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EFFECTS OF GIBBERELLIN ON SEEDLINGS
OF NINE FOREST-TREE SPECIES

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
Frank W. Kearns

1958

**EFFECTS OF GIBBERELLIN ON SEEDLINGS
OF NINE FOREST-TREE SPECIES**

By

FRANK W. KEARNS

A THESIS

**Submitted to the College of Agriculture
Michigan State University of Agriculture
and Applied Science in partial fulfillment
of the requirements for the degree of**

MASTER OF SCIENCE

Department of Forestry

1958

Gibberellin has been tested on a wide range of plants in recent years and has usually been effective in producing significant increases in height growth in most plants.

Starting in May 1957 a study was conducted at Michigan State University to test the effects of gibberellin on the seeds and seedlings of various forest tree species.

The effects of gibberellin on seed germination were tested in a replicated experiment using 1,200 seeds each of Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco), Norway spruce (Picea abies (L.) Karst), Scotch pine (Pinus sylvestris L.), eastern white pine (Pinus strobus L.), jack pine (Pinus banksiana Lamb.), and white spruce (Picea glauca (Moench) Voss). All the seeds were partially excised to insure penetration of the solution to the embryo. They were soaked for periods of 6, 12, or 24 hours in aqueous solutions containing 0, 10, 100, or 1,000 parts per million gibberellin. The differences in germination rate due to treatment were not significant in any of the six species. However, the gibberellin treatment caused significant differences in germination percentage for three of the species; Douglas-fir and Scotch pine were increased whereas Norway spruce was decreased.

In the spring and summer of 1957 three different experiments were established to determine the effects of foliar applications of gibberellin on nursery stock. In the first experiment second-year seedlings of Scotch pine,

jack pine, and white spruce were sprayed on May 6 and May 26 with 0, 10, 100, or 1,000 parts per million gibberellin. In the second experiment seedlings of the same species and of sugar maple (Acer saccharum Marsh.) were sprayed on July 2 with 0, 5,000, 10,000 or 20,000 parts per million gibberellin. In the third experiment seedlings of Scotch pine, jack pine, eastern white pine, red pine (Pinus resinosa Ait.), Douglas-fir, white spruce, black locust (Robinia pseudoacacia L.) and sugar maple were sprayed on July 24 and August 14 with 0, 10, 100, or 1,000 parts per million gibberellin. There were noticeable height growth increases in the sugar maple seedlings sprayed with 20,000 parts per million solution in early July and in the Douglas-fir and Scotch pine seedlings sprayed with the late summer application of 1,000 parts per million. The jack pine treated in early July became noticeably chlorotic and the black locust treated in late summer had smaller leaves than the controls but neither species showed a height growth response. Treatments made at other times and with other species produced no noticeable effects.

In a greenhouse experiment 6-week-old seedlings of eastern white pine, Douglas-fir, and Norway spruce were sprayed with four weekly applications of 0, 100, or 1,000 parts per million gibberellin solution to which Volk oil was added to increase penetration. In all three species the needles of the treated seedlings were significantly yellower than were those of the

FRANK W. KEARNS

ABSTRACT

controls. In addition, the treatments resulted in significantly increased height growth in eastern white pine and in a significant delay in the formation of terminal buds in Douglas-fir.

ACKNOWLEDGEMENTS

The author wishes to express his sincere thanks to his major professor, Dr. Jonathan W. Wright under whose guidance this study and thesis has been completed. He is also greatly indebted to Drs. S. H. Wittwer and M. J. Bukovac of the Horticulture Department for their valuable suggestions in laying the ground-work for the experiments. Grateful acknowledgement is due William E. Taylor, graduate student at Michigan State University, for his assistance in gathering and compiling data. The writer deeply appreciates the contributions of his wife, Sally, in making this thesis a reality.

This work was conducted under a grant from the Chas. Pfizer Co., of Brooklyn, New York.

TABLE OF CONTENTS

	Page
I. Introduction	1
II. Review of literature	3
III. Materials and methods	8
A. Seed treatments	8
B. Seedling treatments	10
IV. Results	14
V. Discussion	24
VI. Summary	25
VII. Literature cited	

LIST OF TABLES

Table	Page
1. Woody ornamental plants that gave positive growth response when sprayed with gibberellin (from McVey and Wittwer 1958)	6
2. Days required for germination of tree seeds soaked in gibberellin solutions	15
3. Germination percentage of tree seeds soaked in gibberellin solutions	16
4. Germination percentage of tree seeds soaked in gibberellin solutions (Summary of Table 3)	17
5. Germination times and percentages for entire and nicked seeds of conifer tree species	19
6. Height growth from July 26 to September 9, 1957 of second-year seedlings sprayed with gibberellin solutions	21
7. Response of first-year seedlings to four weekly applications of gibberellin	23

INTRODUCTION

In 1932, Japanese scientists reported that a plant growth-promoting substance was present in cultured filtrates of the fungus Gibberella fujikuroi (Saw.) Wr., (Kurosawa 1932). This substance was determined to be the causative agent of the "Bakanae" or "foolish seedling" rice disease which caused the affected rice seedlings to grow very rapidly at first, then die. In 1938, this growth-promoting substance was isolated and called gibberellin. In recent years workers in Japan, England, and the United States have isolated four different gibberellin compounds, A_1 , A_2 , A_3 , and A_4 . These compounds differ only slightly from each other chemically, but they do not have similar properties in all physiological systems (Bukovac and Wittwer 1958). All the results quoted in this paper are based on tests made with a mixture of gibberellins A_1 and A_3 .

The obscurity of the original Japanese publications and the occurrence of World War II delayed widespread study of this phenomenal discovery. Since 1954, however, numerous workers in Japan, the United States, England, and other countries have made intensive studies of gibberellin and its biological properties. These experiments have been conducted on a large number of field crops. Few have been made on woody plants. In the majority of cases the gibberellin has stimulated height growth and hastened flowering. In many cases it also stimulated seed germination (Bukovac and Wittwer 1956; Marth, Audia, and Mitchell 1956; Lang 1956; Wittwer and Bukovac 1957).

It has been determined by Kato (1958) that gibberellin is not an auxin. When both gibberellin and auxin were applied to pea seedlings, the gibberellin overcame the inhibition of shoot growth produced by the auxin. At the same time root formation, normally stimulated by the auxin, was inhibited by the gibberellin.

The use of chemicals by foresters to promote desirable responses in trees is not new. They have experimented widely with the various weed-killing chemicals and with those in the auxin group. Forest tree nurserymen are interested in gibberellin seed treatments because of the possibility that they would hasten germination. This would mean higher plant percentages in years of adverse weather conditions. They are also interested in the increased growth rate that often results from gibberellin treatment. It now takes two to four years to produce plantable pine or spruce seedlings. Reduction of this period by a year would mean large nursery savings.

Gibberellin treatments that could be given just prior to outplanting to hasten growth of tree seedlings in the first year in the field would be especially valuable in forestry. Normally such seedlings grow very little the first year after being removed from the nursery and suffer from competition with already established weedy vegetation. Any treatment which would enable the young trees to get off to a good start would not only increase survival but would shorten the rotation age.

REVIEW OF LITERATURE

The class of growth-promoting substance called gibberellins is successful in inducing and accelerating many types of plant growth.

Some 22 years after the original discovery of gibberellins by Japanese scientists, a group of British workers, Brian et al. (1954) noted accelerated stem elongation on seedlings of wheat, pea, oats and other vegetable and field crops when they were grown in nutrient solutions containing 5 parts per million gibberellin. They also observed that chlorosis and leaf roll developed, especially in the low nutrient solutions. Concentrations of gibberellic acid from 10 to 100 parts per million did not increase the acceleration in growth and concentrations of 250 to 1000 parts per million were inhibitory.

Further success in treating vegetables grown in the greenhouse was obtained by applying an aqueous solution of gibberellin directly in the growing tips of the plants. Quantities of 10 to 20 micrograms per plant induced marked elongation of internodes and often caused the leaves to broaden or elongate. In the early growth stages of celery plant heights and petiole lengths were almost doubled, and fresh and dry weights increased by about 50 percent (Bukovac and Wittwer 1956).

It was found that the tedious process of applying the gibberellin solution to the individual growing tips of the plants could be lessened by using an aqueous spray solution applied under pressures of 75 to 100 pounds per square inch. Wittwer and Bukovac (1957a) produced varied growth responses

THEORY OF THE CASE

The following is a summary of the facts and circumstances of the case.

The plaintiff, a resident of the State of New York, is a citizen of the State of New York.

The defendant, a resident of the State of New York, is a citizen of the State of New York.

The plaintiff and the defendant are both residents of the State of New York.

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from many vegetables both in the greenhouse and under field conditions by the application of foliar sprays of varying concentrations.

Lang (1956) found that carrots and other plants which require cold could be induced to flower without cold treatment by the application of gibberellin. Gibberellin treatments also seem to equal the effects of long day exposure in producing rapid flowering response in certain flowering plants.

Barton, Fine, and Chandler (1957) were able to break epicotyl dormancy of tree peony seedlings which had not received cold treatment by the application of 1, 10, or 100 micrograms of gibberellin to the hypocotyl of the germinated seed.

In the treatment of seeds, Wittwer and Bukovac (1957b) showed that gibberellin incorporated with a slurry protectant and applied to the seed coat of peas and beans promoted earlier emergence of the seedlings in both greenhouse and field plantings. Heights, length of hypocotyls, and length of internodes of plants grown from treated seeds were directly related to the concentration of gibberellin in the slurry. Concentrations of gibberellin in a slurry ranging from 500 to 1,000 parts per million promoted early emergence, but resulted in excessive elongation of stems and severe seedling abnormalities.

Various growth responses of woody plants to gibberellin have been obtained. McVey and Wittwer (1958) reported a variety of responses from thirteen different woody ornamentals sprayed with varying concentrations of gibberellin either as a single application or at weekly intervals during a

4-month period. Several of the ornamentals showed marked stem elongation, an increase in node number, continuous growth of shoots and a succession of growth flushes from otherwise dormant buds (Table 1). In only one species, Japanese yew (Taxus cuspidata Sieb. and Zucc.), was there a stunting effect. However, a chlorotic yellowing was noted in many of those which showed marked elongation response.

Scurfield and Moore (1958) treated yellow box (Eucalyptus melliodora A. Cunn.) with 0.1 milliliter of a gibberellin solution by cutting off the tip of the second leaf and immersing the stub in the solution; thereby allowing the solution to be absorbed into the tree. Treatments were made with concentrations of 1, 5, 25, and 100 parts per million, and there were twelve trees in each plot. Results showed that an increase in concentration of gibberellin caused an increase in stem height and diameter, number of leaves and weight of stems, but caused a decrease in weights of leaf and root. Total weight was not significantly affected.

Pfnur (1958) reported that there was no increase in rate of germination of seeds of eastern white pine and Norway spruce after soaking for 12 hours in gibberellin solutions of 5, 10, 25, or 250 parts per million.

Marth, Audia and Mitchell (1956) applied gibberellin to the young stem tissue in a 1.0 percent lanolin paste mixture and noted increases of stem length over that of controls to be 400 percent for Japanese red maple (Acer palmatum L.), 109 percent for sugar maple (Acer saccharum Marsh.), 48 to 119 percent for willow oak (Quercus phellos L.), 40 percent for hybrid poplar

• *Staphylococcus aureus* (Staph aureus) • *Staphylococcus epidermidis* (Staph epidermidis)

• *Streptococcus pneumoniae* (Pneumococcus) • *Streptococcus pyogenes* (Group A streptococcus)

• *Streptococcus agalactiae* (Group B streptococcus) • *Streptococcus dysgalactiae* (Group G streptococcus)
• *Streptococcus mitis* (Group D streptococcus) • *Streptococcus viridans* (Group F streptococcus)

• *Streptococcus thermophilus* (Group C streptococcus) • *Streptococcus salivarius* (Group E streptococcus)
• *Streptococcus faecalis* (Group B streptococcus) • *Streptococcus faecium* (Group A streptococcus)

• *Streptococcus lactis* (Group D streptococcus) • *Streptococcus thermophilus* (Group C streptococcus)
• *Streptococcus salivarius* (Group E streptococcus) • *Streptococcus faecalis* (Group B streptococcus)

• *Streptococcus faecium* (Group A streptococcus) • *Streptococcus lactis* (Group D streptococcus)
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Table 1. Woody ornamental plants that gave positive growth responses
when sprayed with gibberellin
 (from McVey and Wittwer 1958)

6

Species	: Relative height growth (as a percent of the control)				
	: After a single spraying with :			: After weekly spray	
	: gibberellin solutions in			: with gibberellin	
	: concentrations of --			: solutions in con-	
	: concentrations of --			: concentrations of --	
	: 10	: 100	: 1000	: 10	: 100
	: p.p.m.	: p.p.m.	: p.p.m.	: p.p.m.	: p.p.m.
	<u>Percent</u>	<u>Percent</u>	<u>Percent</u>	<u>Percent</u>	<u>Percent</u>
<u>Euonymus fortunei</u> <u>vegetus</u>	155	228	408 ^{1/}	186	465 ^{1/}
<u>Forsythia</u> "Arnold Dwarf"	112	190 ^{1/}	275 ^{1/}	200 ^{1/}	375 ^{2/}
<u>Ligustrum obtusifolium</u> <u>vicari</u>	133	148	202 ^{1/}	160 ^{1/}	338 ^{2/}
<u>Magnolia soulangeana</u>	120	201 ^{1/}	224 ^{2/}	129	198
<u>Phellodendron amurense</u>	92	95	153 ^{1/}	103	215 ^{2/}
<u>Berberis thunbergi</u> "Crimson Pygmy"	124	140	138	157	165 ^{1/}
<u>Thuja occidentalis</u> <u>hoveyi</u>	121 ^{1/}	121 ^{1/}	116	121 ^{1/}	90
<u>Viburnum opulus nana</u>	129	143 ^{1/}	123	129	162 ^{1/}

^{1/} Differ from the control values at the 5 percent level.

^{2/} Differ from the control values at the 1 percent level.

(Northeastern Forest Experiment Station OP-247 Populus sp.), 200 percent for yellow-poplar (Liriodendron tulipifera L.), 28 to 68 percent for Virginia pine (Pinus virginiana L.), 0 to 22 percent for white spruce (Picea glauca (Moench) Voss.), and 0 percent for eastern white pine (Pinus strobus L.). Using a spray of 400 parts per million concentration resulted in a 40 percent height increase for loblolly pine (Pinus taeda L.).

Nelson (1957) used a 1.0 percent lanolin solution of gibberellin applied to the young stems of eleven southern tree species grown in a greenhouse. He found that treatment produced increases in height growth from 75 to 353 percent on eastern cottonwood (Populus deltoides Bartr.), American sycamore (Platanus occidentalis L.), yellow-poplar, sweetgum (Liquidambar styraciflua L.), cherrybark oak (Quercus falcata var pagodaefolia Ell.). White oak (Quercus alba L.), eastern white pine, Arizona cypress (Cupressus arizonica Greene) and water oak (Quercus nigra L.) exhibited no significant increases as a result of treatment.

MATERIALS AND METHODS

Seed Treatments

Two experiments were conducted to test the effect of soaking conifer seeds in a gibberellin solution of varying concentrations.

Experiment I. Experiment I was started in May 1957. Approximately 100 seeds each of red pine (Pinus resinosa Ait.), Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco) and white spruce were soaked in gibberellin solutions of 0, 100, or 1,000 parts per million at room temperature for 120 hours. The seeds were then planted in a flat of vermiculite and placed in the Forestry Department greenhouse. Germination was observed and seedling heights were measured 30 days after the seeds were planted.

Experiment IIa. Experiment IIa was a test of the effects of gibberellin on seed germination rate. It was conducted during January and February 1958. The seed coats were partially removed from 1200 seeds each of Douglas-fir, white spruce, Norway spruce (Picea abies (L) Karst.), jack pine (Pinus banksiana Lamb.), eastern white pine, and Scotch pine (Pinus sylvestris L.). This was accomplished by carefully nicking one end of each seed with a sharp knife. Seeds that had been obviously damaged in the process were discarded. The partial excision was undertaken to insure that the solutions actually penetrated to the embryo.

The nicked seeds were soaked in concentrations of gibberellin of 0, 10, 100, or 1,000 parts per million for 6, 12, and 24 hour periods at 6°C. They

were planted in the Forestry Department greenhouse in a seed bed of unsterilized topsoil and sand covered with one inch of vermiculite. Each of the six species were treated as a separate randomized block experiment with five replications of 20 seeds in each treatment plot. Germination counts were made every day for 20 days, after which no further germination took place.

Experiment IIb. Experiment IIb was designed to test germination rate differences between nicked and entire seeds of the same species that were used in Experiment IIa. Both types of seed were soaked for 12 hours at 6°C in an aqueous solution containing 100 parts per million gibberellin. They were planted in the greenhouse in a randomized block design with 20 seeds in each plot. Germination counts were made every 2 days for 20 days.

Seedling Treatments

Experiment III. Experiment III was set up in the Bogue Nursery on the campus of Michigan State University in May 1957. Its purpose was to test the effect of an aqueous spray of gibberellin on second-year seedlings of white spruce, Scotch pine, and jack pine.

Aqueous solutions of gibberellin were made up on concentrations of 0, 10, 100 and 1,000 parts per million, with 1.5 gram of "Tween 20"^{1/} per liter added as a wetting agent. Each concentration was sprayed on a 1 x 2 - foot plot of seedlings. There were five replications per species. Each plot was sprayed twice, first on May 6 and again on May 26. At the time of the first spraying the buds were just beginning to break dormancy. The spray was directed downward on the tops of the seedlings at a pressure of 100 pounds per square inch. A cardboard shield was used to prevent the spray from blowing on adjacent seedlings. The seedlings remained dry for 12 hours after each treatment. Height measurements and scoring for other growth responses were made on June 27. However, an inadvertent mowing of the trees in the experiment by the nursery crew in early July made further measurements useless.

^{1/} "Tween 20" is the trade name of a non-ionic, polyoxyethylene sorbitan monolaurate distributed by the Atlas Powder Company.

1. The first part of the document discusses the importance of maintaining accurate records of all transactions and activities. It emphasizes that proper record-keeping is essential for transparency and accountability, particularly in financial matters. The text suggests that organizations should implement robust systems to track and document every aspect of their operations, from procurement to sales.

2. The second section addresses the challenges associated with data management and security. It highlights the need for organizations to protect sensitive information from unauthorized access and breaches. The text recommends the use of secure storage solutions and the implementation of strict access controls to ensure that data remains confidential and intact.

3. The third part of the document focuses on the importance of regular audits and reviews. It states that periodic assessments are necessary to identify potential weaknesses and areas for improvement. The text encourages organizations to conduct thorough audits of their financial statements, operational processes, and compliance with relevant regulations.

4. The fourth section discusses the role of technology in enhancing organizational efficiency. It notes that the adoption of modern software and tools can significantly streamline workflows and reduce the risk of human error. The text suggests that organizations should invest in training to ensure that employees are proficient in using the latest technologies.

5. The fifth part of the document touches upon the importance of clear communication and collaboration within an organization. It emphasizes that effective communication is key to ensuring that all team members are aligned with the organization's goals and objectives. The text recommends the use of regular meetings and clear channels of communication to foster a collaborative work environment.

6. The sixth section addresses the importance of staying up-to-date with industry trends and regulations. It notes that organizations must be proactive in monitoring changes in the market and regulatory landscape to remain competitive and compliant. The text suggests that organizations should establish a dedicated team or function to track and analyze these changes.

7. The seventh part of the document discusses the importance of maintaining a strong corporate culture. It states that a positive and values-driven culture can lead to higher employee morale and productivity. The text recommends that organizations clearly define their core values and ensure that these are reflected in all aspects of their operations.

8. The eighth section touches upon the importance of risk management. It notes that organizations should identify potential risks to their operations and develop strategies to mitigate these risks. The text suggests that organizations should conduct regular risk assessments and have contingency plans in place to respond to any unforeseen events.

9. The ninth part of the document discusses the importance of financial planning and budgeting. It states that organizations should have a clear understanding of their financial position and develop a realistic budget for the upcoming period. The text recommends that organizations should review their budget regularly to ensure that they are staying on track.

10. The final section of the document provides a summary of the key points discussed. It reiterates the importance of transparency, security, audits, technology, communication, industry awareness, culture, risk management, and financial planning. The text concludes by stating that these practices are essential for the long-term success and sustainability of any organization.

Experiment IV. Experiment IV was started on July 2 in the Bogue Nursery. In this experiment a single 3 x 5 - foot plot of each of three species was sprayed with 5,000, 10,000 or 20,000 parts per million gibberellin solution to which 1.5 grams of "Tween 20" per liter was added as a wetting agent. The species sprayed were white spruce, jack pine, Scotch pine, and sugar maple. On August 20 the seedlings were measured and scored for color differences.

Experiment V. Experiment V was also conducted in the Bogue Nursery during July and August, 1957. It was similar to Experiment III in concentrations used and method of application. It differed from that experiment in number of species treated, efficiency of experimental design, and date of application.

The species tested were white spruce, Scotch pine, jack pine, red pine, eastern white pine, Douglas-fir, sugar maple, and black locust (Robinia pseudoacacia L.).

A separate experiment was established for each species. Each experiment consisted of five randomized blocks of four plots each. Each plot contained four seedlings with a growing space of 12 square inches per seedling. The treated seedlings were chosen because of their uniformity. Two methods of thinning were used: the seedlings were pulled up by the roots or were cut off at ground level with shears. All trees received an application of soluble fertilizer (20 - 24 - 15 analysis) before treatment.

Spray applications of gibberellin were made on July 24 and on August 14. On both days the spraying was done in the early evening during calm weather and with the additional aid of a cardboard shield to reduce any blowing effects of the spray. Height measurements to the nearest 0.5 centimeter were made prior to each treatment and on September 9.

Experiment VI. Experiment VI, a fourth test of the effect of a gibberellin spray on young tree seedlings, was carried out in the Forestry Department greenhouse. In February 1958 approximately 1,000 seeds each of eastern white pine, Douglas-fir, and Norway spruce were soaked in water for 16 days, treated with the seed protectant "Captan", and planted in a seed bed of topsoil and sand covered with one inch of vermiculite. On April 9, 6 weeks after the first germination, the seedlings were thinned to a density of 1 per 4 square inches and the spray treatments were begun.

The trees in this experiment were sprayed four times at intervals of a week, receiving 0, 100, or 1,000 parts per million gibberellin at each spraying. In addition to the gibberellin the spray solutions contained 1,500 and 5,000 parts per million of "Tween 20" and Volk oil respectively to increase wetting and penetration of the gibberellin into the seedlings. Each treatment was replicated four times with 20 trees per plot. A randomized block design was used.

The heights of the trees were measured to the nearest millimeter on April 9 and May 9 and other characteristics were scored on May 9.

Statistical Procedure

The data from all replicated experiments was subjected to analyses of variance and the results of these analyses are the basis for all statements of statistical significance. In all cases plot means were used in the analyses.

Where data was gathered in percentages the percentages were transformed and the analysis was made of the arc-sine of the percentage square-root.

In the analyses, treatment, time, replicate, and treatment x time interaction variances were calculated. The remainder was used as the error term for the calculation of the F values.

RESULTS

Experiment I. Soaking of red pine, Douglas-fir, and white spruce in varying strengths of gibberellin solution in 1957 resulted in no significant differences in germination rate. Nor were there observable differences among the treated lots and the controls in height or color at the end of 30 days, when the experiment was terminated.

Experiment IIa. The 1958 seed germination tests differed from those performed a year earlier in that a small corner of the seed coat was removed from each seed prior to soaking. This insured that the solution reached the embryo. Even so, the results were similar to those obtained earlier in that germination rate was not affected by treatment (Table 2). However, the gibberellin treatments did result in small but statistically significant effects on germination percentage in three of the species (Tables 3 and 4). In Douglas-fir and Scotch pine the gibberellin-treated seedlings germinated more completely than did the controls. In Norway spruce the reverse was true.

Experiment IIb. This experiment was not truly a part of the gibberellin experimentation but was performed to determine whether seed coat impermeability might have played a significant role in the comparative lack of response of conifer seeds to earlier gibberellin treatments. Also, it would show whether the type of partial seed excision used in performing Experiment IIa was in itself detrimental. The significantly

Table 2. Days required for germination of tree seeds soaked in gibberellin solutions^{1/2/}

15

Species	: Germination time after soaking in : Soaking : gibberellin solutions of concentrations --				
	: time	: 0	: 10	: 100	: 1000
	:	: p.p.m.	: p.p.m.	: p.p.m.	: p.p.m.
	<u>Hours</u>	<u>Days</u>	<u>Days</u>	<u>Days</u>	<u>Days</u>
Douglas-fir	6	11.4	11.1	9.9	11.7
	12	11.6	10.4	12.2	12.9
	24	12.5	10.8	11.8	10.5
Norway spruce	6	11.2	11.3	11.0	12.6
	12	9.9	9.0	10.2	11.4
	24	11.1	11.2	10.7	11.7
White spruce	6	16.4	15.3	13.2	16.5
	12	17.8	14.5	13.3	15.9
	24	14.3	13.7	15.4	16.5
Eastern white pine	6	13.1	13.6	15.9	16.6
	12	18.8	14.2	12.3	13.9
	24	15.6	12.0	13.7	13.1
Scotch pine	6	13.6	12.0	11.8	11.0
	12	12.2	13.6	11.9	11.6
	24	11.3	11.5	10.4	11.6
Jack pine	6	15.0	14.8	14.0	13.9
	12	14.3	14.1	14.8	14.0
	24	16.0	16.1	16.6	14.6

1/ 20 seeds were planted for each treatment in 5 replications for every species tested

2/ No statistical differences were found by analysis of variance.

Table 3. Germination percentage of tree seeds soaked in gibberellin solutions^{1/}

Species	Germination percentage after soaking in gibberellin solutions of concentrations --				
	Soaking Time	0	10	100	1000
		p.p.m.	p.p.m.	p.p.m.	p.p.m.
	Hours	Percent	Percent	Percent	Percent
Douglas-fir ^{2/}	6	24	22	50	26
	12	32	19	19	35
	24	16	19	17	37
Norway spruce ^{3/}	6	36	5	20	10
	12	32	6	17	35
	24	29	18	33	11
White spruce	6	7	22	12	9
	12	18	10	9	12
	24	13	16	18	16
Eastern white pine	6	6	4	6	3
	12	9	8	6	9
	24	14	3	9	7
Scotch pine ^{3/}	6	8	11	14	17
	12	3	17	10	12
	24	3	22	25	15
Jack pine	6	20	29	14	24
	12	18	13	17	8
	24	11	14	10	18

1/ Differences between lots soaked for different times were not statistically significant

2/ Differences between dosages were significant at the 5 percent level

3/ Differences between dosages were significant at the 1 percent level

Table 4. Germination percentage of tree seeds soaked in
gibberellin solutions
(Summary of Table 3)

Species	: Germination percentage after soaking with			
	: gibberellin solutions in concentrations of --			
	: 0	: 10	: 100	: 1000
	: p.p.m.	: p.p.m.	: p.p.m.	: p.p.m.
	<u>Percent</u>	<u>Percent</u>	<u>Percent</u>	<u>Percent</u>
Douglas-fir ^{1/}	24	20	28	32
Norway spruce ^{2/}	32	10	23	18
White spruce	13	16	13	12
Eastern white pine	10	5	7	6
Scotch pine ^{2/}	5	17	16	14
Jack pine	16	18	14	17

^{1/} Differences between dosages were significant at the 5 percent level

^{2/} Differences between dosages were significant at the 1 percent level

faster germination of the nicked eastern white pine seeds indicates that seed coat impermeability may indeed affect the ability of that species to respond to gibberellin treatments involving soaking of the seed (Table 5). However, the generally lower germination percentage of the nicked seeds indicates that although the nicking was carefully done, this method of exposing the embryos to the solutions was an undesirable experimental procedure.

Experiment III. In this first experiment in which a spray solution was used on conifers, unthinned rows of seedlings were sprayed to see if the trees would respond under ordinary nursery conditions. There were no observable differences between the heights of treated plots and the controls for jack pine, Scotch pine, and white spruce. About 3 weeks after the second spray treatment a color difference could be noted in the jack pine. The needles of these seedlings sprayed with 1,000 parts per million solution began to show chlorosis whereas the controls and other treated seedlings did not change color. The color difference disappeared within 2 months of its first occurrence.

Experiment IV. When no height differences were observed in Experiment III the decision was made to increase the concentration range of the gibberellin solutions in order to determine whether low dosage was the factor limiting response to treatment. Height growth differences were not apparent in jack pine, Scotch pine, or white spruce even when the

Table 5. Germination times and percentages for entire and nicked seeds of conifer tree species

Species	Germination time		Germination percentage	
	Entire	Nicked	Entire	Nicked
	Seeds	Seeds	Seeds	Seeds
	<u>Days</u>	<u>Days</u>	<u>Percent</u>	<u>Percent</u>
Jack pine	9.4	8.9	75	44
Eastern white pine ^{1/}	18.4	13.3	58	44
Norway spruce ^{2/}	9.6	12.5	65	14
Scotch pine	10.2	9.6	53	64
Douglas-fir	9.6	9.5	77	67
White spruce	11.7	12.9	45	31

^{1/} Differences of germination time significant at the 1 percent level

^{2/} Differences of germination percentage significant at the 1 percent level

concentration of the spray was increased to 20,000 parts per million. However, as in the previous experiment, the treated jack pine seedlings became noticeably yellower than the controls.

In contrast to the conifers the treated sugar maple seedlings showed a marked growth response to the high gibberellin concentrations. The plot treated with 20,000 parts per million grew approximately 30 percent taller and had narrower leaves than the controls. The increased height growth was due to greater internode length rather than to increased number of internodes.

Experiment V. In the preceding nursery experiments the seedlings had not been thinned and the outer rows showed more marked response to the gibberellin than did the inner rows, perhaps because of the better nutrition and better spray coverage of the outer rows. To overcome this source of experimental error in Experiment V, only inner rows were treated, and all plots were thinned and fertilized prior to treatment.

Douglas-fir and Scotch pine were the only species of the six conifers used in this experiment that showed significant differences in height growth due to treatment (Table 6). In both instances the plots treated with the highest dosage (1,000 parts per million) made the most growth. No chlorosis was evident in any of the conifers.

The black locust responded to treatment by developing more spindly terminal shoots and narrower leaves. The controls and treated plots

Table 6. Height growth from July 26 to September 9, 1957 of
second-year seedlings sprayed with gibberellin solutions

Species	: Height growth after spraying with gibberellin : solutions in concentrations of --			
	: 0	: 10	: 100	: 1000
	: p.p.m.	: p.p.m.	: p.p.m.	: p.p.m.
	<u>Milli-</u> <u>meters</u>	<u>Milli-</u> <u>meters</u>	<u>Milli-</u> <u>meters</u>	<u>Milli-</u> <u>meters</u>
White spruce	0	0	0	.2
Scotch pine ^{1/}	2	2	5	12
Jack pine	36	37	27	34
Red pine	7	4	8	8
Eastern white pine	15	11	9	24
Douglas-fir ^{2/}	37	46	25	64
Sugar maple	2	9	7	6
Black locust	307	385	301	315

^{1/} Differences between treatment means significant at 5 percent level

^{2/} Differences between treatment means significant at 1 percent level

made similar height growth. A high percentage of the treated black locust died during the winter of 1957-58.

Although the sugar maple responded with increased height growth when sprayed with 20,000 parts per million in early July, the late July and early August applications of 10 to 1,000 parts per million failed to produce significant responses.

Experiment VI. In this experiment an attempt was made to create optimum growth conditions and to insure that the gibberellin which was applied could be taken in by the seedlings. Accordingly the first-year seedlings growing in the greenhouse were thinned and fertilized prior to treatment and Volk oil was added as a spreader in the spray solution.

The treated plots were measured 32 days after the first of four weekly applications. The gibberellin treatments were effective in increasing height growth significantly in eastern white pine but not in Douglas-fir and Norway spruce (Table 7). However, the treated seedlings of all three species became significantly more chlorotic than the controls, and in Douglas-fir the treatments caused significant delays in the formation of dormant terminal buds.

Table 7. Response of first-year seedlings to four weekly applications of gibberellin

Dosage	Height	Trees with yellow foliage	Trees with terminal buds
	<u>Millimeters</u>	<u>Percent</u>	<u>Percent</u>
Eastern white pine			
0 p.p.m.	6.5	10	0
100 p.p.m.	10.0 ^{1/}	95 ^{2/}	0
1000 p.p.m.	11.0 ^{1/}	87 ^{2/}	0
Douglas-fir			
0 p.p.m.	5.1	10	62
100 p.p.m.	6.3	83 ^{2/}	18 ^{1/}
1000 p.p.m.	4.3	78 ^{2/}	38 ^{1/}
Norway spruce			
0 p.p.m.	7.8	8	82
100 p.p.m.	7.6	90 ^{2/}	45
1000 p.p.m.	9.4	92 ^{2/}	28

^{1/} Differs significantly from the control means at the 5 percent level

^{2/} Differs significantly from the control means at the 1 percent level

DISCUSSION

The foliar spray application of gibberellin used in this study produced a lower level of response in the species tested than did the lanolin paste application used by Marth, Audia and Mitchell (1956) and Nelson (1957). A possible reason for this lesser response might be that the gibberellin was not being absorbed into the regions of the plant where current growth was taking place. This would appear to be the case where the concentrations of 20,000 parts per million gibberellin did not produce appreciable results in conifers, when it is known that a lanolin paste application of 1000 parts per million has caused significant height growth responses in many of the same species that were tested in this experiment.

Future studies might then be made to find a suitable carrying agent which would allow adequate absorption of gibberellin into the plant growing regions.

There are two other factors which probably affected the response of the seedlings in this experiment: the stage of growth of the trees treated and the season of application. We cannot evaluate these factors in this study as there was so much unaccountable variation in the response of the species in different experiments.

A final factor causing the low level of response of the conifers to the gibberellin spray treatment might be the inherent difference which exist between angiosperms and gymnosperms. They differ quite markedly in some of their physiological responses and it may well be that the foliar spray is effective in treating broadleaf species, but is just not effective on gymnosperms.

SUMMARY

In a study to determine the effects of gibberellin on seeds and seedlings of various forest tree species, experimental work was divided into three parts: (1) greenhouse experiments on the germination of partially excised seeds soaked in gibberellin, (2) greenhouse experiments on the growth of first-year seedlings, sprayed with gibberellin, and (3) nursery experiments on the growth of second-year seedlings sprayed with gibberellin. The species used were Scotch pine, jack pine, red pine, eastern white pine, white spruce, Norway spruce, Douglas-fir, sugar maple and black locust. In all cases the gibberellin was applied as an aqueous solution to which a wetting agent, either "Tween-20" or Volk oil was added. The dosages varied from 0 to 1,000 parts per million except in one trial where dosages up to 20,000 parts per million were used.

Gibberellin-soaked seeds of six conifer species germinated at the same rate as did the controls. However, the germination percent of three of the species was significantly affected by treatment. In Douglas-fir and Scotch pine the percentage was increased, whereas in Norway spruce the reverse was true.

First-year seedlings of three conifer species growing in a greenhouse showed statistically significant responses to four weekly applications of gibberellin when scored 32 days after the first application. The treated seedlings were yellower than the controls in each of the

three species. Also, in eastern white pine there was an increase in height growth whereas in Douglas-fir the treatment caused a delay in the formation of dormant terminal buds.

Unthinned rows of second-year nursery seedlings of three conifer species showed no growth rate responses to late spring gibberellin treatments. Nor did the same species show height growth differences when sprayed in mid-summer with extremely high concentrations (20,000 parts per million). However, jack pine seedlings developed temporary chlorosis after both levels of treatment. Second-year sugar maple seedlings which were sprayed with the 20,000 parts per million solution grew appreciably taller than the controls and had smaller leaves.

In another nursery experiment the plots were thinned and fertilized prior to gibberellin treatment in late summer. Of six conifer species tested in this experiment only Scotch pine and Douglas-fir responded. In both cases the gibberellin-treated seedlings grew significantly faster than the controls. Of the two hardwood species included in this test sugar maple failed to respond to the gibberellin treatment and black locust responded with reduced leaf and stem size.

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