AN ABSTRACT OF THE THESIS OF

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Title: EFFECTS OF THREE PLANT GROWTH REGULATORS UPON GROWTH PATTERNS IN SEEDLINGS OF THREE RACES OF DOUGLAS-FIR, PSEUDOTSUGA MENZIESII (MIRB.) FRANCO.

Abstract approved: Signature redacted for privacy. (Dr. Helge Irgens-Moller)

Populations of Douglas-fir seedlings from interior British Columbia, Vancouver Island, and Arizona received six bi-weekly foliar spray applications of the growth regulators IAA, CCC, and B-995, each at three concentration levels under controlled environmental conditions. Large differences were found among the three populations in their responses to the growth regulators. The responses to IAA treatments were as follows: height growth of interior British Columbia seedlings was reduced markedly by all three concentrations, Vancouver Island seedlings were unaffected by all concentrations, and only the lowest concentration (75 ppm) reduced the height growth of the Arizona seedlings; 300 ppm caused twisting of leaders and a marked increase in the number of periods of dormancy in all seedlings; and top dry weight was affected only

in interior British Columbia seedlings, particularly by 300 ppm. Although the number of periods of dormancy was markedly increased by 300 ppm IAA in the Vancouver Island and Arizona seedlings, height growth was the same as in untreated seedlings. Root dry weights varied in an inconsistent manner throughout the experiment. The responses to CCC treatments were as follows: 2000 ppm reduced height growth and top dry weight of interior British Columbia and Vancouver Island seedlings but had no significant effect on the Arizona seedlings. With increasing concentrations internodes became progressively shorter and chlorosis more intense. CCC had little effect upon number of periods of dormancy. The responses to B-995 were as follows: height growth and number of periods of dormancy were both decreased in Arizona seedlings; interior British Columbia and Vancouver Island seedlings were affected to a lesser degree. B-995 had no effects on top or root dry weight.

In a separate experiment, the effects of the high concentrations of the three growth regulators upon the time of break of dormancy were determined. Dormant 1-0 Douglas-fir seedlings lifted from a nursery bed in mid-February were treated once or twice using either two percent dimethyl sulfoxide (DMSO) or water as carrier. The containers of seedlings were then transferred to a growth chamber and exposed to an environment favorable toward growth initiation (same environment as first experiment). Only 2000 ppm of CCC

applied twice using water as carrier slowed the rate of growth initiation significantly.

EFFECTS OF THREE PLANT GROWTH REGULATORS UPON GROWTH PATTERNS IN SEEDLINGS OF THREE RACES OF DOUGLAS-FIR, PSEUDOTSUGA MENZIESII (MIRB.) FRANCO.

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I. INTRODUCTION

Since the discovery of the biological activity of the compound indoleacetic acid in 1926 by Went in Holland (74, p. 13) there has developed an expanding interest in plant growth regulation. Numerous investigations employing exogenous biologically effective compounds have deepened understanding of endogenous plant regulatory mechanisms. It is currently believed that plant growth is regulated by three classes of endogenous compounds; the auxins, gibberellins, and cytokinins (33,71). Plant growth apparently is in part an expression of an intricate balance of these three hormonal systems among themselves and in cooperation with other known or still unknown factors.

Hundreds of synthetic organic chemicals that have regulating properties on plant growth are currently recognized. These chemicals obviously are related in some way to certain constituents of the natural systems which exist in various types of plants under various conditions. These synthetic chemicals impose some alteration of the natural systems producing the varied responses observed. Similarly, modifications of growth may be produced through

administration of supra-optimal concentrations of endogenous regulating substances to plants. Many of these growth modifications are beneficial from a practical standpoint. As new chemicals for modifications of plant growth have become available, many of the major aspects of development, e.g. stem elongation and flowering, have become subject to chemical manipulation. Applications of chemicals to plants are useful not only from the standpoint of growth regulation but also in the experimental sense of offering a wide range of possibilities for exploring the biochemical mechanisms behind physiological processes in the plant.

A commercially important group of plant growth regulators are the herbicides, some of which are used to selectively kill certain kinds of vegetation. Among the commonly used selective herbicides are synthetic auxins, such as 2,4-D (2,4-dichlorophenoxyacetic acid), which have biological activity more potent and persistent than the endogenous auxin and which exhibit general selective toxicity toward dicotyledonous plants. In addition to inducing the actual death of a plant, growth regulators often are used to stimulate or inhibit growth, hasten or retard flowering and fruit ripening, extend or break dormancy, increase plant tolerance to severe environmental conditions, and in some instances, increase plant resistance to certain pathogens.

A review of the extensive literature in this area of plant physiology reveals a distinction between the Angiospermous and Gymnospermous classes of plants insofar as responses to applied chemicals are concerned. Results from the few investigations with Gymnosperms have been variable, conflicting, and in most cases the responses are smaller in magnitude than in the Angiosperms.

The present investigation will evaluate the individual effects of the regulators indole-3-acetic acid (IAA), (2-chloroethyl) trimethylammonium chloride (CCC or Cycocel), and N-dimethylamino-succinamic acid (Alar, B-995, or B-Nine) upon growth and development of Douglas-fir seedlings. Since different genera, species, and even varieties of plants have been reported to differ in response to growth-regulating substances the comparative responses of three physiologically dissimilar races of Douglas-fir (30, 31) were studied.

II. REVIEW OF LITERATURE

The auxins are the most comprehensively investigated group of plant hormones. Their activity was first clearly demonstrated by F. W. Went who isolated a diffusible substance from oat seedlings which promoted the growth of these seedlings (74). It is now reasonably certain that indole-3-acetic acid (IAA) is the major native indolic auxin in plants. Consequently, the word "auxin" is commonly used when referring to the compound IAA. Other indole compounds have shown growth-regulating activity in the various bio-assay tests, but most of their activity results from their ready conversion to IAA. IAA appears to be almost universally effective in plants, i. e., the growth phenomena in one species which are influenced by this compound are affected in a similar manner in many other species. The presence of IAA has actually been demonstrated in a number of coniferous species (3,16,22,48).

Initially each of the three classes of endogenous regulators was held responsible for very specific aspects of the growth process, e. g. cell enlargement and cell division (71). The current consensus is that a sensitive balance in the levels of endogenous auxin, gibberellin, and cytokinin exists and that plant growth depends on the status of this relationship (72). Moreover, auxin plays a so-called "dualistic" role, in which the actual concentration of auxin present

determines the direction of response, i. e., stimulation or inhibition. Within the plant, each organ may be stimulated by an optimal auxin concentration and inhibited by supraoptimal concentrations, and different organs may be stimulated or inhibited to different degrees by a given concentration. In addition, different genera, species, and even varieties of plants display varying sensitivity to auxin.

Although many physiological processes require auxin, the mechanism of action at the growth site is still uncertain. According to Van Overbeek (72), Skoog was the first investigator to discover that the primary effect of plant hormones was on nucleic acid metabolism. Protein synthesis is generally associated with plant growth. Bonner et al. (5,6,7) have suggested that endogenous growth substances function by directing gene activity. Bonner's group has shown the existence of a histone sheath around DNA (deoxyribonucleic acid) and cites evidence suggesting that this histone sheath is removed during times of intensive growth activity, such as flowering. At this time there is also a relatively high concentration of auxin present. The histone sheath is believed to act as a physical barrier to RNA nucleotides, preventing the DNA molecules from acting as a template to synthesize messenger RNA (ribonucleic acid). Thus, Bonner suggests that the plant growth regulators induce the removal of the histone sheath from the DNA molecule. Datta and Sen (19) have demonstrated that the process of amino acid incorporation into

nuclear protein is stimulated by IAA and that the stimulation is abolished by actinomycin D, which is known to interfere with DNA-dependent RNA synthesis. Moreover, the inhibition due to actinomycin D can be removed by adding higher concentrations of IAA.

The following discussion of exogenous auxin applications is confined mainly to coniferous species. Ostrom (54) treated nursery seedlings of red, loblolly, shortleaf, pitch and table-mountain pines, and red spruce with the growth regulators naphthaleneacetic acid, naphthalenemethylacetate, and naphthalene acetamide individually or in mixtures with or without indolebutyric acid. Methods of application included dipping, soaking, or spraying with water solutions, carrier emulsions of lanolin or commercial wax, and exposure to vapors of the compounds. Several of these treatments resulted in inhibition of shoot elongation, curved leaders, and sometimes death of treated seedlings. The surviving seedlings recovered from these symptoms during the same growing season and were no more resistant than untreated seedlings to artificial drought. Preliminary experiments with IAA on Douglas-fir seedlings in the nursery (38) show no apparent effects of this substance on growth rate, but treated seedlings lifted during the early rest period demonstrated a higher survival potential than control seedlings the following summer. In a pilot study (39) under controlled environmental conditions, height growth of Douglas-fir seedlings was significantly

inhibited by IAA. This evidence suggests the need for further study of responses of Douglas-fir seedlings to IAA to determine its usefulness in improving seedling physiology.

Cathey (13) defines "growth retardants" as "all chemicals that slow cell division and cell elongation in shoot tissues and regulate plant height physiologically without formative effects." Investigations of the effects of growth retardants on plants were initiated relatively recently. The first group of growth-retarding compounds to be discovered, the nicotiniums, was reported upon by Mitchell et al. in 1949 (49). Although these compounds were active in only a narrow range of plants and often caused necrosis or malformation, their discovery opened a new era of research. Subsequently, several new families of chemicals have been reported which are capable of retarding the overall growth of various plant species without causing malformation of the plant. The useful growth-retarding chemicals which are now known can be arranged into several groups according to their chemical characteristics: these are the nicotinium compounds, quaternary ammonium carbamates, phosphonium compounds choline analogues, and succinamic acid derivatives (13).

In 1950 Wirwille and Mitchell (76) reported that some quaternary ammonium carbamates were more active and less toxic than the nicotiums. The compound Amo-1618 (4-hydroxyl-5-isopropyl-2-methylphenyl trimethyl ammonium chloride 1-piperdine

carboxylate) was selected by Wirwille and Mitchell (76) as the most active of this group of growth-retarding chemicals.

The phosphoniums are a group of quaternary compounds which contain a phosphonium cation (13). The most active substance is 2,4-dichlorobenzyl-tributyl phosphonium chloride, designated Phosfon. Two investigations dealing with Phosfon treatment of Douglas-fir seedlings (34,75) reported that moderately high concentrations of Phosfon appeared to stimulate growth in this species. This is remarkable in that the growth retardant Phosfon was active on a Gymnospermous species and that a stimulatory response was produced within a certain concentration range.

The chemical (2-chloroethyl) trimethylammonium chloride (CCC or Cycocel) is an analogue of choline in which the hydroxyl group is replaced with chlorine. The properties of this chemical were first reported by Tolbert (66). CCC is unique among growth-retarding compounds in that it is effective in dwarfing a broad spectrum of plant species without causing apparent injury or necrosis, except at heavy rates of application (40,64,65,66,78). In addition to retarding internode elongation, CCC has been reported to increase resistance to aging (43,44), to salinity (14,45), drought (14,25,45), heat (11,70) and frost (13,14,46,70), and to alter flowering time (10,12,62,69) among other specific responses. This evidence supports the varied activity and usefulness of CCC in

cultivated species of plants.

According to Cathey, woody plants as a group are not adapted to the utilization of growth-retarding chemicals because of their short growing season and extensive root system (11), and those plants which grow in flushes, e.g. oak, respond only to high dosages (10). Further, he reports (13) that growth retardants have been inactive in Gymnosperms. Asher, on the other hand, has reported (1) that CCC is active in retarding seedling height growth in slash pine linearly with the logarithm of concentration. In a preliminary experiment (59) a similar response was observed in Douglas-fir to foliar application of this compound.

Several authors have noted that responses to CCC and its analogues tend to be just the opposite of responses to gibberellin (9,17,20,37,50,51,67). Gibberellin appears to simulate the effects of a long photoperiod of red light and exposure to high temperatures, while CCC tends to duplicate the effects of a short photoperiod of high intensity blue light and low temperatures (78). Most of these opposing effects of growth retardant and gibberellin are mutually antagonized when both chemicals are applied to the same plant (17, 20,23,64). It was therefore suggested that the growth retardants be designated anti-gibberellins. This terminology has caused controversy. Several investigators (20,77,78) thought that the growth retardant must inhibit an enzyme system involved in gibberellin

biosynthesis. Lockhart (42) proposed that the compounds interact competitively with gibberellins, thus retarding stem elongation by partially blocking the system which provides natural gibberellin to the growing points. However, Kuraishi and Muir (35, 36, 37) came to the conclusion that these compounds do not interact directly with gibberellin, but rather with auxin. They observed that CCC reduced the diffusible auxin content in treated pea plants to one-sixth that in untreated plants, and hence proposed that CCC is actually an anti-auxin. Halevy (24) has found that potassium gibberellate inhibits the activity of peroxidase and IAA oxidase, whereas CCC, along with other growth retardants including B-995, stimulates peroxidase and IAA oxidase in cucumber seedlings. Peroxidase usually participates in the IAA oxidase system (56). Therefore Halevy (24) proposed that the growth retardants exert their influence on plant growth by interacting with gibberellin in IAA oxidase and thus affecting the auxin level in the tissues. More recently, Lang et al. (26, 53) have concluded that the mode of action of CCC and its analogues is based on inhibition of the biosynthesis of gibberellins which are required for growth processes. This conclusion is supported by the finding that in barley endosperm, a tissue wholly dependent on exogenous gibberellins, the retardants had no effect upon formation of alpha amylase, a gibberellin-controlled response (55). Thus, it appears that the action of CCC does not result from

competition with or destruction of gibberellic acid, but is more likely involved with interference of gibberellic acid biosynthesis.

There have been reports of a CCC-like material isolated from plant tissue (47) and from a plant pathogen Tilletia sp. (52). The symptoms of Tilletia sp. infection are very similar to those of CCC treatment.

A new class of chemically different growth retardants includes the organic acids N-dimethylaminomaleamic acid (C-011 or DMAM) and N-dimethylaminosuccinamic acid (Alar, B-995 or B-Nine) (58). B-995 was found to be more stable in aqueous solution than the substituted maleamic and effective in retarding growth of the same species (18). These compounds are chemically somewhat similar to maleic hydrazide (63). B-995 has been found by several investigators (2,3,8,12,13,14,32,70) to be active in retarding the overall growth of several species of plants of agronomic and horticultural importance. Details are not yet available on the plant spectrum of B-995 (58), but it is apparent that many different kinds of plants are responsive, perhaps the widest range of plants yet observed. Many side effects of the chemical may occur with or without growth retardation: increased drought (14), salt (14), frost (14,46), and smog (14) tolerance; alteration of flowering time (12,61); prevention of fruit drop (21); and improvement of fruit color (21) and longivity of storage (63). No reports could be found in the literature on the

effects of B-995 on coniferous species.

As a possible mode of action of B-995, Reed, Moore, and Anderson (57) correlated the retardation of shoot elongation in peas induced by B-995 with the inhibition of tryptamine oxidation by diamine oxidase, and proposed that B-995 may influence auxin concentration in vivo by inhibiting its synthesis. Bennett (4) has suggested that B-995 may share the same mode of action as maleic hydrazide due to the structural similarity of these two chemicals. Leopold and Klein (41) have shown that maleic hydrazide causes a reversible inhibition of auxin.

This review of literature has indicated the decisive and important effects of a variety of plant growth regulators on Angiospermous species of plants. However, there is an obvious scarcity of literature pertaining to the responses of Gymnospermous species to treatment with these compounds. Existing reports and pilot studies have suggested a definite effect of certain compounds in coniferous species. Hence, it would appear on the basis of the evidence presented that growth-regulatory substances IAA, CCC, and B-995 may influence the growth of Douglas-fir seedlings. The purpose of the present investigation was to study this possibility.

III. EXPERIMENTAL

Materials and Facilities

Controlled Environment Chamber

A walk-in controlled environment chamber at the Oregon State University Forest Research Laboratory was used for the experiments. The temperature regime of 78°F. during the day (14 hours) and 70°F. during the night, was accurately regulated throughout the study period. Lighting was provided by a combination of "cool white" fluorescent and incandescent lamps, yielding an average intensity of 1000 foot-candles at the seedling shoot tips. The incandescent lights were turned off about ten minutes after the fluorescent lights so that the seedlings were exposed to light with energy both in the red and far-red regions at the end of each day.

Sources of Seeds

Ecological races of a species arise as a result of different selection pressures under various combinations of environmental factors (28). Irgens-Moller (29) reported that considerable variation existed in seedling responses to temperature and photoperiod among various provenances of Douglas-fir. When Douglas-fir seedlings of several provenances were grown under a common

environment, considerable variation in total height was evident at the end of a single growing season. Currently, two varieties of Douglas-fir---Pseudotsuga menziesii var. menziesii and Pseudotsuga menziesii var. glauca (Beissn.)(Franco.)---are recognized, which are commonly termed the Pacific Coast and the Rocky Mountain varieties, respectively, of Douglas-fir.

Because of the possibility of racial variation in response to applied chemicals, Douglas-fir seed from three widely separated origins was used. These were: (1) northern coastal type (variety menziesii), collected from southern Vancouver Island; (2) northern interior type (variety glauca), collected near Salmon Arm, British Columbia; and Rocky Mountain type (variety glauca), collected near Flagstaff, Arizona. Seed collections were made from several open-pollinated trees at each origin. In this paper the seed sources will be referred to by their provenance, i. e. Vancouver Island, interior British Columbia, and Arizona, rather than the broader regions they represent.

In previous experiments on the comparative physiology of different provenances of Douglas-fir, Irgens-Moller (30, 31) noted that these three provenances consistently differed by significant margins. If variations in response to applied chemicals exist among varieties or seed sources of Douglas-fir, they most likely would be detected in seedlings from these three areas.

Soil

Forest soil from the Burnt Woods area west of Corvallis, Oregon was used. The dark mineral soil from the surface one foot below the duff layer was screened through a one-half inch mesh in the field. At the laboratory the soil was further screened through a one-fourth inch mesh and thoroughly mixed to insure uniform texture and moisture content.

Containers

A total of 120 metal pots measuring eight inches in diameter at the top and eight inches deep was used. These containers held an average of 7.6 pounds each of oven-dry soil. Drainage holes in the base of each pot were plugged with cotton.

Methods

The study consists of two experiments: (1) a detailed experiment following the title of this manuscript, and (2) a short experiment on the effects of exogenous growth regulators upon time of break of dormancy. A description of the second experiment is found at the end of this chapter. Both experiments were conducted in the same controlled environment chamber under identical conditions.

Design of Experiment

Each of the three growth regulators was applied at three concentrations to seedlings of the three seed collections of Douglas-fir. Each of the 30 treatment combinations was represented by a sample size of 64 seedlings divided equally between two pots treated alike (replications). The number of untreated control pots was the same as that for each chemical. The experimental design is outlined in Table 1.

Table 1. Design of experiment with growth regulators. The 10 treatments are repeated for each seed source, producing 30 treatment combinations. Each treatment combination was represented by two replications, each containing 32 seedlings.

	Growth Regulator and Concentrations (ppm)			
Treatment Level	IAA	CCC	B-995	Control
1	75	500	1000	0
2	150	1000	2000	
3	300	2000	4000	

Experimental Procedures

The pots were numbered and assigned to treatment before they were filled with soil. A sample of 20 empty pots was weighed and the mean pot weight was determined to be 0.65 pounds. The

pots were filled with soil so that the total weight of each pot was 10 pounds. Soil moisture samples were taken frequently while the pots were filled to measure variation in weight of soil moisture content (and therefore soil quantity) and to enable computation of a watering schedule (Appendix I p. 53) According to this calculation, the pots contained an average of 7.6 pounds of oven-dry soil. With this information, a corresponding range of total pot weight for a desired moisture range of 33% to 55% of oven-dry weight was calculated to be 10.7 pounds to 12.4 pounds. This technique permitted frequent control over soil moisture content by placing a pot on a scale and adding water until the total weight reached 12.4 pounds.

The soil surface in each container was covered by a layer of Perlite approximately three-fourths inch thick. Perlite has the advantages of reducing water loss by acting as a mulch and decreasing the incidence of damping-off of young seedlings. The additional weight of Perlite was considered to be uniform. The seeds were stratified by first soaking in an excess of water at room temperature for forty-eight hours and then storing at 35°F. in the dark for two weeks. They were then germinated on filter paper in petri dishes at a constant temperature of 68°F. and a photoperiod of 14 hours. The seeds were considered germinated and ready for transplanting when their radicles had achieved a minimum length of one-half inch. Approximately six days were required for

germination, and age of planted seedlings differed by no more than two days. Thirty-two seedlings were planted in each pot; a total of 24 pots of each seed source was planted. The seedling populations were raised in the growth chamber during a 34 day period prior to treatment. The pots were watered and rotated systematically at seven day intervals during the course of the experiment.

The seedlings were 35 days old and averaged 13 millimeters in height from cotyledons to shoot tip when the treatments were initiated. Six foliar spray treatments at the concentration levels outlined in Table 1 were applied to the corresponding seedlings at bi-weekly intervals. The preparation procedures for the treatments are given in Appendix 1, p. 55). Approximately .025% Tween 20(polyoxyethylene soibitan monolaurate) was added to each treatment solution to reduce runoff. One day prior to each treatment the soil moisture in each of the pots was adjusted to 55% of oven-dry weight. Treatments were applied as aqueous foliar sprays using a Hudson hand operated chemical sprayer under dimly lighted, well aerated conditions. Control seedlings were sprayed with a solution of .025% Tween 20 in distilled water. The seedlings remained under these conditions for approximately ten hours before they were returned to the growth chamber.

During the late winter of 1966, a short experiment was conducted to determine the influence of IAA, CCC and B-995 treatments

on the time of growth initiation in Douglas-fir seedlings. According to Vegis (73), by that time the seedlings have passed the "main rest" period and have entered a period of "after rest" where the dormant seedlings are ready to start their growth when exposed to very favorable external conditions. At the beginning of the "after rest" the limits of external conditions for stimulating growth are very narrow, but during this phase of the rest period the limits gradually widen.

Dimethyl sulfoxide (DMSO) has been reported to be useful as a potentiator of agricultural and medicinal chemicals (27). When employed as a solvent, DMSO has the desirable properties of enhancing penetration of applied chemicals into and transport within plant tissues. Sciuchetti and Born (60) found that plant responses to CCC and B-995 combined with DMSO were greater than treatment with the growth retardants alone. DMSO was employed in the present experiment to test its carrier properties with treatments applied to dormant seedlings.

The design of the experiment is given in Table II. The high concentration of each growth regulator from the previous experiment was applied once or twice using either two percent DMSO or water as a carrier. Each treatment combination was represented by a sample of 70 seedlings, divided approximately equally among four replications.

At the State Forest Nursery north of Corvallis, Oregon, dormant one-year-old Douglas-fir seedlings of local seed origin were carefully lifted in mid-February with clumps of soil and planted in eight-inch diameter metal pots. A total of 48 pots was used. Care was taken to lift seedlings of uniform size and to plant a uniform number of seedlings in each pot.

Table II. Design of experiment to test the effects of three growth regulators upon break of dormancy. Each of the treatments was replicated four times. Individual pots contained approximately 18 seedlings.

Number of Applications and Carrier	Growth Regulator and Concentration (ppm)			
	IAA	CCC	B-995	Control
1 - DMSO	300	2000	4000	0
2 - DMSO	300	2000	4000	0
2 - water	300	2000	4000	0

At the laboratory the seedling population was thinned so that each pot contained 18 $\pm$ 4 seedlings and each treatment was represented by a total of 70 $\pm$ 5 seedlings. The pots of seedlings were maintained out of doors for a total of two weeks before they were moved to the growth chamber.

Preparation procedures for the treatments in this experiment are the same as those described in Appendix I, p. 55), except, when two percent DMSO was used as the solvent, no wetting agent

was employed. The first foliar spray treatment was applied to all seedlings one week after lifting; a second treatment followed one week later. The treatments were applied under dimly lighted and well aerated conditions.

On the day following the second treatment, the seedlings were moved to the growth chamber and exposed to the environment previously described on p. 13. The numbers of seedlings falling into the following classifications (15) were recorded for each pot at two day intervals.

1. Tight bud - sharp-tipped, solid in texture, dark reddish-brown in color, elliptical in shape.
2. Swelling bud - soft in texture, showing yellowish scales, oval in shape.
3. Needles exposed to air - showing green color through opening of bud scales at top.
4. Brush state - bud scales still visible, however needles exposed more than one-half of their length.
5. Elongation of shoot.

Inspections for bud-burst were made every two days until all of the seedlings had passed stage three, which was considered to be the most clearly discernible stage. This was reached after eight observations. The eight sets of observations were combined into a single set of values as follows: the eight sets were numbered

in reverse order, so that the first observation was given number eight. Then for each observation, the ascribed number was multiplied by the number of seedlings entering stage three on that date. The eight products were then totaled and the sum was divided by the total number of seedlings in the container. According to this method, the treatments having a large proportion of seedlings undergoing early initiation of growth obtained a correspondingly higher value. Further details and an example of these calculations are given in Appendix II. In addition to these data, any differences in seedling appearance during a period following bud-burst were noted.

IV. RESULTS

The findings of the experiments will be reported individually for the types of data taken, i. e. height growth, seedling appearance, terminal bud initiation, and dry weight. Some interrelationships will be discussed as the data are presented. The effects of the growth regulators on break of dormancy are discussed at the end of this chapter.

Effects Upon Height Growth

Measurements, in millimeters, of seedling height between the cotyledons and base of the terminal bud or shoot tip were started one day prior to the first treatment. Three subsequent measurements of seedling height were made at approximate one month intervals. Table III gives information on the relationship of the four measurements and the six applications of treatments to each other and to seedling age and height.

Seedlings from the three seed sources were found to differ significantly in height at the .1% level as early as 34 days of age (Table III). In order of decreasing height, the three seed sources were ranked as follows: (1) Vancouver Island, (2) interior British Columbia, and (3) Arizona. The differences in seedling height persisted throughout the course of the experiment. The photograph in

Figure 1 shows these differences at age 120 days. Comparative rates of growth of control seedlings from the three sources are shown in Figure 2. Seedlings from Vancouver Island grew considerably faster than the other seedlings and also demonstrated greater variability.

Table III. Schedule of treatments and height measurements. Mean height of untreated seedlings is given at four different ages.

Seedling Age (days)	Measurement Number	Treatment Number	Seed Source and Mean Height of Untreated Seedlings (mm)		
			Interior British Columbia	Vancouver Island	Arizona
34	1		14.7	16.1	7.9
35		1			
49		2			
61	2		27.9	32.4	17.3
63		3			
77		4			
89	3		46.2	61.2	30.9
94		5			
101		6			
114	4		68.6	103.0	51.8

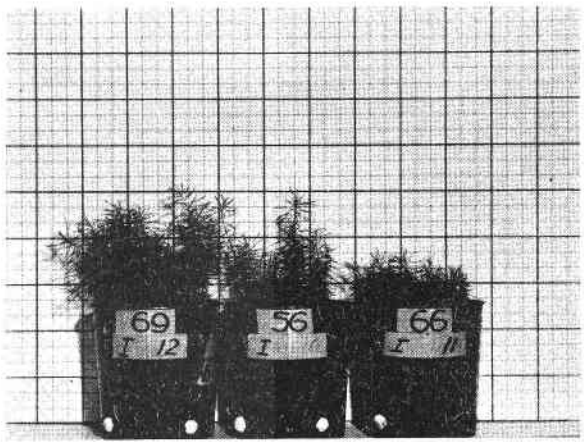

Figure 1. Untreated seedlings from the three seed sources at age 120 days. Left to right: Vancouver Island, interior British Columbia and Arizona.

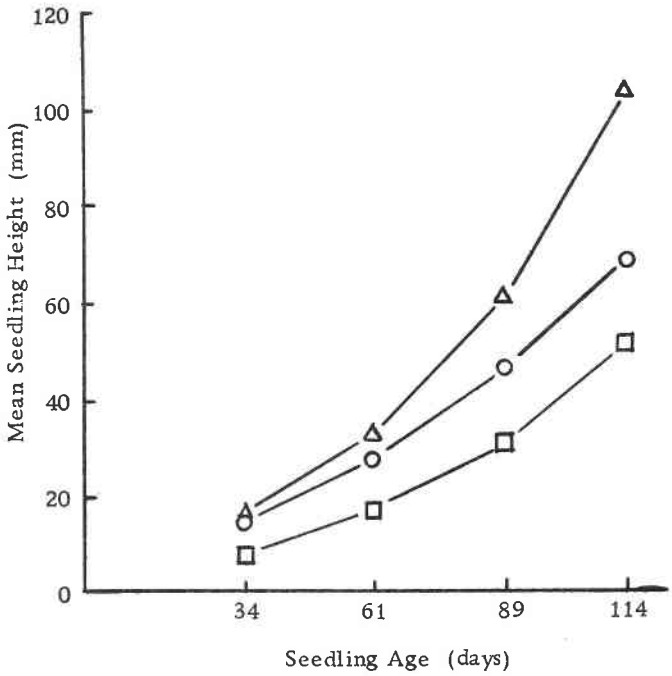

Figure 2. Growth rates of untreated seedlings from the three seed sources. Top to bottom: Vancouver Island (△), interior British Columbia (○) and Arizona (□).

Upon termination of the experiment the mean seedling height was determined for each container for each of the four measurements. Differences in mean seedling heights among treatments were tested by analysis of variance according to the model given in Appendix II, p. 58 . The significance of differences between means of treatment combinations and the means of pooled controls was determined by calculated LSD values.[1] These data are summarized in Table IV. Identical statistical methods were also used to test differences in number of periods of dormancy and in dry weight determinations.

To evaluate the uniformity in height of the seedling populations before treatment the data of measurement one were analyzed statistically. The various treatment combinations and pooled controls were found to be uniform at the beginning of the experiment with one exception---the seedlings from Vancouver Island assigned to the CCC, 1000 ppm treatment were initially significantly taller (1% level) than the pooled control. However, the difference diminished as the experiment proceeded. This could possibly be attributed to the treatment.

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[1] One container of interior British Columbia seedlings and one of Vancouver Island seedlings were damaged by defoliating insects during the experiment. Both containers received the 4000 ppm B-995 treatment. These data were replaced by substitute "dummy" values equal to the similarly treated replication and were assumed to have one replication when the treatment means were compared with pooled control means.

Table IV. Mean seedling heights. The data given under each seed source are mean seedling heights (mm) of nine treatments and pooled control for each of four measurements.

Growth Regulator	Concentration (ppm)	Seed Source: Interior British Columbia — Seedling Age (days) 34	61	89	114	Vancouver Island — Seedling Age (days) 34	61	89	114	Arizona — Seedling Age (days) 34	61	89	114
IAA	75	14.5	25.9	40.9*	55.7***	16.7	32.1	63.0	101.9	7.7	15.6*	27.2*	45.0**
	150	14.5	26.5	38.6**	55.1***	17.0	31.1	59.0	100.2	8.4	17.0	32.6	51.7
	300	14.3	25.7	37.8**	54.8***	17.3	32.3	58.2	95.7	8.3	17.3	33.9	50.9
CCC	500	13.9	25.3*	43.1	63.2	16.8	31.1	60.7	99.2	8.1	17.0	34.6	53.2
	1000	13.4	24.7*	44.8	64.2	18.4**	35.2	70.1	104.9	8.4	17.0	34.1	52.1
	2000	13.2	24.8*	39.2*	51.7***	15.7	30.2	53.8	80.4**	8.4	17.8	34.7*	49.8
B-995	1000	14.7	27.5	46.8	64.6	17.3	32.9	65.6	100.8	8.6	17.1	32.8	49.3
	2000	14.3	26.1	42.3	58.1**	16.7	32.9	67.3	101.5	8.1	16.6	29.5	45.2**
	4000	14.4	25.4	47.0	65.2	16.5	29.6	54.0	86.1*	8.0	16.5	27.8	42.6***
Pooled Control	0	14.7	27.9	46.2	68.6	16.1	32.4	61.2	103.0	7.9	17.3	30.9	51.8
s^2		0.78	1.81	9.17	10.54	.72	5.27	40.90	61.28	.15	.81	4.68	5.96
LSD (5%)		1.6	2.4	5.3	5.7	1.4	4.0	11.2	13.7	0.7	1.61	3.8	4.3
LSD (1%)		2.2	3.4	7.4	7.9	2.0	5.6	15.6	19.0	0.9	2.2	5.3	5.9
LSD (.1%)		3.0	4.6	10.2	11.0	2.8	7.8	21.6	26.5	1.3	3.0	7.3	8.3

* Differs significantly from control at 5% level.
** " " " " " 1% " "
*** " " " " " .1% " "

Most of the responses to the treatments consisted of reductions in growth rate. Four instances of significant growth inhibition were present at age 61 days, 12 days after treatment two. The interior British Columbia seedlings were apparently sensitive to all three concentrations of CCC at age 61 days (measurement two), but only the response to the third level persisted in succeeding measurements. This early response to CCC was not evident in seedlings from the other two sources. IAA at 75 ppm (only) caused a significant and persistent growth inhibition in Arizona seedlings beginning at age 61 days.

At age 89 days, 12 days after treatment four, the interior British Columbia seedlings showed significant growth inhibition which appeared to increase linearly with increasing concentration of IAA. After six applications, age 114 days, the reduction in growth rate of these seedlings was approximately equal for all three concentrations of IAA. There was no significant reduction in growth rate in the Vancouver Island seedlings resulting from IAA treatment.

At age 89 days the growth of the Arizona seedlings was enhanced slightly by 2000 ppm of CCC. These responses bear some resemblance to those at age 61 days in the interior British Columbia seedlings in that the responses appeared and then disappeared in succeeding measurements. Perhaps certain stages occur in the seedling physiology at different times in which the

seedlings are more sensitive or insensitive to CCC treatment. However, it is also likely that these responses are spurious and not related to the treatments.

At age 114 days, after six treatments of 2000 ppm of CCC the reduction in growth in interior British Columbia and Vancouver Island seedlings was highly significant. No reduction occurred in the Arizona seedlings.

No significant responses to B-995 treatment were demonstrated prior to the fourth measurement. At age 114 days height growth of the interior British Columbia seedlings was reduced only by 2000 ppm, the Vancouver Island seedlings only by 4000 ppm, and the Arizona seedlings by both the 2000 ppm and 4000 ppm treatments.

The measurements at age 114 days are also presented graphically in Figure 3. The various treatment means were expressed as a percentage of the pooled control mean.

From the foregoing results it appears that considerable variation exists among the three seed sources in timing and degree of response. When the responses are considered in terms of the two varieties of Douglas-fir, i. e. Vancouver Island seedlings are variety menziesii and interior British Columbia and Arizona seedlings represent variety glauca, no clear trends can be seen in the data of Table IV.

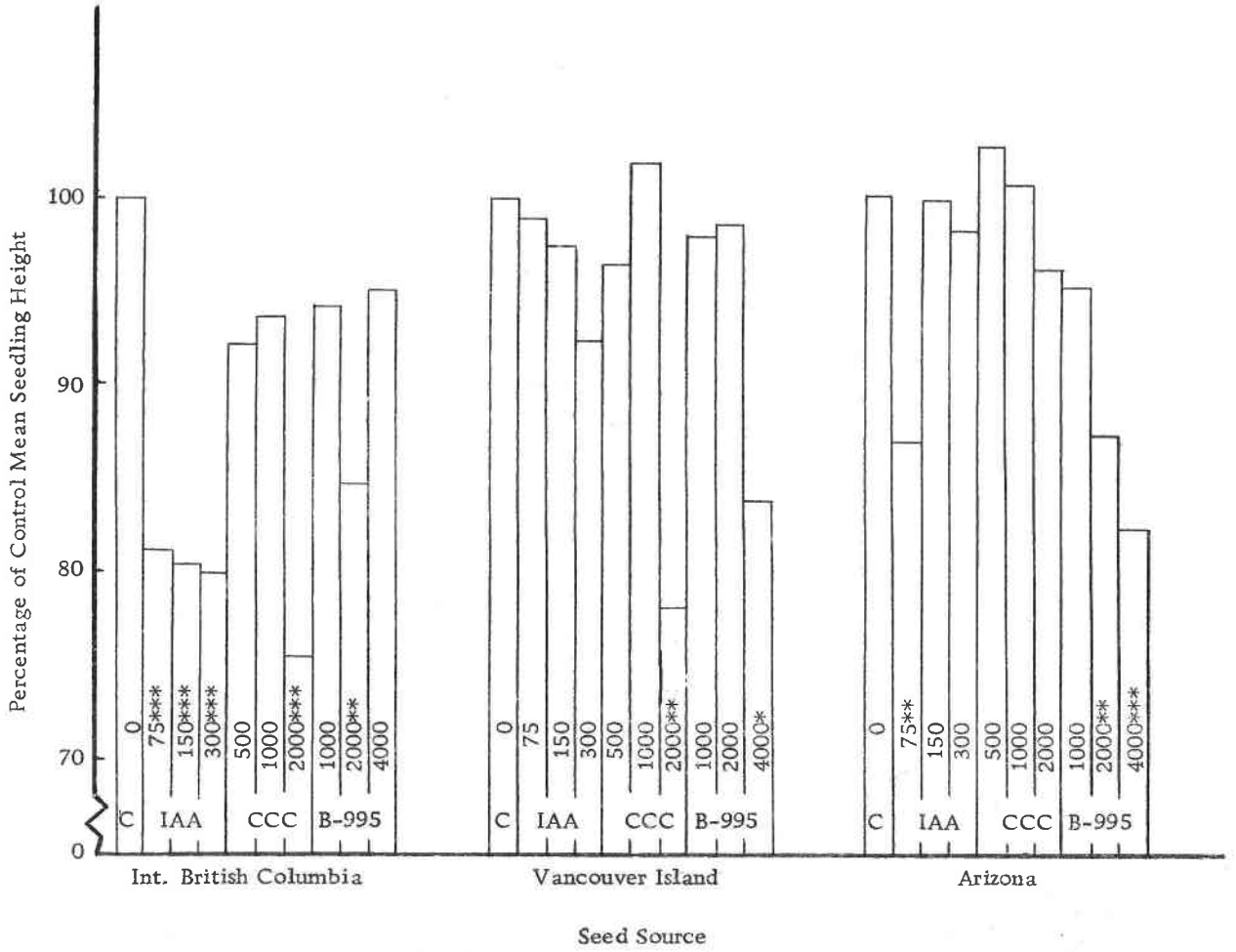

Figure 3. Mean seedling heights at age 114 days expressed as a percentage of the control. Significance of differences from control: 5% level (*), 1% level (**) and .1% level (***).

It was hypothesized that differences in surface-layer anatomy, i. e. cuticle and outer epidermal wall thickness, may contribute to the varying responses among the three races through differential uptake of the applied chemicals. Slides of untreated needle tissue were examined for differences in surface-layer anatomy. Photographs taken through a Zeiss phase-contrast microscope are shown in Figure 4. An earlier study of this type (68) noted that the cuticle layer of Douglas-fir seedlings grown in a growth chamber was commonly thinner and apparently of a different chemical composition than in seedlings grown out-of-doors. Upon examination of the surface layers of needles from seedlings from the three seed sources, little correlation could be seen between the trends in responses to treatments and surface layer thickness. The Vancouver Island seedlings had the thinnest cuticle and epidermal layers, presenting the least impediment to chemical penetration, but they also responded less to the treatments than seedlings from the other two sources. Arizona seedlings had the thickest surface layers, and exhibited an intermediate response to treatments. British Columbia seedlings had intermediate surface layer thickness and demonstrated a decidedly greater sensitivity to the applied chemicals. Therefore, the differences in responses among the seed sources cannot justly be attributed to only anatomical features affecting penetration. More likely, the differences in response are attributable to several

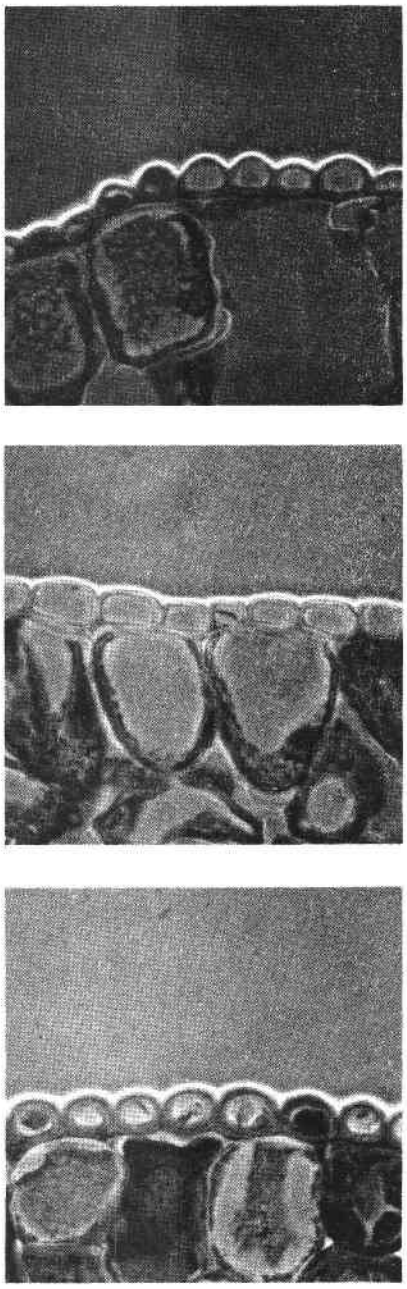

Figure 4. Cross-sections of needles from untreated seedlings. Top to bottom: interior British Columbia, Vancouver Island and Arizona. Photographs taken through a Zeiss phase-contrast microscope, phase setting 2, magnification 640X.

physical and physiological factors in combination which remain unknown.

Effects Upon Seedling Appearance

Seedlings treated with 300 ppm IAA developed a distinct twisting or distortion of the leaders during the course of the experiment. This condition was observed in all of the seed sources, with or without inhibition of height growth. There were no other observable differences aside from growth reduction between IAA treated and control seedlings.

Chlorosis and shortened internodes were characteristics of CCC treated seedlings. The symptoms were confined to actively growing tissue at the time of treatment. Older needles present before treatment were little affected. The symptoms intensified with increased concentration and repeated application of CCC. The appearance ranged from slight chlorosis at 500 ppm to extremely chlorotic, compact seedlings at 2000 ppm. The most severely affected seedlings exhibited a "bushy" appearance with many white, chlorotic needles, and some brown needles. In a few seedlings, repeated application of the 2000 ppm concentration caused die-back. In a previous pilot study involving CCC treatment of Douglas-fir seedlings (36), treatment with 2400 ppm CCC caused death of some seedlings and surviving seedlings recovered only slightly. Less

seriously affected seedlings eventually recovered. At lower concentrations, the needles turned yellow rather than white and chlorosis was often confined to the basal two-thirds of the needle or to the outside margin. The reported symptoms did not appear to be as severe in the Arizona seedlings as in the Vancouver and British Columbia seedlings for a given concentration of CCC. No differences could be observed between B-995 treated seedlings and control seedlings.

Effects Upon Periods of Dormancy

Upon termination of the experiment, the number of bud scale traces on each seedling was counted. This number is indicative of the frequency of dormancy periods, or flushes, during the study period. The mean number of periods of dormancy per seedling was calculated for each treatment and pooled control and compared by analysis of variance.

IAA treated seedlings had more periods of dormancy than the pooled control, especially at the highest concentration. Vancouver Island seedlings responded to all three levels of concentration. The CCC treatments, on the other hand, had little influence upon the number of periods of dormancy. In only one instance, the 1000 ppm treatment of interior British Columbia seedlings, was the average number of periods of dormancy slightly higher (5% level) than the

Table V. Mean number of periods of dormancy per treatment at age 122 days. Seedlings from three sources were treated with growth regulatory substances at ages 35, 49, 63, 77, 94, and 101 days.

Growth Regulator	Concentration (ppm)	Seed Source: Interior British Columbia	Seed Source: Vancouver Island	Seed Source: Arizona
IAA	75	1.22	0.26*	0.88
	150	1.50	0.29**	0.91
	300	1.88***	0.65***	1.22**
CCC	500	1.34	0.21	0.97
	1000	1.54*	0.12	0.89
	2000	1.21	0.05	0.47
B-995	1000	1.28	0.11	0.67
	2000	1.43	0.08	0.39*
	4000	1.47	0.25*	0.27**
Pooled Control	0	1.20	0.11	0.74
s^2		0.0341	0.0055	0.0255
LSD (5%)		0.32	0.13	0.28
LSD (1%)		0.45	0.18	0.39
LSD (.1%)		0.62	0.25	0.54

* Differs significantly from control at 5% level.
** " " " " " 1% " .
*** " " " " ".1% " .

control. Only Arizona seedlings responded to B-995 treatment and the response appeared to be opposite from IAA and CCC treatments.

Effects Upon Dry Weight

The seedlings were harvested by severing at the soil surface and determining dry weight of tops and roots for each container. The tops and roots were dried in an oven at 158°F. for 72 hours. Mean dry weights (Table VI) were determined by dividing the total weight by the total number of seedlings in each container.

In general, the trends in top dry weight were closely related to those in height growth. For the IAA treatments, top dry weight was significantly reduced only in the interior British Columbia seedlings. Although all three concentrations of IAA were effective, the response to 300 ppm was somewhat greater. Mean root dry weight was reduced only by 300 ppm of IAA in the interior British Columbia and Vancouver Island seedlings. Neither top nor root dry weight of the Arizona seedlings was affected, although seedling height was reduced by 75 ppm.

Two thousand ppm of CCC caused highly significant reductions in top dry weight of interior British Columbia and Vancouver Island seedlings, while the Arizona seedlings were unaffected. Root dry weight varied in an inconsistent manner with 500 ppm causing reduced dry weight of interior British Columbia seedlings. While both

Table VI. Mean top and root dry weights of 122-day old seedlings from three seed sources. The seedlings received six applications of the growth regulators.

Growth Regulator	Concentration (ppm)	Seed Source: Interior British Columbia Mean Top Dry Weight (grams)	Interior British Columbia Mean Root Dry Weight (grams)	Vancouver Island Mean Top Dry Weight (grams)	Vancouver Island Mean Root Dry Weight (grams)	Arizona Mean Top Dry Weight (grams)	Arizona Mean Root Dry Weight (grams)
IAA	75	0.150**	0.125	0.250	0.150	0.175	0.100
	150	0.155*	0.125	0.245	0.170	0.185	0.090
	300	0.135***	0.110*	0.235	0.135*	0.180	0.090
CCC	500	0.160	0.110*	0.255	0.125*	0.195	0.090
	1000	0.165	0.120	0.270	0.145	0.195	0.140***
	2000	0.140***	0.125	0.200**	0.115**	0.190	0.105
B-995	1000	0.165	0.155	0.260	0.155	0.195	0.095
	2000	0.160	0.120	0.270	0.150	0.180	0.090
	4000	0.180	0.140	0.220	0.180	0.180	0.090
Pooled Control	0	0.173	0.155	0.248	0.170	0.186	0.097
S^2		0.000071	0.000578	0.000345	0.000385	0.000059	0.0001
LSD (5%)		0.015	0.042	0.033	0.034	0.013	0.018
LSD (1%)		0.021	0.058	0.045	0.048	0.019	0.024
LSD (.1%)		0.029	0.081	0.063	0.066	0.026	0.034

* Differs significantly from control at 5% level.
** " " " " " 1% " .
*** " " " " " .1% " .

500 ppm and 2000 ppm reduced root dry weight of Vancouver Island seedlings, and 1000 ppm caused a substantial increase in root dry weight of Arizona seedlings. The B-995 treatment was without effect on either top or root dry weight in any of the seed sources.

In general, the root dry weight data seem to have little meaning. This may reflect the fact that at the time of measurement the roots had reached the bottom of the container and in many instances the plants might be termed "pot bound".

Effects Upon Break Of Dormancy

The individual values in Table VII represent the mean rate of growth initiation, i.e. the mean time of attainment of stage three over the four pots of seedlings (replications) representing each treatment combination.

There was no overall effect of carrier or number of applications when the rates of bud-burst were averaged over growth regulators. Only one treatment combination differed significantly from its control. This was the CCC, Water (2) treatment, which differed from the control and from the DMSO (2) treatments at the 1% significance level, and from the DMSO (1) treatment at the 5% significance level.

Apparently this treatment was effective in slowing the rate of growth initiation. The CCC treatments had no effect upon the

color appearance of new tissue following bud-burst. Seedlings representing the other treatments in this experiment broke dormancy at a more or less uniform rate.

Table VII. Effects of three growth regulators upon rate of bud burst in 1-0 Douglas-fir seedlings from Corvallis, Oregon. See Appendix II p. 60 for method used to calculate rate of bud burst.

Growth Regulator	Concentration (ppm)	Carrier and Number of Applications		
		DMSO (1)	DMSO (2)	Water (2)
IAA	300	4.58	4.88	5.11
CCC	2000	4.74	5.00	4.19**
B-995	4000	4.81	4.80	5.14
CONTROL	0	5.03	5.09	4.98

** Differs significantly from control at 1% level.

V. DISCUSSION

It is clear that the three different races of Douglas-fir did indeed differ in their sensitivity to the three growth regulators. This is particularly true with respect to the effect of IAA upon height growth (Table IV). The reason for the lack of effect in the Vancouver Island seedlings as compared to those from interior British Columbia, where the effect was highly significant, is difficult to explain. It may possibly reflect the fact that the experimental conditions, particularly the thermoperiod, differed considerably from the conditions of the native habitat in interior B. C. and that these abnormal conditions somehow increased the sensitivity to exogenously applied IAA.

However, if that were the case one should also expect that the Arizona seedlings would react more strongly than they did. Further experiments under different thermo- and photoperiods would be needed to clarify this. Since IAA is the only one of the three growth regulators which is known to occur naturally in plants, the differences in response to exogenously applied IAA may also be due to differences in the levels of endogenous auxin contained in seedlings from the three sources.

It is of interest to note that although no effect of IAA on growth rate was observed in the Vancouver Island seedlings, there

were significant effects on the number of dormancies. Apparently the more frequent formation of terminal buds did not affect the total height growth. Possibly IAA induced some change(s) in the metabolic system of the seedlings which brought about successive periods of dormancy. The environment may have brought about a prompt reversal of this reaction during the period between treatments so that an active growth rate was maintained. However, appreciating the diverse nature of IAA activity, this is spectulative.

Unlike the IAA treatments, CCC had little effect upon terminal bud initiation (Table V). Only in one case, 1000 ppm of CCC on interior British Columbia seedlings, was the number of periods of dormancy per seedling slightly increased. The effects of CCC seem to be confined to shortening of internodes and interference with chlorophyll synthesis.

It should be pointed out that the results of the experiments must be qualified by the environmental conditions and methods under which they were conducted. For example, in an earlier attempt of the experiment involving three seed sources, it was found that under a twelve hour photoperiod the within seed source variation was so great that treatment differences were not detectable. Under the fourteen hour photoperiod used in the present experiment seedling growth was more uniform and responses to treatments could be detected more easily. Apparently many of the treatment effects

were masked by the unfavorable twelve hour photoperiod. In another experiment (59) similar treatments applied under a nine hour photoperiod and 78°F. during the day and 40°F. during the night did not yield as clear a response as under the environment employed in the present experiment.

The results of the experiment with break of dormancy must also be qualified in terms of environment. At the time the experiment was conducted, the seedlings were in a physiological condition ready for growth initiation as soon as external conditions became favorable. The environment used in the growth chamber was very favorable toward growth resumption since growth was resumed in many seedlings following only one week in the growth chamber. The effects of the chemicals might have been greater if the plants had been treated earlier in the dormancy period. No explanation can be offered for the fact that 2000 ppm of CCC was effective with water as a carrier while not with DMSO. DMSO may possibly react chemically with CCC, change its ionic state, or affect the availability of CCC at the site of action. In any event, DMSO seems to have no role as a potentiator of chemical treatments under the conditions and manner of application employed in this experiment.

VI. CONCLUSIONS

Because of the general nature of these investigations, it would be meaningless to arrive at very specific conclusions. However, the data have demonstrated some definite trends which would support the following conclusions:

1. Seedlings from the three seed sources differed markedly in their sensitivity to the applied growth regulators. The general level of sensitivity was considered to be low compared with other plants, e.g. many Angiosperms.
2. Each of the three growth regulators produced an individual pattern of response in treated seedlings. Seedlings treated with B-995 exhibited little change, while IAA and CCC caused distortion of treated seedlings which intensified with increasing concentration.
3. Only the CCC treatment using water as carrier significantly slowed the initiation of growth in dormant seedlings. No significant change was observed when DMSO was used as carrier for the same treatment.

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APPENDICES

APPENDIX I

Calculation of Watering Schedule

Data Required

1) Mean weight of empty containers..... 0.65 lb.

2) Total weight of pot plus soil. .....10.00 lb.

3) Soil moisture samples..... 8 samples.

Step One:

Soil moisture was calculated through completion of the following table.

Pot Numbers	Wt. of Soil can	Wt. Can, Soil H_2O	Wt. Can, Soil	Wt. Soil	Wt. H_2O	H_2O%
1 - 10	77.80	186.77	165.16	87.36	21.61	24.7
11 - 20	78.40	155.90	140.99	62.59	14.91	23.8
21 - 30	75.94	146.76	133.51	57.57	13.25	23.0
31 - 40	77.35	169.85	152.93	75.58	16.92	22.4
41 - 50	77.44	159.87	144.74	67.30	15.13	22.5
51 - 60	77.42	174.80	156.57	79.15	18.23	23.0
61 - 70	77.86	161.80	146.33	68.47	15.47	22.6
71 - 72	78.33	153.53	139.05	60.72	14.48	23.8

Step Two:

Calculate total pot weight at desired moisture percentages according to the following table.

1 Pot Numbers	2 Wt. Pot, Soil, H_2O(lb)	3 Wt. Pot(lb)	4 Wt. Soil H_2O(lb)	5 H_2O(%)	6 Wt. Oven-Dry Soil(lb)	7 Wt. Soil, 55% H_2O(lb)	8 Wt. Soil, 33% H_2O(lb)
1 - 10	10.00	0.65	9.35	24.7	7.5	11.63	9.98
11 - 20	"	"	"	23.8	7.6	11.78	10.11
21 - 30	"	"	"	23.0	7.6	11.78	10.11
31 - 40	"	"	"	22.4	7.6	11.78	10.11
41 - 50	"	"	"	22.5	7.6	11.78	10.11
51 - 60	"	"	"	23.0	7.6	11.78	10.11
61 - 70	"	"	"	22.6	7.6	11.78	10.11
71 - 72	"	"	"	23.8	7.6	11.78	10.11

The final four columns are computed as follows:

(5) from step one.

$$(6) = \frac{(4)}{100\% + (5)}$$

(7) = (6) X 1.55

(8) = (6) X 1.33

The average of columns seven and eight are 11.76 pounds and 10.09 pounds respectively. The mean empty container weight of 0.65 pound was added to each of these values to produce total pot weights of 12.41 pounds and 10.74 pounds at the desired soil moisture percentages of 55 percent and 33 percent respectively.

Preparation of Treatments

Stock Solutions

The following stock solutions were prepared at 20 degrees Centigrade. Two percent DMSO was used as solvent in place of distilled water for treatments of the break of dormancy experiment.

Growth Regulator	Concentration (ppm*)	When Prepared
IAA	300	each treatment
CCC	2000	every other treatment
B-995	4000	every other treatment
CONTROL	0	each treatment

Dilutions

The following dilutions were made at 20 degrees Centigrade for each treatment. Approximately 0.025 percent Tween 20 was added (growth patterns experiment) after dilutions were made.

*ppm = parts per million, grams chemical per 1,000,000 grams solution.

Growth Regulator	Concentration (ppm*)	Ml. Stock Solution	Ml. Total Solution
IAA	75	50	200
IAA	150	100	200
IAA	300	200	200
CCC	500	50	200
CCC	1000	100	200
CCC	2000	200	200
B-995	1000	50	200
B-995	2000	100	200
B-995	4000	200	200
CONTROL	0	200	200

*ppm = parts per million, grams chemical per 1,000,000 grams solution.

APPENDIX II

Analysis of Variance

Growth Patterns Experiment

The following model was employed for evaluating seedling height, number of periods of dormancy, and top and root dry weight data. Because the three seed sources did not contribute equally to experimental error, a separate error mean square was calculated and used in testing data for each seed source.

Source of Variation	Degrees of Freedom
Total	71
Seed Source	2
Growth Regulator in Seed Source	27
Growth Regulator in B. C.	9
Growth Regulators	3
Within IAA	2
Within CCC	2
Within B-995	2
Growth Regulator in Van. Island	9
Growth Regulators	3
Within IAA	2
Within CCC	2

Source of Variation (Con't.)	Degrees of Freedom
Within B-995	2
Growth Regulator in Arizona	9
Growth Regulators	3
Within IAA	2
Within CCC	2
Within B-995	2
Error in British Columbia	14
Error in Vancouver Island	14
Error in Arizona	14
Combined Error	42

The significance of differences between treatment means and pooled control means was determined by the method of least significant difference (LSD). Controls were pooled because of differences between replications. These calculations were as follows:

If: $\left| \bar{y}_{pc} - \bar{y}_{tc} \right| > t_{12 \text{ d.f.}} \, (\%) \sqrt{s^2 \left(\frac{1}{n_{pc}} + \frac{1}{n_{tc}} \right)}$, then the difference is significant.

Where:

$\bar{y}_{pc}$ = pooled control mean

$\bar{y}_{tc}$ = treatment combination mean

$t_{12\ d.f.}^{(\%)}$ = Student's t - distribution, 12 d.f., 2-tailed test, 5%, 1%, or .1% sig. level.

s^2 = error mean square for seed source

n_{pc} = number replications of pooled control mean = 6

n_{tc} = number replications of treatment combination mean = 2

Break of Dormancy Experiment

The following is an example calculation involved in the method of combining eight sets of two day observations of bud-burst into a single set of values. For a container of 18 seedlings, typical data were as follows:

	Observation Date							
	8	7	6	5	4	3	2	1
Stage of bud-burst	3-10	3-12	3-14	3-17	3-19	3-21	3-23	3-25
1	16	9						
2	2	9	13	5	1			
3			5	13	15	9	1	
4					2	6	8	2
5						3	9	16

The observation dates are numbered in reverse order beginning with number eight. The value representing the speed of budburst of the seedlings in this pot is calculated as follows:

$$0 \times 8 + 0 \times 7 \div 5 \times 6 + 8 \times 5 + 4 \times 4 + 1 \times 3 + 0 \times 2 + 0 \times 1 \; / \; 18 = 89/18 = 4.94$$

Values calculated for each container were compared by analysis of variance according to the following model:

Source of Variation	Degrees of Freedom
Total	47
Treatment	11
Growth Regulator	3
Carrier and Number of Applications	2
Growth Regulator x Carrier & No. Applications	6
Error	36

The significance of differences between treatment means was determined by the method of least significant difference (LSD). These calculations were as follows:

If: $\bar{y}_c - \bar{y}_{gr} > t_{36 \text{ d.f.}}(\%) \sqrt{\frac{2 s^2}{n}}$, then the difference is significant.

Where:

$\bar{y}_c$ = mean of a control treatment

$\bar{y}_{gr}$ = mean of a growth regulator treatment

$t_{36\ d.f.}(\%)$ = Student's t-distribution, 36 d.f., 2-tailed test, 5%, 1% or .1% level of significance

s^2 = error mean square

n = number replications in treatment means = 4