PRESENT AND POTENTIAL USES CF GROWTH REGULATORY SUBSTANCES IN DOUGLAS-FIR

by

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The present uses of growth regulatory substances in Douglas-fir seedlings can be dealt with very quickly. There are no practical uses for growth regulators on Douglas-fir seedlings at the present time. The potential uses for growth regulators is a different matter, however.

Douglas-fir, in common with most conifers, is characterized by extremely slow seedling growth and by very heterogeneous populations. Obviously it does not recommend itself as an experimental organism to physiologists studying basic processes in plant growth. It is not surprising, then, that there are little data describing chemical growth regulation of this species nor that the great majority of the existing information is derived from highly empirical trials. The first part of this paper will be concerned with a summary of empirical trials; the second part with current studies designed to identify endogenous regulators in Douglas-fir seedlings.

Table I is a compilation of the chemicals reported which have been employed in studies of growth regulation of Douglas-fir. It does not include, however, such synthetic plant growth regulators as the phenoxy group which have been used primarily as silvicides.

The term "growth retardants" is defined by Cathey as "chemicals that slow cell division and cell elongation in shoot tissues and regulate plant height physiologically without formative effects." Optimum applications of these materials will result in reduced plant size but not reduced vigor or development.

The first such chemical, B995, is a member of a new class of growth retardants which are somewhat similar to maloic hydrazide. It has been shown to retard the growth of apples, pears, cherries, and other plants. In our laboratory one-month-old Douglas-fir seedlings wore sprayed to the drip point six times at biweekly intervals with aqueous concentrations up to a maximum of 4,000 ppm. When the four-month-old seedlings were harvested, no treatment effect upon dry weight was found and only the highest concentrations reduced stem elongation. No significant effect of an 8,000 ppm soil drench was found either.

The second compound, a quarternary ammonium compound known as cycocel, abbreviated CCC, is an analogue of choline. It has been shown to retard the growth of the majority of plants tested. The mode of action of CCC appears to be inhibition of the biosynthesis of gibberellins which are required for growth processes. Workers at the Earhart Plant Research Laboratory report that CCC applied as a 5,900 ppm soil drench twice weekly for two months had no significant effects upon the growth of two-year-old Douglas-fir seedlings. Similarly we have found that this chemical is not effective in retarding the growth of Douglas-fir seedlings when employed as a soil drench. However, seedlings sprayed at bi-weekly intervals with concentrations up to 2,500 ppm active ingredient developed marked chlorosis and greatly shortened crowns. The plants treated with 2,500 ppm were very bushy and weighed less than one-third of the control seedlings at the end of the four month study.

A second quarternary compound, Phosphon-D, has been reported to reduce internode growth and to produce dark green leaves for a number of test plants. In common with the previous two retardents, this chemical has been most effective when applied to dicotyledons. Several trials with Douglas-fir seedlings have demonstrated either no effect or erratic height growth response, but increasing levels of chemical in the soil up to a maximum of five grams of active material per quart of soil resulted in increasing chlorosis of the seedling foliage.

In contrast to the above compounds, Cathey describes maleic hydrazide as a "growth inhibitor," a class of compounds which may suppress growth corn, pletely in treated plants. Maloic hydrazide suppressses apical dominance and frequently results in plants with greatly shortened internodes and dark green foliage. One-year-old Douglas-fir seedlings in nurseries in England were sprayed with maleic hydrazide during the period of bud swell in the spring. The purpose of the treatment was to control seedling size and late season flushing, but no significant response in seedling growth was noted. In contrast, seedlings sprayed with maleic hydrazide in late summer in the Nisqually Forest Nursery failed to form terminal buds and subsequently died during the winter-1/

The next two compounds, naringenin and abscisic acid, also known as abscisin II or "dormin," have been shown to be associated with the biochemistry of the dormant period of perennial plants. Naringenin is one of the flavenoids reported to occur naturally in the flowers, but not in other tissues of Douglas-fir (21). Two-months-old Douglas-fir seedlings were sprayed to drip point at our laboratory with aqueous solutions of naringenin at bi-weekly intervals. A similar second trial employed 1 naringenin in lanolin applied to seedling epicotyls. No effect of the treatment on height growth or on initiation of dormancy was noted in either experiment.

Abscisic acid has been found in birch and sycamore, cotton, and a wide range of other higher plants. Waring and co-workers have shown this substance to be associated with growth inhibition or dormancy in both birch and sycamore. In our laboratory, two-month-old Douglas-fir seedlings were sprayed to drip point one, two or three times at bi-weekly intervals with concentrations of from 0 to 25 ppm. No treatment effects upon seedling crown length, total dry weight, or initiation of dormancy were found. And no effects of this chemical

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1/ Personal communication from Dr. J. W. Duffield, 12/62.

applied in lanolin were noted in a parallel trial. The data may reflect the low concentrations of active material employed, although one part of abscisic acid per billion has been reported to cause detectable inhibition of Lemna minor growth. Without definitive data upon the absorption and translocation of this material by Douglas-fir seedlings it is impossible to determine if the lack of response was due to the inactivity of abscisic acid in Douglas-fir or the failure of the plant to absorb or translocate the material to an active site.

The remaining compounds in Table I are generally considered to be growth promoters and are termed "cytokinins" or "phytokinins," "gibberellins" or auxins.

Cytokinins have held a fascination for plant physiologists ever since their discovery a few years ago. Incidentally, kinetin was the first of this group to be isolated. One of their disappointing properties, however, is that they do not seem to be translocated in the plant. If applied to a leaf, they tend to remain in that leaf. J. Van Overbeek at Shell Development Laboratory in Modesto, California, attempted to formulate a cytokinin which would be translocated in plants. The result was SD 8339, the code number for 6-benzylamino-9-(tetrahydropyrany1)-9H-purine. This compound d-d appear to be translocated in plants and appeared to be a plant growth regulator. When applied to grapes, it increased fruit set and increased the size of the berries.

Two-month-old Douglas-fir seedlings were treated with concentrations of SD 8339 in both aqueous foliar sprays and in lanolin paste at concentrations ranging from 500 ppm to 10,000 ppm. The next few slides illustrate some of the effects of this substance.

A member of the second major class of plant growth promoting chemicals, gibberellic acid, has been shown to be effective growth promoter for a wide range of plants, but, in general, the greatest response is demonstrated by herbaceous angiosperms (24). Gymnosperms have generally demonstrated little or no response to applications of this compound (29). It should be noted that all these studies used gibberellic acid. It may be that one of the more recently isolated gibberellins will be found to be effective on Douglas-fir.

Indoleacetic acid, the major native indole auxin in plants, is thought to be universally present in higher plants, but the only recorded data on its occurrence in Douglas-fir are the inconclusive chromatogtaphic studies of Dinus. Evidence that growth regulators might hasten the onset of dormancy of Douglas-fir seedlingsi/ prompted trials with indoleacetic acid at our Corvallis nursery. No effects upon seedling phonology wore noted after treatment with aqueous sprays of 125 ppm indoleacetic acid in Nay, Juno, July, and August. However, trials in a controlled environment chamber demonstrated that one-month-old Douglas-fir seedlings produced twisted, rigid shoots when sprayed with indoleacetic acid solutions of 200-300 ppm. Shoot elongation and shoot dry weight were generally reduced by this treatment.

Although alpha-naphthaleneacetic acid is not a natural plant hormone, it has been shown to produce many of the growth responses engendered by the application of indoleacetic acid. However, it is, in general, somewhat less effective.

1/ Personal communication from Dr. J. W. Duffield, 1962.

Dr. J. W, Duffield found that aqueous sprays of 125 to 250 ppm applied in August produced early dormancy in Douglas-fir seedlings. Dr. Duffield also reported that similar spray treatments appeared to increase the root regeneration of Douglas-fir seedlings lifted in November and December (2). And Hoitmubllor (17) notes that Douglas-fir cuttings soaked for twenty-four hours in a 500 ppm solution of the potassium salt of alpha-naphthaleneacotic acid rooted vigorously, while a six hour period of soaking in a solution of 2,000 ppm alpha-naphthaleneacetic acid and 2,000 ppm indoleacotic acid yielded slightly loss favorable results.

The last 5 compounds shown in Table I have been employed in rooting trials of Douglas-fir cuttings at Oregon State University's North Marion Experiment Station. Reports indicate only erratic success with indolebutyric acid and virtually no success with the remaining compounds.

We can conclude from our discussion so far that Douglas-fir is much less responsive than many angiospermous plants to the major classes of plant growth regulating compounds. This may reflect a more primitive physiology which would be consistent with the generally accepted scheme of phylogony for higher plants. That is, the conifers in general and Pinaceae in particular are more primitive than the angiosperms. This primitive physiology is also reflected by the nature of the pigments in Douglas-fir flowers which are much less complex than the pigments of angiosperms.

At this point we might ask, if Douglas-fir is not very responsive to the known classes of growth regulators for angiosperms, is there any hope that the growth of Douglas-fir can ever be controlled by chemical growth regulators? We believe the answer is yes. But we must use compounds to which Douglas-fir is sensitive and we do not have these compounds in hand at this time.

Our approach is to isolate and identify chemically the naturally-occurring growth regulators in Douglas-fir, to determine how these regulators control growth processes, and finally how we can use those regulators to control the growth of Douglas-fir. Let us now consider some studies currently in progress at Oregon State University.

In his review, "Dormancy in Woody Plants," Samish suggests that the dormant period of perennials is not a homogeneous phenomenon, but rather a series of distinctly different physiological states. Ho terms these periods as °quiescence," "preliminary rest," "mid-rest," and "after-rest." Each state is defined by the growth response produced by an environment favorable to growth. The growth which may be expected during quiescence, preliminary rest, or after-rest is much more vigorous than that which occurs during mid-rest.

Interest in the natural and potential artificial regulation of the dormancy of Douglas-fir was stimulated at Oregon State University by the above evidence and by evidence that seedlings disturbed during routine nursery lifting procedures in the period from late September until early December were much less able to withstand stress than were plants lifted from December to March (20). One tenable hypothesis for these data is that physical disturbance during the "mid-rest" phase of dormancy results in a severe delay in the normal sequence of concentrations of growth regulators.

The first of a series of experiments expected to establish the validity of the above hypothesis was designed to define the seedling tissues which are the sites of growth regulator synthesis during the dormancy period. Data from

this study indicated that: (1) seedling buds are the major site of shythcsis of growth regulatory material; (2) the growth stimulatory substance (or substances) produced by active buds are not translocated to dormant buds; (3) lateral moristom growth is stimulated by materials exported by active buds higher on the shoot; and (4) root growth is independent of shoot activity (23).

The second series of experiments was conducted to ascertain whether application of growth regulatory materials to decapitated seedling apices could change the regulatory system for the plant as a whole. These materials, indoleacetic acid and gibberellic acid, did not affect the activity of the roots or buds. Neither did they stimulate activity of lateral meristoms in shoots, except in the period of transition from mid-rest to after-rest. The effect of indoleacetic acid in this period of transition provides a clue to the manner in which the growth-regulatory system may work. In the fall, buds may contain such an accumulation of inhibitors that moristems cannot be activated even with the application of exogenous growth promoters. At the end of mid-rest, the biological activity of the inhibitors seems to diminish but synthesis of intrinsic auxin is not sufficient to stimulate the growth of lateral meristoms as much as does the application of exogenous indoleacetic acid. It is during this period, also, that the effect of long photoperiods in stimulating bud activity first begins to lessen, and that the foliage appears to export materials which stimulate bud activity (23). In after-rest , concentrations of inhibitors in the buds are very probably sharply reduced and the production of auxin increased to a level where addition of exogenous auxin fails to stimulate meristomatic activity (20).

The third series of experiments was designed to measure the effects of girdling, defoliation, and debudding of seedlings together with applications of indoleacotic acid and gibberellic acid (19). Results from this study indicate that: (1) the activity of seedling root systems, although apparently independent of measurable shoot growth is, in fact, absolutely dependent upon materials exported from the shoot; these materials may or may not include growth regulators; (2) the lateral meristoms of seedlings which were defoliated produced no now xylem elements until the growth of buds higher on the stem had produced fully expanded foliage. It appears, then, as though the leaves produce a substance necessary for lateral moristem activation. This substance may be a growth regulator.

Another series of experiments approached the problem from a different direction. We extracted Douglas-fir buds throughout the dormancy period and measured the levels of growth regulatory substances with the Avena colooptile section bioassay. This experiment was disappointing in that we wore not able to detect any substantial changes in growth regulators during the dormancy period. We did detect a very potent growth inhibitor in the bud extracts, however.

One disadvantage of using Avena plants to detect growth regulators from Douglas-fir is that the Avena plants may not be sensitive to the same regulators that Douglas-fir is. Since we are interested in growth regulators for Douglas-fir, we devised an assay using Douglas-fir plants. This assay can detect 0.1 microgram (10^{-7} g) of indoleacetic acid. The response is somewhat variable but it is comparable in sensitivity to the commonly used Avena coleoptilo section assay. The next slide shows a treated Douglas-fir seedling. The distance between the cotyledons and uppermost portion of the shoot is measured; approximately 1 mg of lanolin containing the 'substance to be measured is applied to

the side of the shoot; the plant is grown for 4 weeks in the greenhouse, and the distance between cotyledons and uppermost portion of the shoot is again measured. The increase in shoot length is a measure of the effectiveness of the substance contained in the lanolin in promoting or retarding growth in the Douglas-fir seedling. The next slide shows some results we have obtained with this assay using the well-known growth promoter, indoleacetic acid.

The next slide is a graph showing the separation of regulators obtained from a bud extract on a paper chromatogram using the Avena coleoptile section assay as a detector. The area of strong inhibition appeared in all of our bud extracts. When this same chromatogram was assayed using Douglas-fir plants instead of the Avena test, the reverse reaction was obtained. That is, the area on the chromatogram which was inhibitory for Avena coleoptiles apparently stimulated the growth of Douglas-fir seedlings. Here is a graph of the results. The area of growth promotion for Douglas-fir appears to be the same as that which inhibited the growth of oat coleoptiles.

These data can be explained in one of several ways. There may be several substances involved, one of which inhibits Avena growth but not Douglas-fir and one substance which promotes Douglas-fir but has no effect on Avena coleoptile growth; or there may be only one active component which both inhibits Avena coleoptile growth but promotes Douglas-fir growth. We are currently attempting to identify chemically the growth substances in our extracts and define those which are active on Douglas-fir.

Once we know the identity of the natural growth regulators in Douglas-fir, we can then attempt to control the growth of Douglas-fir by applying these regulators directly on the seedlings. I don't believe it is necessary to describe in detail to this group the advantages of being able to control dormancy or height growth or root growth in Douglas-fir seedlings. I believe we will be able to do these things in the foreseeable future.

TABLE I

Growth Regulatory Chemicals Applied
to Douglas-Fir Seedlings

B-995 (N-dimethylaminosuccinamic acid)

CCC (2-chloroethyl trimethylammonium chloride)

Phosphon-D (tributyl-2,4-dichlorobenzylphosphonium chloride)

Maleic hydrazide

Naringenin (4', 5, 7-trihydroxyflavanone)

Abscisic Acid (Abscisin II) (Dormin) (3-methyl-5-(2', 6', 6'-trimethl-1' hydroxy-4'-keto'cyclo-hexa-2'enyl) cis-trans-2,4-pentadicnoic acid)

SD 8339 (6-Benzylamino)-9-(2-tetrahydrophyranyl)-9H-purine)

Gibberellic acid

Indoleacetic acid

Alpha-naphthaleneacetic acid

Indoiebutyric acid

Kinetin (6-furfuryaminopurine)

MDB (2-methoxy-3,6-dichlorobenzoic acid)

TPP (2,4,5-trichlorophenoxyproprionic acid)

Bibliography

1. Allen, R. M. Changes in acid growth substances in terminal buds of Longleaf Pine saplings during the breaking of winter dormancy. Physiologia Plantarom 13(3) 555-B. 1960.

2. Austin, R. C. and Robert Strand. Date of Lifting Symposium. Minutes by R. C. Austin, Crown Zellerbach Corp. 5p. mimeographed. 1961.

3. Batjer, L. P., Max W. Williams and George C. Martin. Effects of N-dimethyl amino succinamic acid (B-Nine) on vegetative and fruit characteristics of apples, pears, and sweet cherries. Proceedings of the American Society for Horticultural Science 85:11-16. 1963.

4. Bonner, James. Plant Biochemistry. Academic Press, Inc., New York, N.Y. 1950. 537 p.

5. Cathey, Henry 11. Physiology of Growth Retarding Chemicals. Annual Review of Plant Physiology. Vol. 15 p. 271-300. 1964.

6. Cathey, H. N. and Y. W. Stuart. 1961. Comparative Plant growth retarding activity of AM)-1618, Phosfon and CCC. Botanical Gazette 123(1): 51-57.

7. Ching, K. K. and T. M. Ching. Extracting Douglas-fir Pollen and Effects of Gibberellic on its Germination. Forest Science Vol. 5(1) p. 74-80. 1959.

8. Corms, William G. 14th Annual North Central Weed Control Conference 1957 Research Report p. 144-45.

9. Cornforth, J. W., B. V. Milborrow and G. Ryback. Synthesis of (+) Abscisin II). Nature -4895 p. 715. 1965. Vol. 206.

10. Cornforth, J. U., B. V. Milborrow and G. Ryback. Nature 210, 628. 1966.

11. Cornforth, J. W., B. V. Milborrow, G. Ryback, M. Rothwell and R. L. Main. Nature 211, 742 (1966).

12. Dinus, Ronald John. Chromatographic Studies of the Growth Hormones Present in the Buds of Different-Aged Douglas-Fir (Pseudotsuga menziesii (Mirb.). Franco) Trees. M.S. Thesis. University of Washington. 1963. 82p.

13. Eagles, C. F. and P. F. Wareing. Experimental Induction of Dormancy in Betula pubescens. Nature 199:3745(4396). 1963.

14. Flemion, Florence. A Rapid Method for Determining the Viability of Dormant Seeds. Contribution from the Boyce Thompson Institute for Plant Research p. 339-351. Volume 9. 1938.

15. Report on Forest Research for the year ending March, 1955. Forestry Commission, London 1956. 140 p.

16. Harada, H. and A. Lang. Effect of some (2-chloroethyl) trimehtylammonium chloride analogs and other growth retardants on gibberellin biosynthesis in Fusarium moniliforme. Plant Physiology 40:176-183. 1965.

17. Hoitmuller, H. H. Untersuchungen uber die Wirkung synthetischer Wuchsstoffe auf die Secklins bewurzelung bei Waldbaurmen. Zeitschrift fur Forst genot. 1(4), 1952 p. 100-7) F. A. 14-1. 1953. #296.

18. Hendershott, C. H. and D. R. Walker. Identification of a growth inhibitor from extracts of dormant peach flower buds. Science, 130, 798-800. 1959.

19. Hermann, R. K. and D. P. Lavender. Effects of Debudding, Defoliating and Girdling on the Activity of the Shoot and Root Meristems in Douglas-Fir Seedlings. Abstr. in Northwest Science 47(1) p. 52. 1967.

20. Hermann, Richard K. and Denis P. Lavender. Physiological Changes in Dormant Douglas-Fir Seedlings and Their Implications for Nursery and Planting Practice. Paper presented at the International Union of Forest Research Organizations Meeting at Munich, Germany. 1967.

21. Highly, Terry L. The Flavenoid Compounds of Three Floral Phenotypes of Douglas Fir. MS Thesis, Oregon State University, 1964. 69 p.

22. Kung, Peter F. S. and Kim K. Ching. Effects of Phesion-D on the Growth of Douglas-fir Seedlings. In preparation. 1967.

23. Lavender, D. P. and R. K. Hermann. Further Observations on the Dormancy Period of Douglas Fir Seedlings. Journal of Forestry 65(3) 225. 1967.

24. Marth, Paul C., William V. Audia and John W. Mitchell. Effects of Gibberellic Acid on Growth and Development of Plants of Various General and Species. Botanical Gazette Vol. 118(2) p. 106-111. 1956.

25. Ninnemann, H. D., A. D. Zeevaart, H. Kende and A. Lang. The plant growth retardant CCC as inhibitor and gibberellin biosynthesis in Fusarium moniliforme. Plants 61:229-235. 1964.

26. Overbeck, J. L. van, and J. E. Loeffler and M. Iona R. Mason. Dormin (Abscisin II), Inhibitor of Plant DNA Synthesis. Science 156 p. 1497-1499. 1967.

27. Pharis, Richard P., Manfred Ruddat, Cornell Phillips. Response of Conifers to Growth Retardants. Botanical Gazette 128(2) 105-9. 1967.

28. Phillips, I. D. J. Some Interactions of Gibberellic Acid with Naringenin (5,7,4'-trihydroxy flavanone) in the Control of Dormancy and Growth in Plants. Journal of Experimental Botany 13, 213-225. 1962.

29. Rediske, John H. Gibberellic Acid tested on Douglas-Fir Seedlings. Weyerhaeuser Company. Forestry Research Note Number 35. 1960. 8p.

30. Richardson, S. D. Radicle Elongation of P. menziesii in Relation to Light and Gibberellic Acid. Nature, Lond. 181(4606) p. 429-30. 1958.

31. Richardson, S. D. Germination of Douglas-fir Seed as Affected by Light, Temperature and Gibberellic Acid. Forest Science 5(2):174181. 1959.

32. Rietveld, Willis James. Effects of Three Plant Growth Regulators upon Growth Patterns in Seedlings of Three Races of Douglas-fir, Pseud menziesii (Dario.) Franco. M.S. Thesis, Oregon State University, 1967. 62 p.

33. Robinson, P. M. and P. F. Wareing. Chemical Nature and biological properties of the inhibitor varying with photoperiod in sycamore (Ater pseudoplantanus). Physiologia Plantarum 17(2) 314-323. 1964.

34. Sarnish, R. N, Dormancy in woody plants. Ann. Rev. Plant Physiology 5:183-204. 1954.

35. Strand, R. F. and R. C. Austin. Influence of Gibberellins on Height Growth of Douglas Fir and Western Hemlock – Three Trials. Crown Zellerbach. Central Research Department. 1958. 11 p. mimeo.

36. Weaver, R. J., J. van Overbeek, and R. M. Pool. Induction of fruit set in Vitis vinifera L. by a kinin. Nature 206:952-953 (1935).

37, Went, F. W. and Kenneth V. Thimann. Phytohormones. New York, The MacMillan Company, 1937. 294p.

38. Wheat, J. G. Effect of Phosion-D on potted Douglas fir. Tree Planters Notes 70 p. 15-16. 1965.

39. Young, David Alfred. Experiments with the Diffusion Method of Auxin Removal from Douglas Fir (Pseudotsuga menziesii (Mirb.) Franco) Seedlings. M.S. Thesis, University of Washington, 1961.