cool one hastened it. This is in contrast to the findings for treatments without a cool root temperature. This effect was more pronounced in

Table 15. Effect of cool root temperature as an additional factor on elongation (cm) during study.[1,2]

Treatment (t--pp--ms)	Low Elevation Seedlings	High Elevation Seedlings
W S L	2.53a	2.44a
W S M	2.46a	2.14b
C S L	1.74b	2.80c
C S M	2.05b	2.30a

[1]Values within each elevation followed by the same letter do not differ significantly (p = 0.05) by Duncan's test.
[2]Each observation is the average of 25 seedlings.

Table 16. Effect of cool root temperature as an additional factor on weeks until seedlings within 1 mm of final length.[1,2]

Treatment (t--pp--ms)	Low Elevation Seedlings	High Elevation Seedlings
W S L	3.6a	3.6ab
W S M	3.8a	3.8a
C S L	3.4b	3.6ab
C S M	3.3b	3.4b

[1,2]See Table 15.

the low elevation seedlings. High elevation seedlings were still more sensitive to warm shoot temperature although cool root temperature tended to reduce this sensitivity to some degree.

The results of the follow-up study to ascertain the effect of nutrient level on initiation of dormancy are shown in Tables 17 and 18. Unfortunately, the possible effect of reduced nutrient level could not be shown to be significant in any of the pairs of treatments. At the start of the study foliar nitrogen level was between 2.45 and 2.60% dry

Table 17. Effect of nutrient level on elongation (cm) and weeks until seedlings within 1 mm of final length.[1,2]

Nutrient Level	Elongation	Weeks until w/i 1 mm
High	2.82a	5.5a
Low	2.58a	5.4a

[1]Values within each column followed by the same letter do not differ significantly (p = 0.05) by Duncan's test.
[2]Each observation is the average of 100 seedlings.

Table 18. Effect of treatments on elongation and weeks until seedlings within 1 mm of final length in second initiation study.[1,2]

Treatment (pp-ms-t-nut)	Elongation (cm)	Weeks until w/i 1 mm
L L W H	6.47a	9.6a
L L W L	5.58a	8.7a
CS M W H	2.62b	5.4b
CS M W L	2.23b	5.5b
S M W H	0.76c	3.1c
S M W L	1.17d	3.6cd
S M C H	1.40d	3.9d
S M C L	1.35d	3.8cd

[1]Values within each column followed by the same letter do not differ significantly (p = 0.05) by Duncan's test.
[2]Each observation is the average of 25 seedlings.

weight for all seedlings. After five weeks of treatments, nitrogen analyses showed that seedlings receiving the low level of nutrient application had not shown a reduction in foliar nitrogen content and still had nitrogen contents similar to those in the high nutrient level treatments. At this point, all nutrient applications to the low level treatments were stopped for the remainder of the study. By this time,

however, the effects of the other factors such as photoperiod, temperature, and moisture stress, had already initiated dormancy in most of the treatments. Foliar nitrogen levels did decline in seedlings receiving no additional nutrients by the end of the study and were generally 0.25 to 0.45% lower than seedlings receiving the high nutrient level.

The pair of nutrient treatments which had a long photoperiod, low moisture stress, and a warm temperature were still actively growing at the time application of nutrient solution was stopped in the low level treatment and the effect of reduced nutrient availability could be seen. The high nutrient level seedlings averaged 3.35 cm of elongation from this time until the end of the study while the low nutrient level seedlings only averaged 2.19 cm of growth during the same period. The growth rate of these two treatments was significantly different after nutrient solution was withheld from the low level treatment. In addition, only 4% of the seedlings in the high nutrient level treatment stopped elongation prior to the conclusion of the study whereas 40% of the seedlings in the low nutrient level treatment had stopped elongating. In an unrelated study, nutrients were withheld from hemlock seedlings growing under otherwise favorable conditions. Under these conditions, the seedlings stopped growing and produced dormant buds but were very chlorotic. These observations lend support to the findings of Cheung (1973) that withdrawal of nitrogen, phosphorus, and potassium or of nitrogen alone is capable of initiating dormancy in western hemlock.

An interesting finding from the present study, which was not expected, was a high mortality of seedlings which received a high nutrient level in conjunction with a short photoperiod, a warm temperature, and moderate moisture stress. Seedlings under the same conditions except that they received the low level of nutrient solution showed no signs of mortality. This leads to the belief that a mineral toxicity problem was the cause of the mortality. This may have been a result of the high nutrient level available to the seedlings coupled with reduced photosynthate production and increased respiration. The problem has not yet been further investigated.

The seedlings which were over-wintered under natural conditions to observe subsequent spring growth actually showed the necessity of low temperature conditioning to develop frost hardiness in western hemlock. Shortly after seedlings were placed outside, a series of below freezing days occurred. Seedlings which had received the long photoperiod or the continuously shortening photoperiod suffered near 100% mortality. Seedlings which had received the short photoperiods also had high mortality, although not has high as seedlings which received the longer photoperiods. Seedlings which received a cool temperature with the short photoperiod had slightly higher survival than seedlings which had received the warm temperature. Thus, while photoperiod can induce some hardiness, western hemlock probably has a two phase acclimation period to develop maximum hardiness as outlined by Weiser (1970) with the second phase dealing with low temperatures being necessary for survival of the seedlings. Future research in the area of cold acclimation of

western hemlock is indicated as this is of great importance to nursery seedlings.

Harvest data from these dormancy induction studies as well as all other studies reported in this thesis have been included in the Appendices.

These studies provide some interesting findings on dormancy induction in western hemlock. The effectiveness of a short photoperiod in inducing dormancy in western hemlock certainly agrees with the bulk of literature on the subject of photoperiodically sensitive woody plants. The ability of moisture stress to initiate dormancy in western hemlock seedlings indicates that this may be one of the natural controlling factors of dormancy in this region of the country and is in agreement with work on other species native to the region. The findings relative to the effect of temperature on dormancy induction, however, are somewhat in disagreement with portions of the literature.

Cheung (1973) has previously shown that short photoperiods and moisture stress are able to induce dormancy in container grown western hemlock and my data are in agreement with his findings. He further found that low temperature also induced dormancy which is in contrast with my results. However, his low temperature treatment consisted of moving seedlings previously grown at 22°C in a greenhouse into a growth room set for a constant 12°C for three weeks followed by five weeks at 17°C and was therefore dissimilar to the methodology I used and may account for the contrast in our results. In addition, the moving of the seedlings from a greenhouse into a growth room may have produced a light intensity effect, although this was not discussed.

Work with other species also presents an unclear picture of the effect of temperature as has been noted in the literature review section. In some instances, other environmental variables within the studies can be used to explain some of the variation in results. The limited work available on any individual species also raises the possibility that each species has a distinct response to temperature as it affects dormancy induction. Thus, the species used in the research may account for the seemingly contradictory findings.

Lavender and Overton (1972) studied the effect of cool root temperatures on Douglas-fir and found that cool soil temperatures promoted dormancy in seedlings maintained under a long photoperiod but had little effect on seedlings maintained under a short photoperiod. All the treatments I conducted which included cool root temperature also had a short photoperiod and little effect of the root temperature on initiating dormancy in western hemlock was found.

From an operational standpoint, variables like photoperiod and root temperature are difficult and expensive to control and nutrient level alone is not a strong enough variable to be used to initiate dormancy. The combination of temperature and moisture stress level is easily controlled in greenhouse production of seedlings and has been shown to be effective in initiating dormancy in Douglas-fir (Lavender, Ching, and Hermann, 1968). They found that the combination of low night temperatures and moisture stress was effective in initiating dormancy, although the temperature effect was not consistent and was not confirmed by Lavender and Overton (1972). Steinbrenner and Rediske (1964) found that shoot growth of both ponderosa pine and Douglas-fir

was reduced by low temperatures and increased moisture stress and that temperature had more influence than moisture stress. The cool temperature they used was 13°/17°C and is considerably below the optimum for growth of the two species, which was contained in their warm temperature treatments.

Because of the lower optimum temperature for growth of 18°C for western hemlock (Brix, 1971), my warm temperature treatments were above this optimum and might account for the reduced growth and dormancy initiation I found. The combination of moisture stress and warm temperatures was successfully used to initiate dormancy in western hemlock. Cool temperatures might be used to initiate dormancy in western hemlock although they would probably have to be less than 13°C in order to produce the response. Since dormancy initiation would be desired in mid to late summer, a large expenditure of energy would be required to maintain these cool temperatures in a greenhouse and would be less desirable at the operational level.

This research has thus shown that an ideal regime to initiate dormancy in containerized western hemlock seedlings would consist of an 8 hour photoperiod, a 25°/20°C temperature regime, a plant moisture stress of 12 to 15 bars, and reduced nutrient availability. Under this regime cool soil temperatures had little effect. A regime consisting of the temperature, moisture stress, and reduced nutrient level was also found to be effective under a 16 hour photoperiod. While this second regime did not induce dormancy as rapidly as the first, it does provide the nursery manager with a feasible method at the operational level. The timing of this treatment should be set to

coincide with having the seedlings in the proper stage of dormancy when lifting or planting are desired, as will be discussed later.

Chilling Requirements

The rapidity with which terminal and lateral buds resumed growth and the number of buds that broke per seedling after the chilling period were the primary criteria for assessing the effectiveness of each pretreatment-treatment sequence in fulfilling the chilling requirement of western hemlock seedlings. The number of days to terminal and lateral bud burst within each sequence was remarkably consistent, with the exception of treatments which received 4 weeks of chilling. At least two-thirds of the seedlings within each of the treatments broke bud within a 4 day span and the time span between the first and the last seedling to break bud was generally less than 11 days. Lateral buds were found to resume growth slightly faster than terminal buds and were generally 1 to 4 days faster.

Tables 19 and 20 show the effect of the length of chilling received and the effect of the pretreatment prior to chilling on the response variables. When the chilling treatments were combined to assess the effect of the pretreatment no significance could be shown, although the effect of the pretreatments on the individual chilling lengths will be discussed shortly. The length of the chilling period had a definite effect on the speed of bud break. While 4 weeks of chilling was sufficient to cause bud break in many of the seedlings, it was slower and occurred over a longer period of time than did the longer chilling periods. Six and eight weeks of chilling produced

Table 19. Effect of length of chill on growth responses.[1,2]

Chilling	Days to Terminal Bud Break	Days to Lateral Bud Break	Number of Broken Buds
4	30.6a	27.2a	9.6a
6	23.4b	21.9b	17.6b
8	21.9b	21.3b	17.0b
12	19.3c	18.3c	18.6b

[1]Values within each column followed by the same letter to not differ significantly (p = 0.05) by Duncan's test.

[2]Each observation is the average of 150 seedlings.

Table 20. Effect of pretreatment on growth responses after chilling.[1,2]

Pretreatment	Days to Terminal Bud Break	Days to Lateral Bud Break	Number of Broken Buds
None	26.1a	23.2a	15.1a
3 wk. SD	25.1a	23.5a	14.6a
6 wk. SD	22.6a	21.7a	16.3a
6 wk. Nat. D	20.7a	19.7a	16.8a
6 wk. LD	24.5a	22.9a	15.8a

[1]Values within each column followed by the same letter do not differ significantly (p = 0.05) by Duncan's test.

[2]Each observation is the average of 120 seedlings.

similar rates of bud burst and 12 weeks produced the most rapid bud break. Number of broken buds per seedling was significantly lower in the 4 week treatment and was similar in the 6, 8, and 12 week treatments.

Figure II and Tables 21, 22, and 23 show the effect of the interaction of the pretreatment and chilling length on the seedlings. After

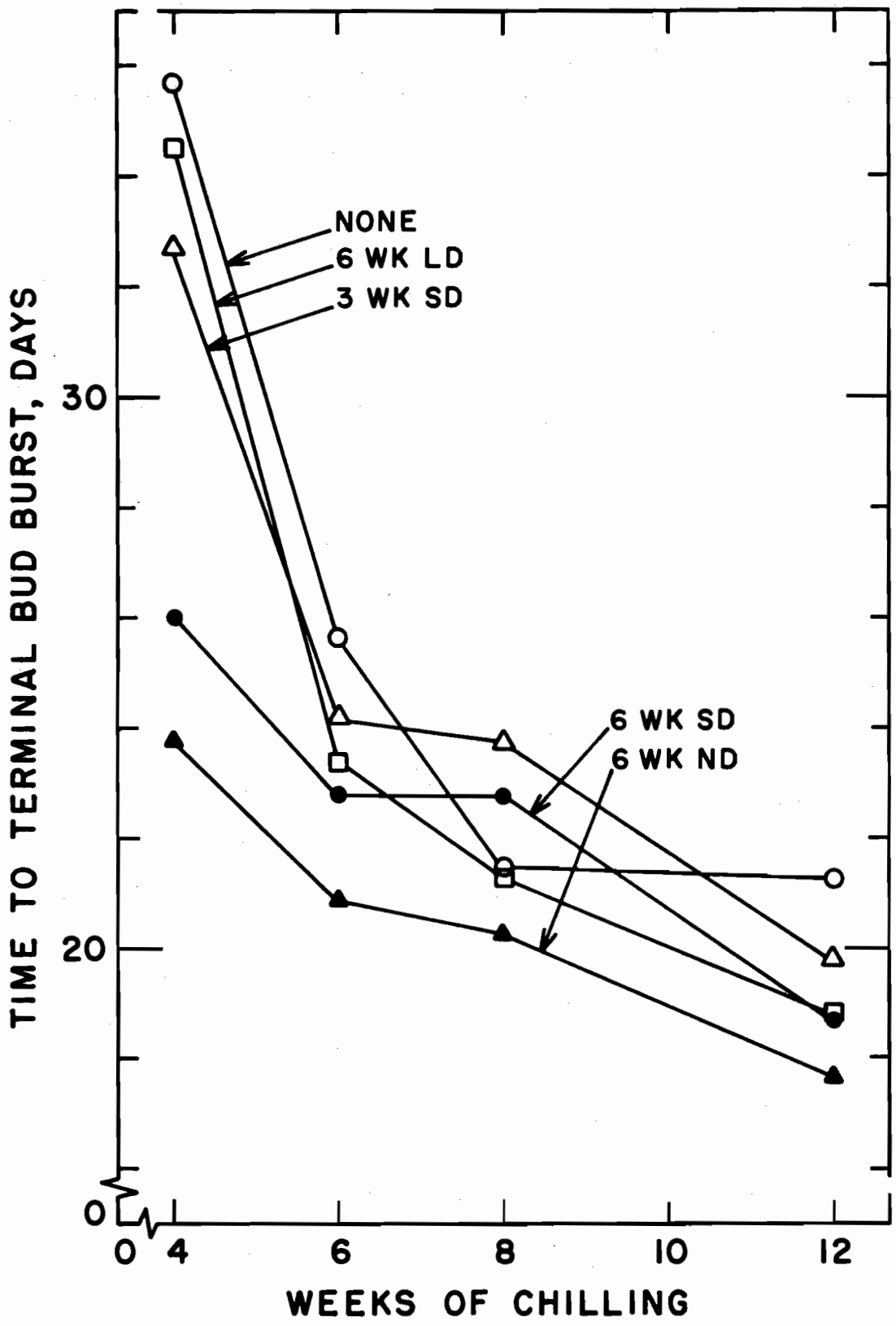

Figure II. Effect of pretreatment and length of chill on days to terminal bud break.

Table 21. Effect of pretreatment and chill length on days to terminal bud break.[1,2]

Weeks of Chilling	Pretreatment				
	None	3 wk. SD	6 wk. SD	6 wk. ND	6 wk. LD
4	35.7a	32.7a	26.0b	23.8b	34.5a
6	25.7b	24.2bc	22.8ce	20.9d	23.4bc
8	21.5cf	23.8be	22.8e	20.3d	21.3f
12	21.3c	19.8d	18.7d	17.8f	18.8d

[1]Values within each column or row followed by the same letter do not differ significantly (p = 0.05) by Duncan's test.

[2]Each observation is the average of 30 seedlings.

Table 22. Effect of pretreatment and chill length on days to lateral bud break.[1,2]

Weeks of Chilling	Pretreatment				
	None	3 wk. SD	6 wk. SD	6 wk. ND	6 wk. LD
4	29.3a	29.3a	25.3e	22.5d	29.7a
6	21.7b	22.7bc	22.0bc	20.4e	22.8c
8	21.5b	22.7c	21.9bc	19.3e	21.1b
12	20.2d	19.3df	17.8f	16.5f	17.8f

[1,2]See Table 21.

Table 23. Effect of pretreatment and chill length on average number of broken buds per seedlings.[1,2]

Weeks of Chilling	Pretreatment				
	None	3 wk. SD	6 wk. SD	6 wk. ND	6 wk. LD
4	7.1a	8.7ac	11.3c	13.6bc	7.2a
6	17.6b	16.7b	16.4b	19.7b	17.7b
8	15.8b	17.2b	19.2b	15.3b	18.6b
12	19.7b	16.8b	18.1b	18.7b	19.8b

[1,2]See Table 21.

4 weeks of chilling, seedlings which received 6 weeks of short days or natural days after setting bud under short days and moisture stress but prior to the chilling resumed growth significantly more rapidly than seedlings which received 6 weeks of long days, 3 weeks of short days, or no pretreatment after bud set. The pretreatments that broke bud more rapidly were the ones which were conducive to bud maturation prior to chilling. With this short duration of chilling, the environment between the time of bud set and the beginning of chilling definitely had an effect on growth responses of the seedlings when they were placed in the growth room after chilling. The magnitude of the influence of the pretreatment was reduced if the seedlings were given 6 weeks or longer of chilling, although it was still evident in treatments which received 6 weeks of chilling. Differences in days to bud break between the 6 and 8 weeks of chilling treatments were generally insignificant. Twelve weeks of chilling generally produced significantly faster bud break than 8 weeks and again showed the same general trend with some minor variations. Lateral bud break was slightly faster than terminal bud break. The number of buds breaking per seedlings was not affected by pretreatment if 6 weeks of chilling was received.

These data supporting a 6 to 8 week chilling requirement of western hemlock compare favorably with early work on eastern hemlock (T. canadensis (L.) Carr.) (Olson and Nienstaedt, 1957; Olson, Stearns, and Nienstaedt, 1959). These papers reported a 5 to 8 week chilling requirement for eastern hemlock to resume growth when returned to favorable temperatures and a 12 hour photoperiod. They also found that a 12 hour photoperiod was not able to compensate for the chilling

requirement of that species. They reported that about 10% of seedlings chilled for 2 weeks were able to break bud under the 12 hour photoperiod and that a 20 hour photoperiod with "strong" light (400 foot-candles) was able to induce bud break of unchilled seedlings. If a 12 hour photoperiod is unable to compensate for chilling in western hemlock, seedlings which have been properly preconditioned will have had most of their chilling requirement fulfilled by 4 weeks of chilling. It must be kept in mind that studies of this nature deal with exposure to a continuous low temperature and that this is much more efficient at fulfilling the chilling requirement than the fluctuating temperatures which seedlings are exposed to under natural conditions. Minor variations in the chilling requirement of western hemlock seedlings from different seed sources probably are present as has been shown for other species.

Western hemlock's response to pretreatment prior to chilling and the length of chilling are considerably different than those found for Douglas-fir. Douglas-fir has been shown to have a chilling requirement of 12 weeks of constant low temperature to rapidly resume growth in response to warm temperatures, although 8 weeks of chilling induced almost as rapid a response (Wommack, 1964; Lavender, and Hermann, 1970) and is longer than the chilling requirement of western hemlock. Douglas-fir is also much more sensitive to the prechilling environment than is western hemlock. If treated with long days prior to chilling, 12 weeks of constant chilling are required before bud break will occur when seedlings are moved into a favorable environment and the speed of bud break is greatly reduced in comparison with seedlings which received short days prior to chilling. Chilling Douglas-fir immediately after bud set shows

a similar response. Western hemlock, however, did not show this magnitude of pretreatment effect if 6 weeks of constant chilling was provided. Pretreatment of western hemlock with long days or with no pretreatment did have an effect on bud break after 4 weeks of chilling, although about 50% of the seedlings in each case did break bud and resume growth after being placed in the growth room. The reduced influence of the pretreatment may be due to the shorter chilling requirement of western hemlock, although this cannot provide an adequate explanation of the large difference between the two species.

The shorter chilling requirement and the reduced effect of the environment prior to chilling in comparison to Douglas-fir means that scheduling of greenhouse conditions for production of containerized western hemlock seedlings may not be as rigorous as that necessary for Douglas-fir. For seedlings grown for fall planting, dormancy should be induced about mid-June to insure proper condition of the stock. Natural chilling in the field will then fulfill the requirement. Seedlings for spring planting often are grown later into the season before dormancy is induced. In this situation, the photoperiod is naturally shortening and no supplemental lengthening of it would be desired. After dormancy is induced, greenhouse temperatures should be maintained in order to allow maturation of the bud prior to beginning the chilling. At this time the greenhouse should be opened up to allow natural chilling of the seedlings if possible. Samples of the stock could periodically be moved into a warm greenhouse under a 12 hour photoperiod to determine their rate of bud break as a measure of how much chilling the seedlings had received in the greenhouse. If this system has not provided enough

chilling to get vigorous, rapid bud burst of the seedlings by late January or early February, a period of constant chilling could be used to supplement the natural chilling which the seedlings received. A period of 4 weeks of constant low temperatures should be adequate to fulfill the remaining chilling requirement and provide stock which will break bud rapidly in the spring.

On sites where early bud break and growth are required for survival, such as sites which experience early drought, the effect of the pretreatment may become more important than on moist sites. While pretreatment usually caused less than 2 days difference on bud break, this was in conditions favorable for resumed growth and not under natural spring conditions. This was the subject of the next study.

Effect of Fall Photoperiod Upon Spring Bud Break

The dates of terminal and lateral bud break were used as the primary criteria for assessing the effect of the fall photoperiod upon spring bud break of seedlings which received natural chilling and natural conditions in the spring. The results of this study indicate there is a slight but significant effect on the fall photoperiod the seedlings experienced on their subsequent responses (Table 24). The effect was shown in all three measures of the response, although it varied somewhat among the measures. The natural photoperiod proved to be the most beneficial one in all cases. An 8 hour photoperiod increased the date of terminal and lateral bud break and also reduced the amount of growth during the following season. A 16 hour photoperiod

Table 24. Effect of fall photoperiod on date (in May) of spring bud break and new growth during the season.[1,2]

Fall Photoperiod	Date of Terminal Bud Break	Date of Lateral Bud Break	Growth (cm)
Natural	9.1a	7.8a	6.78a
8 hour	10.8b	8.9a	5.41b
16 hour	13.4c	12.3b	4.95b

[1]Values of each response followed by the same letter do not differ significantly (p = 0.05) by Duncan's test.

[2]Each observation is the average of 30 seedlings.

slowed terminal and lateral bud break and new growth to the greatest extent.

The difference of about 4 days in the date of bud break between the natural and long day photoperiod treatments is in sharp contrast to data on Douglas-fir. Lavender (1974) found that bud break of Douglas-fir was delayed up to 15 days by exposing the seedlings to long days in the fall rather than to the natural photoperiod. He also found that bud break occurred 12 days earlier if seedlings were exposed to a 9 hour photoperiod rather than the natural photoperiod. His fall photoperiod treatments ceased at about the same time as mine but the subsequent effect on Douglas-fir was much greater than was found in western hemlock.

Also in contrast to the findings of Lavender (1974) for Douglas-fir is the relative effect of a short or natural photoperiod. He found a 9 hour photoperiod during the fall to be more beneficial than the natural photoperiod on subsequent bud break. My work with western hemlock has shown that the natural photoperiod was more beneficial than an

8 hour photoperiod during the fall. The natural photoperiod during the treatment period started at around 12 hours and was around 9-1/2 hours at the time treatments were stopped and thus was never as short as the 8 hour photoperiod. Both treatments were carried out in a greenhouse and thus there was little difference in the light intensity received by the seedlings. The benefit of the natural photoperiod was also seen in the chilling requirement study. Seedlings pretreated with the natural photoperiod for 6 weeks prior to chilling broke bud more rapidly than those which had received 6 weeks of short days prior to chilling for all lengths of chilling.

Apparently an 8 hour photoperiod during the fall is not sufficiently long to fulfill some physiological requirement of western hemlock seedlings during this period. The increased growth of the natural photoperiod treatment as compared with the short day treatment may indicate that an 8 hour photoperiod adversely affected needle primordia development or maturation due to a reduced availability of photosynthate. This reduced photosynthate production could lead to arrested development of the next years growth prior to what would normally occur under natural conditions. This effect could then be carried over into the date of spring bud break. A slightly longer constant photoperiod of perhaps 10 hours may be more beneficial than the natural one and might provide the required photosynthate, although this was not investigated. The long day photoperiod treatment would provide adequate photosynthate but would have the seedling in the wrong phase of dormancy to receive the benefits of chilling and this would lead to the delayed date of bud break and reduced growth.

These findings have practical implications on the production of nursery stock. The data show that a natural photoperiod in the fall was most beneficial for the amount of spring growth produced. This is, of course, an important consideration in the production of planting stock used for regeneration. The slightly delayed date of bud break may be of little importance in many instances. However, on sites which experience early moisture stress the delayed bud break could reduce survival. On sites of this nature, stock which breaks bud earlier and completes its current years growth earlier will have the best chance for survival. If the use of supplemental light to extend the photoperiod is avoided, there should be little effect on the subsequent growth of the seedlings in the spring. Seedlings grown into the fall before dormancy is initiated may require some special treatment as outlined in the prior section but should be ready for rapid growth after spring planting if chilling has been fulfilled and long photoperiods have been avoided after dormancy is induced.

Effect of Cold Storage Upon Growth Response

This study was designed to ascertain the effect of date of cold storage on subsequent growth of western hemlock seedlings when they were either placed in a growth room immediately after cold storage or planted into a cold frame after cold storage. The effect of a daily photoperiod during cold storage and the effect of transplanting seedlings into larger containers prior to cold storage were also examined. The primary criteria used in assessing treatment effect were the number of days until bud break occurred in the growth room and the date of

spring bud break and mortality in the cold frame.

Tables 25, 26, and 27 show the individual effects of date, photoperiod, and transplanting on days to bud break in the growth room. The

Table 25. Effect of date of cold storage on days to terminal and lateral bud break in the growth room.[1]

Date of Storage	Terminal Bud Break	Lateral Bud Break
October[2]	30.2a	29.7a
December[2]	26.3b	24.7b
February[3]	20.6c	19.8c

[1]Values within each bud type followed by the same letter do not differ significantly (p = 0.05) by Duncan's test.

[2]Averate of 100 seedlings.

[3]Average of 150 seedlings.

Table 26. Effect of photoperiod during cold storage on days to terminal and lateral bud break in the growth room.[1]

Photoperiod	Terminal Bud Break	Lateral Bud Break
None[2]	26.7a	25.8a
8 hour[2]	25.3b	24.3b
16 hour[3]	18.7c	17.8c

[1]See Table 25.

[2]Average of 150 seedlings.

[3]Average of 50 seedlings.

date of storage was significant for all dates tested. The more rapid bud break after December or February storage is probably a reflection of the increased natural chilling which had taken place prior to the start of cold storage. The seedlings stored in February had had their chilling requirement naturally fulfilled prior to storage and indicates

Table 27. Effect of transplanting prior to cold storage on days to terminal and lateral bud break in the growth room.[1,2]

Container	Terminal Bud Break	Lateral Bud Break
Original	26.9a	26.0a
Transplant	24.5b	23.4b

[1]Values within each bud type followed by the same letter do not differ significantly (p = 0.05) by Student's t-test.

[2]Each observation is the average of 175 seedlings.

that cold storage during the post dormant phase but prior to bud break did not adversely affect the seedlings. All photoperiod treatments during cold storage also had a significant effect on speed of bud break. A 16 hour photoperiod, which was used only during the February storage, produced the most rapid bud break. Use of a 16 hour photoperiod during prolonged storage should be avoided, however, since Lavender (1977) had shown that Douglas-fir seedlings will break bud in response to a 16 hour photoperiod even under cold storage conditions. Transplanting the seedlings into CZ Dee-Pots from their original plant blocks the day prior to the start of cold sotrage had a significant effect on the speed of bud break after cold storage. Lateral bud break generally occurred slightly earlier than did terminal bud break, as was the case in the other studies.

When the effects of photoperiod or transplanting are broken down by date of cold storage (Tables 28 and 29), similar results are found. As the date of cold storage was delayed, the speed of bud break in the growth room was increased for all storage treatments. In addition, transplanting prior to cold storage gave significantly more rapid bud break for all dates tested. The magnitude of this effect was greatest

Table 28. Effect of photoperiod during storage or transplanting prior to storage by date of storage on days to terminal bud break in the growth room.[1,2]

	Date		
	October	December	February
Photoperiod			
Dark	30.5	27.2	22.4
8 hour	29.9	25.4	20.7
16 hour	--	--	18.7
Container			
Original	32.0	27.4	21.3
Transplant	28.4	25.2	19.8

[1]All values are significant (p = 0.05) by Duncan's test except photoperiod during October.

[2]Each observation is the average of 50 seedlings except container in February which is 75.

Table 29. Effect of photoperiod during storage or transplanting prior to storage by date of storage on days to lateral bud break in the growth room.[1,2]

	Date		
	October	December	February
Photoperiod			
Dark	30.4	25.3	21.7
8 hour	28.9	24.1	19.9
16 hour	--	--	18.7
Container			
Original	31.6	25.8	20.7
Transplant	27.7	23.6	18.8

[1]All values are significant (p = 0.05) by Duncan's test.

[2]See Table 28.

during October storage and decreased for later storage. Seedlings which received a daily photoperiod during cold storage broke bud significantly more rapidly than those which were stored in the dark. The only exception was after October cold storage when a daily photoperiod did not produce a significant difference in speed of terminal bud break, although it was more rapid than seedlings stored in the dark. Seedlings stored during February with a 16 hour photoperiod had the fastest bud break when placed in the growth room.

The breakdown of the treatments into the three factors examined (Table 30) again showed that the later storage began the more rapid was bud break in the growth room after storage. After October cold storage, transplanted seedlings showed significantly faster bud break and photoperiod had no significant effect. After December storage, photoperiod had a significant effect only if the seedlings had been transplanted and the effect of transplanting was reduced in comparison to the effect on seedlings stored in October. After February storage, photoperiod during storage was significant and transplanting was significant only if a daily photoperiod was received by the seedlings. The effectiveness of a daily photoperiod during cold storage in stimulating rapid bud break in the growth room clearly increased as the date of storage was delayed. Conversely, the effectiveness of transplanting seedlings into larger containers prior to storage was reduced as the date of cold storage was delayed.

The effect which transplanting seedlings had on speed of bud break prompted a follow-up study of this phenomenon. The study utilized seedlings originally grown in either black polyethylene plant blocks

Table 30. Effect of transplanting and photoperiod on days to terminal and lateral bud break in the growth room.[1,2]

Treatment[3]	Terminal Bud Break	Lateral Bud Break
October		
Dk	32.7a	32.1a
8	31.4a	31.2a
T-Dk.	28.4b	28.8b
T-8	28.4b	26.6b
December		
Dk	27.6a	26.1a
8	27.1a	25.4a
T-Dk.	26.7a	24.4b
T-8	23.6b	22.8c
February		
Dk	22.9a	22.3a
8	21.5b	21.0a
16	19.6c	18.7b
T-Dk.	21.9ab	21.0a
T-8	19.8c	18.7b
T-16	17.8d	16.8c

[1]Values within each storage date and bud type followed by the same letter do not differ significantly (p = 0.05) by Duncan's test.

[2]Each observation is the average of 25 seedlings.

[3]Treatment photoperiod preceded by T- indicate transplants.

or "styroblocks" and which received various treatments designed to provide root disturbance prior to cold storage such as transplanting seedlings using soil or a bark mix (T-Soil and T-Bark in the tables), removing them and replacing them in the same container (Pulled), removing them from the container and storing them in clear polyethylene bags (Out), and not disturbing them at all (Control). These treatments received an 8 hour photoperiod during storage. In addition, seedlings which were transplanted into bark (T-Bark Dk) or received no disturbance (Dark) were stored without a photoperiod. The results of the study are summarized in Tables 31 and 32. No significance was found due to root treatment for the "styroblock" seedlings and only limited significance was present in the polyethylene block seedlings.

Table 31. Effect of root disturbance on days to terminal and lateral bud break after December cold storage of seedlings originally grown in polyethylene blocks.[1,2]

Treatment	Terminal Bud Break	Lateral Bud Break
Control	26.7a	23.6a
T- Bark	27.0a	23.8ab
T- Soil	27.8ab	24.0ab
Dark	28.0ab	24.6ab
Out	28.3ab	24.7ab
Pulled	29.6b	24.3ab
T- Bark Dk	30.6b	25.4b

[1]Values within each bud type followed by the same letter do not differ significantly (p = 0.05) by Duncan's test.

[2]Each observation is the average of 20 seedlings.

Table 32. Effect of root disturbance on days to terminal and lateral bud break after December cold storage of seedlings originally grown in "styroblocks."[1,2]

Treatment	Terminal Bud Break	Lateral Bud Break
Control	28.5a	25.1a
Pulled	29.3a	25.3a
T- Soil	29.8a	26.8a
Dark	29.8a	26.9a
T- Bark	30.2a	26.1a

[1,2]See Table 31.

The study failed to clarify the influence of transplanting prior to cold storage on the subsequent response of the seedling when placed in a favorable environment. A number of explanations for the effect are possible, such as the change in rooting volume, the degree of root aeration, or biochemical changes due to the physical disturbance of the roots. This is an area which deserves further research.

The aspects of this study detailed so far have dealt with the response of the seedlings to a favorable environment for growth immediately after cold storage and have provided valuable information about the effect of cold storage. From a practical standpoint, however, this is rarely the case. Seedlings placed in cold storage are generally planted in the field prior to the time of spring bud break and must still be able to withstand the environmental stresses placed upon them before a natural environment favorable to growth is present. It was for this reason that the treatments were duplicated and the seedlings planted into a cold frame after cold storage to observe their response to the following spring.

Tables 33, 34, and 35 show the effect of date of storage, the photoperiod during storage, and transplanting the seedlings prior to storage on the date of spring bud break and seedling mortality. As a point of comparison, seedlings which were over-wintered under natural

Table 33. Effect of date of cold storage on date (in April) of terminal and lateral bud break and mortality in the cold frame.[1,2]

Date of Storage	Terminal Bud Break	Lateral Bud Break	Mortality (%)
October[3]	32.8a	31.5a	65a
December[3]	19.5b	18.0b	4b
February[4]	20.6b	21.6b	0c

[1]Values within each bud type or mortality followed by the same letter do not differ significantly (p = 0.05) by Duncan's test.

[2]Values greater than 30 indicate dates in May.

[3]Average of 100 seedlings.

[4]Average of 150 seedlings.

Table 34. Effect of photoperiod during cold storage on date (in April) of terminal and lateral bud break and mortality in the cold frame.[1]

Photoperiod	Terminal Bud Break	Lateral Bud Break	Mortality (%)
None[2]	23.6a	22.6a	29a
8 hour[2]	24.3a	24.0a	17b
16 hour[3]	19.1b	21.8b	0c

[1]See Table 33.

[2]Average of 150 seedlings.

[3]Average of 50 seedlings.

Table 35. Effect of transplanting prior to cold storage on date (in April) of terminal and lateral bud break and mortality in the cold frame.[1,2]

Container	Terminal Bud Break	Lateral Bud Break	Mortality (%)
Original	24.9a	24.1a	18a
Transplant	21.2b	22.0b	21a

[1]Values within each bud type or mortality followed by the same letter do not differ significantly ($p = 0.05$) by Student's t-test.

[2]Each observation is the average of 175 seedlings.

conditions in their original containers and received no cold storage had an average date of terminal bud break of April 30. Seedlings planted after December storage had the earliest spring bud break, although it was not significantly faster than seedlings stored in February. Seedlings stored in October had a much later date of bud break and had the highest mortality rate. Seedlings planted after December or February storage had excellent survival rates at the end of the growing season. A 16 hour photoperiod during cold storage was found to have a significant effect on the date of spring bud break while the difference between seedlings receiving an 8 hour photoperiod and those stored in the dark was not significant. Mortality was significantly reduced as the photoperiod during storage was increased. Transplanting seedlings into large containers prior to storage had a significant effect on the date of bud break but had no effect on seedling mortality.

The effect of photoperiod during storage or transplanting prior to storage on the individual storage dates of the seedlings are shown in Tables 36, 37, and 38. Transplanting had an effect on date of

Tables 36. Effect of photoperiod during storage or transplanting prior to storage on date (in April) of terminal bud break in the cold frame.[1,2,3]

	Date		
	October	December	February
Photoperiod			
Dark	37.1a	18.5b	22.0c
8 hour	31.5e	20.5f	20.8cf
16 hour	--	--	19.1d
Container			
Original	34.6a	20.0c	21.7c
Transplant	30.9b	19.0c	19.5c

[1]Values followed by the same letter do not differ significantly (p = 0.05) by Duncan's test.

[2]Each observation is the average of 50 seedlings except container in February which is 75.

[3]Values greater than 30 indicate dates in May.

Table 37. Effect of photoperiod during storage or transplanting prior to storage on date (in April) of lateral bud break in the cold frame.[1,2,3]

	Date		
	October	December	February
Photoperiod			
Dark	31.3a	17.4b	21.5c
8 hour	31.7a	18.6b	21.6c
16 hour	--	--	21.8c
Container			
Original	34.3a	18.1c	21.2c
Transplant	27.6b	17.9c	22.0c

[1,2,3]See Table 36.

Table 38. Effect of photoperiod during storage or transplanting prior to storage on mortality (%) in the cold frame.[1,2]

	Date		
	October	December	February
Photoperiod			
Dark	84a	2c	0c
8 hour	46b	6c	0c
16 hour	--	--	0c
Container			
Original	60a	4b	0b
Transplant	70a	4b	0b

[1,2] See Table 36.

spring bud break only for seedlings stored during October and had no effect on mortality of any of the test dates. Seedlings stored during December had earlier bud break than seedlings stored during February but mortality between the two dates was not significant for any storage treatment and was generally very low. A daily photoperiod during October storage had a strong influence on spring bud break and mortality and seedlings which received a photoperiod during storage had earlier bud break and lower mortality than those which were stored in the dark. Although mortality was high for all October stored seedlings, a photoperiod during storage produced a reduction of mortality by nearly 50%. A photoperiod during February cold storage generally yielded seedlings with the earliest date of bud break, while a photoperiod during December storage yielded seedlings with a later date of bud break as compared with seedlings receiving dark storage. Photoperiod during

December or February storage had no significant effect on mortality of the seedlings.

The effect of the combination of the daily photoperiod during cold storage and transplanting seedlings prior to cold storage for the dates tested is shown in Table 39. As was the case with the growth room study, transplanting had a greater effect than photoperiod during October on date of bud break. Transplanting seedlings and giving them an 8 hour photoperiod produced the earliest bud break and the lowest mortality rate for seedlings stored at this time. The mortality rate after October storage was strongly influenced by the photoperiod received during storage. Seedlings which were transplanted and received dark storage had the highest mortality rate. Little significance due to storage conditions was found for seedlings stored in December. Seedlings which were transplanted and stored in the dark broke bud earliest in the spring and seedlings which received dark storage generally broke bud earlier than those which had received a daily photoperiod. Mortality of seedlings stored in December or February was quite low and not significant. The effect of February storage conditions was dominated by the photoperiod received during storage with a 16 hour photoperiod providing the earliest bud break. Seedlings transplanted prior to storage generally had earlier dates of bud break, although they were not significant. The combination of transplanting seedlings and exposing them to a 16 hour photoperiod during storage gave the earliest date of bud break after February storage. It seems that a short photoperiod was not effective in speeding bud break after

Table 39. Effect of transplanting and photoperiod on date (in April) of bud break and mortality in the cold frame.[1,2,3]

Treatment[4]	Terminal Bud Break	Lateral Bud Break	Mortality (%)
October			
Dk	37.1a	35.5a	72b
8	32.1a	33.1a	48ab
T-Dk[5]	--	--	96c
T-8	30.9b	30.2b	44a
December			
Dk	19.8ab	18.8ab	4a
8	20.2ab	17.4ab	4a
T-Dk	17.2a	16.0a	0a
T-8	20.7b	19.8b	8a
February			
Dk	23.2a	22.9ab	0a
8	21.6ab	21.1bc	0a
16	20.3bc	19.7d	0a
T-Dk	20.8ab	20.0cd	0a
T-8	20.0bc	22.1bc	0a
T-16	17.8c	23.8a	0a

[1]Values within each storage date and bud type or mortality followed by the same letter do not differ significantly ($p = 0.05$) by Duncan's test.

[2]Each observation is the average of 25 seedlings.

[3]Values greater than 30 indicate dates in May.

[4]Treatment photoperiod preceded by T- indicates transplants.

[5]Only 1 seedling survived.

December or February storage whereas a 16 hour photoperiod during February was able to speed the date of bud break. The reason why a short photoperiod was able to reduce mortality after October storage but was not beneficial during December or February storage is not clear. Cold storage for one month in December or February consistently produced earlier bud break than seedlings which over-wintered naturally, regardless of the storage conditions. The date of bud break was between 7 and 13 days earlier than for seedlings which received natural conditions and varied with the treatment received.

Many of the findings from this study compare favorably with data on other species and western hemlock. The bulk of literature relating to cold storage of seedlings, however, has dealt with bare-root stock and few data are available for containerized seedlings. The poor survival of fall lifted Douglas-fir has been recognized for many years (Lavender and Wright, 1960; Walters and Soos, 1961) but the reason for this phenomenon is still not clear. The problem of fall lifting has been found to occur in other species native to the Pacific Northwest, such as ponderosa pine (Stone and Schubert, 1959a) and western hemlock (Brown, 1976), and species native to this region seem to be much more sensitive than species native to other regions of the country.

Many authors have examined the possibility that the seedlings roots are the site at which fall lifting and planting exerts its deleterious effect on seedling survival. The root regenerating potential of ponderosa pine (Stone and Schubert, 1959a) and Douglas-fir (Stone, Jenkinson, and Krugman, 1962; Lavender, 1964) is characteristically low during the fall and does not reach a high level until

December. Brown (1976) has recently shown a similar trend for wildling western hemlock seedlings, with high potentials for root growth occurring from late winter through early spring. Container-grown seedlings generally had a lower root regenerating potential than did bare-root stock. He found a good correlation between the root regenerating capacity of the seedlings and their survival.

Hermann (1964) found Douglas-fir seedlings to be most sensitive to exposure of their roots to drying conditions during the fall and less sensitive during the winter. He also noted that cold storage during any of the test dates increased the sensitivity of the roots to exposure. While extended periods of root exposure are common for bare-root regeneration stock, containerization has practically eliminated this problem and exposure of roots would not account for the rate of mortality found for western hemlock in this study.

Fall lifting followed by cold storage has been found to further reduce the survival rate of ponderosa pine (Stone and Schubert, 1959a) and Douglas-fir (Lavender, 1964; Lavender, Hermann and Zaerr, 1969; Zaerr and Lavender, 1972). Seedlings lifted after mid-November and placed in cold storage for up to 6 weeks had much better survival than seedlings which were lifted prior to this time. Aldhous (1964) found that western hemlock lifted and stored in January were more vigorous than seedlings stored at the end of March. He further noted that western hemlock could be safely stored after December and that it could be stored for periods of up to 6 months without suffering detrimental effects. In my findings, December cold storage appears to be more beneficial than February for providing rapid bud break in the spring.

The slight variation of optimum dates for storage between my data and that of Aldhous can probably be accounted for by the normal variations which occur from year to year in transition from one stage of dormancy to another and by the difference in the location of the tests since his work was done in England.

Brown (1976) found that the root regenerating potential of bare-root western hemlock nursery seedlings or container-grown seedlings was not deleteriously affected by 4 weeks of dark cold storage in the fall. Seedlings receiving cold storage in October or November were found to have higher root regenerating potential than seedlings lifted at the same time but not stored. After transplanting into the field, however, seedlings stored in October had a high mortality rate. Seedlings lifted after December and stored had good survival rates and Brown's general trend of date of lifting and storage as it affected survival was very similar to that which I observed. Although fall cold storage of western hemlock may not be as detrimental to the seedlings root regenerating potential, it is clear that survival is not merely based on the root regenerating potential at the time of lifting.

A daily photoperiod during fall cold storage of Douglas-fir seedlings has been found to reduce the mortality of the seedlings after planting (Lavender and Wareing, 1972; Hermann, Lavender, and Zaerr, 1972). The results of my study indicate that the effect of photoperiod during fall storage of western hemlock on mortality is similar to that found for Douglas-fir. While seedlings receiving a photoperiod during storage still had high mortality, it was considerably less than for seedlings receiving dark storage. The reason for the effectiveness of

a daily photoperiod in reducing mortality is still unclear. The light intensity used in all studies was relatively low and would seem to have had little effect on seedling photosynthesis during storage.

Lavender and Wareing (1972) have suggested that part of the detrimental effect of fall cold storage may be due to the cool temperature experienced by the roots. Seedlings stored with a root temperature of 15°C and a shoot temperature of 2°C had a much higher survival rate than seedlings whose roots experienced the 2°C temperature. This appears to be a reasonable hypothesis and is supported to some extent by my findings on container-grown western hemlock seedlings. Because of the nature of containerized seedlings, little or no physical disturbance of the root system occurred prior to cold storage. Seedlings which were transplanted prior to cold storage were planted as plugs and only the outer portion of the root system would have been disturbed. The disruption of seedling physiology due to disturbance and exposure of the roots during lifting and cold storage of bare-root stock as an explanation of mortality thus appears inappropriate for containerized seedlings. Seedlings which received October cold storage experienced a rapid change of root temperature since they were moved from a greenhouse directly into cold storage. Seedlings which received December or February cold storage had experienced natural fall and winter temperatures prior to cold storage and did not experience the magnitude of temperature change that the October trial did. Whether this large temperature change was in any way responsible for the mortality rate of the October stored seedlings is not clear. Because cold storage of western hemlock seedlings has not been found to seriously affect root

regenerating potential, it can only be stated that the effect of cool root temperatures during the fall cold storage is exerted through some as yet undetermined mechanism.

The lack of mortality of seedlings receiving October cold storage followed by 6 weeks in a growth chamber further complicates an interpretation. While bud break of these seedlings was slower than for later storage dates, this was probably due to the reduced amount of natural chilling received prior to cold storage. Several of the studies previously mentioned have indicated that Douglas-fir and ponderosa pine seedling mortality occurred after fall cold storage even when seedlings were placed into a favorable environment for growth. Brown (1976), however, found that good survival of western hemlock seedlings occurred if they were placed under a favorable environment for growth after fall cold storage and this result is supported by my current findings. The response of the three species to planting of non-stored and stored stock in the fall is similar but the response to a favorable environment after storage is evidently quite different. The reason for the discrepancy among the response of that species is not clear. It is possible that fall cold storage of Douglas-fir or ponderosa pine causes irreversible physiological changes in the seedlings which cannot be countered by placing them in a favorable environment and that storage during this period of western hemlock is less severe and can be overcome or reduced by the favorable environment. The expression of any deleterious effects of fall cold storage on western hemlock would then become dependent on the subsequent environment. With the stresses placed on the seedlings by planting them into

a cold frame, the effect of cold storage manifest itself in the form of a high mortality rate of the seedlings. It is, however, not yet clear that the mechanism responsible for the reduced survival of the three species is the same. This area is one which will require a great deal of research to elucidate the reason for reduced survival after fall cold storage to seedlings.

From an operational standpoint, western hemlock seedlings will require the same type of precautions relating to cold storage which are known to be necessary for Douglas-fir. Proper storage conditions, such as appropriate temperature, moisture, and humidity, are similar for the two species and must be present to insure quality seedlings at the time of planting. These conditions have been adequately reviewed (Hocking and Nyland, 1971; Brown, 1973) and deviation from them can cause injury to the seedlings. If fall planting of western hemlock is necessary, the stock should not have received any cold storage prior to outplanting. With the containerized stock, this should present no problem. A daily photoperiod during fall cold storage will reduce seedling mortality, although it will still be high and may lead to insufficient stocking of the areas to be reforested. Cold storage of containerized western hemlock seedlings after the beginning of December will have no detrimental effects and will, in fact, produce seedlings with early, vigorous spring growth. This could be used as a valuable technique in the production of stock to be used on sites which experience early moisture stress, since early spring bud break will give the seedling a better chance of survival under these circumstances. The data presented here are for seedlings which were planted after one

month of cold storage, although indications are that western hemlock can be stored for longer periods without suffering any damage. Cold storage during this period may be especially helpful if seedlings have received insufficient natural chilling in a greenhouse. Work with Douglas-fir has shown that cold storage at 2°C is effective in fulfilling the chilling requirement of that species (van den Driessche, 1977) and this is also true for western hemlock. Cold storage starting in February was found to have little detrimental effect on seedling survival if planted after one month of storage. Here again, longer storage periods are probably possible and could be used to provide seedlings which have not broken bud for planting in the late spring. If cold storage is used to prolong the date of bud break, long photoperiods during storage should be avoided since this may lead to bud break of the seedlings while in cold storage, as has been shown for Douglas-fir (Lavender, 1977).

Depth of Dormancy Phases

The studies thus far reported have dealt with small areas of the physiology of dormancy of western hemlock. While they provide valuable information on their respective areas and have practical applicability for nursery production of high quality seedlings for reforestation, they have only hinted at the distinct physiological stages which occur within the seedlings. The existence of separate physiological stages during dormancy is recognized to occur in many woody species, including Douglas-fir, and determination of their existence in western hemlock and their general timing was the purpose of this study. By determining

their timing and understanding the general physiology associated with each of them, a certain degree of predictability of seedling response to various cultural practices can be achieved.

A number of different terms have been used to identify the various phases of dormancy in the literature. For the purposes of this paper, the terminology of Lavender and Cleary (1974) has been adopted which consists of four separate phases of dormancy. These phases are termed dormancy induction, dormancy deepening, dormant, and post dormancy and will be described more fully shortly. Generally they are based on the seedling's response to an environment favorable for growth.

Western hemlock seedlings are capable of a second flush of growth if favorable environmental conditions are present shortly after a terminal bud has been formed. This is easily observed if seedlings are maintained in a growth room with a long photoperiod and adequate moisture and is common in nature if a sufficient amount of rainfall occurs during the summer when natural bud set is occurring. These facts substantiate the presence of a dormancy induction (predormancy) phase.

The initial series of tests confirmed the existence of the other three phases of dormancy. Photoperiods during the test dates included a 16 hour day of high intensity, a 16 hour day composed of 8 hours of high intensity and 8 hours of 20 foot-candle intensity (labeled 8+8 in tables and figures), and an 8 hour day of high intensity. The results (Figure III, Table 40) showed that during the October trial the seedlings were in the dormancy deepening phase due to the inability of the long photoperiods to stimulate bud break. During the December trial, both of the long photoperiod treatments were able to cause bud break,

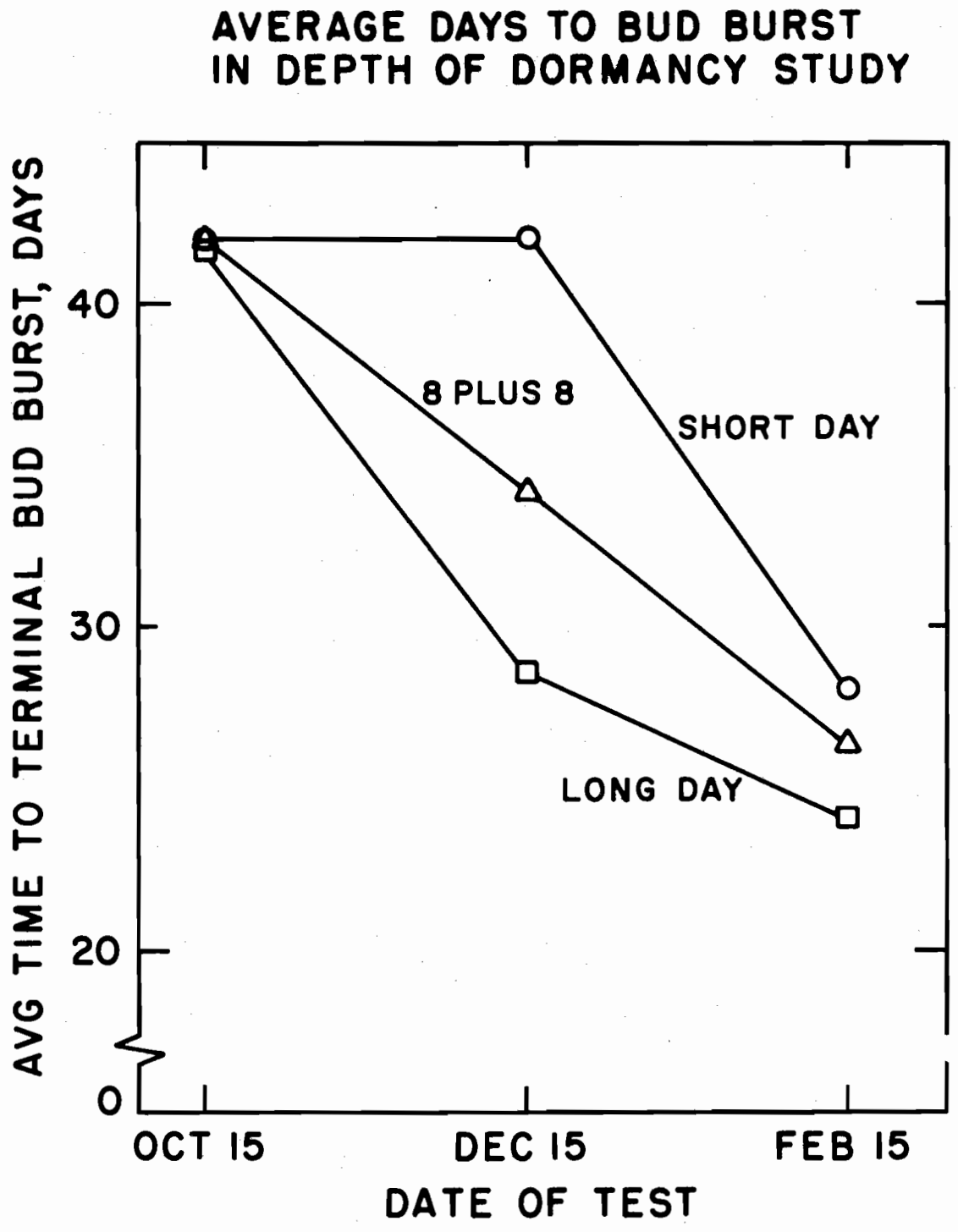

Figure III. Average days to terminal bud break of seedlings in initial study of the phases of dormancy.

Table 40. Effect of date seedlings were moved into the growth room on days to bud break (initial study).[1,2]

	Terminal Bud Break			Lateral Bud Break		
	Photoperiod			Photoperiod		
	Long Day	8+8	Short Day	Long Day	8+8	Short Day
Oct 15	41.7a	--[3]	--	41.7a	--[3]	--
Dec 15	28.6b	34.2c	--	27.5b	32.6c	--
Feb 15	24.0d	26.3e	28.0be	23.2d	25.0de	26.6be

[1]Values within each bud type followed by the same letter do not differ significantly (p = 0.05) by Duncan's test.

[2]Each observation is the average of 25 seedlings.

[3]No bud break occurred within 42 days of test.

whereas the short day treatment was not able to produce bud break. This is indicative of the dormant phase and that during this phase there is a photoperiodic effect, with a long photoperiod being able to overcome the remaining chilling requirement. In the February trial, all treatments broke bud and there appeared to be little photoperiodic effect, which is indicative of the post dormancy phase. By this time the chilling requirement had been fully met and bud break became a temperature dependent process. Date of test and photoperiod were generally found to be significant on the speed of bud break, although the photoperiod effect was reduced during the February trial. Lateral buds were generally found to resume growth slightly faster than terminal buds, as was the case in the previous studies.

With this evidence of the distinct phases of dormancy similar to Douglas-fir, the study was again carried out the following year using the same methodology but with more frequent test dates. From this it was hoped that transition dates from each phase of dormancy to the next

could be more closely defined. The results (Figure IV, Table 41) showed that seedlings were in the deepening dormancy phase from about

Table 41. Effect of date seedlings were moved into the growth room on days to bud break.[1,2]

	Terminal Bud Break			Lateral Bud Break		
	Photoperiod			Photoperiod		
Date	Long Day	8+8	Short Day	Long Day	8+8	Short Day
Oct 1	--[3]	--	--	41.3a	--	--
Oct 15	--	--	--	--	--	--
Nov 1	--	--	--	--	--	--
Nov 15	41.9a	--	--	38.2b	41.4a	--
Dec 1	33.6b	40.4d	--	27.3d	39.0b	--
Dec 15	30.2c	37.5e	--	25.8d	32.2c	--
Jan 1	29.4c	34.1b	41.5a	25.4d	29.8e	39.9b

[1]Values within each bud type followed by the same letter do not differ significantly (p = 0.05) by Duncan's test.

[2]Each observation is the average of 25 seedlings.

[3]No bud break occurred within the 42 days of the test.

October 1 until November 15, since long photoperiods were unable to stimulate bud break of the seedlings during this period. From the period of late November until about January 1 the seedlings were in the dormant phase, since long photoperiods were able to cause bud break of the seedlings and short photoperiods were not. The ability of long photoperiods to substitute for the remaining chilling requirement, which is being fulfilled during this phase, was clearly seen during this period. The speed of bud break increased as the date of the test was postponed and indicated the increased chilling received prior to

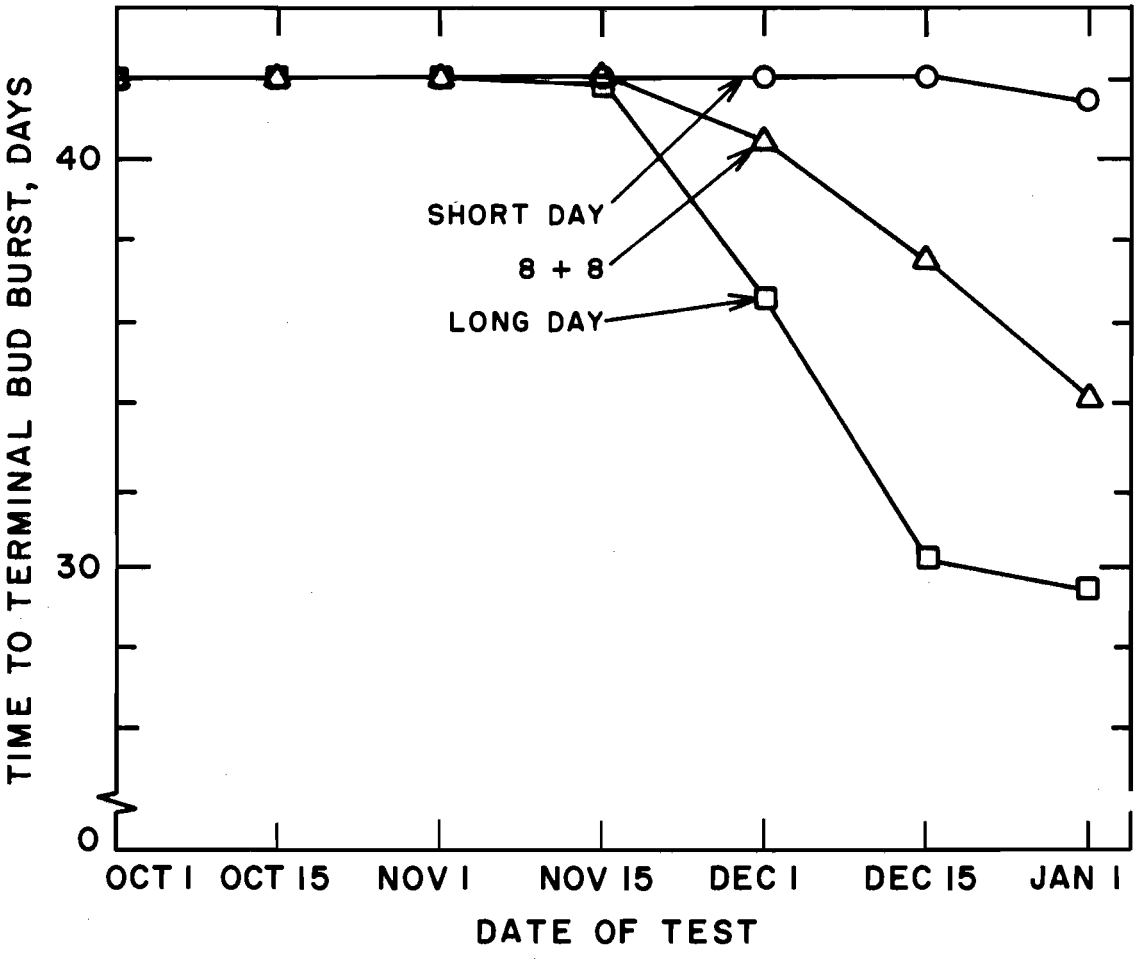

Figure IV. Average days to terminal bud break of seedlings in study of the phases of dormancy.

the start of the test. By the January 1 test, seedlings began to break bud under short photoperiods and indicates that the chilling requirement had been fulfilled. The long photoperiod during the January 1 test produced no significant increase in the speed of bud break as compared to the December 15 test. Bud break during this test date had become a temperature dependent phenomenon and is indicative of the seedling having moved into the post dormant phase.

While it must be kept in mind that the dates of transition between phases are gradual and will vary from year to year and with location, the results of these studies made it possible to propose general dates for western hemlock (Figure V). Seedlings will begin dormancy induction in mid-summer and this phase will remain until around the beginning of October when they enter the dormancy deepening phase. This phase lasts about 6 weeks and around the middle of November they enter the dormant phase. They will remain in this phase until their chilling requirement is fulfilled and then enter the post dormancy phase in which they remain until the stimulus for spring bud break occurs. These dates of transition are similar to those reported for Douglas-fir (Lavender and Cleary, 1974) with the exception that western hemlock enters the post dormancy phase about one month earlier than does Douglas-fir. This is probably due to the much shorter chilling requirement of western hemlock.

The first phase is dormancy induction and is characterized by metabolic changes within the seedlings and by the formation of winter buds. Under natural conditions in the Pacific Northwest, this phase is probably initiated by the moisture stress experienced during the

		JULY 15
I.	DORMANCY INDUCTION	↓
		OCT 1
II.	DORMANCY DEEPENING	↓
		NOV 20
III.	DORMANT	↓
		JAN 20
IV.	POST DORMANT	↓
		SPRING

Figure V. Phases of western hemlock seedling dormancy and approximate dates.

summer and to some degree by warm temperatures. The ability of these two factors to initiate dormancy under a long photoperiod was confirmed in the study of factors initiating dormancy of western hemlock seedlings. Seedlings in this phase are in an environmentally imposed dormancy and are capable of resuming growth under favorable environmental conditions, especially adequate moisture and long photoperiods.

The second phase is dormancy deepening and is characteristic of the period from late September or early October until late November. Seedlings in this phase will generally fail to break bud in response to a favorable environment for growth. During this phase needle primordia initiation and maturation are continuing to take place and seedlings are beginning to acquire frost hardiness. It is during this time that a period of short, mild days prior to receiving chilling has been found to be beneficial for Douglas-fir and to a lesser extent for western hemlock. This response, presumably, is due to the favorable conditions for maturation of the winter bud and the ability of the short photoperiod to induce the first phase of cold hardiness (Weiser, 1970). Seedlings in this phase characteristically are very sensitive to environmental stresses and high mortality rates are observed if seedlings are cold stored during this period. The mechanism by which the detrimental response is brought about is not clear, although cool temperature appears to be the environmental stimulus in containerized western hemlock. It is possible that the mortality experienced after cold storage during this phase may reflect the large, sudden change of temperature which the roots experienced. Seedlings stored at later dates had received natural temperatures prior to cold storage and would

have received gradually cooler temperatures. Therefore, the roots were not subjected to the rapid temperature change which the fall stored seedlings received. It is possible that some temperature dependent physiological process is disrupted during this period. Hermann, Lavender, and Zaerr (1972) investigated the possibility that the adverse effect of fall lifting of Douglas-fir was due to physical disturbance that resulted in a severe delay in the normal sequence of concentrations of growth regulators. Although their findings were inconclusive, disruption of the normal hormonal balance of the seedling is still a feasible hypothesis. Due to the lack of physical disruption of the containerized seedlings, this would not be the reason for hormonal changes. It is possible that the cool temperature due to fall cold storage slowed down or stopped the normal maturation process of the winter buds and that this was responsible for a detrimental imbalance of growth promoters and inhibitors which could affect the seedlings ability to withstand environmental stresses after the time of planting. By delaying cold storage until proper maturation of the buds had occurred, the proper hormonal balance to withstand the storage and natural stresses might be achieved. Alternatively, the cool temperature could be affecting growth regulator production in the roots and be the reason for an imbalance. The ability of a daily photoperiod during storage to reduce the detrimental effect is not clearly understood, although it suggests that an interaction of photoperiod and temperature may exist. These phenomena are ones which clearly will required a great deal of future research to properly determine their cause.

With the transition of the seedling into the third phase of dormancy, the physiology and response of the seedling is markedly changed. The dormant phase of western hemlock is generally from mid-November until late January or early February and is the period when the chilling requirement of the seedling is being fulfilled. A long photoperiod can overcome part of the chilling requirement of the seedlings and seedlings moved into the growth room during this phase will break bud in response to a long photoperiod. The speed of bud break will be modified by the amount of natural chilling which has taken place prior to being placed under the long photoperiod. During this phase seedlings will achieve their maximum cold hardiness and will be resistent to environmental stresses placed upon them. Cold storage of seedlings during this phase had no detrimental effect on survival of the seedlings after they were outplanted and resulted in seedlings which broke bud more rapidly in the spring than seedlings which did not receive a cold storage period. Because of their resistance to stress, this is the most appropriate time for cultural practices, such as outplanting or cold storage of western hemlock container stock.

Unfortunately, seedlings in the dormant phase are indistinguishable morphologically from those in the dormancy deepening phase and the only way to determine that the transition has taken place is to observe the seedlings response to a favorable environment for growth. Cold storage of seedlings could be delayed until well after the general date of transition proposed, but it must be remembered that this date will vary from year to year, with location, and with the previous cultural regime received by the seedlings. The finding of a rapid,

accurate method for determining the phase of dormancy a seedling is in is the subject of several continuing investigations. When successful procedures are determined, a new method will prove invaluable at the operational level as well as to research in the area of dormancy.

As mentioned earlier, due to the shorter chilling requirement of western hemlock, it will make the transition from the dormant phase to the post dormant phase up to one month earlier than Douglas-fir if they both have received similar natural environments. Transition to the post dormant phase occurs with the fulfillment of the chilling requirement and occurs around late January under natural conditions and this phase will last until the stimuli for spring bud break are received. Bud break of western hemlock appears to be a temperature dependent process and seedlings placed in a growth room will break bud even if maintained on a short day regime. During this period the seedlings begin a gradual loss of resistance to environmental stresses. Seedlings will become sensitive to disturbances caused by cultural practices and this sensitivity will increase as the date of bud break approaches. Seedlings receiving February cold storage had enough resistance that this treatment still had a high survival rate, although date of bud break was slightly delayed. Seedlings stored later than this and outplanted in the spring may have a reduced survival rate, although this was not tested.

Nursery Production of Seedlings

The greenhouse production of containerized seedlings for use in regeneration has greatly increased the potential for environmental

manipulation compared with that which is possible for bare-root stock. The recognition of the physiological phases of dormancy and the constraints which they place on nursery operations must be taken into account in the planning of seedling production if stock with a high survival potential is to be produced. Because of the flexibility which greenhouse production allows, especially in regard to photoperiod, temperature, and moisture, quality container stock of western hemlock for spring planting can be easily obtained. Seed could be germinated beginning in February and actively grown until late July or early August. Supplemental light during the early period will be required to extend the natural photoperiod and proper temperatures, adequate moisture and fertilization will be necessary for rapid growth. Around the beginning of August dormancy should be induced using increased moisture stress, warmer temperatures, and reduced nutrient availability. By mid-September the seedlings should be into the dormancy deepening phase and mild temperatures should be maintained in the greenhouse until mid-November to allow seedlings to progress into the dormant phase. The natural photoperiod during this time will be shortening and no manipulation will be required. Seedlings could then be overwintered in an unheated greenhouse until the time they are needed in the winter or spring for planting. For seedlings to be planted in the spring, the chilling requirement will have to have been fulfilled and a period of cold storage will be able to accomplish this if the natural temperatures received have not satisfied the chilling requirement.

Production of seedlings for fall planting, which will be necessary in some instances and cannot be totally avoided, will require more extensive planning. Seed will need to be germinated during December and seedlings actively grown until mid-June. This will require extending the natural photoperiod with supplemental light during much of this growth period and the additional cost of heating the greenhouse due to the normal weather conditions. Induction of dormancy should be started no later than mid-June or early July and would consist of a regime of moderate moisture stress, warm temperature, and reduced nutrient applications. Natural photoperiods during this time will be long and care must be taken not to provide enough moisture to allow seedlings to produce a second flush of growth. Preliminary results of Cheung (1975) indicate that ethrel (2-chloroethyl phosphinic acid) has considerable potential for dormancy induction and delay of bud break of western hemlock seedlings. When an optimum level of treatment and the long term effects are found, the use of ethrel for dormancy induction could become a valuable part of the production of seedlings for fall planting. Seedlings grown in this manner should be in the deepening dormancy phase by early August and be maintained under mild conditions until planting to allow the proper physiological progression into the dormant phase by mid-October. Slight moisture stress should be maintained during the early part of this period due to the long natural photoperiod to assure that seedlings are in the deepening dormancy phase. During the latter period, cooler temperatures should be received by the seedlings to increase their cold hardiness in preparation for the field environment. While this manipulation of the

dates of the various phases of dormancy may provide higher survival of seedlings than if dormancy initiation was delayed, survival will probably still be less than for winter or early spring planted seedlings. Seedlings conditioned in this manner will be exposed to long photoperiods during the time which would be synonymous with fall and this may delay the date of spring bud break. Ideally, a short photoperiod would be used during dormancy induction and the dormancy deepening phases but this is not generally feasible on an operational level.

While having seedlings in the proper phase of dormancy at the time of out-planting or cold storage is definitely necessary for good survival, the site on which the seedlings are planted will also have a considerable influence on the seedlings' survival and proper planting of the seedlings is essential. The seedling must be able to adapt quickly to its new surroundings and be able to tolerate the new environment to which it will be exposed. In the spring, the seedling will need to initiate new root growth and have rapid, vigorous bud break and early growth, and the necessity of this increases with the time and severity of summer drought which the seedling will experience. In the final analysis, then, the seedling's being in the proper phase of dormancy at the time it is introduced into its new environment is essential for the seedling's ability to adapt to and withstand its new environment and will determine the success of any regeneration operation using western hemlock nursery stock.

Growth Regulators

The importance of the several classes of plant hormones on many aspects of growth and development has become appreciated over the past 50 years. Evidence has accumulated which supports the hypothesis that dormancy is to some extent also under hormonal control. As methods of analysis have become more sophisticated and sensitive, it has become clear that the control of dormancy is not due to any single hormone, but, rather to the balance of two or more hormones. Abscisic acid (ABA) has been the primary inhibitor studied and its interrelationships with a number of promoters, including indole-3-acetic acid (IAA), the gibberellins, and the cytokinins, have been examined as a possible explanation of the dormancy phenomenon. The general topic of hormones and dormancy has been reviewed by a number of papers (Smith and Kefford, 1964; Galston and Davies, 1969; Perry, 1971; Wareing and Saunders, 1971) and is an area which is still being intensively investigated. The implication of hormones on controlling dormancy has come about primarily as the result of the correlation of changes in hormone levels with plants entering and breaking dormancy. It must be remembered, however, that this correlation by no means establishes a causal relationship between the two. Before this can be established, the mechanism of action of the balance of hormones will need to be evaluated.

A qualitative study to identify IAA, a growth promoter, and ABA, a growth inhibitor, in western hemlock and to determine seasonal changes in the levels of each of the hormones was undertaken. A study to determine the effect of various environmental factors on the level

of ABA present in western hemlock seedlings was also carried out, since changes in hormone levels due to the environment would be necessary if they were to function as an inducer and terminator of dormancy.

Occurrance and Seasonal Variation of IAA and ABA

IAA and ABA were qualitatively identified in most of the extracts prepared from western hemlock seedlings growing in the wild. Identification of the trimethylsilyl derivative of IAA on the SE-30 (4.7%) column was not possible due to interfering substances at the appropriate retention time (R_t). This derivative was resolved better on the XE-60 (3.5%) column and the methyl ester of IAA was clearly resolved on both columns. Identification of IAA present in the extracts was therefore possible for 3 of the 4 combinations of derivatives and columns. The qualitative determination of ABA in the extracts was accomplished using the methyl ester of ABA and the XE-60 (3.5%) column attached to an electron capture detector. Due to the high sensitivity of this system for ABA and the lack of interferring compounds, no other detection system was needed. Purification and analysis of the extracts for the presence of IAA and ABA was done using techniques which have been used successfully on Douglas-fir and which have been confirmed by identification using mass spectrometry (DeYoe, 1974; Webber, 1974). Due to the past success of the procedures and the positive results of the various supplemental techniques used to provide identification of IAA and ABA, mass spectrometry was not used to identify the hormones. It is therefore possible that the identification of either IAA or ABA in the extracts may be in error, although this seems very unlikely.

This work indicates the endogenous presence of IAA and ABA in the shoots of western hemlock. Since extracts were made over the period of an entire year it was possible to show the seasonal changes in the endogenous levels of these hormones in the shoot (Figure VI). The level of IAA was found to be at a maximum during April and May and to decrease during late summer. It remained low throughout the fall and early winter until it began to increase in February. In terms of the phases of dormancy, the IAA level was low during the dormancy induction and deepening dormancy phases, began to increase during the dormant phase, and reached a maximum during the post dormant phase and during spring bud break and early elongation. The level of ABA in the seedlings was lowest during the period immediately prior to bud break and during early spring growth. Its level increased during the summer and was at its maximum in December after which it steadily declined. The ABA content of western hemlock was increasing during the deepening dormancy phase and reached its highest level at the time of transition into the dormant phase. During the dormant phase the ABA level declined and was low during the post dormant phase and at the time of bud break. The seasonal variation of IAA and ABA in the shoots of western hemlock are similar to the trends of IAA (DeYoe, 1974) and ABA (Webber, 1974) in the shoots of Douglas-fir as well as many other species.

The ratio of levels of IAA and ABA throughout the year proves to be of more value in terms of explaining the phases of dormancy and is shown in Figure VII. This ratio of promoter to inhibitor correlates very well with the phases which seedlings go through during the year.

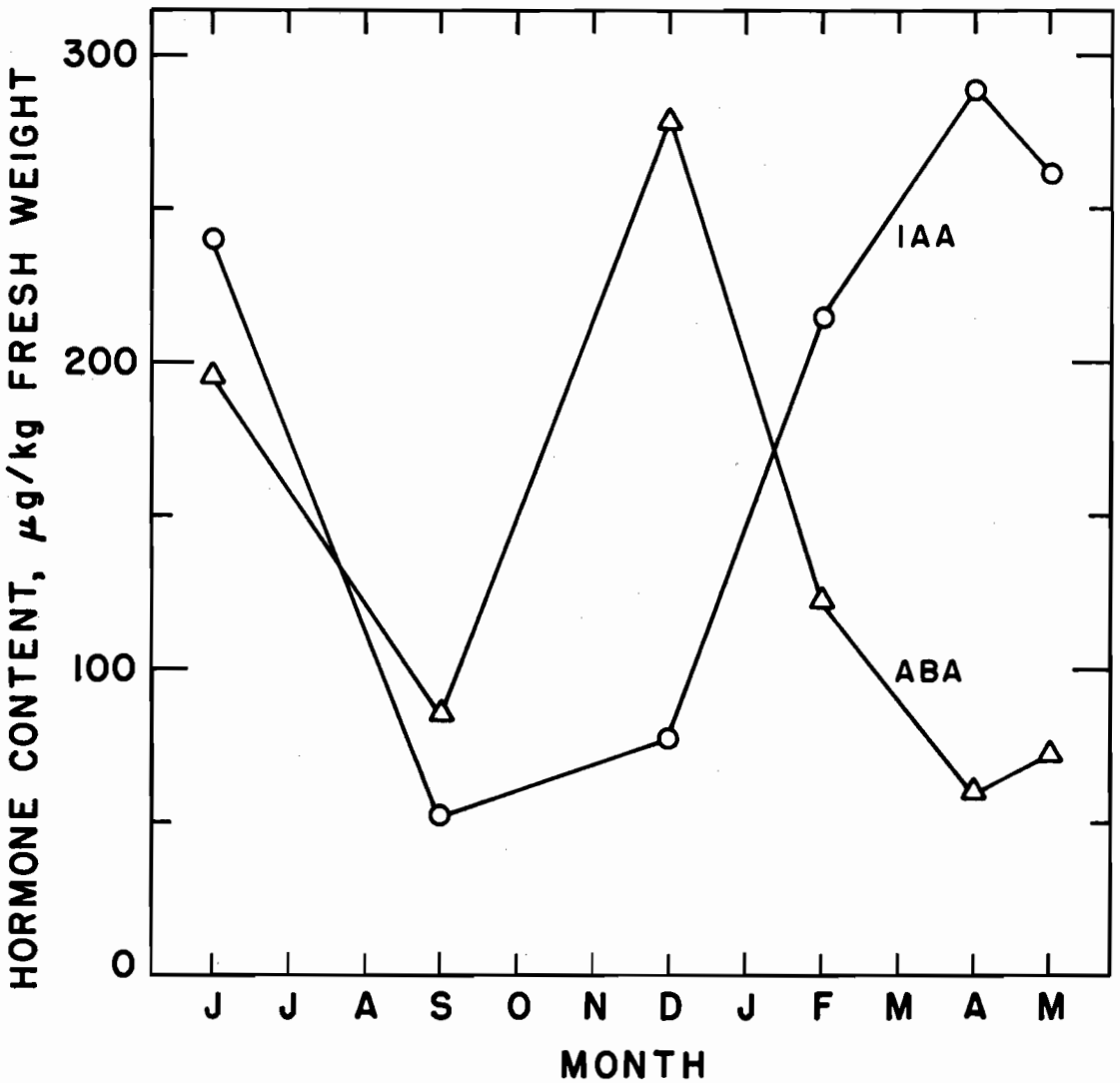

Figure VI. Changes in endogenous levels of IAA and ABA in western hemlock shoots.

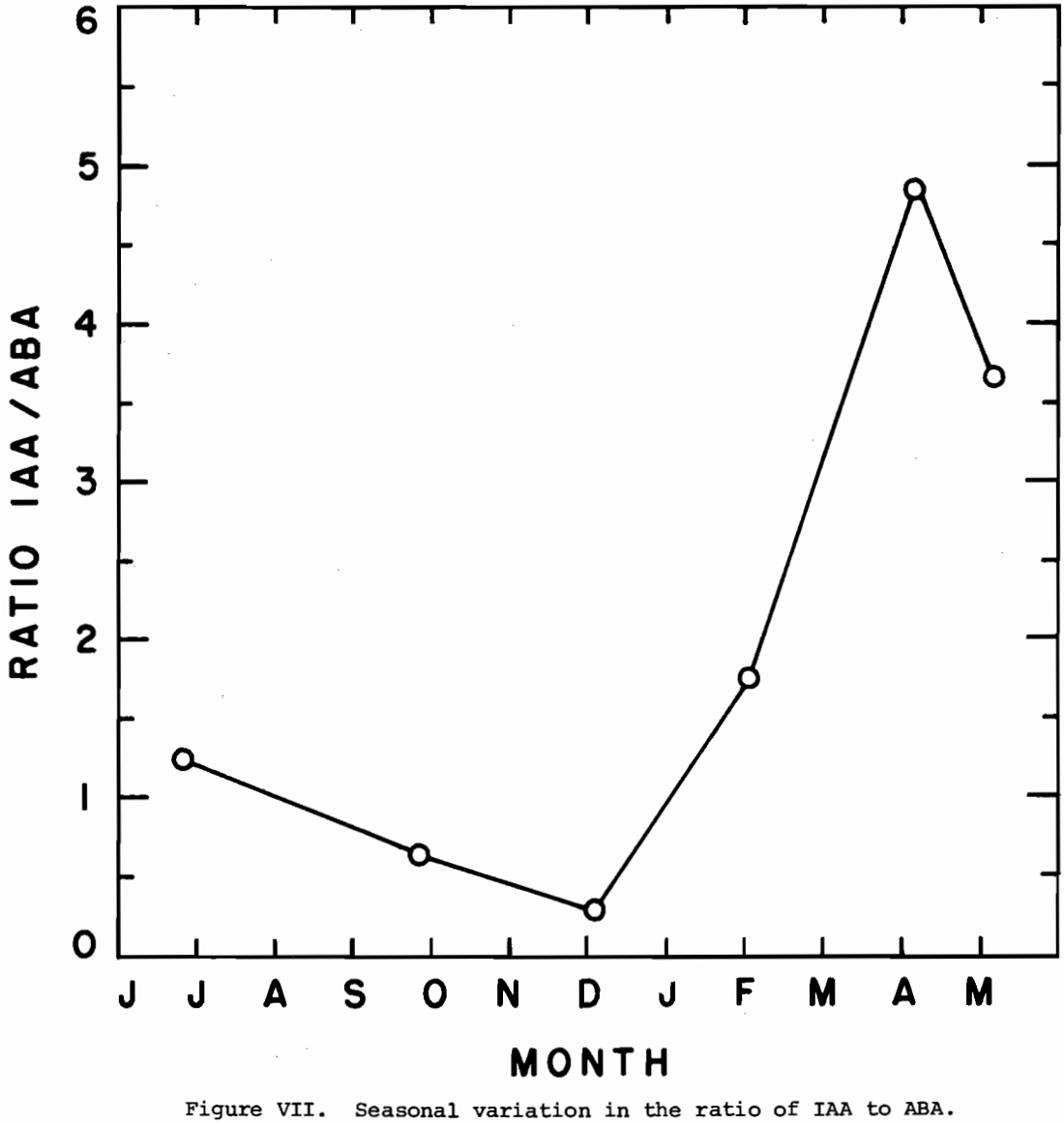

Figure VII. Seasonal variation in the ratio of IAA to ABA.

At the time of natural dormancy initiation the ratio falls below one and continues to decrease during the dormancy induction and dormancy deepening phases. The ratio reaches a minimum in December at the time of transition into the dormant phase and begins to rise during this phase. The most rapid increase is during the post dormant phase and reaches a maximum immediately prior to bud break. A high ratio of IAA to ABA is maintained during the early portion of bud break and growth after which it again declines. Because of this good correlation between the IAA/ABA ratio and the phases of dormancy, more research should be undertaken to confirm and refine this relationship. The potential for developing a rapid method of determining the transition into the dormant phase based on the rapid increase of the ratio appears to be promising and would certainly be an asset in the scheduling of nursery operations.

Comparison of my results with published literature in this area is difficult. The main problem arises from the fact that the vast majority of work on endogenous hormone levels has been carried out using bioassays. In many instances, the results can be drastically altered by compounds which interact or antagonize the substance being tested without the realization of their presence. Only recently has much of the methodology and instrumentation necessary to measure the low levels of hormones been developed and utilized, especially for IAA and ABA. It is unfortunate that articles still occasionally appear which have used bioassays to determine IAA and ABA levels. Comparison of results obtained by "similar" methodology can also be deceiving since minor modifications of the method used for extraction and

purification as well as the method of identification and quantification can often lead to large differences in the results obtained. My results for the maximum endogenous concentrations of IAA and ABA are, however, of a similar magnitude as those reported for other coniferous species. The levels of both hormones in western hemlock are slightly lower than those reported in Douglas-fir (DeYoe and Zaerr, 1976a; Webber, 1974) and the level of IAA is slightly higher than has been reported in Pinus silvestris (Aldén, 1971).

Comparing general trends reported is somewhat easier as long as the methodology within each study remained constant throughout the time span monitored. As mentioned earlier, the seasonal trend of IAA and ABA in the shoots of western hemlock reported here are in general agreement with those of other woody species which have been examined. Due to the length of time between extraction dates, these results indicate only a general trend for IAA and ABA. Rapid fluctuations occurring between sampling dates could not be deduced from this study, although the effect of environmental variables over a short period of time on ABA levels were examined more closely in the subsequent hormone studies.

Working with western hemlock roots, Brown (1976) found a somewhat different pattern of IAA and ABA variation throughout the year than I have shown for western hemlock shoots. He showed ABA levels in the roots to be highest during the fall and early summer and to be lowest during the period of December to April. IAA was found to be at a maximum in the fall, decreased slightly during the winter, and lowest in June. The ratio of IAA to ABA in the roots was also different than

that I calculated for the shoots. He found that this ratio in the roots was high in the period from December to April after which it declined rapidly while I found that this ratio did not begin to increase in the shoots until after December and did not reach its maximum until April. Brown suggested that the ratio of promoter to inhibitor may in some way be responsible for potential root growth since it closely follows the root regenerating potential of the seedlings. Roberts and Fuchigami (1973) suggested that the balance of promoter to inhibitor might be responsible for the rooting of cuttings of Douglas-fir. It appears that the seasonal balance of hormones is different in the shoots and roots. This seems to be a reasonable hypothesis if hormones do in fact have any control over growth since the active period of growth of roots is generally prior to and after the normal period of shoot growth. This difference could be due to preferential rates of transport of hormones to the shoots or roots throughout the year, or it could be due to differential rates of destruction of the hormones in the shoots or roots. Whatever the reason for this difference in the balance of IAA and ABA, it does seem to correlate well with the physiological processes occurring in the roots and shoots.

While the correlation of ABA levels with the phases of bud dormancy is good, it remains unclear that there is a causal relationship between the two. Webber (1974) suggested that ABA may, in fact, only play a secondary role in bud dormancy of Douglas-fir, and conifers in general. This confusing role of ABA is complicated by erratic findings reported for the effect of application of exogenous ABA since conifers

appear to show less response than hardwoods. For exogenous applications to have any effect, however, the substance must be incorporated into the plant and then transported to its active site. In some instances, this sequence of events is not clearly apparent.

Work by Irving (1969) indicates that an increase in ABA level may be responsible for the development of cold hardiness in Acer negundo. Since development of cold hardiness occurs during the deepening dormancy phase and a corresponding increase in ABA level in western hemlock was noted during this phase, the function of ABA and its influence on dormancy appears to be essential, at least for the increased frost hardiness acquired during this phase. The decrease in ABA level with chilling and its low level at the time of bud break also suggests its influence during these phases as well, since high levels of ABA can greatly reduce the incidence of bud break.

While current thought suggests that IAA is not directly related to the breaking of dormancy, it is clear that IAA, as well as the other classes of promoters, do affect shoot growth in some manner. It is probable that one or more of these classes of promoters is interacting with ABA in the control of dormancy. There is considerable interest at the present time on the effect of changing levels of gibberellins or cytokinins on controlling dormancy. Lavender, Sweet, Zaerr, and Hermann (1973) have postulated that spring bud break of Douglas-fir may be due to gibberellins synthesized in the roots in response to warming soil temperature and transported into the shoot at a time when the ABA level is low. Other work with sugar maple (Dumbroff and Brown, 1976) found a large increase in the level of cytokinins during the period of

root growth prior to bud break and a corresponding increase of cytokinins in the shoot. It is becoming clear that the physiology of the whole plant rather than the isolated organs must be examined in order to study dormancy. The evidence of hormones produced in and transported from the roots and the possibility of their control of bud break has been the primary reason for this expanded view. Bachelard and Wightman (1974) have postulated a mechanism of dormancy release based on the levels and interaction of ABA, gibberellins, and cytokinins. As techniques for isolation and identification of the gibberellins and cytokinins improve, perhaps a clearer picture of their possible correlation with the various phases of dormancy will emerge. The area of hormones and dormancy is one which will take a great deal of work to elucidate but one which will produce valuable information to explain a wide variety of plant processes.

Effect on Environment on ABA Level

Extracts for ABA analysis were prepared in an attempt to determine the effect of two environmental variables which were shown to strongly influence dormancy induction of western hemlock seedlings, namely photoperiod and moderate moisture stress. Extracts made after two or four weeks of treatment with short photoperiods or moderate moisture stress were compared with extracts of control seedlings which had received long photoperiods and little or no moisture stress and the level of ABA present in each control assigned the value of 100% for comparison purposes. Results showed that moving seedlings from long day to short day conditions had little effect on the ABA level present in the

seedlings, even after four weeks (Figure VIII). Applying moderate moisture stress to seedlings maintained under a long day regime, however, had a dramatic effect on the ABA level and an increase of almost 6 fold occurred within a four week period. The moisture stress received by the seedlings was not constant throughout the period. When seedlings reached approximately 15 bars of plant moisture stress the planting mix was watered to about one-half of field capacity and again allowed to begin drying. Thus there was a cycling of the stress received by the seedlings and no indication of a threshold level of moisture stress leading to an ABA increase could be discerned. The magnitude of the increase in ABA level found, however, certainly indicates that moisture stress is at least one of the factors which controls the endogenous ABA level in western hemlock seedlings. The lack of effect of the photoperiod on the ABA level would seem to indicate that this factor has little influence over the level of ABA.

In an effort to determine if a threshold moisture stress was present in western hemlock seedlings above which the ABA level increased, a series of extractions were made after the pre-dawn plant moisture stress of the seedlings was measured. This series of extracts was made from mature needles, which are considered to be the primary site of ABA synthesis (Milborrow, 1973). The results show that there is, indeed, a range of plant moisture stress which led to a rapid increase in the endogenous ABA level of the seedlings (Figure IX). An increase in the ABA level was seen by the time seedlings reached a plant moisture stress of 12 bars and the increase was most rapid between 16 and 22 bars of stress. At plant moisture stresses above 22 bars, the rate

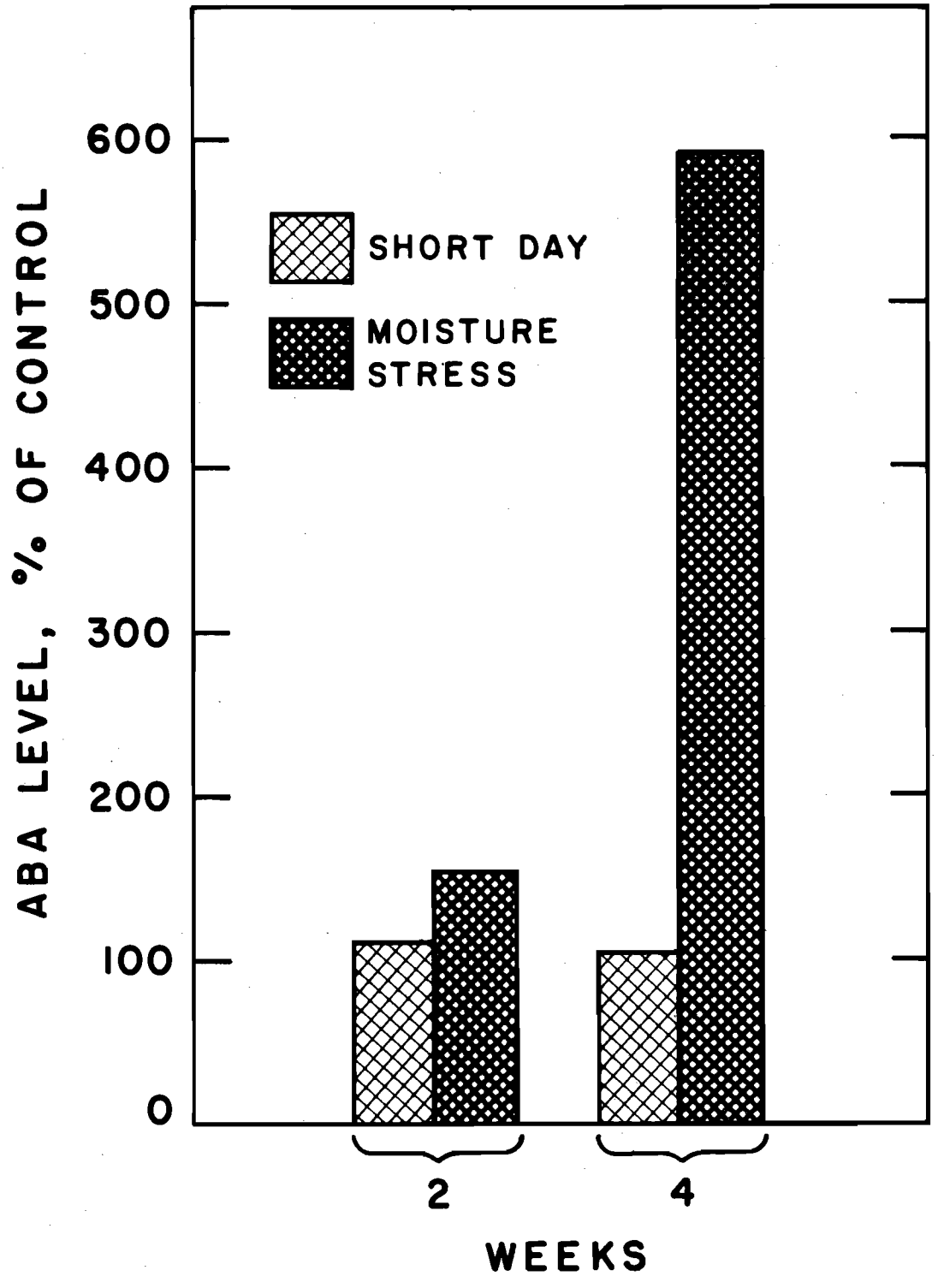

Figure VIII. Effect of short photoperiods or moderate moisture stress on ABA levels.

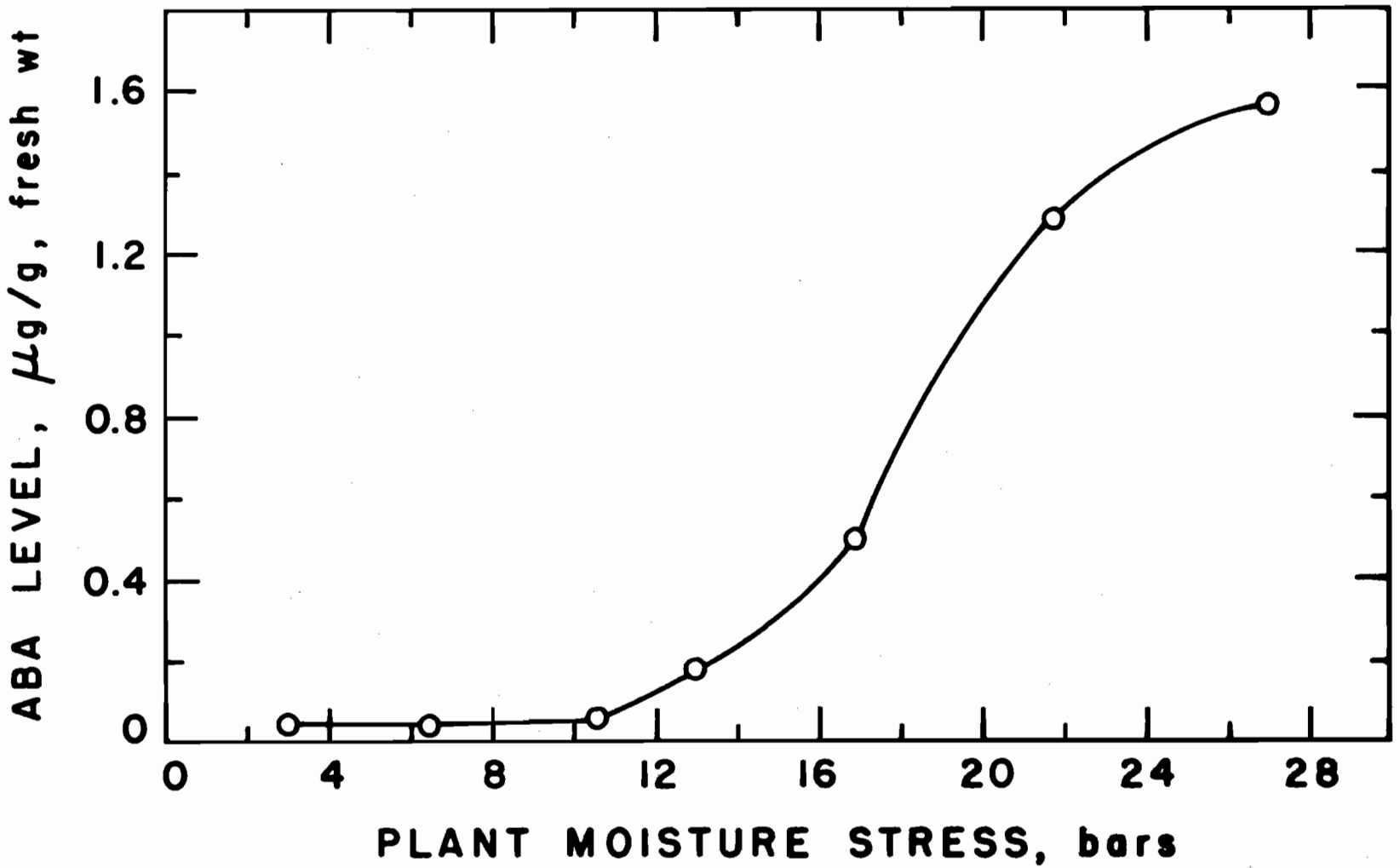

Figure IX. Effect of plant moisture stress on ABA level.

of increase of the ABA levels in the needles was slowed. This reduced increase could be due to a number of reasons, such as a reduced rate of synthesis or an increased rate of transport out of the needles. The quantity of endogenous ABA present by the time seedlings reached these higher moisture stresses was over 30 times greater than that present in seedlings which had received little moisture stress. This build-up would certainly be adequate to exert any hormonal effect it might have after its transport to the shoot tip. It is clear that the moisture stress which seedlings experience has a definite influence on the ABA level of the mature needles. The mechanism by which this influence is carried out, however, still remains unclear.

The data showing the lack of influence of the photoperiod on the ABA level of western hemlock seedlings supports most current literature in this area. Early work prior to the initial isolation and identification of ABA indicated that some growth retarding component or components within the β-inhibitor complex increased when plants received short day treatments (Phillips and Wareing, 1959; Robinson and Wareing, 1964). With the characterization of ABA as a component of the β-inhibitor complex, ABA was considered to be the inhibitor which varied with the photoperiod. This early work was carried out using bioassay tests which were not specific for ABA, and with the adaptation of more sophisticated analytical techniques, such as gas-liquid chromatography, it became clear that the endogenous ABA level was not increased by short photoperiods (Lenton, Perry, and Saunders, 1972). It is possible that some as yet uncharacterized inhibitory substance is present in the β-inhibitor complex which does vary with the photoperiod. However,

from my findings and the results reported in current literature, it is evident that ABA level is unaffected by photoperiod, at least in western hemlock.

The fact that photoperiod had little effect on the ABA level of western hemlock seedlings casts some doubt on the hypothesis that ABA has an integral function in the initiation of dormancy of this species. Since a short photoperiod was found to have the greatest effect on the initiation of dormancy of western hemlock, the lack of an increase in the ABA level under short day conditions would indicate that this hormone was not responsible for induction of dormancy. It is not inconceivable, however, that there may be several mechanisms which lead to dormancy of the plant. Thus, the induction of dormancy by a short photoperiod may be mediated through a separate series of mechanisms, such as the phytochrome system, of which ABA is not a part. This hypothesis would require the existence of several separate systems of dormancy induction, each of which might be controlled by a separate environmental variable, which would all lead to the same final result. If this is the case, the role of ABA in dormancy induction could not be entirely ruled out because it was not a member of the photoperiodically mediated system since it could be a member of a system which was mediated by another environmental factor, such as temperature or moisture stress.

Moisture stress was found to be capable of inducing dormancy of western hemlock seedlings and increased moisture stress was also found to be associated with an increased endogenous ABA level in the seedlings. The ABA level began to increase when seedlings experienced a

plant moisture stress of 12 bars and increased as the stress increased beyond this value. My findings that moisture stress directly affects ABA level in western hemlock seedlings is in agreement with literature which has examined the relationship between these two entities. A number of papers have reported increased ABA levels when leaves of plants are subjected to moisture stress (Hoad, 1973; Zeevaart, 1974; Beardsell and Cohen, 1975; Rasmussen, 1976). A variety of methods to induce water stress in plants have been examined, such as subjecting the plant to wilting or low relative humidity or excising the leaf, and all have resulted in increased ABA levels in the plant. These papers have attributed the role of increasing stomatal resistance to the build-up of ABA, thus allowing the plant to reduce its water loss. The regulatory role of ABA on stomatal closure seems to be well established and it would be assumed that this role of ABA also functions in western hemlock. Recent findings by Newville (1978), however, cast some doubt on the cause and effect relationship between ABA and stomatal behavior in Douglas-fir during drought. When seedlings were subjected to a second drought after recovery from the first, stomatal behavior was unrelated to ABA level in seedlings from xeric seed sources. Seedlings from mesic seed sources were found to retain a high ABA level after recovery from the first drying cycle. Based on his findings, he suggested that ABA may not be involved in the rapid short term control of stomatal behavior.

The increase in ABA content of naturally growing western hemlock seedlings was clearly indicated in the study of the seasonal variation of this hormone. In the late summer when the seedlings were beginning

to experience the effects of moisture stress, the ABA level was found to increase. Webber (1974) has shown that the buds of Douglas-fir contain the highest concentration of ABA and that the concentration of ABA in the needles is highest at the time when dormancy is being initiated in that species. It could be argued that a similar situation occurs in western hemlock under natural conditions where moisture stress is the primary factor in dormancy induction. As moisture stress begins to increase the needles act as the sensor and increase ABA production. This ABA would then be transported to the forming buds where it would concentrate. It is not clear, however, whether this increased ABA level is merely a related phenomenon or a causal factor of dormancy induction. Under the hypothesis that several separate systems exist which are able to induce dormancy in plants, the relationship between ABA and water stress could suggest its function as a causal agent in the dormancy of western conifers where water stress is most often the critical factor on growth. The presence of an ABA increase in response to water stress in a number of annual plants, such as spinach, tomato, maize, and sorghum, suggests that its primary role may be to control water loss rather than as an initiator of dormancy, although the latter should not be ruled out. It is clear, in any case, that a great deal of future research will need to be conducted with the definitive answer lying in the discovery of the mechanisms involved in dormancy induction.

The existence of a threshold value of moisture stress above which ABA levels increase which was shown for western hemlock has also been reported for other species, although the threshold appears to vary

slightly with the species being studied (Zabadal, 1974; Beardsell and Cohen, 1975). Blake and Ferrell (1977) found a relationship between ABA content and plant moisture stress in Douglas-fir similar to that which was found for western hemlock. Douglas-fir, however, appears to have a more rapid increase at lower moisture stresses than did western hemlock with the maximum rate of increase occurring between 12 and 15 bars in Douglas-fir and between 16 and 22 bars in western hemlock. Each species showed a reduced rate of increase at stresses above the point where the maximum rate was found. Blake and Ferrell also showed that soil water potential was more closely related to ABA level than was plant moisture stress and suggested that the roots were the drought sensing organ and that the message was then acted upon in the needles by stomatal closure and increased ABA levels. Whether this is the case for western hemlock remains to be determined. Newville (1978) found that Douglas-fir seedlings from xeric seed sources exhibited the threshold phenomenon of ABA increase during a second drying period, although this was not correlated with stomatal behavior.

The presence of a threshold of moisture stress above which ABA levels increase provides an additional piece of indirect evidence to implicate this hormone in the phenomenon of moisture stress induced dormancy. The increased ABA level when a plant experiences specific moisture stress appears to be an ideal sensing mechanism to avoid the detrimental effects of drought. Saunders, Harrison, and Alvin (1973) have suggested that high ABA levels induced by water stress may contribute to the cessation of shoot growth of trees during the summer, and ultimately in the formation of winter buds. If this is true, and

because of the importance of water stress in inducing dormancy under natural conditions in the Pacific Northwest, there remains the possibility that ABA is in some way regulating the process of moisture induced dormancy. The evidence is, admittedly, only circumstantial but deserves further study.

If ABA level is a controlling factor in moisture stress induced dormancy, this fact could also be used to explain the reason for the longer growing season of western hemlock as compared to that of Douglas-fir. Western hemlock commonly grows on sites which are moister than those which support Douglas-fir. The longer growing season of western hemlock may therefore be a reflection of its moister environment. A study to determine whether western hemlock has a longer growing season than Douglas-fir on the same site might provide additional evidence supporting the possible role of ABA on dormancy induction. The endogenous ABA level in western hemlock was found to begin increasing at a slightly higher moisture stress and showed maximum rates of increase at higher moisture stress than has been reported for Douglas-fir. This higher threshold level would allow western hemlock to continue growth later into the summer before dormancy was induced by moisture stress. This explanation rests on the assumption that several separate systems capable of inducing dormancy are present in plants and that ABA is an integral part of the moisture stress mediated system.

The mechanism of action and the range of processes mediated by hormones remains one of the great unanswered questions in plant physiology. Only by solving these problems will a number of "cloudy" areas,

such as the function of hormones in mediating the dormant period of plant growth, be fully understood. The step forward which the field of plant physiology will take when this occurs will, indeed, be a gigantic one and looms on the horizon as one of the most exciting periods in this field.

SUMMARY

With the increasing demand for wood products, western hemlock is beginning to play a larger role in forestry in the Pacific Northwest and information about this species is necessary for successful management. One area of physiology which has been shown to affect the potential survival of seedlings is that of dormancy. Several aspects of the physiology of dormancy of western hemlock seedlings were investigated in the current study. Many responses of western hemlock during dormancy were found to be similar to those of Douglas-fir, although dissimilarities were also found. These dissimilarities must be recognized to insure production of quality western hemlock planting stock and justify the use of caution in extrapolating data on Douglas-fir to western hemlock.

Dormancy of western hemlock seedlings was initiated most rapidly under a regime consisting of an 8 hour photoperiod, a warm temperature regime (25°/20°C), and a moderate moisture stress (12-15 bars, PMS). A short photoperiod had the greatest influence of all environmental factors examined on dormancy induction. Seedlings from a high elevation seed source were more sensitive to warm temperatures as a dormancy inducing factor than were seedlings from a low elevation seed source. Moderate moisture stress had approximately equal effects on initiation of dormancy of seedlings from both seed sources. A regime of a warm temperature and moderate moisture stress was able to induce dormancy under a 16 hour photoperiod, although not as rapidly as with the short photoperiod. This latter regime can easily be adopted in the

production of containerized western hemlock planting stock on an operational level where control of the photoperiod would be difficult or expensive. The photoperiod experienced by the seedling in the fall was found to have a slight but significant effect on the date of spring bud break after natural overwintering, with a long photoperiod during the fall delaying the date of bud break. Seedlings which received a short or a natural photoperiod during the fall broke bud at about the same time the following spring.

The chilling requirement of western hemlock was found to be considerably shorter than that of Douglas-fir. If seedlings were preconditioned with six weeks of mild, short days the requirement was met by four weeks of a constant 5°C temperature. This preconditioning effect is similar to that of Douglas-fir and this period is probably necessary for the normal maturation of the bud prior to chilling. Seedlings not receiving this beneficial pretreatment generally had their chilling requirement fulfilled by six to eight weeks of constant chilling. Fulfillment of the chilling requirement of western hemlock seedlings maintained through the winter in unheated greenhouses should be no problem except when mild winters occur. In this case, a period of cold storage after December can be used to meet the chilling requirement and assure vigorous spring growth.

Cold storage of western hemlock seedlings for four weeks during October resulted in delayed bud break if they were then planted in a growth room or very high mortality if they were planted in a cold frame. A daily photoperiod during cold storage reduced mortality of seedlings stored in October, although it was still high. Cold storage

during December or February was found to have no adverse effect on the seedlings. A 16 hour daily photoperiod during February storage provided seedlings which resumed growth most rapidly in the growth room and earliest in the cold frame. Seedlings which received December or February cold storage followed by transplanting into the cold frame generally broke bud one to two weeks earlier in the spring than seedlings which had received natural conditions during the winter. Requirements for successful cold storage of western hemlock appear to be similar to those which have been found for Douglas-fir.

Western hemlock was found to have four distinct phases of dormancy similar to those which occur in Douglas-fir. The second phase, during which bud maturation is completed and frost hardiness is acquired, is the time when seedlings are most susceptible to environmental stresses, such as those imposed by lifting or cold storage. Nursery practices during this period should be designed to take this into account. The third phase during which the chilling requirement is being fulfilled is shorter for western hemlock and results in the transition into the fourth phase earlier than Douglas-fir. The phase of dormancy which the seedling is in appears to have a controlling influence on the response of the seedling to a variety of treatments and cultural manipulations. The recognition and understanding of this fact will dictate appropriate nursery scheduling of operations to produce high quality seedlings for regeneration.

Indole-3-acetic acid and abscisic acid were identified as endogenous hormones in western hemlock. The seasonal variation of the level of these two hormones in seedlings growing under natural

conditions was found to be similar to those reported for other woody species in which they have been identified. The ABA level was highest in the fall and early winter after beginning to rise late in the summer and lowest at the time of spring bud break. The IAA level was highest in the spring and lowest during the summer and early fall. The ratio of IAA to ABA correlated well with the phases of dormancy which the seedlings went through, although no causal relationship was established. The ABA level was found to increase with increasing moisture stress experienced by the seedling, while the photoperiod experienced by the seedling had little effect on the ABA level. A threshold value of approximately 12 bars of plant moisture stress was found to be the level above which ABA concentration began to increase in the seedlings, with the maximum rate of increase occurring between 16 and 22 bars of stress. It appears that moisture stress experienced by western hemlock seedlings will lead to an increase in the ABA level which may be responsible, in some way, for initiating dormancy. Moisture stress, however, is probably not responsible for maintaining the high ABA level since fall and early winter are not commonly periods when seedlings are under moisture stress in the Pacific Northwest. While these data and those reported in the literature provide material for speculation, our understanding of the function, if there is any, of hormones in controlling the dormant period of a plant's life is far from complete.

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APPENDICES

APPENDIX A. Stock Solutions and Nutrient Solution Composition.

Stock A	g/l
KNO_3	60.6
KH_2PO_4	16.3
$MgSO_4 \cdot 7H_2O$	61.0
H_3BO_4	0.69
$CuCl_2 \cdot 2H_2O$	0.13
$MnCl_2 \cdot 4H_2O$	0.43
$Zn(NO_3)_2 \cdot 6H_2O$	0.055
MoO_3	0.004
NH_4Cl	32.1

Stock B	
$Ca(NO_3)_2 \cdot 4H_2O$	112.0

Stock C	
KOH	15.0
EDTA	26.1
$FeCl_2 \cdot 4H_2O$	17.8

To make nutrient solution

Stock	ml/4.8 l
A	200
B	200
C	100

Nutrient Concentration in Working Solution

Nutrient	Concentration (ppm)
N	1400
P_2O_5	357
K_2O	1655
Fe	100
Ca	933
Mg	250
Zn	0.5
Mn	5.0
Cu	0.2
B	5.0
Mo	0.1

APPENDIX B. Weight of Material Used on Each Extraction Date.

Date	Fresh Weight (grams)
June 26	437.6
September 24	475.4
December 4	327.8
February 3	324.1
April 6	382.6
May 6	318.2

APPENDIX C. Weight of Material Extracted in Study of the Effect of Environmental Factors on ABA Level.

Treatment	Fresh Weight (grams)
Control	
Start	107:6; 93.1
2 weeks	118.1; 92.4
4 weeks	115.5; 135.6
Moderate Moisture Stress	
2 weeks	89.0; 106.1
4 weeks	107.8; 105.0
Short Day	
2 weeks	98.7; 104.8
4 weeks	87.4; 85.8

APPENDIX D. Weight of Material Extracted in Study of Effect of Moisture Stress on ABA Level.

Moisture Stress (bars)	Fresh Weight (grams)
3.08	1.16
6.43	1.28
10.53	1.13
12.93	1.28
16.90	1.13
21.83	1.15
27.00	1.14

APPENDIX E. Harvest Data for Low Elevation Seedlings in Initiation of Dormancy Study.

Treatment (T-Pp-MS)	Diameter	Shoot Length	Root Length	Shoot Weight	Root Weight	S/R	Root Activity
		(cm)		(g)			
W L L	0.150	15.15	19.36	0.280	0.059	4.75	1.16
W L M	0.138	15.88	18.95	0.259	0.056	4.63	1.08
W S L	0.131	14.48	16.96	0.188	0.040	4.70	1.00
W S M	0.116	13.98	16.23	0.170	0.035	4.86	1.00
C L L	0.199	19.84	19.15	0.474	0.119	3.98	1.48
C L M	0.157	16.20	19.79	0.319	0.074	4.31	1.04
C S L	0.156	16.70	20.24	0.253	0.066	3.83	1.04
C S M	0.118	12.05	17.66	0.147	0.042	3.50	1.04
With Cool Root Temperature							
W S L	0.131	14.06	20.07	0.208	0.082	2.54	1.28
W S M	0.136	13.26	19.02	0.170	0.072	2.36	1.24
C S L	0.144	13.02	20.05	0.229	0.106	2.16	1.56
C S M	0.144	13.45	21.17	0.247	0.111	2.23	1.60

APPENDIX F. Harvest Data for High Elevation Seedlings in Initiation of Dormancy Study.

Treatment (T-Pp-MS)	Diameter	Shoot Length (cm)	Root Length (cm)	Shoot Weight (g)	Root Weight (g)	S/R	Root Activity
W L L	0.130	15.03	17.48	0.258	0.048	5.38	1.32
W L M	0.146	15.33	18.25	0.288	0.050	5.76	1.16
W S L	0.124	11.99	16.47	0.144	0.043	3.52	1.00
W S M	0.104	12.90	16.50	0.119	0.031	3.84	1.00
C L L	0.210	20.28	20.22	0.614	0.128	4.80	1.20
C L M	0.157	14.59	18.44	0.304	0.072	4.22	1.00
C S L	0.138	12.14	18.06	0.172	0.050	3.44	1.08
C S M	0.136	12.76	18.45	0.169	0.047	3.60	1.04
With Cool Root Temperature							
W S L	0.141	13.33	17.89	0.182	0.071	2.56	1.20
W S M	0.139	13.21	18.95	0.175	0.063	2.78	1.40
C S L	0.157	14.48	17.65	0.251	0.106	2.37	1.20
C S M	0.160	13.32	18.08	0.234	0.088	2.66	1.16

APPENDIX G. Harvest Data for Continuation of Initiation of Dormancy Study.

Treatment (Pp-MS-T-Nut)	Diameter	Shoot Length	Root Length	Shoot Weight	Root Weight	S/R	Root Activity
		(cm)		(g)			
L L W H	0.220	23.34	18.94	0.804	0.174	4.62	1.40
L L W L	0.235	23.50	20.06	0.880	0.198	4.44	2.00
CS M W H	0.189	16.16	16.94	0.420	0.098	4.29	1.00
CS M W L	0.189	16.20	17.02	0.420	0.134	3.13	1.40
S M W H	0.177	17.66	17.82	0.524	0.120	4.37	1.00
S M W L	0.184	16.82	16.64	0.428	0.104	4.12	1.00
S M C H	0.166	18.52	16.74	0.454	0.082	5.54	1.40
S M C L	0.168	16.78	17.24	0.486	0.102	4.77	1.20

APPENDIX H. Harvest Data for Chilling Requirement Study.

Pretreatment	Treatment (wk)	Diameter	Shoot Length (cm)	Root Length	Shoot Weight (g)	Root Weight	S/R	Root Activity
None	4	0.183	12.32	28.50	0.335	0.198	1.69	1.2
	6	0.185	13.87	28.32	0.319	0.148	2.15	1.2
	8	0.145	13.36	27.71	0.300	0.118	2.55	1.2
	12	0.196	14.68	29.44	0.390	0.141	2.76	1.6
3 SD	4	0.155	12.47	28.55	0.315	0.214	1.47	1.2
	6	0.173	13.49	30.48	0.355	0.176	2.02	1.5
	8	0.170	12.67	27.91	0.297	0.133	2.23	1.2
	12	0.201	13.92	32.77	0.327	0.181	1.81	1.5
6 SD	4	0.178	12.45	30.35	0.314	0.190	1.65	1.3
	6	0.183	13.11	31.80	0.317	0.166	1.91	1.1
	8	0.196	13.72	33.25	0.401	0.224	1.79	1.3
	12	0.201	14.38	28.91	0.347	0.143	2.43	1.4
6 Nat	4	0.173	13.21	29.39	0.339	0.220	1.54	1.2
	6	0.201	14.50	29.31	0.418	0.234	1.78	1.1
	8	0.173	12.93	34.01	0.298	0.203	1.47	1.3
	12	0.203	14.25	29.01	0.408	0.204	2.00	1.4
6 LD	4	0.198	12.12	30.20	0.333	0.280	1.19	1.3
	6	0.208	13.67	32.72	0.387	0.250	1.55	1.1
	8	0.193	13.54	34.98	0.374	0.293	1.28	1.5
	12	0.196	14.10	28.88	0.360	0.160	2.25	1.5

APPENDIX I. Harvest Data for Effect of Fall Photoperiod Study.

Fall Photoperiod	Diameter	Shoot Length (cm)	Root Length	Shoot Weight (g)	Root Weight	S/R	Root Activity
Natural Day	0.290	18.72	36.30	1.095	0.513	2.14	1.8
Short Day	0.277	17.58	33.99	1.014	0.450	2.25	1.9
Long Day	0.282	16.43	34.87	0.902	0.421	2.14	1.8

APPENDIX J. Harvest Data for Cold Storage Study (growth room).

Date	Treatment	Diameter	Shoot Length (cm)	Root Length	Shoot Weight (g)	Root Weight	S/R	Root Activity
October	D	0.142	14.16	18.85	0.257	0.102	2.52	1.92
	8	0.151	14.42	20.03	0.310	0.114	2.72	2.16
	T-D	0.148	15.46	27.06	0.366	0.148	2.47	2.56
	T-8	0.151	14.78	27.30	0.359	0.125	2.87	2.16
December	D	0.164	15.52	20.84	0.334	0.116	2.88	1.16
	8	0.168	16.24	20.96	0.337	0.102	3.30	1.36
	T-D	0.181	15.77	27.51	0.412	0.166	2.48	2.00
	T-8	0.182	16.58	30.10	0.412	0.143	2.88	1.68
February	D	0.146	14.82	23.50	0.292	0.098	2.99	1.00
	8	0.157	15.18	25.42	0.331	0.104	3.18	1.00
	16	0.164	16.19	23.07	0.402	0.124	3.24	1.12
	T-D	0.162	15.59	29.52	0.370	0.130	2.85	1.28
	T-8	0.170	16.56	28.93	0.430	0.131	3.28	1.20
	T-16	0.177	17.25	29.10	0.494	0.166	2.98	1.32

APPENDIX K. Harvest Data for Cold Storage Study (cold frame).

Date	Treatment	Diameter	Shoot Length	Root Length	Shoot Weight	Root Weight	S/R	Root Activity
October	D	0.276	23.90	25.66	1.388	0.418	3.32	1.00
	8	0.383	34.46	27.82	2.570	0.886	2.90	1.00
	T-D	0.213	20.85	18.20	0.850	0.365	2.33	1.00
	T-8	0.289	35.18	16.34	2.366	0.750	3.16	1.00
December	D	0.277	27.12	33.22	1.742	0.784	2.22	1.00
	8	0.248	26.26	35.22	1.344	0.620	2.17	1.00
	T-D	0.265	28.20	32.36	1.706	1.016	1.68	1.00
	T-8	0.227	23.64	36.02	1.238	0.876	1.43	1.00
February	D	0.264	29.06	39.24	1.460	0.830	1.76	1.00
	8	0.298	26.92	33.06	1.674	1.034	1.62	1.00
	16	0.290	28.60	32.36	1.504	0.984	1.53	1.00
	T-D	0.296	30.08	29.68	1.972	1.148	1.72	1.00
	T-8	0.208	19.76	31.98	0.902	0.670	1.35	1.00
	T-16	0.233	26.28	36.14	1.048	0.594	1.76	1.00

APPENDIX L. Harvest Data for Continuation of Cold Storage Study.

Treatment	Diameter	Shoot Length (cm)	Root Length	Shoot Weight (g)	Root Weight	S/R	Root Activity
Polyethylene							
Control	0.214	20.78	23.36	0.812	0.284	2.86	2.60
Pull	0.210	20.00	21.04	0.634	0.310	2.05	2.80
Dark	0.190	20.46	22.04	0.686	0.294	2.33	2.40
T-Bark	0.250	21.16	26.10	0.964	0.344	2.80	3.20
T-Soil	0.217	21.46	25.46	0.872	0.348	2.51	3.00
T-Bk-Dk	0.204	19.08	24.64	0.652	0.298	2.19	3.20
Out	0.203	19.14	27.00	0.662	0.328	2.02	3.00
Styroblock							
Control	0.153	13.94	14.88	0.460	0.206	2.23	1.80
Pull	0.164	14.22	14.10	0.478	0.208	2.30	1.40
Dark	0.148	13.86	14.54	0.368	0.164	2.24	1.60
T-Bark	0.134	11.40	20.98	0.270	0.136	1.99	2.60
T-Soil	0.144	12.58	19.30	0.388	0.156	2.49	2.60

APPENDIX M. Harvest Data for Original Depth of Dormancy Study.

Date	Treatment	Diameter	Shoot Length (cm)	Root Length	Shoot Weight (g)	Root Weight	S/R	Root Activity
October	SD	0.135	13.39	19.85	0.221	0.082	2.70	1.80
	8+8	0.147	13.72	22.12	0.243	0.111	2.19	2.12
	LD	0.158	14.06	22.17	0.308	0.168	1.83	2.16
December	SD	0.147	13.51	19.90	0.200	0.087	2.30	1.08
	8+8	0.153	14.47	20.69	0.245	0.096	2.55	1.12
	LD	0.162	15.67	20.62	0.310	0.126	2.46	1.32
February	SD	0.143	12.50	23.18	0.202	0.090	2.26	1.00
	8+8	0.153	16.17	23.03	0.298	0.098	3.04	1.00
	LD	0.156	16.12	22.36	0.304	0.100	3.06	1.12

APPENDIX N. Harvest Data for Second Year of Depth of Dormancy Study.

Date	Treatment	Diameter	Shoot Length (cm)	Root Length	Shoot Weight (g)	Root Weight	S/R	Root Activity
October 1	LD	0.232	17.40	18.78	0.574	0.254	2.26	2.60
	8+8	0.201	18.96	17.84	0.546	0.172	3.17	1.40
	SD	0.185	18.04	19.22	0.480	0.140	3.43	1.00
October 15	LD	0.220	19.22	16.52	0.678	0.356	1.90	2.40
	8+8	0.176	17.52	19.84	0.436	0.202	2.16	2.20
	SD	0.178	18.24	17.80	0.418	0.170	2.46	1.20
November 1	LD	0.207	18.04	19.66	0.574	0.346	1.66	2.80
	8+8	0.193	17.90	20.60	0.518	0.260	1.99	2.40
	SD	0.200	16.54	19.58	0.546	0.268	2.04	1.00
November 15	LD	0.222	19.04	19.12	0.578	0.380	1.52	2.60
	8+8	0.213	19.20	21.20	0.630	0.318	1.98	1.80
	SD	0.188	16.36	21.28	0.404	0.238	1.70	1.00
December 1	LD	0.197	16.38	21.40	0.560	0.292	1.92	2.00
	8+8	0.208	17.34	22.16	0.600	0.314	1.91	1.60
	SD	0.163	16.92	19.24	0.500	0.172	2.91	1.00
December 15	LD	0.192	19.28	22.14	0.668	0.286	2.34	2.20
	8+8	0.177	17.84	19.02	0.588	0.196	3.00	1.60
	SD	0.160	16.32	20.16	0.456	0.224	2.04	1.40

APPENDIX N (continued)

Date	Treatment	Diameter	Shoot Length (cm)	Root Length	Shoot Weight (g)	Root Weight	S/R	Root Activity
January 1	LD	0.198	19.70	22.86	0.764	0.430	1.77	2.40
	8+8	0.194	17.78	19.48	0.586	0.354	1.66	2.40
	SD	0.176	17.16	19.48	0.456	0.182	2.51	1.80