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FLORAL ENHANCEMENT OF THE PICEA GENUS
THROUGH HORMONAL AND CULTURAL TREATMENTS

presented by

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of the requirements for

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FLORAL ENHANCEMENT OF THE *PICEA* GENUS THROUGH HORMONAL AND CULTURAL TREATMENTS

By

Robert Douglas Marquard

A DISSERTATION

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ABSTRACT

FLORAL ENHANCEMENT OF THE *PICEA* GENUS THROUGH HORMONAL AND CULTURAL TREATMENTS

By

Robert Douglas Marquard

White and blue spruce are commercially important species in much of North America, but they have a relatively long generation time that slows genetic improvement. Applied plant hormones and root-pruning were tested for their ability to enhance strobili production. Three weekly treatments with the gibberellin mixture $A_{4/7}$ significantly enhanced female strobili on 8- and 9-year-old white spruce, but treatment success was dependent on the time of treatment, crown position, and GA concentration. In general, uppermost crown regions were less responsive to treatment, and GA treatments initiated after meristematic differentiation began were ineffective. A GA concentration of 500 ppm had a supraoptimal effect on female strobili production when compared to a concentration of 250 ppm. Seed yield was not affected by $GA_{4/7}$ treatment and male strobili production was not enhanced.

Root-pruning, exogenous $GA_{4/7}$ treatment, and naphthalene acetic acid (NAA) were tried alone and in combination as a floral enhancement treatment on juvenile white spruce. When compared to the untreated plantation average, 500 ppm GA plus NAA plus root-pruning increased female strobili

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174-fold on 6-year-old trees. As individual treatments, 500 ppm GA, 250 ppm GA, and root-pruning increased female strobili production 52-fold, 13-fold, and 12-fold, respectively. Two-year-old trees given identical treatments did not initiate strobili of either sex.

When a 500 ppm GA treatment was applied to branches of mature white spruce identified as strictly male and transitional in sexuality, both male and female strobili were enhanced. On male-zone branches, female strobili were induced and male strobili production increased 6-fold. On branches transitional in sexuality, female and male strobili production increased 6.2- and 2.4-fold, respectively.

Mechanical root-pruning on 2 of 4 sides of 11-year-old blue spruce was ineffective as a floral enhancing treatment. Severity of the treatment was apparently inadequate to enhance strobili production.

A continuum of tree height and fecundity were observed in a plantation of white spruce and related to a shading pattern provided by an adjacent hardwood stand. Ambient temperature, photon flux density, and spectral distribution were measured within the spruce plantation. Of the environmental parameters measured, only photon flux density was correlated with gradients in tree size and fecundity.

ACKNOWLEDGEMENT

I wish to thank Dr. James W. Hanover (committee chairman) who provided guidance, encouragement, and the opportunity to pursue a doctoral degree at Michigan State University; Drs. J.A.D. Zeevaart, F.I. Dennis and D.I. Dickmann who served on my guidance committee; Dr. Royal Heins who provided technical assistance in collecting environmental data reported in Chapter 5 and who kindly substituted for F.I. Dennis during the oral defense; Walter Lemmien for complete cooperation in the use of the spruce plantations located in the Kellogg Experimental Forest; George Elson and the Imperial Chemical Industries who graciously supplied the gibberellin; and finally to my wife, Liz, whose continuous support, devotion, and good humor helped sustain me throughout.

TABLE OF CONTENTS

page

LIST OF TABLES

FIG

1

2

3

4

5

LIST OF FIGURES

FIGU

1

12

1

1

1

1

1

1

1

FIGURE page

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CHAPTER I

FLOWER ENHANCEMENT OF WOODY PLANTS: A REVIEW OF THE LITERATURE

Flowering is an extremely dynamic physiological process influenced by environmental stimuli, genotype, and plant age. Inherent differences exist between generative initiation of herbaceous plants and woody plants in that many herbaceous plants initiate flowers in response to changes in photoperiod which can evoke a translocatable stimulus. In contrast, flowering of woody plants is generally not affected by photoperiod (Mirov, 1956; Wareing, 1958) and endogenous stimuli generally are not translocated. Rather, flowering of woody plants may be regulated by endogenous chemical balances maintained at each apical meristem (Jackson and Sweet, 1972). This review summarizes flower enhancement of woody plants with emphasis on the *Coniferales*.

Flower enhancement (equivalent to strobili enhancement in conifers) of some tree species has been achieved through cultural, environmental, and hormonal treatments either alone or in combination. Cultural treatments that can enhance flower production include: trunk and branch girdling, strangulation or banding, fertilization, thinning, and horizontal training of branches (see reviews by Mathews, 1963; Jackson and Sweet, 1972). Additional cultural treatments that enhance flower production include pollarding

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(Copes, 1973; Matheson and Willcocks, 1976; Nienstaedt, 1981; Masters, 1982;), root-pruning (Hoekstra and Mergen, 1957; Stephens, 1961; Holst, 1968; Silen, 1973a; Gregory and Davey, 1977), and pot culture or root-binding (Ebell, 1967; Quirk, 1973; Greenwood, 1977; Ross, 1977; Wheeler *et al*, 1982).

Several hypotheses have been proposed to explain the effects of cultural treatments on flower production. These include changes in endogenous carbohydrate levels (Shoulders, 1970), nitrogen levels (Schmidtling, 1974), or hormonal levels (Jackson and Sweet, 1972). An observed change in one endogenous factor, however, may be too simplistic to explain flower enhancement. Rather, cultural treatments may modify several metabolic pathways that in combination evoke a generative response.

Environmental Factors

A modification of environmental conditions can influence flower production. Durzan *et al*, (1979) inhibited flowering of white spruce (*Picea glauca* (Moench) Voss) by night interruptions with red light, and Kosinski and Giertych (1982) used optical fibers to promote strobili production of *Pinus sylvestris* L. and *Picea abies* (L.) Karst. by allowing a greater portion of red light to reach apical meristems.

Irradiance can influence flower production. Moderate to heavy shading reduced flowering of several woody species (Migita, 1960; May and Antcliff, 1963; Silen, 1973b; and Jackson and Palmer, 1977a). Whereas shading can reduce

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flower production, a relative increase in irradiance may enhance flowering. Waldron (1965) found a strong relationship between a tree's crown class and strobili production; the majority of female strobili were initiated on trees of a dominant crown class. In addition, strobili often are non-randomly distributed within tree crowns. Female strobili frequently are located on upper crown positions or on distal branch positions that receive a relatively greater amount of direct solar radiation (Winjum and Johnson, 1964; Smith and Stanley, 1968; Powell, 1972; Kosinski and Giertych, 1979).

Shading may influence patterns of strobili production by altering internal bud temperatures. Under conditions of direct sunlight, Pukacki (1980) found internal bud temperatures of *Picea abies* and *Pinus sylvestris* to be 7°C higher than ambient temperature. Internal temperatures were always higher than ambient temperatures on sunny days and internal bud temperature was related to crown location.

Likewise, shading individual buds reduced flowering of *Vitis vinifera* (May, 1965) and *Pinus sylvestris* in some years (Giertych and Kosinski, 1978). Since shading of individual buds would not reduce photosynthesis, it was suggested internal bud temperature may influence flower production of some tree species. Tompsett and Fletcher (1977), Luukkanen (1979), Pollard and Portlock (1981) and Chalupka *et al*, (1982) promoted strobili production by elevating air temperature surrounding tree crowns of conifer spec

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An increase in crown temperature has also been shown to elevate endogenous gibberellin-like levels in *Picea abies* (Chalupka *et al*, 1982). A change in gibberellin-like levels following an increase in crown temperature is compatible with numerous studies that show strobili production to be enhanced following exogenous gibberellic acid (GA) treatments.

Attempts have been made to relate meterological data to yearly flucuations in flower production. Warm and sunny weather during summer months often benefits flower production (Mathews, 1955; Maguire, 1956; Fraser, 1958; Daubenmire, 1960; vanVrendenbuch and LaBastide, 1969; Eis, 1973; Fober, 1976). Likewise, dry conditions preceding meristematic differentiation may also favor flower production of some tree species (Dewers and Moehring, 1970; Eis, 1973). However, extrapolation from meterological data is difficult in that each tree species may have different environmental requirements and some species may be influenced by environmental conditions only during a specific portion of the growing season.

Some tree species flower more profusely when moved to more southerly latitudes (Wright *et al*, 1966; Ganzel, 1973; Schmidtling, 1979; Rudolph, 1981) or when grown on a southerly aspect (Simpson and Powell, 1981). Greater flower production on trees grown in more southerly latitudes may be a response to more favorable climate or to a change in phot

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photoperiod. Recently, Longman (1982) found *Pinus contorta* Dougl. produced more strobili under a 10-hour photoperiod as compared to a 19.5-hour photoperiod.

Flowering can also be enhanced by altering growth regimes. Accelerating early seedling growth has significantly shortened the juvenile period of some woody plants (Rudolph, 1966, 1979; Lepisto, 1973; Aldwinckle, 1975; Fraser, 1975; Young and Hanover, 1976; Cecich, 1981). In addition, Greenwood (1978, 1981) enhanced strobili production of *Pinus taeda* L. by imposing out-of-phase dormancy and Larson (1961) enhanced strobili production of *Pinus banksiana* Lamb. by altering the date of spring bud break.

Chemical Treatments

Of the experimental treatments tried, exogenous treatment with plant hormones has been most successful in enhancing strobili production of species in the *Coniferales*. Of the major phytohormones, the GAs are used most frequently in flower enhancement studies. Due to availability, the polar GA_3 and the less-polar GA mixture $A_{4/7}$ are most commonly used. Structural differences between these GAs include the number of carboxyl side chains and the saturation of the ent-gibberellane skeleton. Chemical structures of GA_3, GA_4, and GA_7 are given in Figure 1.

During the 1950's, Japanese workers used GA_3 to enhance strobili production of several species within the *Cuppressaceae* and *Taxodiaceae* families. Pharis and others er

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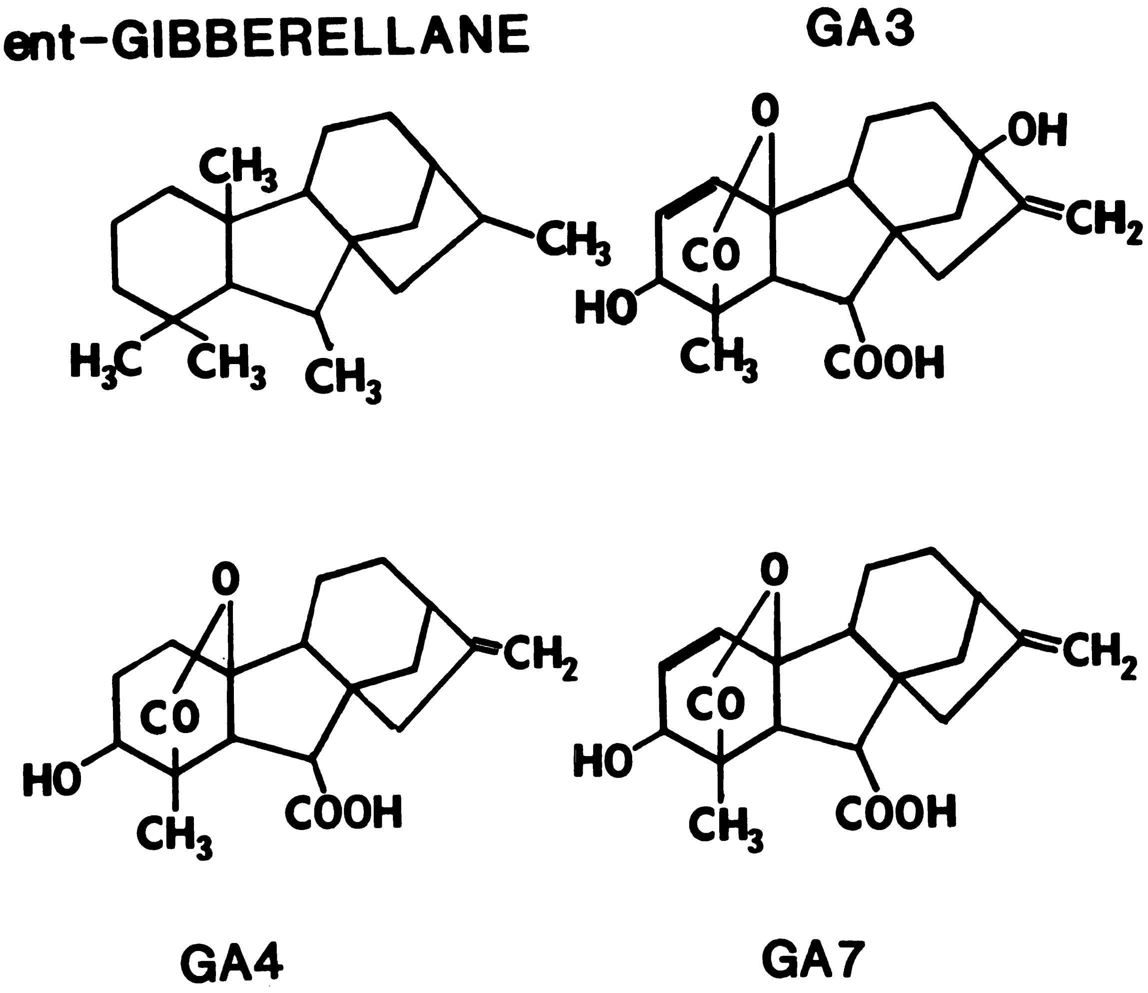
ent-GIBBERELLANE

GA3

GA4

GA7

Figure 1. Chemical structure of the gibberellins A_3, A_4, and A_7 and of the ent-gibberellane skeleton.

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subsequently demonstrated the efficacy of GA_3 to enhance strobili production of other species within the aforementioned families (see review by Pharis and Kuo, 1977). However, GA_3 is generally ineffective when *Pinaceae* species are treated but has enhanced strobili production of *Picea abies* (Bleymuller, 1976; Chalupka, 1979). Presently, the less-polar GAs are recognized as the more active subgroup of GAs for strobili enhancement of *Pinaceae* species.

Strobili production of at least 12 species within the *Pinaceae* family has been enhanced by $GA_{4/7}$ treatments. These species include: *Pseudotsuga menziesii* (Ross, 1976; Ross and Pharis, 1976; Puritch *et al*, 1979; McMullan, 1980; Pharis *et al*, 1980), *Tsuga heterophylla* (Raf.) Sarg. (Ross *et al*, 1981; Brix and Portlock; 1982), *Pinus banksiana* (Cecich, 1981), *Pinus taeda* (Ross and Greenwood, 1979; Greenwood, 1981), *Pinus sylvestris* (Chalupka, 1978; Luukkanen and Johansson, 1980a,b), *Pinus radiata* D. Don (Sweet, 1979), *Pinus palustris* Mill (Hare, *et al*, 1979), *Pinus contorta* (Wheeler *et al*, 1980; Longman, 1982), *Picea sitchensis* (Carr.) Bong. (Tompsett, 1977, 1978; Tompsett *et al*, 1980), *Picea abies* (Chalupka, 1979, 1980; Dunberg, 1980; Luukkanen and Johansson, 1980a), and *Larix decidua* Mill. and *Larix leptolepsis* (Sieb. and Zucc.) Gord. (Bonnet-Masimbert, 1982).

In general, exogenous treatment with less polar GA favors enhancement of female strobili. However, this trend

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is not ubiquitous in that some studies have shown greater enhancement of male strobili (Chalupka, 1978; Luukkanen and Johansson, 1980b). Within the *Pinaceae* family, strobili are typically enhanced 2- to 4-fold by $GA_{4/7}$ treatments, but under optimum conditions and with adjunct treatments (i.e., root-pruning, fertilization, girdling) a 100-fold increase in strobili production has been obtained (Ross *et al*, 1981).

Whereas GA is the preferred phytohormone in flower enhancement studies, naphthalene acetic acid (NAA) is often used as an adjunct treatment with GA. However, the benefit of NAA as an adjunct treatment remains in question. NAA in combination with $GA_{4/7}$ has enhanced female strobili production (Ross *et al*, 1980; Pharis *et al*, 1980), reduced female production and increased male strobili production (Tompsett, 1977; Luukkanen and Johansson, 1980b), or has had no effect (Dunberg, 1980; Cecich, 1981). Low levels of NAA with $GA_{4/7}$ enhanced female strobili production of Douglas-fir, but a higher NAA level suppressed female strobili production (Ross *et al*, 1980).

Cultural treatments are also effective in combination with less polar GAs. Specifically, cultural treatments that impose a water deficit (i.e., root-pruning, pot culture, and girdling) have been most effective (Ross and Pharis, 1976; Ross, 1977; Chalupka, 1978; Greenwood, 1977; Ross *et al*, 1980; Greenwood, 1981).

Exogenous GA treatments may influence meristematic differentiation by changing endogenous hormone balances, by a

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directing translocation of metabolites, or by modifying the rate of cell division. Pharis and Owens (1966) and Pharis and Morf (1967) hypothesized that vegetative growth in the *Cuppressaceae* and *Taxodiaceae* families is maintained by the endogenous GA concentration which remains low in juvenile trees. Furthermore, strobili are initiated when vegetative growth is duly suppressed or when endogenous GA levels are adequately increased. Some support for this hypothesis exists in the literature. Some species require higher exogenous GA concentrations when younger to elicit a flowering response (Pharis *et al*, 1965), and Pharis and Morf (1967) found that flower buds of *Crytomeria arizonica* Greene aborted when exogenous GA treatments were stopped.

GA can also mobilize photosynthate (see Pharis and Kuo, 1977). Several researchers have discussed the possibility that high levels of photosynthate at the apical meristem may evoke a flowering response (see Zeevaart, 1976).

GA_3 has been reported to increase cell division in a wide variety of plant species (Jones, 1973). Tompsett (1978) believes that differentiation in *Picea sitchensis* is influenced by bud vigor. By increasing mitotic division through exogenous GA treatments, generative production could be favored.

Benefits of Enhanced Flower Production

Great strides in genetic improvement of tree species could be made if treatments were available that consistently enhanced flower production. Economic gain would be expected

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to accompany treatments that increase seed production of trees with superior phenotypes. Whereas genetic improvement of agronomic crops has been advanced through intensive breeding programs, tree breeders are hampered by the long generation time of most commercially important tree species (often 10 to 25 years). In contrast, crop breeders have the luxury of growing 1 to 4 generations in 24 months. Flower enhancement treatments could also be used to reduce the yearly variation in flower and seed production (Wenger, 1957; Waldron, 1965; Powell, 1977).

Recently, researchers have recognized the potential benefit of intensively managed seed orchards as a method to maximize seed production (Fraser, 1977; Sweet and Krugman, 1977; Sprague *et al*, 1979; Masters, 1982; Ross and Pharis, 1982). In the future, management techniques to enhance seed production may include pot culture, hedging, greenhouse culture, and chemical treatments.

Presently, more than 700 tree seed orchards are located throughout the United States (Anonymous, 1982). However, 46% of these seed orchards were reported as juvenile and therefore unproductive. As the need for wood products increases, tree improvement programs will become an increasingly important source of genetically superior seed.

Flower production is a prerequisite for tree improvement, and cultural and chemical treatments can now be used experimentally to enhance flower production of some tree species. Further work is needed to determine

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management systems capable of maximizing seed production for each major species.

Perspectives and Objectives

The Department of Forestry at Michigan State University has a progressive tree improvement program that emphasizes genetic improvement of conifer species. During 1981, 19.7 million spruce and 46.5 million pine seedlings were grown commercially in Michigan (Levenson, personal communic.). Combining the importance of conifer species in Michigan with my interest in reproductive biology, a research project was developed to test the efficacy of plant hormones and cultural treatments to enhance strobili production of trees within the *Picea* genus.

Picea glauca was chosen as the primary experimental species because hormonal effects on strobili production have not been previously published for this species. [However, an unpublished report of $GA_{4/7}$ enhancing strobili production was mentioned by Pharis and Kuo (1977)]. In addition, genetic breeding of white spruce has progressed the farthest of any one species in Michigan and white spruce is commercially important and a dominant species in much of Canada.

Blue spruce (*Picea pungens* Engelm.) was chosen as a secondary experimental species because of its value as a Christmas tree and ornamental. Secondly, a range wide provenance-progeny test of blue spruce established at Augusta, Michigan, in 1970 remained juvenile and available fo

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for experimentation.

To be operationally feasible, treatments that enhance strobili production must be cost effective. Hormonal treatments should be short in duration and correctly timed. For white spruce, Owens and Molder (1977) hypothesized that the final phase of shoot elongation would be the most effective time for treatment. From this working hypothesis (based only on anatomical work), I realized many researchers who attempt to enhance strobili production neglect relating meristematic activity to the treatment period. Additionally, some hormonal treatments are indiscriminately applied over a significant portion of the growing season or on a large portion of the tree crown.

The main objective of my research was to develop effective and efficient techniques for increasing strobili production of white and blue spruce. A self-imposed criteria on research direction was that experimentation should be applied in scope and beneficial to the tree breeding program at Michigan State University.

Specific objectives included:

1) Examination of the relationship between crown position, time of $GA_{4/7}$ treatment, meristematic activity, shoot elongation, and GA concentration on strobili enhancement of mature white spruce.

2) To examine the effect of $GA_{4/7}$ treatment on the pattern of meristematic differentiation of selected branches of white spruce.

3) To test the efficiency of $GA_{4/7}$, and root-pruning (alone and in combination) to induce strobili production on juvenile white spruce.

4) To examine the effects of exogenous $GA_{4/7}$ treatments on branches that naturally produce only male strobili and on branches that have the natural capacity to produce strobili of both sex types.

5) To quantify and relate environmental parameters within a *Picea* plantation to observed differences in fecundity and tree height.

6) To test the effect of elevated air temperature surrounding tree crowns on strobili production of mature white spruce.

7) To examine the effect of root-pruning on strobili production, vegetative phenology, and terminal shoot elongation of juvenile blue spruce.

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CHAPTER II

RELATIONSHIP BETWEEN GA CONCENTRATION, TIME OF TREATMENT, AND CROWN POSITION ON STROBILI PRODUCTION OF *PICEA GLAUCA*

Abstract

Female strobili production of mature 8- and 9-year-old white spruce (*Picea glauca*) (Moench) Voss) treated with the non-polar gibberellin mixture $GA_{4/7}$ was significantly influenced by time of treatment, crown position, and GA concentration. GA treatments initiated before the onset of meristematic differentiation (late-June) enhanced female strobili whereas treatments initiated after meristematic differentiation were ineffective. A 250 ppm GA treatment was more effective than 500 ppm GA in enhancing female strobili. Female strobili naturally predominated on central positions of individual shoots. GA treatments that enhanced female strobili production altered the distribution of female strobili to favor more distal shoot positions. Male strobili were not enhanced and average seed yield per strobili was not affected by GA treatment.

Introduction

Cultural treatments, fertilization, and hormonal treatments have been used to enhance strobili production of some conifer species. The less-polar gibberellin mixture $GA_{4/7}$ has been successfully used to promote strobili production of species within the *Pinaceae* family (see Pharis and Kuo, 1977; Tompsett, 1977; Chalupka, 1979; Ross and Greenwood, 1979; Tompsett and Fletcher, 1979 Ross *et al*, 1980; Dunberg, 1980; Cecich, 1981; Bonnet-Masimbert, 1982). While treatment results are often reproducible, many questions remain about the control of flowering of tree species.

A recent trend in floral enhancement studies toward applied research should be continued to develop operationally feasible treatments that benefit tree breeding programs. To be efficient, exogenous chemical treatments need to be correctly timed and reasonably short in duration. In addition, species and age are important parameters that determine optimum treatment frequency, concentration, and mode of chemical application.

This study dealt with the relationship between the concentration of exogenously applied $GA_{4/7}$, time of treatment, crown position, and strobili production of white spruce. Meristematic activity was also related to shoot elongation and the success of specific GA treatments in enhancing strobili production. To determine if exogenous $GA_{4/7}$ treatments influenced the differentiation pattern of individual branches, the distribution of female strobili on untreated branches was compared to the distribution of strobili on branches treated with GA.

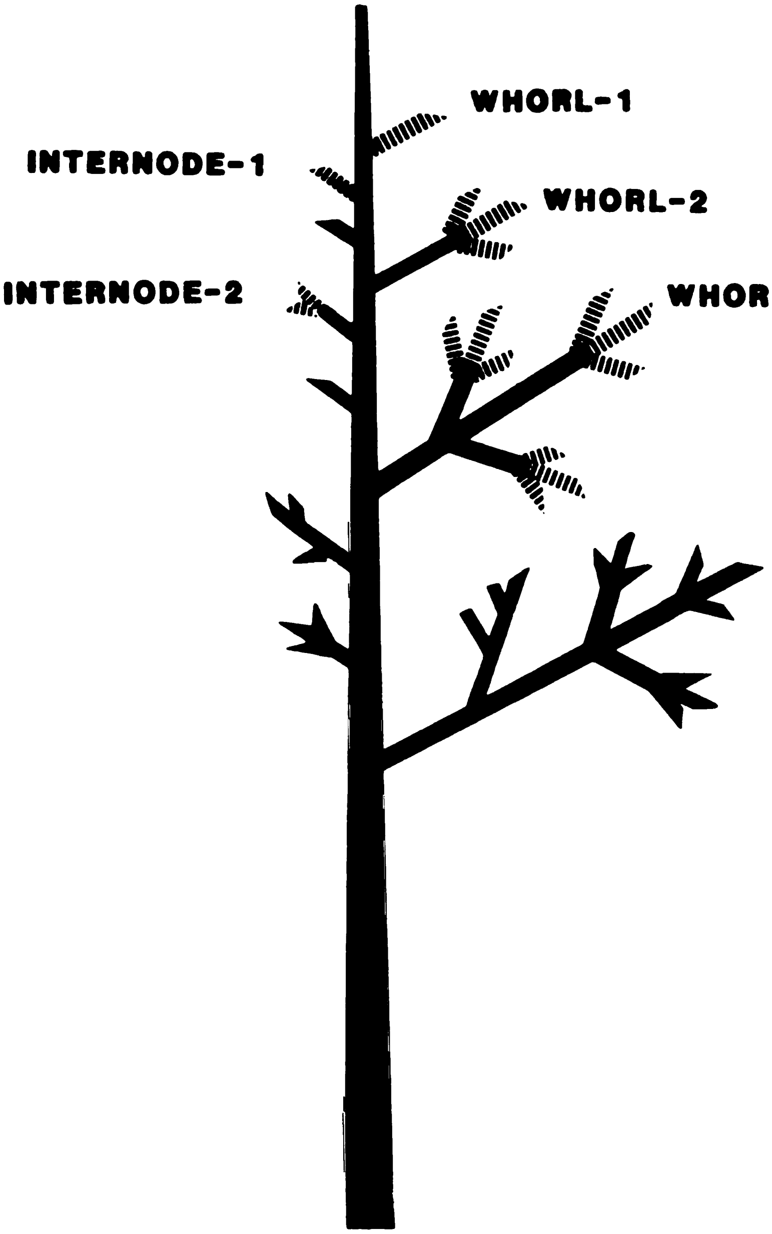
Materials and Methods

During the spring of 1981, 51 8- and 9-year-old white spruce were randomly selected for experimentation within a *Picea* plantation located in southwestern Michigan. Selection was based on maturity (e.g., having produced strobili in the past) and good crown form. Two experiments tested the effects of the GA mixture $A_{4/7}$ on strobili production. With one exception, both experiments were conducted in an identical manner. In the first experiment, $GA_{4/7}$ was used at a concentration of 500 ppm, whereas a concentration of 250 ppm was used in the second experiment. Both experiments followed methods described below.

To test the effect of the time of treatment, 24 of the 51 trees were randomly chosen for hormonal treatments beginning on either May 13, June 2, June 23, or July 15, 1981. Individual branches served as experimental units to remove inherent differences between individual trees and crown positions. On each tree, five crown positions were selected for treatment: whorl-1, internode-1, whorl-2, internode-2, and whorl-3 (Figure 2). At each crown position, 3 branches were randomly selected to receive either a GA treatment, solvent treatment, or to act as a control. Branches were treated 3 times at weekly intervals.

GA was dissolved in ethanol and the final carrier was a

Figure 2. Representation of the uppermost 5 crown regions of 8- and 9-year-old white spruce treated with gibberellin.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19:1 (v:v) water:ethanol solution. The ratio of GA_4 to GA_7 was 19:12 by weight and the surfactant used was Aromox C-12w (0.02% active ingredient by volume). The solvent treatment was a water-ethanol solution with surfactant added while control branches remained untreated. Household atomizers were used to apply chemical treatments to newly expanding shoots as a foliar spray until run-off. Individual branches were cupped with a modified funnel to eliminate chemical drift during treatment. Location of treated shoots is shown in Figure 2.

Shoot elongation of branches in the uppermost two whorls was measured, and apical meristems were collected periodically from the 3 remaining untreated trees during the 1981 growing season. The uppermost two whorls represent the crown region where female strobili most frequently are initiated (Chapter 4). Collected apices were killed and fixed under vacuum in FAA and dehydrated in tertiary-butyl alcohol (Berlyn and Miksche, 1976). Apical meristems were imbedded in paraplast, longitudinally sectioned at 10µ, and stained in hematoxylin and safranin (Johansen, 1940). Meristematic activity was related to initiation of the 4 GA treatments and the treatment effect on strobili production was assessed during the spring, 1982. Female strobili initiated on experimental branches were open pollinated and mature female cones were collected from treated branches in the fall, 1982. Filled seed from collected cones were extracted and counted and the average number of filled seed per cone was calculated. An analysis of variance (AOV) determined if seed production was affected by GA treatment. For analysis, seed yields from solvent and control branches were combined as the control.

An AOV determined treatment effects on female strobili production based on a split-split-block design and male strobili production based on a split-block design. Male strobili production was pooled by tree since few crown positions bore male strobili. Differences between treatment means were tested at the 5% level of significance unless otherwise noted.

During the 1982 spring, 27 untreated whorl-1 and 18 untreated whorl-2 shoots were randomly collected from 20 white spruce excluded from experiments 1 and 2. Collected shoots had elongated during the 1981 growing season and bore one or more female strobili during 1982. Shoots collected from whorl-2 branches were the most distal portion of the branch. To quantify patterns of differentiation, each shoot was linearly divided into tenths. The number of vegetative and generative buds initiated on each 10% of the shoot was tabulated and analyzed by a chi-squared test of independence. Similiarly, the location of female strobili was quantified on all whorl-1 and whorl-2 shoots treated with GA (from experiments 1 and 2). Chi-squared was used to test if exogenous GA treatments influenced the pattern of female strobili distribution on whorl-1 and whorl-2 shoots when compared to untreated shoots.

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Results

Shoot Elongation and Meristematic Activity

Bud break on the study trees began during the last week of April, 1981. On May 13, June 2, June 23, and July 15, vegetative shoots had elongated 16, 72, 99, and 100% of their final length (Figure 3). These dates corresponded to the initiation of the 4 GA timing treatments. Bud scale primordia were initiated from late-April until mid-June (Figure 4a,b). Meristematic differentiation began during late-June and by June 30, needle primordia were apparent on the basal portion of apical meristems (Figure 4c). Therefore, GA treatments initiated on May 13 and June 2 preceded meristematic differentiation and treatments initiated on June 23 and July 15 followed differentiation. Needle primordia were initiated from late-June through the end of the GA treatment (Figure 4d).

Effects of GA Treatment on Strobili and Seed Production

Male strobili production was not enhanced by GA treatment (Table 1). Overall, branches treated with 500 ppm GA averaged 1.2 male strobili, solvent treated branches averaged 1.2 strobili and control branches averaged 0.7 strobili. Branches that received 250 ppm GA averaged 1.6 male strobili, solvent treated branches averaged 1.5 strobili, and control branches averaged 1.2 strobili.

Treatment with 500 ppm GA significantly increased female strobili production (Table 1). Hereafter, 'strobili' will denote female strobili unless otherwise noted. Both time of

Figure 3. Relationship between shoot elongation, meristem activity, and initiation of the 4 gibberellin treatments.

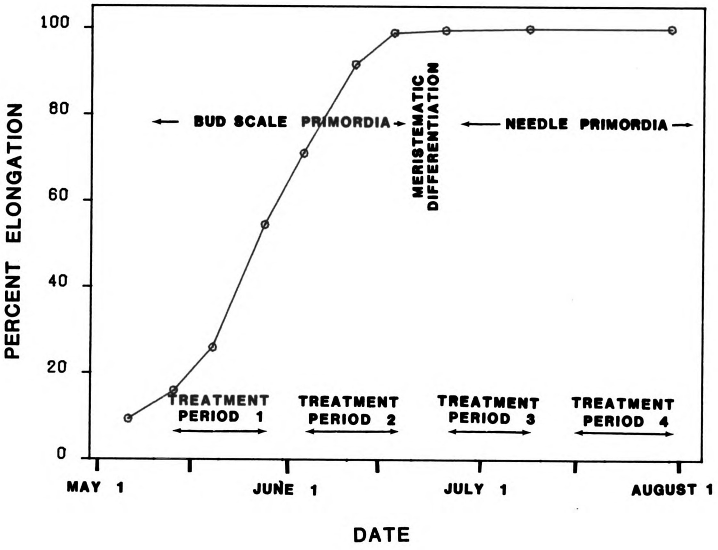
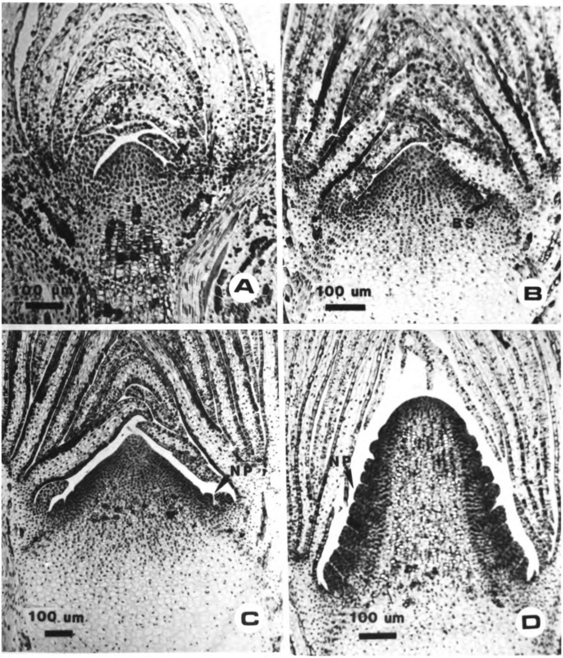

PERCENT ELONGATION
100
80
60
40
20
0
MAY 1
JUNE 1
JULY 1
AUGUST 1
DATE
← BUD SCALE PRIMORDIA →
MERISTEMATIC DIFFERENTIATION
← NEEDLE PRIMORDIA →
TREATMENT PERIOD 1
TREATMENT PERIOD 2
TREATMENT PERIOD 3
TREATMENT PERIOD 4

Figure 4. Median longitudinal sections of apical meristems of white spruce collected during the 1981 growing season [BS=bud scale;NP=needle primordia] A) May 19 B) June 2 C) June 30 D)July 29.


BS
100 um
A
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100 um
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Table 1. Significance levels for time of treatment, branch position, GA treatment, and interactions of main effects on male and female strobili production of white spruce.

SOURCE OF	MALE PRODUCTION		FEMALE PRODUCTION	
VARIATION	250 PPM	500 PPM	250 PPM	500 PPM
Time of Treatment	n.s.	n.s.	*	**
Branch Position	-	-	***	***
Time X Position	-	-	**	***
Treatment	n.s	n.s	***	***
Time X Treatment	n.s	n.s	***	***
Position X Treatment	-	-	**	***
Time X Position X Treatment	-	-	**	***

*,**,*** Denotes sigificance at the 10%, 5%, and 1% level respectively
n.s. Denotes non-significance

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treatment and crown position significantly influenced strobili production and all interactions between main factors were significant (Table 1).

Strobili production on whorl-2 and whorl-3 branches was enhanced by 500 ppm GA applied from May 13 to May 26. The remaining three crown positions, though not statistically enhanced by GA treatment, showed a strong tendency to produce more strobili than their respective controls (Figure 5a). Within any given crown region, strobili production of solvent treated branches did not differ significantly from control branches. When pooled by treatment, branches that received 500 ppm $GA_{4/7}$ from May 13 to May 26 averaged 6.0 strobili which was significantly greater than average strobili production of solvent treated (1.1 strobili) and control branches (0.7 strobili).

A 500 ppm GA treatment from June 2 to June 17 statistically enhanced strobili production on internode-1, internode-2, and whorl-3 (Figure 6a). Strobili production on these three crown positions was enhanced by a minimum of 290, 190, and 360% over their respective controls. With one exception (whorl-2), strobili production of solvent treated and control branches did not significantly differ within a crown position (Figure 6a). When pooled by treatment, branches that received 500 ppm GA from June 2 to July 15 averaged 13.7 strobili which was statistically greater than solvent treated (6.9) and control branches (5.1).

In general, branches treated with 500 ppm GA from June

Figure 5. Mean female strobili production of gibberellin (GA) treated, solvent treated, and control branches on 5 crown regions of white spruce treated from May 13 to May 26, A) 500 ppm GA B) 250 ppm GA.

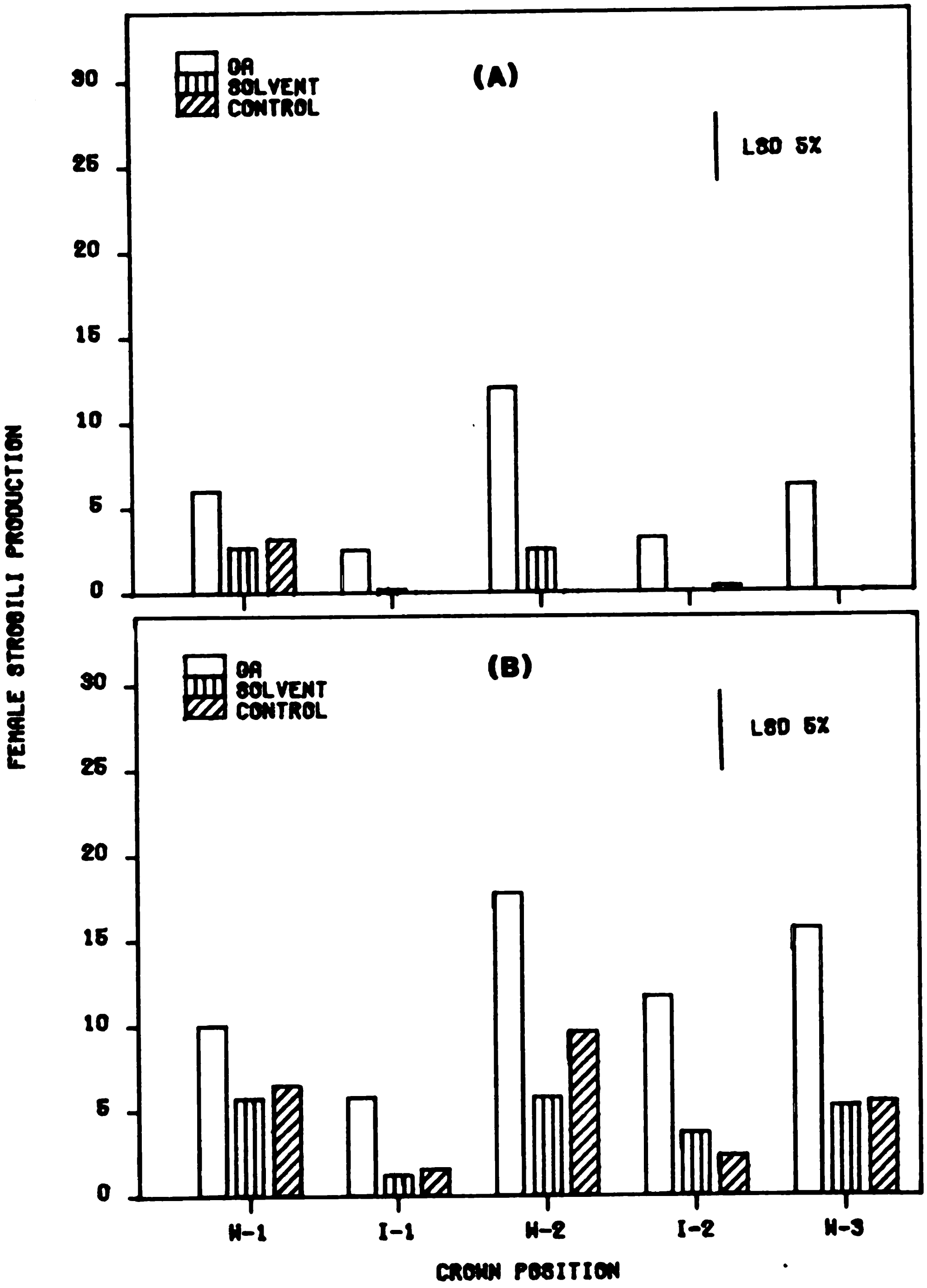
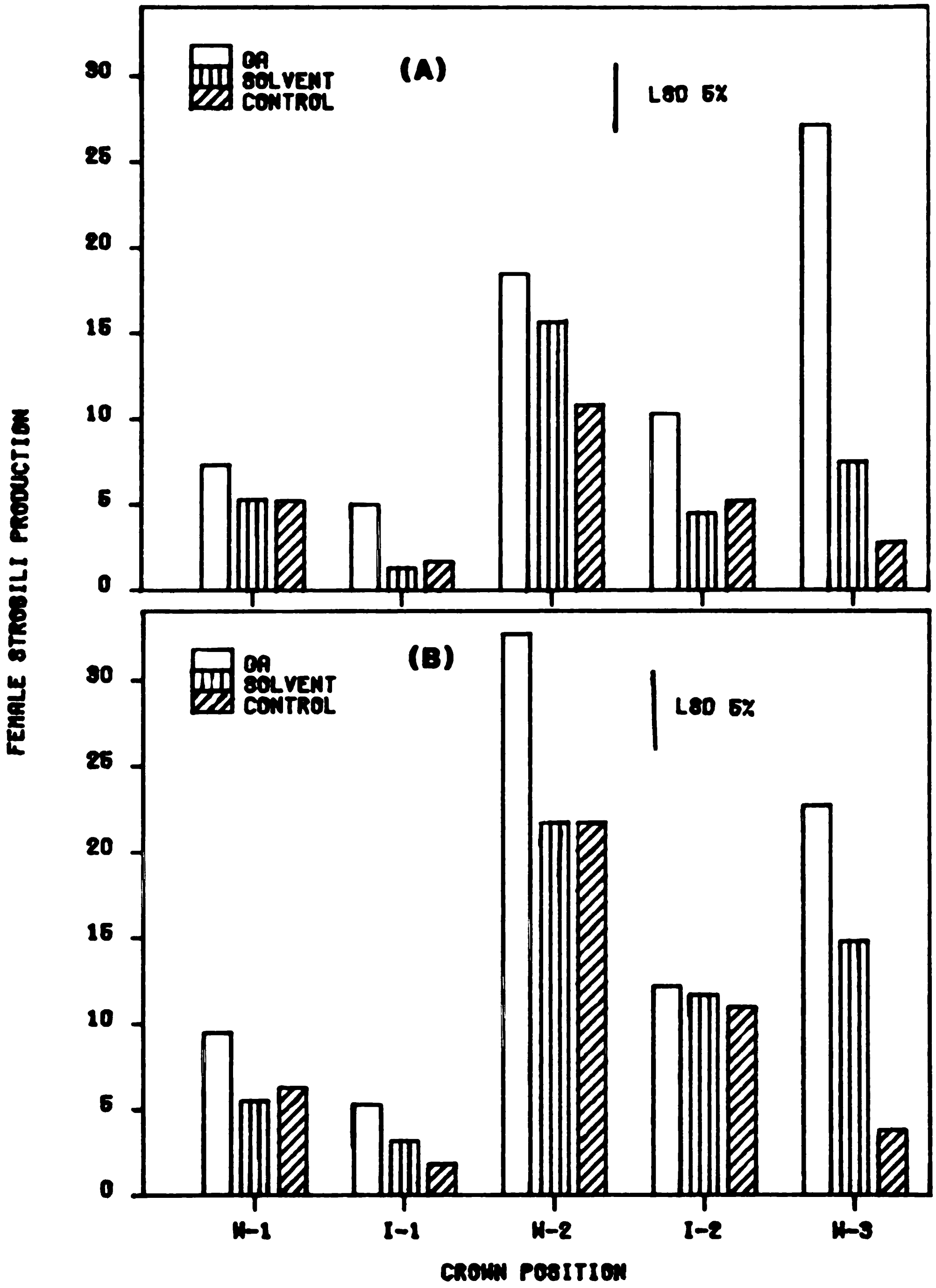

(A)
(B)
GA
SOLVENT
CONTROL
LSD 5%
FEMALE STROBILI PRODUCTION
0
5
10
15
20
25
30
W-1
I-1
W-2
I-2
W-3
CROWN POSITION

Figure 6. Mean female strobili production of gibberellin (GA) treated, solvent treated, and control branches on 5 crown positions of white spruce treated from June 2 to June 17, A) 500 ppm GA B) 250 ppm GA.


(A)
(B)
GA
SOLVENT
CONTROL
LSD 5%
30
25
20
15
10
5
0
FEMALE STROBILI PRODUCTION
W-1
I-1
W-2
I-2
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CROWN POSITION

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23 to July 7 did not produce more strobili than their respective controls (Figure 7a). However, whorl-2 branches treated with GA produced more strobili than whorl-2 control branches, but this was considered an anomaly since GA treated branches did not produce more strobili than both solvent treated and control branches.

Branches treated with 500 ppm GA from July 15 to July 29 did not produce more strobili than their respective control branches. Within individual crown positions, treatment means differed by less than 1.5 strobili (Figure 8a).

Statistical results from experiment 2 were similiar to experiment 1 (Table 1). Both GA treatment and crown position significantly influenced female strobili production. Time of treatment was significant at the 10% level and all interactions between main factors were significant (Table 1).

A 250 ppm GA treatment from May 13 to May 26 statistically enhanced strobili production on whorl-2, internode-2, and whorl-3 branches (Figure 5b). GA treatment enhanced strobili production on the two remaining crown positions over their respective controls at the 10% level. When pooled by treatment, branches treated with 250 ppm GA from May 13 to May 26 averaged 12.2 strobili which was significantly greater than strobili production on solvent treated branches (4.3) and control branches (5.1).

A 250 ppm GA treatment from June 2 to June 15 significantly enhanced strobili production on whorl-2 and

Figure 7. Mean female strobili production of gibberellin (GA) treated, solvent treated, and control branches on 5 crown positions of white spruce treated from June 23 to July 7, A) 500 ppm GA B) 250 ppm GA.

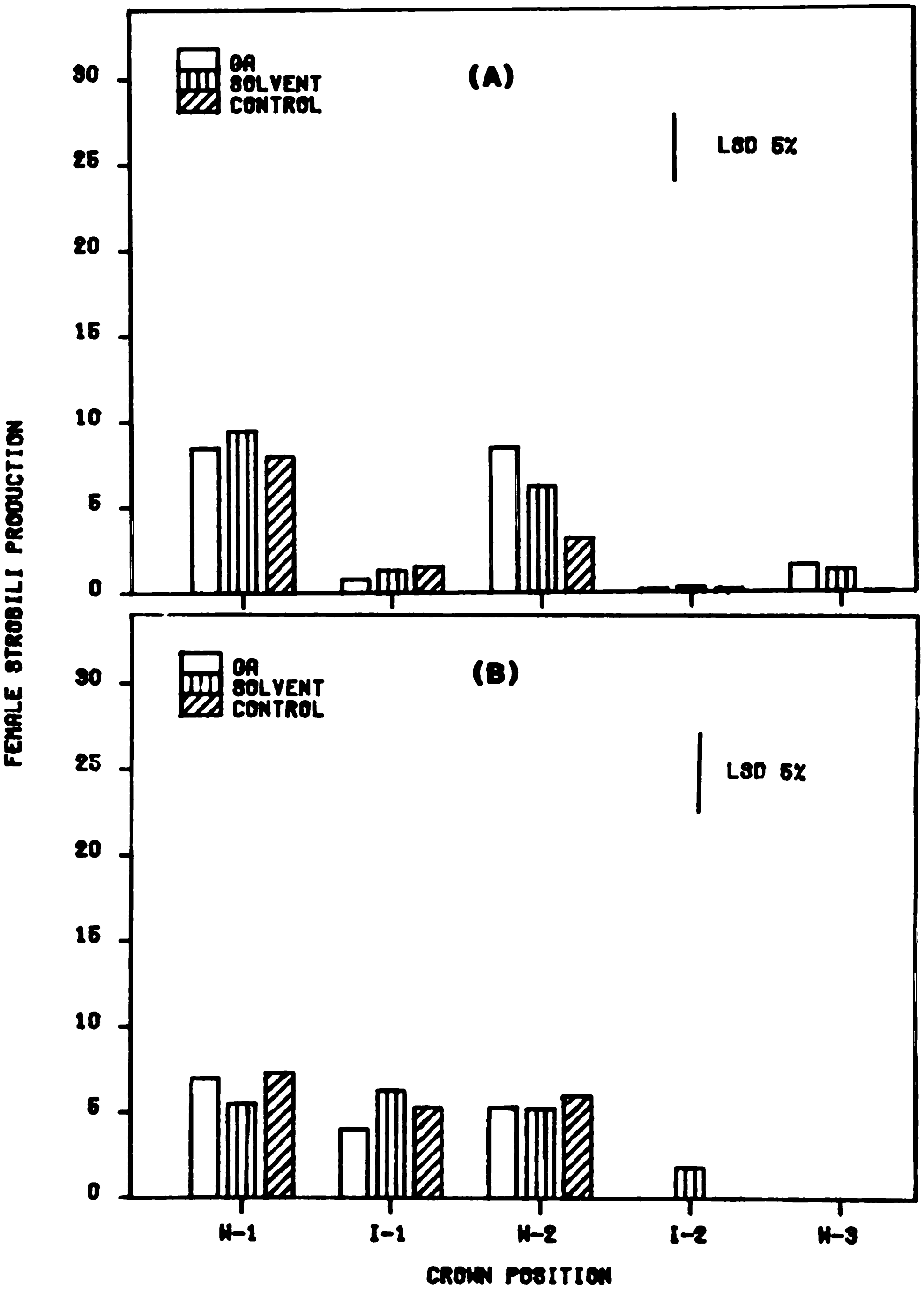
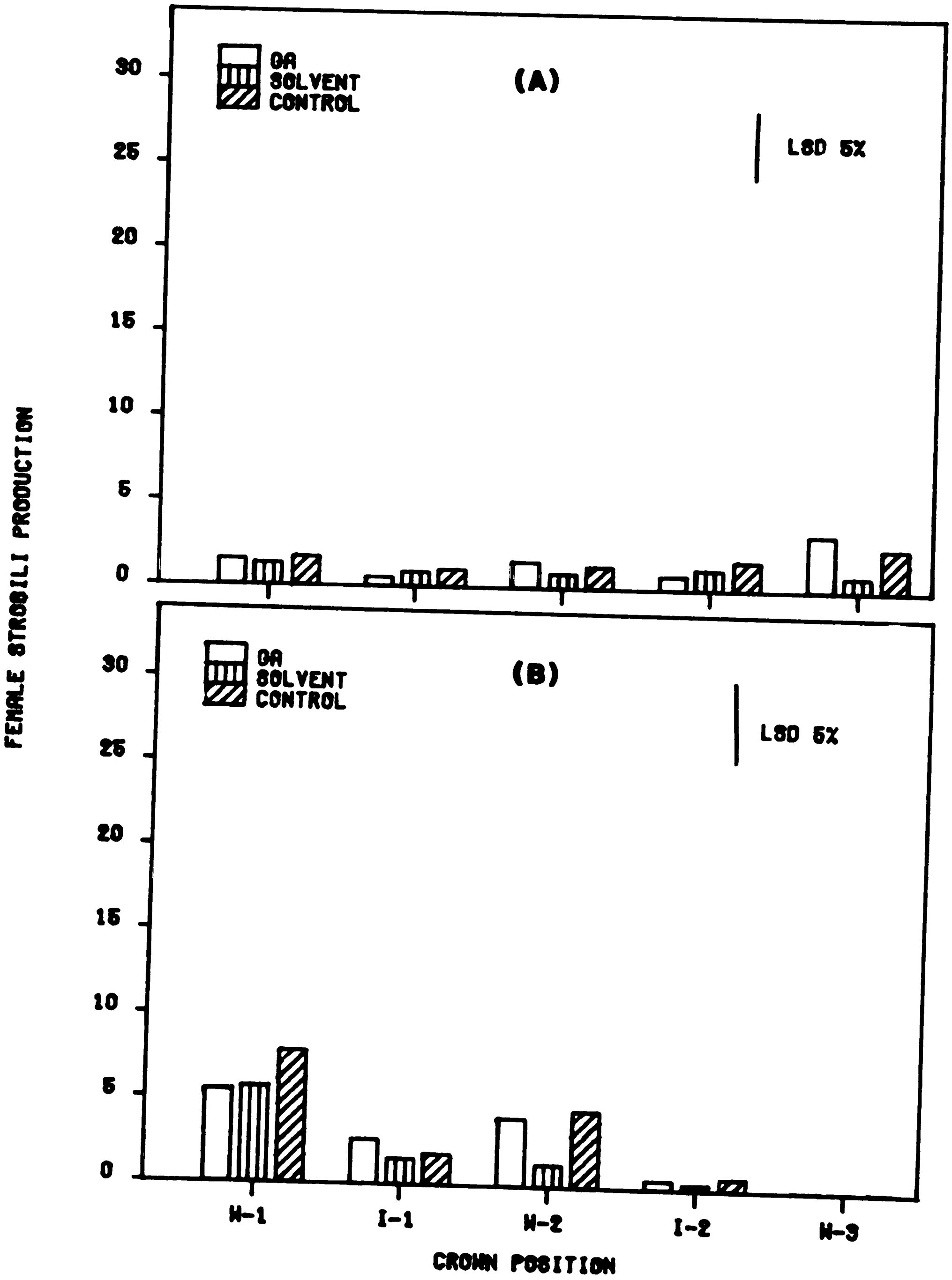

(A)
(B)
GA
SOLVENT
CONTROL
LSD 5%
FEMALE STROBILI PRODUCTION
0
5
10
15
20
25
30
W-1
I-1
W-2
I-2
W-3
CROWN POSITION

Figure 8. Mean female strobili production of gibberellin (GA) treated, solvent treated, and control branches on 5 crown positions of white spruce treated from July 15 to July 29, A) 500ppm GA B) 250 ppm GA.


(A)
(B)
GA
SOLVENT
CONTROL
LSD 5%
FEMALE STROBILI PRODUCTION
0
5
10
15
20
25
30
W-1
I-1
W-2
I-2
W-3
CROWN POSITION

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whorl-3 branches (Figure 6b). In contrast, GA treatment from June 23 to July 7 had no statistical affect on strobili production (Figure 7b). Similiarly, a 250 ppm GA treatment from July 15 to July 29 was ineffective in promoting female strobili production (Figure 8b).

Seed yield (expressed as filled seed per cone) was not affected by $GA_{4/7}$ treatment. Seed yield from cones enhanced by GA was 11.3 seeds per cone and the yield from cones on control branches was 13.8 seeds.

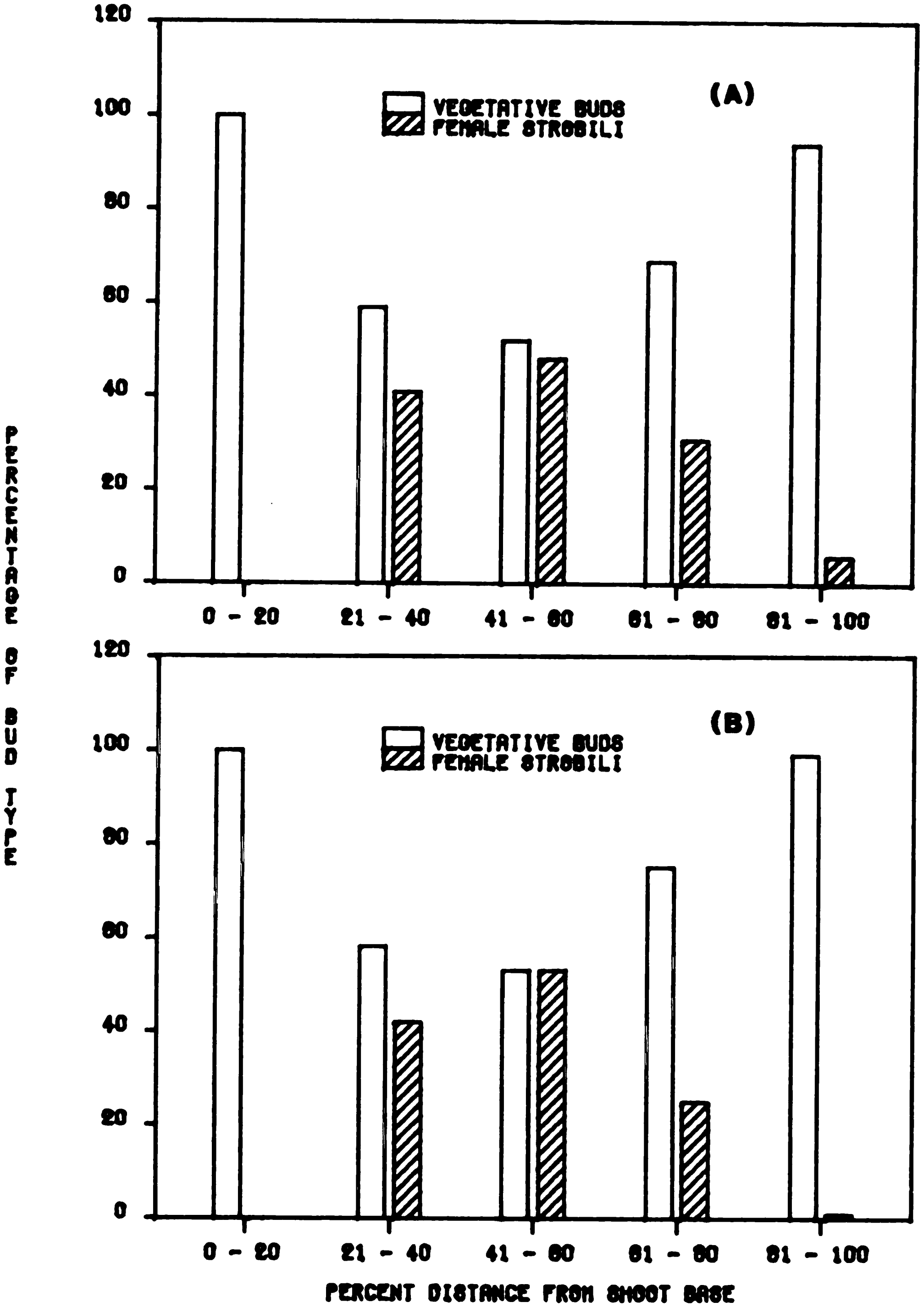
Patterns of Meristematic Differentiation

Differentiation on whorl-1 and whorl-2 shoots was significantly associated with individual bud location (Figure 9a,b). Specifically, all buds initiated on the most proximal 20% of whorl-1 and whorl-2 shoots became vegetative. Likewise, 94% and 99% of buds initiated on the most distal 20% of whorl-1 and whorl-2 became vegetative (Figure 9a,b). Female strobili most commonly differentiated from buds centrally located on whorl-1 and whorl-2 shoots.

The frequency of lateral meristems increased with distance from the basal portion of individual shoots (Figure 10). Biologically, initiation of lateral meristems on more distal branch positions would maximize crown enlargement and minimize mutual shading. Thirty-two and 34% of the meristems initiated by whorl-1 and whorl-2 shoots, respectively, were located on the most distal 20% of the shoot (Figure 10).

Exogenous GA treatments significantly altered the

Figure 9. Frequency of vegetative meristems and female strobili initiated on A) the uppermost whorl B) the second uppermost whorl of white spruce.


(A)
VEGETATIVE BUDS
FEMALE STROBILI
120
100
80
60
40
20
0
0 - 20
21 - 40
41 - 60
61 - 80
81 - 100
(B)
VEGETATIVE BUDS
FEMALE STROBILI
120
100
80
60
40
20
0
0 - 20
21 - 40
41 - 60
61 - 80
81 - 100
PERCENTAGE OF BUD TYPE
PERCENT DISTANCE FROM SHOOT BASE

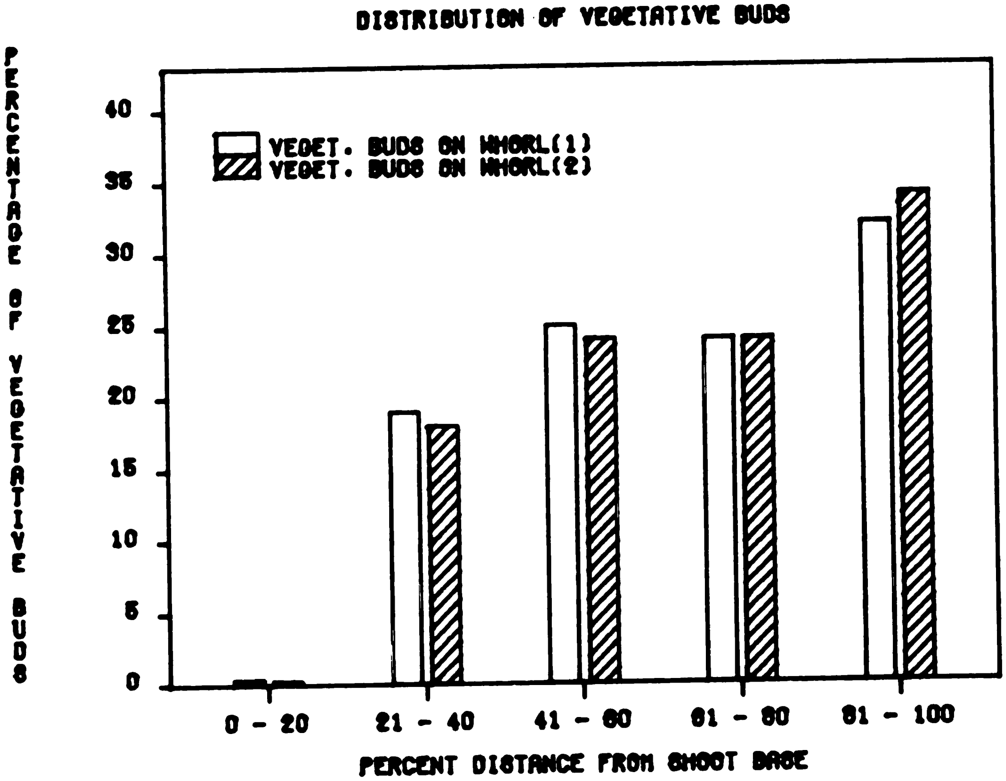


Figure 10. Frequency of lateral meristems on the uppermost whorl (whorl-1) and on the second uppermost whorl of white spruce.

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pattern of meristematic differentiation of whorl-1 branches. GA treatments that preceded meristematic differentiation caused female strobili distribution to be shifted to more distal branch positions (Figure 11). The distribution of female strobili on shoots treated with GA from June 2 to June 17 was intermediate between untreated shoots and shoots treated with GA from May 13 to May 26. Conversely, GA treatments initiated after meristematic differentiation did not influence female strobili distribution (Figure 11).

Similiarly, GA treatment from May 13 to May 26 significantly altered the distribution of strobili on whorl-2 shoots (Figure 12). The distribution of strobili on whorl-2 shoots treated with GA from June 2 to June 17 was intermediate between untreated shoots and those that received GA treatment during May (Figure 12). Whorl-2 shoots treated with GA after meristematic differentiation produced too few strobili to establish a meaningful distribution.

Discussion

While work with Douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco) (Silen, 1973) and sitka spruce (*Picea sitchensis* (Bong.) Carr.) (Tompsett, 1977; Tompsett and Fletcher, 1979) indicated some treatments can enhance strobili production after differentiation has begun, trees in this study responded only to GA treatments that preceded differentiation. Lukkannen (1979) showed that maximum enhancement of female strobili of *Picea abies* (L.)

Figu

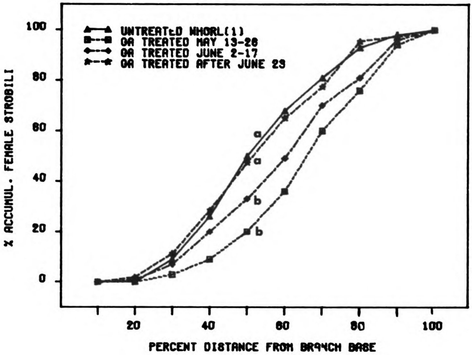


Figure 11. Comparison between the distribution of female strobili on uppermost branches of white spruce treated with gibberellin and untreated branches.

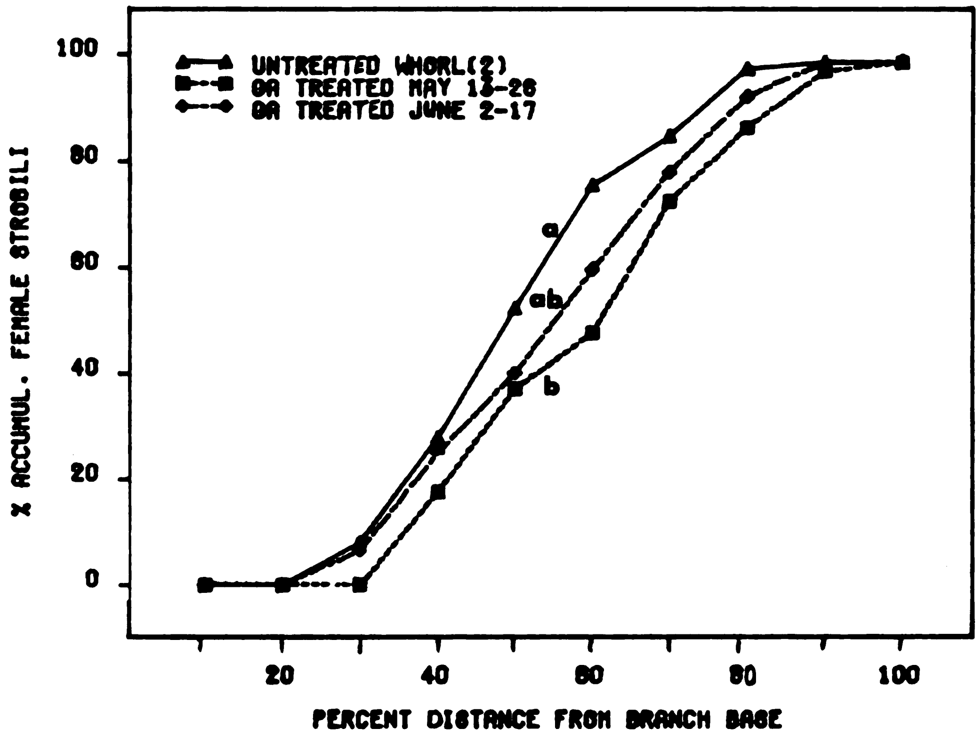


Figure 12. Comparison between the distribution of female strobili on second uppermost whorl branches of white spruce treated with gibberellin and untreated branches.

Karst. resulted from GA treatments that preceded meristematic differentiation by a short period of time. In this study, branches treated with GA beginning on June 2 tended to produce more female strobili than branches treated with GA beginning on May 13. This supports Owens and Molders hypothesis (1977) that the final phase of shoot elongation may be the most responsive time for floral enhancement treatments of white spruce.

However, GA treatment from May 13 to May 26 (which preceded meristematic differentiation by 3-4 weeks) also enhanced strobili production. Non-polar GAs apparently can be readily converted to polar forms which are considered ineffective in enhancing strobili production of most species within the *Pinaceae* family (Wample *et al*, 1975). If exogenously applied $GA_{4/7}$ in this study was rapidly converted to polar GA forms, then female strobili may have been enhanced indirectlty by endogenous metabolites other than GA.

The inability of GA treatments to consistently enhance female strobili on uppermost crown positions may be a result of endogenous chemical gradients. The uppermost whorl of 8- and 9-year-old white spruce was frequently the region where female strobili production was most prolific (Chapter 4). Perhaps conditions for generative production on uppermost crown branches cannot readily be improved by GA treatment. Furthermore, treated branches were closest in proximity on uppermost crown regions. Close proximity could have resulted in translocation of GA from treated to control branches thereby masking treatment effects on uppermost crown positions. Evidence of GA translocation was reported in other flower enhancement studies (Chalupka, 1978; Pharis *et al*, 1980; Wheeler *et al*, 1980).

Some physiological processes are not suppressed by high exogenous GA concentrations. However in this study, GA at a concentration of 500 ppm was supraoptimal for female strobili production ($p < .05$). Branches treated with a concentration of 250 ppm GA beginning on May 13 averaged 12.2 strobili, whereas branches that received 500 ppm GA averaged 6.0 strobili. Likewise, branches treated with 250 ppm beginning on June 2 averaged 16.5 strobili while those treated with 500 ppm averaged 13.7 strobili. Similiarly, a supraoptimal concentration of GA was found to exist for male strobili enhancement of sitka spruce (Tompsett and Fletcher, 1979).

The reduction in female strobili at a higher GA concentration contrasts with 6-year-old juvenile white spruce that tended to produce more female strobili in response to 500 ppm (Chapter 3). Within the *Cuppressaceae* and *Taxodiaceae* families, juvenile trees apparently lack the ability to synthesize adequate amounts of GA required to meet vegetative growth demands and to simultaneously differentiate generative structures (Pharis and Owens, 1966; Pharis and Morf, 1967). Perhaps increased age reduces the level of exogenous GA needed to maximize strobili production in white spruce. Long term studies should be undertaken to address this hypothesis.

Timing of GA application determined treatment success. However, dates used in this study are not suitable landmarks for initiation of GA treatments since genotype, latitude, and yearly variations in spring weather strongly influence time of bud break. At a given locale, the yearly fluctuation in spring bud break of white spruce can vary by 3 weeks (Baldwin, 1931; Cook, 1941). As suggested by Owens and Molder (1977), shoot elongation is a good indicator of meristematic activity, and GA treatments may be most beneficial after shoots have elongated to 50-85% of previous years' growth. In this study, female strobili production was enhanced by 3 weekly GA treatments, but the most effective duration and frequency of treatment remains to be determined.

Changes in differentiation patterns of female strobili following GA treatment support an hypothesis that endogenous chemical gradients may exist within individual shoots of white spruce. The proposed endogenous gradient of individual branches appeared dynamic in that the pattern of differentiation was related to the time of GA treatment. Likewise, seasonal changes in the level of exogenous gibberellin-like substances have been found in *Picea abies* (Dunberg, 1977).

Lateral meristems located on distal branch positions were more responsive to GA treatments. This observation could be explained if distal branch positions had a suboptimal concentration of an endogenous factor that became elevated by exogenous GA treatment. In contrast, proximal branch positions could have a supraoptimal concentration of an endogenous factor. Further study is necessary to explain these changes in patterns of differentiation.

The inability of GA to promote male strobili initiation was unexpected. Trees grown under similiar conditions produced significantly more male strobili on whorl-3 and whorl-4 branches after 5 weekly applications of $GA_{4/7}$ (Chapter 4). Likewise, GA has preferentially enhanced male strobili production in other studies (Chalupka, 1978; Luukkanen and Johansson, 1980). Treatment duration required to cause a positive response may be greater for male strobili than for female strobili.

In conclusion, to maximize strobili production of white spruce, hormonal treatments must be properly timed and of the correct concentration. Crown position influences treatment success and $GA_{4/7}$ treatment apparently does not influence seed yield. The optimum duration and frequency for $GA_{4/7}$ treatments remains to be tested.

CHAPTER III

EFFECTS OF GROWTH REGULATORS AND ROOT-PRUNING ON STROBILI PRODUCTION OF JUVENILE *PICEA GLAUCA*

Abstract

Root-pruning (RP), naphthalene acetic acid (NAA), and the gibberellin mixture $GA_{4/7}$ were used in a factorial design to test their effects on enhancing strobili production of juvenile white spruce *Picea glauca*. A 174-fold increase in female strobili production was achieved on 6-year-old trees treated with 500 ppm $GA_{4/7}$ and 25 ppm NAA as a foliar spray plus RP over the untreated plantation average. When used alone, NAA had no effect on strobili production, RP caused a 12-fold increase, 250 ppm $GA_{4/7}$ caused a 14-fold increase, and 500 ppm GA caused a 52-fold increase in female strobili production. However, as individual treatments, only 500 ppm $GA_{4/7}$ plus NAA plus RP significantly enhanced strobili. Interactions between treatments were additive in their effect on female strobili production with the exception of NAA X RP. Regardless of treatment, no male strobili were produced on any of the 6-year-old trees and two year-old trees did not produce strobili of either sex.

Introduction

Experimental use of the gibberellins (GAs) to enhance flowering of gymnosperms began during the 1950's within the *Cuppressaceae* and *Taxodiaceae* families (see Pharis and Kuo, 1977). These classical experiments consistently showed strobili production to be enhanced by application of the polar gibberellin A_3. However, flowering results were discouraging when species in the *Pinaceae* family were treated with GA_3. With the availability of other GAs, numerous species in the *Pinaceae* family were shown to responded favorably to less polar GAs (see Pharis and Kuo, 1977). In addition, attempts are frequently made to enhance the effect of GA with adjunct treatments.

Naphthalene acetic acid (NAA) is one growth regulator which has been combined with GA. However, this treatment combination has given inconsistent results. Dunberg (1980) found no detectable difference on the flowering response of Norway spruce (*Picea abies* (L.) Karst.) when NAA was used alone or combined with GA_3, $GA_{4/7}$, or GA_9. Similiarly, Cecich (1981) found the addition of NAA to $GA_{4/7}$ did not change the effect of $GA_{4/7}$ on female strobili production of jack pine (*Pinus banksiana* Lamb.) However, Tompsett (1977) found that NAA with $GA_{4/7}$ and GA_3 reduced female strobili production and increased male strobili production of sitka spruce (*Picea sitchensis* (Carr) Bong.). Luukkanen and Johansson (1980b) also observed a suppression in female strobili production of Scots pine (*Pinus sylvestris* L.)

treated with NAA and GA. Low levels of NAA were found to enhance female strobili production of Douglas-fir (*Pseudotsuga mensiezii* (Mirb.) Franco) but higher NAA levels reduced female production (Pharis *et al*, 1980; Ross *et al*, 1980)

As a cultural treatment, root-pruning can enhance strobili production. Studies with Douglas-fir (Silen, 1973a), white spruce (*Picea glauca* (Moench) Voss) (Holst, 1968), and loblolly pine (*Pinus taeda* L.) (Gregory and Davey, 1977) are notable examples of floral stimulation by root-pruning. Additionally, strobili production of some conifer species can be increased when an imposed water-stress is combined with a GA treatment (Greenwood, 1977; Ross *et al*, 1981, Brix and Portlock, 1982).

In the *Picea* genus, *P. sitchensis* and *P. abies* have been preferentially used in floral promotion research. This study was undertaken to examine the effects of $GA_{4/7}$, NAA, and root-pruning on strobili production of juvenile *P. glauca*.

Materials and Methods

Experiment 1

During the spring of 1981, sixty 6-year-old white spruce trees were selected for experimental treatment based on size and site uniformity. Selected trees comprised a portion of a young seed orchard located in southwestern Michigan. While some trees in the plantation produced female strobili in previous years (about 1%), selected trees showed no signs of maturity. Each tree was randomly assigned one of 12 treatments which comprised a 3 X 2 X 2 factorial experiment. Three levels of $GA_{4/7}$ (0, 250 and 500 ppm) were factored with 2 levels of NAA (0 and 25 ppm) which were factored with a root-pruning and control treatment. Five trees replicated each treatment.

Root-pruning was conducted on April 1, 1981, well in advance of bud break. Tree roots were severed to a depth of 40cm using a hand spade. Root-pruning began at the drip-line of each tree and continued at a 45^{o} angle to produce a cone shaped aggregate of undisturbed roots.

Household atomizers were used to apply growth regulators as a foliar spray until run-off. The solvent used was a 19:1 (v:v) water:ethanol solution with the surfactant Aromox C-12w added (0.02% active ingredient by volume). Composition of the $GA_{4/7}$ mixture was 19:12 by weight. Growth regulators were applied at weekly intervals from June 3 until June 30, 1981. The treatment period was chosen to bracket the time meristematic differentiation was thought to occur. Spraying with growth regulators was limited to new shoots comprising the uppermost whorl and internode where female strobili are most frequently initiated (Chapter 4).

On days of hormonal treatment, shoot elongation of the uppermost whorl was measured on 3 untreated trees and apical meristems were collected to quantify shoot elongation and meristematic activity in relation to the treatment period. Collected meristems were killed and fixed under vacuum in

FAA, dehydrated in tertiary-butyl alcohol (Berlyn and Miksche, 1976), imbedded in paraplast, longitudinally sectioned at 10µ, and stained in hematoxylin and safranin (Johansen, 1940). To assess the effect of root-pruning on water potential and vegetative growth, a pressure bomb (PMS Instrument Co., Corvallis, Oregon) was used to measure afternoon shoot water potential on June 10, 1981. Sampled branches were from the uppermost internode of control trees. Terminal shoot elongation was measured after cessation of growth following the 1980 and 1981 growing season. Growth measurements were normalized by expressing terminal shoot elongation as a percentage of total tree height.

During the spring of 1982, strobili counts were made on all trees in the plantation. An analysis of variance separated treatment differences based on female strobili production. The percentage of trees that flowered was transformed to arc-sine and analyzed by a t-test.

Experiment 2

A total of 192 white spruce were grown under accelerated-optimum-conditions (Hanover, 1976) from February 28, 1980, when seeds were sown, until early summer, 1980, when seedlings were moved out-of-doors. Seedlings were grown in plant bands measuring 5cm X 5cm X 27cm until March 30, 1981. On this date, the lower half of the root system was pruned from 96 trees and all tree seedlings were transplanted into larger plant bands measuring 7.6cm X 7.6cm X 27cm.

Trees were randomly assigned to 1 of 12 treatments as described in experiment 1. Treatment with growth regulators began on June 5 and continued at weekly intervals until July 7, 1981. Growth regulators were applied as described in experiment 1, with the exception that entire seedlings were treated. Each treatment was replicated by 16 trees. Trees were evaluated for strobili production during the spring of 1982.

Results

Experiment 1

Effects of Root-Pruning on Vegetative Growth

Root-pruning significantly reduced shoot water potential. Two months after root-pruning, pruned trees had an average water potential of -16.9 bars, whereas unpruned trees averaged -14.1 bars ($p <.05$). Excessive deficits in shoot water potential may reduce photosynthesis. Beadle and Jarvis (1977) recorded a rapid reduction in CO_2 uptake at shoot water potentials below -12 bars in sitka spruce.

Water stress of root-pruned trees was also manifested in terminal shoot elongation. Following root-pruning, terminal shoot elongation of the treated group was significantly reduced ($p <.01$). Terminal shoot elongation of unpruned trees averaged 42.7% of total height, whereas root-pruned trees elongated 26.1% of total height. The reduction in terminal shoot elongation amounted to 39%. During the growing season that preceded the root-pruning treatment, unpruned trees elongated 39.9% of their total height and root-pruned trees elongated 40.0% of their total height.

Treatment Effects on Generative Production

Treatment by GA and root-pruning enhanced the percentage of trees that flowered ($p < .01$), but the two levels of GA did not differ significantly in their effect. NAA did not effect the percentage of trees that flowered. Seventy-two percent of root-pruned trees bore 1 or more female strobili compared to 21% for the pooled controls. In addition, 57% of the GA treated trees flowered compared to 25% for the pooled controls. All trees in experiment 1 that flowered had been root-pruned or had received a $GA_{4/7}$ treatment.

Hereafter, 'strobili' will denote female strobili production since no male strobili were produced in either experiment. $GA_{4/7}$, NAA, and root-pruning as main treatments significantly enhanced strobili production (Table 2). When pooled by GA treatment, trees that received 500 ppm and 250 ppm averaged 15.8 and 11.0 strobili per tree, respectively. Numerically, the responses represent a nearly 7- and 5-fold increase in strobili production over the pooled control. However, only the pooled 500 ppm treatment was statistically different from the pooled control. Root-pruned trees produced an averaged of 13.8 strobili per tree and the pooled control trees averaged 5.0 strobili.

The combination of the three main treatments (GA, NAA, and root-pruning) were additive in their effect on strobili production with the exception of the NAA by root-pruning

Table 2. Analysis of variance based on female strobili production of white spruce following treatment with gibberellin, naphthalene acetic acid, and root-pruning.

SOURCE OF VARIATON	DF	MS	F
Total	59	325.5	
GA	2	1006.9	3.99**
NAA	1	1058.4	4.20**
Root-Pruning	1	1421.1	5.63**
GA X NAA	2	215.5	0.85
GA X Root-Pruning	2	123.0	0.49
NAA X Root-Pruning	1	1215.0	4.82**
GA X NAA X Root-pruning	2	610.4	2.42
Error[1]	46	252.2	

[1] Two degrees of freedom were subtracted from the error since two missing values were calculated

** Significant at the 5% level

interaction (Table 2). Strobili production decreased slightly on unpruned trees treated with NAA but increased on trees that were root-pruned and received NAA. NAA and GA were additive in their effect on strobili production as was root-pruning with GA (Table 2) Statistically, however, only one individual treatment produced more strobili than the control group: 500 ppm GA plus NAA plus root-pruning (Table 3).

Meristematic Activity and the Treatment Period

To ensure success in floral promotion, suitable chemical and cultural treatments should precede meristematic differentiation. In addition, the final phase of shoot elongation appears to be the optimum time to enhance strobili production of white spruce (Owens and Molder, 1977). In this study, growth regulator treatments began on June 3rd when shoot elongation was 66% of final length and apical meristems were initiating bud scale primordia. Shoots had completed elongation and meristems were initiating needle primordia when the treatment period concluded. Therefore, hormonal treatments did bracket the differentiation period.

Experiment 2

The 2-year-old seedlings used in this experiment, which received identical treatments as trees in experiment 1, did not produce a single strobili of either sex. Presumably, white spruce at such an early age cannot readily be induced to initiate generative structures by treatments used in this study.

Table 3. Mean female strobili production and percentage of flowering trees after treatment with gibberellin, naphthalene acetic acid, and root-pruning.

	NON-ROOT-PRUNED		ROOT-PRUNED		POOLED BY GA TREATMENT
	NAA		NAA		
GA level	0 ppm	25 ppm	0 ppm	25 ppm	
0 ppm	0.0 (0)[1]	0.0 (0)	3.0 (40)	6.6 (60)	2.3 (17)
250 ppm	3.4 (0)	8.6 (20)	9.8 (80)	22.4 (80)	11.0 (30)
500 ppm	12.6 (60)	5.5[2] (25)	5.4 (80)	41.8[2,3] (100)	15.8 (43)
POOLED BY ROOT-PRUNING	5.0 (20.7)		13.8 (72.4)		

[1] Percentage of trees that produced female strobili.
[2] Treatments replicated by 4 trees.
[3] Significantly different from the control at the 5% level.

Discussion

The flowering response of treated trees in experiment 1 was more dramatic when compared to strobili production of trees in the plantation excluded from experimentation. Trees outside of the study group averaged 0.24 female strobili per tree (58 strobili were produced on 19 of 241 trees). Using this average, the 2 treatments that combined GA, NAA, and root-pruning increased strobili production 174- and 93-fold. Previously, the largest increase in female strobili production reported was 100-fold on *Tsuga heterophylla* (Raf.) Sarg. following calcium nitrate fertilization in combination with $GA_{4/7}$ (Ross *et al*, 1981).

Due to the large variation in strobili production within individual treatments, only one individual treatment was statistically different from the control. However, every treatment except NAA alone could be argued to have had a biological effect on strobili production. Root-pruning alone produced the fewest number of female strobili (greater than zero) (Table 3). However, this treatment produced a 12-fold increase in strobili production above the plantation average. For tree improvement programs hampered by inadequate strobili production, such an increase would be beneficial. Likewise, the 250 ppm $GA_{4/7}$ plus NAA plus root-pruning treatment was not statistically different from the control but increased strobili production 93-fold over the plantation average.

The ability to induce strobili production on 6-year-old trees contrasted with that of 2-year-old seedlings. With increasing age, trees become more complex and the number of meristems available for generative production increases. However, physiological changes that occur concurrently with increasing age perhaps are more important. For example, juvenile trees may lack the ability to synthesize adequate amounts of GA necessary to maintain vegetative growth and to signal generative production.

How root-pruning influenced strobili production remains speculative. Perhaps root-pruning increased abscisic acid levels, known to increase in concentration following water stress and thereby alterating the endogenous hormonal balance. Vegetative suppression following root-pruning may have signalled floral initiation through a reallocation of available metabolites.

A natural phenomenon, perhaps analogous to vegetative suppression without lowering photosynthetic rates, is the shortening of the vegetative growth period as trees age. The duration of vegetative growth of species within the *Picea* genus decreases with age (Nienstaedt, 1972; Hanover, 1981). Older trees with a shorter growth phase may provide adequate levels of metabolites necessary for generative initiation. Whereas carbohydrate accumulation is implicated in cultural treatments that promote flowering (thinning, girdling, and strangulation), results in the literature do not unequivocally support high carbohydrate levels as beneficial to flower promotion.

The inability to induce male strobili was unexpected. Whereas hormonal treatments were restricted to the uppermost crown region of the tree, auxin and GA are thought to be readily translocated. Male strobili are also known to occur as high in the crown as the uppermost whorl in white spruce (personal observation), and GA treatments can preferentially enhance male strobili production (Luukkanen and Johansson, 1980b; Chalupka, 1978). Additional work needs to be conducted to identify the requirements that influence sex determination in conifer species.

From this study, white spruce was shown to respond to suitable hormonal and cultural treatments. Although inherent variation between trees masked individual treatment differences, foliar application of $GA_{4/7}$ in combination with root-pruning can be recommended as an experimental treatment to enhance strobili production of white spruce. Whereas NAA was additive in combination with $GA_{4/7}$ in this study, further experimentation with NAA is necessary to verify its merit as an adjunct treatment with $GA_{4/7}$.

CHAPTER IV

VERTICAL DISTRIBUTION OF STROBILI AND EFFECTS OF GIBBERELLIN TREATMENT ON FLOWERING OF SELECTED BRANCHES OF *PICEA GLAUCA*

Abstract

The distribution of strobili on crowns of 8-year-old white spruce (*Picea glauca* (Moench) Voss) was evaluated. The uppermost region of the tree crown was limited to female strobili whereas male strobili predominated on lower crown regions. A transitional crown region was defined where both sex types frequently occurred. Hermaphroditic strobili produced by one tree were in the transitional zone. The gibberellin mixture $GA_{4/7}$ was applied as a foliar spray to branches in the transitional zone and strictly male-zone. Treatment with 500 ppm $GA_{4/7}$ increased female strobili production 6.2-fold and male strobili production 2.4-fold on branches in the transitional zone. On male-zone branches treated with $GA_{4/7}$, female strobili were induced and male strobili production increased 6-fold.

Introduction

The gibberellin mixture $GA_{4/7}$ has been used successfully to induce or enhance strobili production on tree species within the *Pinaceae* family (see Paris and Kuo, 1977; Ross *et al*, 1980; Cecich, 1981; Bonnet-Masimbert, 1982). With few exceptions (Chalupka, 1978, 1980; Tompsett *et al*, 1980; Bonnet-Masimbert, 1982), the effects of crown location have not been considered when GA treatments are applied to conifer species. Crown location is an important factor since most conifers exhibit sexual zonation within their crowns.

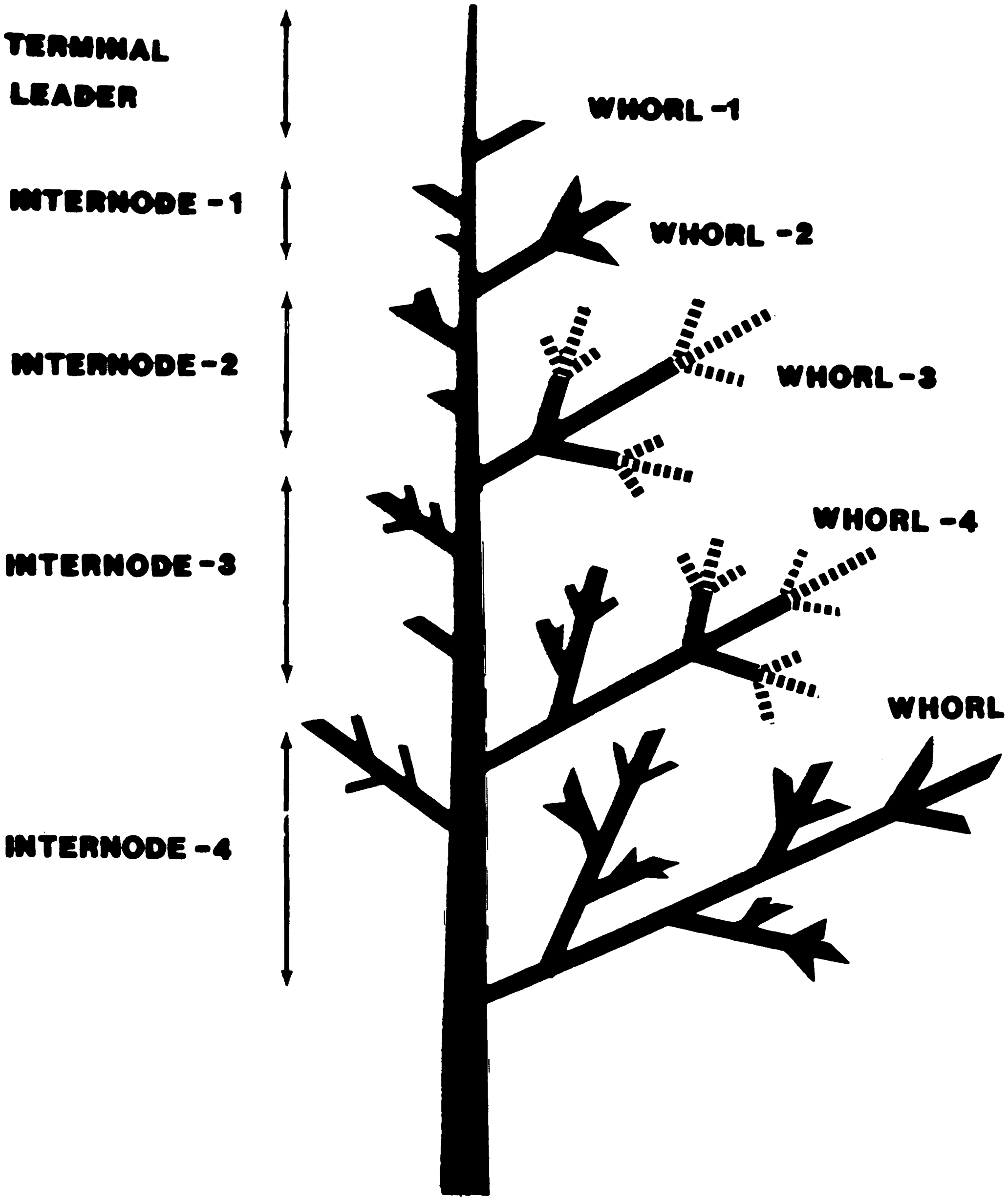
White spruce (*Picea glauca* (Moench) Voss) is one species that shows sexual zonation. Typically, female strobili are produced on uppermost whorls and internodes of the tree crown, whereas male strobili usually predominate on lower portions of the female zone and on whorls and internodes below the female zone. This stratification of male and female strobili results in a transitional area of the crown which has the potential to bear both sex types. The transitional zone at one point in time is an area of the crown that in the past was exclusively a female region. With aging and height growth, the female zone becomes elevated so that the portion of the crown which was predominately female transforms into one capable of producing mostly male strobili. One explanation for the zonal patterns of strobili distribution is that endogenous chemical gradients exist which effect generative production.

The object of this study was to determine the effects exogenous GA treatments have on sex differentiation of white spruce branches in the transitional zone and in the strictly male-zone. This study addressed the questions: can female strobili production be induced in a region which naturally produces only male strobili; and how is sexual differentiation in the transitional zone modified when $GA_{4/7}$ is exogenously applied?

Materials and Methods

Eight- and nine-year-old white spruce of seed origin, located in a spruce plantation in southwestern Michigan, were selected for study of their vertical strobili distribution. During the spring of 1981, 75 of 93 sexually mature trees were evaluated for female strobili distribution. Due to the ephemeral nature of male strobili, their distribution could be accurately evaluated on only 24 trees. To facilitate quantification, tree crowns were divided into distinct regions. Regions were defined as: (A) the terminal leader, (B) all branches comprising an individual nodal whorl, and (C) all branches comprising an individual internode. Whorls were numbered in a basipetal direction beginning at the whorl subtending the terminal leader. Internode regions were numbered the same as the whorl which they subtended (Figure 13). From one tree which possessed 5 hermaphroditic strobili, the location of all strobili produced was recorded.

Figure 13. Diagrammatic representation of the uppermost 8 crown regions of white spruce and the portion of whorl-3 and whorl-4 branches treated with gibberellin.


TERMINAL LEADER
WHORL -1
INTERNODE -1
WHORL -2
INTERNODE -2
WHORL -3
INTERNODE -3
WHORL -4
WHORL -5
INTERNODE -4

After sexual zonation of the study trees was quantified, nine trees were randomly selected to receive GA treatment. To minimize translocation of GA, only two branches on each tree received GA. On selected trees, one whorl-3 and whorl-4 branch were randomly chosen to receive a foliar spray of 500 ppm of the GA mixture $A_{4/7}$ (Figure 13). During treatment, branches were cupped with a modified funnel to eliminate chemical drift. Control branches were randomly selected from the remaining whorl-3 and whorl-4 branches. Whorl-3 and whorl-4 branches represented a portion of the transitional zone and the male zone, respectively. The ratio of A_4 to A_7 was 19:12 by weight and the foliar spray was a 19:1 (v:v) water-ethanol solution with the surfactant Aromox C-12w added (0.02% active ingredient by volume). The GA solution was applied until run-off using a household atomizer. Trees were treated at weekly intervals from June 3 until July 1, 1981 and the treatment period was chosen to bracket meristematic differentiation. Strobili produced on the treated and control branches were counted during the spring of 1982.

Analysis of variance separated sources of variation and determined the effect of GA on strobili production. Inherent differences in flowering potential between individual trees and between the two crown locations were removed by a split-plot experimental design.

Results and Discussion

Distribution of Strobili

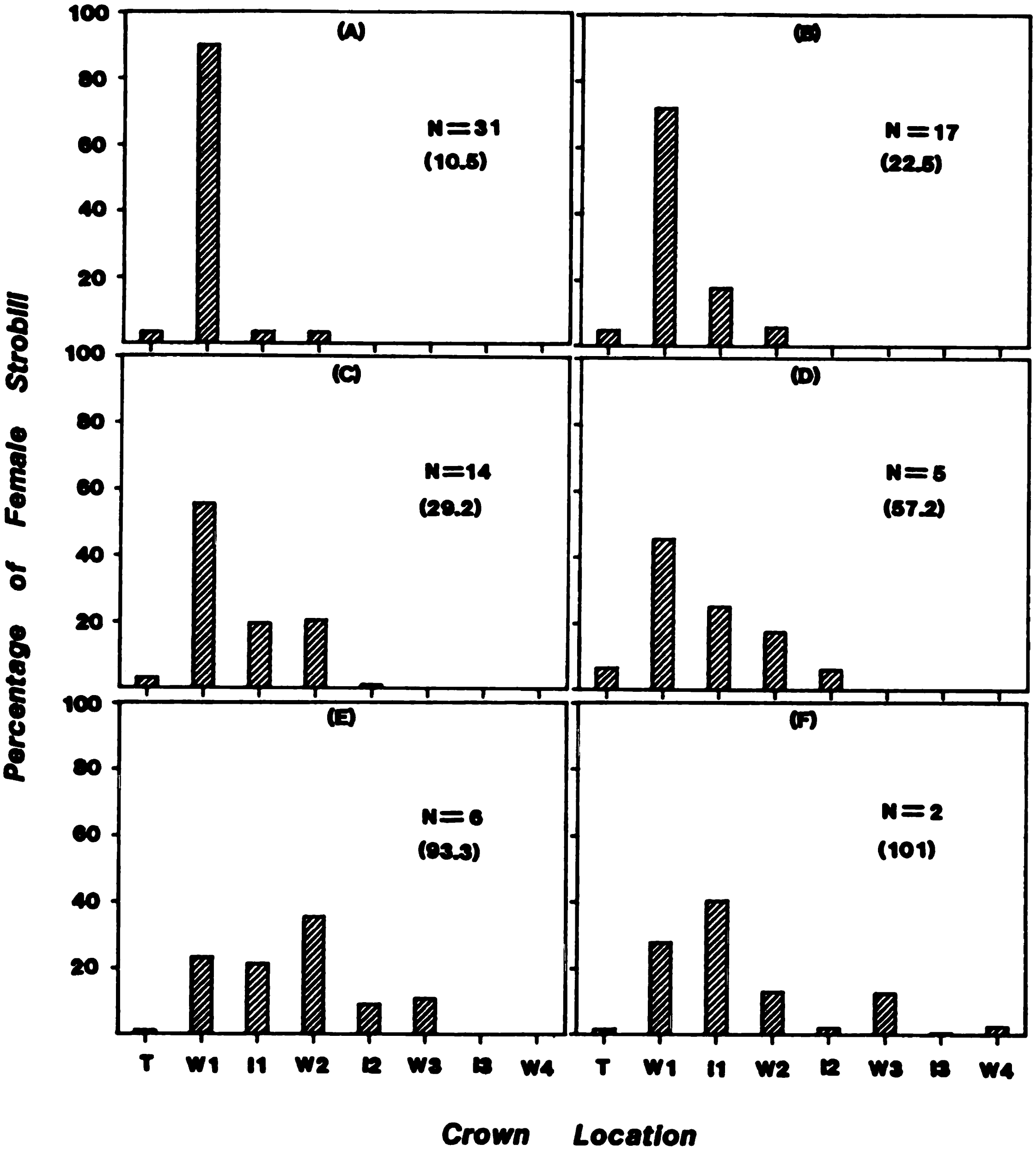
Six histograms were generated to show the relationship between size of the female bearing zone and female strobili distribution (Figure 14). Size of the female zone was the number of crown regions across which female strobili were produced. The majority of trees evaluated (31 of 75) produced female strobili on only one crown region (Figure 14a). Of the female strobili produced by those trees, 90% were located on whorl-1. The remaining 10% of the strobili were equally distributed on the terminal leader, internode-1, and whorl-2.

Seventeen trees produced female strobili across two crown regions (Figure 14b). Again, the majority of strobili produced (72%) were found on whorl-1 with an additional 18% produced on internode-1. The remaining strobili were located on the terminal leader and whorl-2 branches. No female strobili were produced below whorl-2.

The distribution of female strobili on trees having a larger female zone is shown in Figure 14c-f. As the size of the female zone increased, there was a concurrent increase in fecundity (Figure 14). Simple correlation between the size of the female zone and the average number of female strobili per tree was highly significant ($r = 0.96$, $p < .005$, d.f. = 4)

For each group of trees that comprised a histogram, one

Figure 14. Vertical distribution of female strobili in relation to crown position and size of the female zone of 8- and 9-year-old white spruce. N = number of trees comprising each histogram and average strobili production is given in parenthesis. T denotes terminal leader, W-1 denotes the uppermost whorl, and I-1 denotes the uppermost internode. A) Trees bearing female strobili on 1 crown region B) across 2 crown regions C) across 3 crown regions D) across 4 crown regions E) across 5 crown regions E) across 6 or more crown regions.


(A)
N=31
(10.5)
(B)
N=17
(22.5)
(C)
N=14
(29.2)
(D)
N=5
(57.2)
(E)
N=6
(93.3)
(F)
N=2
(101)
100
80
60
40
20
Percentage of Female Strobili
T
W1
I1
W2
I2
W3
I3
W4
Crown Location

crown region appeared most favorable for female strobili production. This apparent "optimum" producing region shifted from whorl-1 on trees having relatively low fecundity (Figure 14a-d) to internode-1 or whorl-2 on trees having relatively high fecundity (Figure 14e,f). In addition, production of female strobili decreased rapidly with an increase in the vertical distance from these apparent optimal regions, especially on trees with a small female zone (Figure 14).

A composite distribution of both male and female strobili is shown in Figure 15. Whorl-3 produced the largest portion of male strobili (almost 34%) on the 24 trees evaluated. While 1% of the male strobili were produced as high in the crown as internode-1; whorl-2 defined the upper limit of the male zone for most trees. No male strobili were produced below whorl-5. Of the male strobili produced, 98% occurred in the crown from whorl-2 to internode-4 (Figure 15). Similiar to female zonation, there appeared to be a crown region that was most favorable for initiation of male strobili. Of the trees studied, almost 55% of the male strobili were initiated on internode-2 and whorl-3. The crown region above whorl-2 was almost exclusively female and the crown region below whorl-3 was almost exclusively male (Figure 15). The intermediate region, whorl-2 to whorl-3, represented the transitional zone where both sex types frequently occurred. This sexual pattern could be explained based on an endogenous chemical

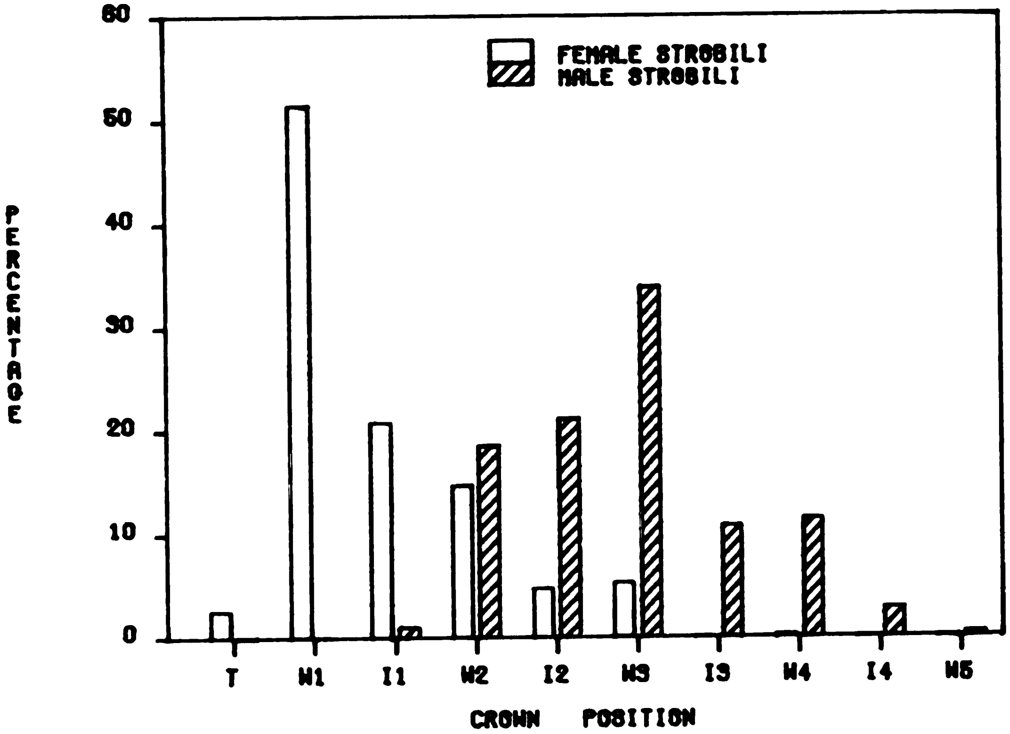


Figure 15. Distribution of female and male strobili in relation to crown position of 8- and 9-year-old white spruce.

balance which influenced differentiation.

Female and male strobili produced by one white spruce having 5 hermaphroditic strobili were well distributed in their respective zones, as defined above. Hermaphroditic strobili were located on whorl-2 and whorl-3, which corresponded to the transitional zone. The presence of 5 hermaphroditic cones and the change in differentiation from microsporophylls to ovuliferous scales indicated a subtle physiological balance may exist in the transitional zone. Hermaphroditic strobili are uncommon in the *Picea* genus and are apparently caused by environmental factors (Santamour, 1959).

Effects of Gibberellin Treatment on Strobili Production

Treatment with $GA_{4/7}$ on whorl-3 and whorl-4 branches had a significant effect on production of both male and female strobili (Table 4). Whorl-3 branches treated with GA averaged 21.0 female strobili which represented a 6-fold increase over control branches that averaged 3.4 strobili. Whorl-3 branches treated with GA averaged 11.8 male strobili per branch and control branches averaged 4.9 strobili (Table 4).

Similiarly, GA enhanced strobili production on whorl-4 branches. While whorl-4 was exclusively male on untreated branches (Table 4), an average of 12.8 female strobili were induced on GA treated branches. In response to exogenous GA treatment, whorl-4 branches no longer exhibited strict maleness, but rather became transitional in sexuality. Male

Table 4. Male and female strobili production of selected branches of white spruce treated with gibberellin. Whorl-3 branches represented the third nodal whorl below the terminal leader.

	FEMALE STROBILI PRODUCTION				MALE STROBILI PRODUCTION			
	WHORL-3		WHORL-4		WHORL-3		WHORL-4	
tree #	GA	Cont.	GA	Cont.	GA	Cont.	GA	Cont.
1	39	8	34	0	0	0	2	0
2	31	11	17	0	21	1	7	3
3	7	0	5	0	29	4	24	2
4	15	0	10	0	7	0	13	0
5	32	11	23	0	0	2	2	0
6	15	1	8	0	9	4	7	2
7	27	0	13	0	9	7	5	0
8	5	0	3	0	0	0	0	0
9	18	0	2	0	31	26	26	7
mean	21.0***	3.4	12.8***	0.0	11.8**	4.9	9.6**	1.6

,* Significantly different from the control at the 5% and 1% level, respectively.

production on whorl-4 was also significantly enhanced by GA treatment. Whorl-4 branches treated with GA averaged 9.6 male strobili and control branches averaged 1.6 strobili (Table 4).

As mentioned earlier, there was a distinct area of the tree crown which appeared optimal for initiation of strobili of each sex. As the vertical distance from these optimal areas increased, enhancement of strobili production by GA decreased. The ability to enhance female strobili decreased from whorl-3 to whorl-4. After GA treatment, whorl-3 branches averaged 21.0 female strobili and whorl-4 branches averaged 12.8. These two means were significantly different at the 1% probability level. The ability to enhance male strobili by GA decreased numerically from 11.7 strobili per branch on whorl-3 to 9.6 on whorl-4. Whereas the number of strobili enhanced by GA treatment decreased with distance from the optimal regions, the efficiency (defined as " -fold increase") of the GA treatments to enhance strobili production apparently increased. For example, whereas male strobili production increased 2.4-fold over controls on whorl-3, a 6-fold increase was realized on whorl-4. Female strobili production responded in a similiar way. Treatment of whorl-3 branches with GA produced a 6-fold increase in female strobili. Whorl-4 control branches completely lacked female strobili whereas the treated branches averaged 12.8 strobili.

This apparent increase in the efficiency of GA with

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increased distance from an optimum strobili-producing zone supports the hypothesis of an endogenous chemical gradient. Limiting chemical concentrations along the proposed gradient could be a barrier to generative differentiation and phytohormones are the most likely chemicals to be involved.

If endogenous GA is involved in meristematic differentiation, exogenously supplied GA could help to maintain relatively high endogenous levels. Tompsett (1978) has proposed the hypothesis that differentiation of sitka spruce (*Picea glauca* (Bong.) Carr) is based on early apical growth rate. Thus, GA treatments may modify individual bud vigor to produce the observed changes in strobili production. GA_3 has been shown to increase cell division in a wide variety of plant species (Jones, 1973). The decrease in the ability of GA treatments to enhance flowering of progressively older branches corresponds well with Fraser's (1962) finding that vigor of black spruce (*Picea mariana* (Mill.) B.S.P.) branches decreases with the age of tree whorls.

Branches treated with GA in the transitional zone can be stimulated to produce both male and female strobili. Similiarly, branches in the male zone can be stimulated to increase male strobili production and to 'induce' female strobili. Similiarly, Bonnet-Masimbert (1982) induced female strobili on branches in the male region of *Larix* crowns with exogenous $GA_{4/7}$ treatments.

From a practical standpoint, the ability to induce such strobili production may be very significant. If the size of the female zone can be enlarged by chemical treatment, seed production of selected trees may be increased. Likewise, an increase in male strobili production of selected trees would be helpful when pollen supply is limited. Both of these responses would benefit tree breeding programs.

CHAPTER V

SHADE EFFECTS ON ENVIRONMENTAL PARAMETERS AND THE BIOLOGICAL RESPONSE OF FIELD GROWN *PICEA GLAUCA*

Abstract

Environmental and biological parameters were measured within a *Picea* plantation located adjacent to a hardwood stand that created a continuum of light and associated environmental conditions within the plantation. White spruce (*Picea glauca* (Moench) Voss) trees planted most closely to the hardwood stand (in the most shaded region of the plantation) were significantly shorter. Likewise, fecundity of white spruce increased 8-fold with increasing distance from the hardwood stand. Photon flux density was significantly correlated with fecundity and tree height. While gradients in spectral quality and ambient temperature were measured, their effect on biological differences observed in the study plantation appeared negligible.

Introduction

Tree improvement programs that can adequately supply genetically superior seedlings will help meet the increasing demand for wood products. However, tree improvement programs are frequently hampered by the long generation interval of many commercially important species. A problem closely linked to the long generation time is the sporadic and often times meager flowering of mature trees. Recently, methods have been proposed to increase seed orchard production through intensive management (Sprague *et al*, 1979; Masters, 1982; Ross and Pharis, 1982). However, to maximize seed production it is imperative to first identify the edaphic and environmental requirements that influence maturation and fecundity of the species of interest.

Shading is one environmental factor known to cause a reduction in floral initiation. Migita (1960) found *Cryptomeria* did not flower when subjected to shade regimes of 15% of full sunlight. Heavily shaded apple trees showed a significant reduction in flower production (Jackson and Palmer, 1977a) in addition to the number, weight, and length of vegetative shoots (Jackson and Palmer, 1977b).

Female strobili are frequently initiated on crown positions which receive relatively more direct solar radiation. Female strobili on *Pinus sylvestris* L. were consistently initiated on distal whorl sectors regardless of the cardinal direction to which shoots were oriented (Kosinski and Giertych, 1979). Smith and Stanley (1968) found a significant difference in the number of female strobili on various crown positions of *Pinus elliottii* Engelm. and concluded that light is probably the major factor modifying reproductive patterns in pine.

Shading can significantly influence reproductive initiation in at least three ways. First, moderate shading can reduce net photosynthesis, thereby reducing metabolite availability. Secondly, direct sunlight can significantly increase internal bud temperatures above ambient temperatures (Pukacki, 1980). Finally, shading by an overstory will change light quality received by understory vegetation (Holmes and Smith, 1977).

The influence of temperature on meristematic regulation is implicated from flower promotion studies utilizing increased air temperature surrounding tree crowns to promote strobili production (Luukkanen, 1979; Pollard and Portlock, 1981; Chalupka *et al*, 1982). Likewise, Tompsett and Fletcher (1977) were able to increase male strobili production 15-fold and female strobili production 43-fold by using polyethylene greenhouses to increase air temperature surrounding grafts of *Picea sitchensis* (Bong.) Carr. Increasing air temperature surrounding individual branches also increased endogenous levels of gibberelin-like substances in *Picea abies* (L.) Karst. (Chalupka *et al*, 1982), which is congruent with flower enhancement results following GA treatment (see Pharis and Kuo, 1977).

This study is a compilation of biological and environmental data collected from a *Picea* breeding plantation. The study plantation was established in 1974 directly west of a mature hardwood stand which created a continuum of light and associated environmental conditions within the plantation. In addition, the effect elevated temperature surrounding white spruce branches (*Picea glauca* (Moench) Voss) had on strobili production was evaluated.

Materials and Methods

During 1981, 149 8- and 9-year-old white spruce in a *Picea* breeding plantation were evaluated for total tree height, terminal shoot elongation during 1981, bud break, and female strobili production. To quantify bud break, each tree was assigned to 1 of 5 phenological classes based on vegetative bud characteristics on April 30 (Table 5). Twenty-four of the 149 trees were evaluated for male strobili production.

An adjacent hardwood stand shaded portions of the *Picea* plantation until late morning. To quantify environmental and biological parameters, the study plantation was divided into 4 sun regions (SRs) based on the time of day that each region first received direct solar radiation (Figure 16).

Spectral quality was measured on May 5, 13, and June 8, 1982, in direct sunlight and in the shade of the hardwood stand. Measurements were taken using an ISCO spectroradiometer traceable to the National Bureau of Standards. Dates of measurement corresponded to pre-bud break, half-leaf expansion, and full-leaf expansion of the

Table 5. Phenological index and biological characteristics used to quantify bud break of white spruce.

NUMERIC VALUE	CHARACTERISTICS
1	All tree buds remain dormant
2	Buds on uppermost whorl are dormant Buds on lower whorls have swollen
3	Buds on uppermost whorl have swollen
4	Uppermost whorl has broken bud and shoots are less that 3 centimeters
5	Uppermost whorl has broken bud and shoots are more than 3 centimeters.

hardwood stand. Spectral quality was measured at 380nm and begining at 400nm was measured at 25nm intervals to 750nm. Measurements were replicated three times at each light regime and date. Zeta, the ratio of the quantum flux in 10nm wide wavelengths bands at 660nm and 730nm, respectively, was calculated for each date and light regime.

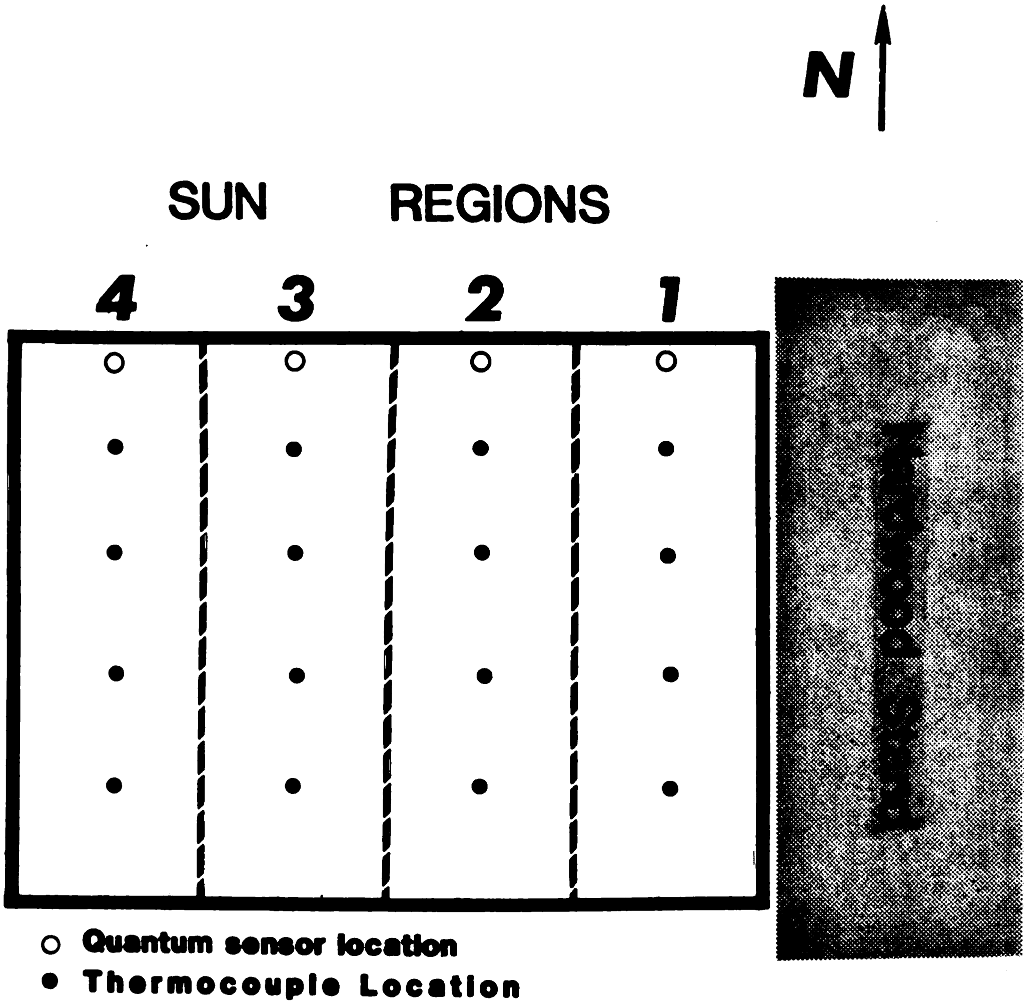
On June 23, 1982, photosynthetically active radiation (PAR) and ambient temperature were meaured within each SR. PAR represents that portion of the electromagnetic spectrum from 400nm to 700nm. Data was collected on June 23 because of clear weather and this date represented a summer day that could impart environmental influence on meristematic differentiation. In each SR, one Li-Cor terrestrial quantum sensor measured PAR and 4 cromel-alumel type KX thermocouples measured ambient temperature. During data collection, thermocouples were shaded and measured ambient temperature at about 1m above the soil surface. Quantum sensors and thermocouples were wired to a Digistrip II datalogger and data were collected at 10 minute intervals over a 24 hour period.

Quantum sensors were equilibrated using 10 instantaneous readings taken over a three day period. For equilibration, quantum sensors were placed within 20cm of each other and correction factors were calculated for each quantum sensor based on the grand mean of the 10 equilibration readings. Likewise, thermocouple junctions were immersed in distilled ice water to ensure their accuracy to ± .5°C. Diagrammatic representation of the SRs and the location of quantum sensors and thermocouples is given in Figure 16. PAR accumulation and a daily heat sum (degree-hour) were calculated for each SR from data collected on June 23.

Forty-three white spruce were selected to assess the potential of enhancing female strobili production by increasing air temperature surrounding tree crowns. Trees were randomly chosen from a group that did not produce strobili during 1981 (an average cone year) and therefore were considered 'poor' flowering individuals. To increase air temperature of tree crowns, the uppermost whorl and internode of selected trees were enclosed in a polyethylene bag. Ten small holes were made in each bag to allow some air exchange. Fourteen trees were 'bagged' from June 3 to June 23, 11 trees were bagged from June 24 to July 15, and 18 trees served as controls. From anatomical work reported in Chapter 2, meristematic differentiation was known to occur during late June.

Using a pressure bomb (PMS Instrument Co., Corvallis, Oregon), shoot water potential of bagged and control trees was measured during the afternoon of June 11, July 7, and July 15, 1981. Water potential was measured on shoots from the uppermost internode of each tree. A Sargent-Welch thermometer was used to measure afternoon temperature of the enclosed crown region and ambient temperatures on June 11, 18, July 7, and July 15, 1982. Strobili production following treatment was evaluated during the spring of 1982.

Figure 16. Representation of the 4 sun regions established in a *Picea* plantation in relation to a hardwood stand, and the placement of thermocouples and quantum sensors used to quantify environmental parameters.


N
SUN REGIONS
4
3
2
1
○ Quantum sensor location
● Thermocouple Location

Results and Discussion

Biological differences clearly existed between SRs. Trees growing in SR4 (the sunniest region of the plantation) were significantly taller than trees in SR1 or SR2 (Table 6). Average tree height increased progressively from SR1 to SR4.

The effect of shade on fecundity was more dramatic. The percentage of trees which flowered in 1981 was significantly related to SR location ($p < .05$) using a chi-squared test of independence. When the data from SR1 were removed and the test of independence rerun, chi-square was insignificant. This lack of significance indicated SR1 was the major contributor to the association, and that the remaining plantation was statistically homogeneous.

Female strobili production of individual trees was related to tree location. Average female strobili production of flowering trees in SR1 to SR4 showed an increasing trend, but was not significant (Table 6). The diversity of genotypes known to occur in the plantation may explain the large variation in strobili production between SRs (Table 6) and account for the non-significant difference between strobili production. Regardless of the statistical insignificance, successful tree breeding depends on adequate strobili production.

Theoretical production of female strobili for each SR was calculated from the percentage of flowering trees in

Table 6. Biological parameters measured in 4 sun regions established in a spruce plantation.

SUN REGION	TIME(EST) REGIONS 1ST RECEIVED DIRECT SOLAR RADIATION	NUMBER OF TREES	MEAN TREE HEIGHT[1] (dm)	MEAN PHENOLOGY[2]	PERCENT MATURE TREES[3]	FEMALE STROBILI / MATURE TREE (st. dev)	THEORETICAL FEMALE PRODUCTION / 100 TREES
1 (most shaded)	10:15	17	18.4_{a}	2.9	30.0	30.5 (8.9)	285
2	8:30	53	21.7_{b}	3.1	58.8	17.8 (22.7)	1,064
3	7:45	32	23.8_{bc}	3.4	66.7	23.3 (29.6)	1,554
4	7:00	47	24.7_{c}	3.3	76.1	30.7 (40.7)	2,336

---

[1] Different letters denote a significant difference at 5%.

[2] Trees were assigned a numeric value based on vegetative phenology on April 30, 1981. Trees were assigned a value between 1 (buds remained dormant) to 5 (buds had broken and shoots had elongated more than 3 centimeters.

[3] Chi-squared test of independence showed a significant relationship between sun region and the number of mature trees in each run region. However, only sun region (1) significantly contributed to the association.

each SR and average female strobili production. Based on 100 trees, SR1 theoretically would produce 285 female strobili compared to 2,336 produced by SR4 (Table 6). This 8-fold increase in strobili production was attributed solely to plantation location without the benefit of floral promoting treatments.

Simple correlations were calculated between evaluated parameters (Table 7). Terminal shoot elongation was correlated with SR location ($r = .59$, $p <.001$), which can be explained in part by the difference in total tree height and by vegetative phenology. In general, trees growing in SRs that received more hours of direct solar radiation were larger and tended to break bud earlier than trees in more shaded SRs (Table 6). Tree height was correlated with the number of male plus female strobili ($r = .40$, $p = .029$). However, female strobili production was not significantly correlated with tree height (Table 7).

Juvenile trees (i.e., not having produce strobili in the past) tended to break bud earlier in the spring than mature trees (Figure 17). The mean phenological value of mature trees was 3.0 (based on characteristics in Table 5) and the phenological mean of juvenile trees was 3.5. Less than 23% of the juvenile trees were assigned a phenological value of 1 or 2, compared to 40 for the mature trees (Figure 17). Likewise, 47% of the juvenile trees and 31% of the mature trees were assigned a phenological value of 4 or 5. Similiarly, Nienstaedt (1972) found bud break of white

Table 7. Simple correlation, probability level, and degrees of freedom between vegetative, reproductive, and environmental parameters measured in a spruce plantation.

RELATIONSHIP	r	p	d.f
Tree height X Male + Female strobili Production	0.40	.029	23
Tree height X Female Strobili Production	0.12	n.s.	87
Terminal Shoot Elongation X Female Strobili Prod.	-0.04	n.s.	87
Terminal Shoot Elongation X Sun Region Location[1]	0.59	.001	141
PAR Accumulation[2] / SR X Mean Tree height / SR	0.988	.050	2
PAR Accumulation / SR X Female Strobili Prod./ SR	0.958	.050	2
PAR Accumulation / SR X Percent Mature Trees / SR	0.999	.001	2
Heat Sum[3] / SR X Female Strobili Production	0.39	n.s.	2
Heat Sum / SR X Mean Tree Height	0.58	n.s.	2

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[1] Four sun regions (SR) were established. SR(1) was the most shaded region and SR(4) was the sunniest region of the study plantation.

[2] Photosynthetically active radiation ($mol m^{-2} day^{-1}$) measured on June 23, 1982.

[3] The sum of hourly mean temperatures (centigrade) for the 24 hour period beginning June 23, 1982.

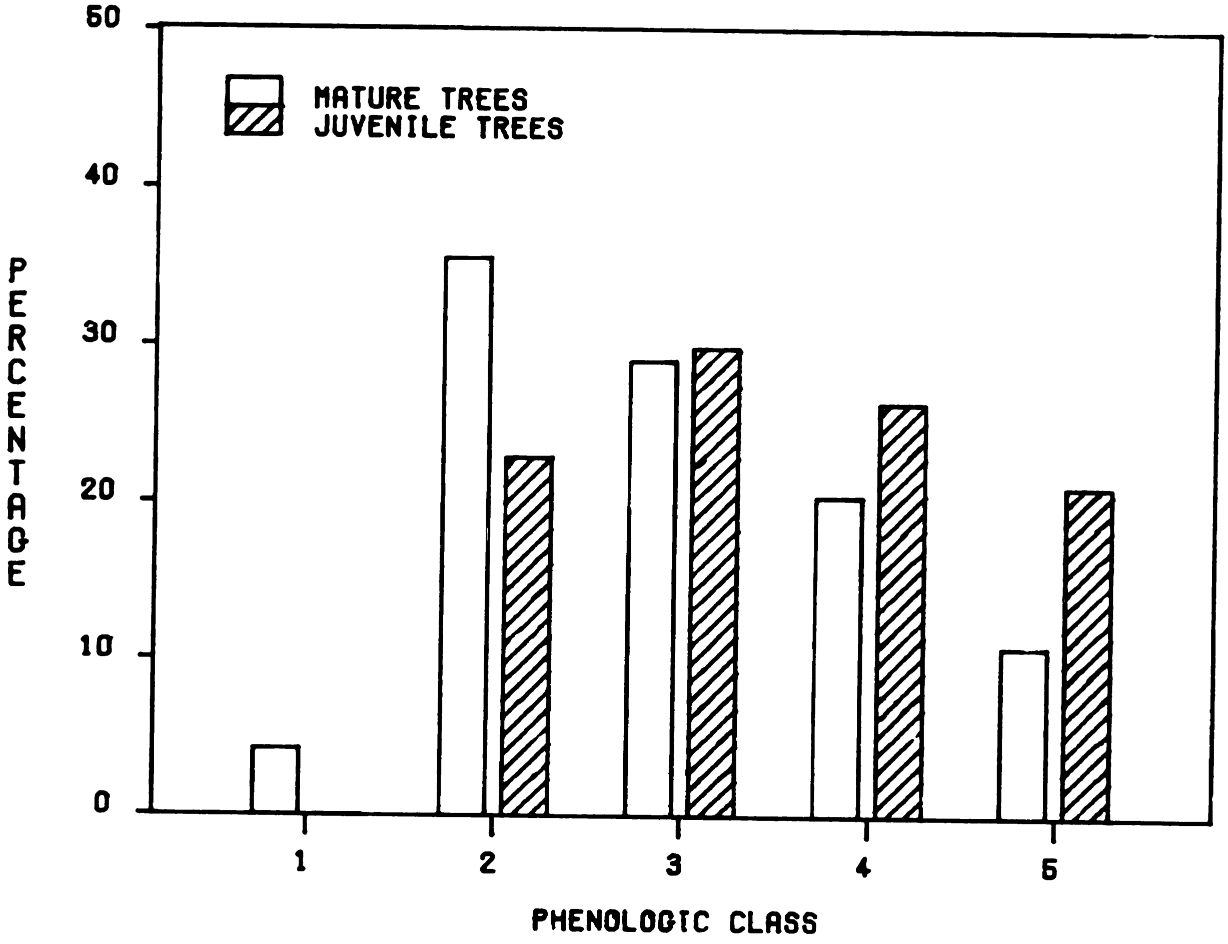


Figure 17. Distribution of juvenile and mature white spruce by phenology class. Trees assigned to phenology class '1' were the slowest to break bud in the spring.

spruce delayed with increased age. Conclusions about the relationship between vegetative phenology and the gain in reproductive maturity, however, are difficult to make. It is not known if delayed bud break precedes the gain in reproductive maturity or if genotypes that delay bud break have a reproductive advantage due to differences in meristematic development. Further experimentation is needed to explain this observed phenomenon.

Environmental Parameters

PAR accumulation increased with distance from the hardwood stand. SR1 (the most shaded region) accumulated 41.1 $molm^{-2}day^{-1}$ on June 23, 1982. In comparison, SR4 accumlated 50.1 $molm^{-2}day^{-1}$. The difference in PAR received by SR1 was 9 $molm^{-2}day^{-1}$ and represented an 18% reduction in PAR. SR2 and SR3 received 46.9 and 48.5 $molm^{-2}day^{-1}$, respectively.

PAR accumulation was significantly correlated with the percentage of flowering trees, average female strobili, and tree height produced by trees in each SR (Table 7). Differential shading of the study trees for 7 years would explain differences in tree height within the plantation. However, the gradient in female strobili production was more strongly related to daily (therefore yearly) PAR accumulation than to tree size. Yearly differences in fecundity within the plantation apparently are contingent on sunny weather preceding meristematic differentiation. Cloudy weather during early summer would be expected to equilize PAR received and subsequent generative production of each sun region.

Ambient temperature in each SR closely paralleled PAR levels during morning hours. However, by late morning, temperature of SR4 was unexpectedly lower than all other SRs (Figure 18). The relative decline in temperature of SR4 was a response to a gradual rise in topography (8%) from SR4 to SR1, which probably led to convective heat loss by SR4. SR4 was also the coolest SR after sunset and trees in SR4 experienced the lowest minimum temperature of the 4 SRs (Table 8). In addition, trees in SR4 experienced the lowest maximum temperature of any SR.

The heat sum calculated for SR4 on June 23 was 373.7 degree-hrs. Only SR1 had a lower heat sum than SR4 and both heat sums for SR2 and SR3 were greater than SR1 and SR4 (Table 8). Whereas temperature gradients existed in the plantation, heat sums were not correlated with fecundity or tree height (Table 7).

Light quality received by trees in the study plantation changed with season and time of day. Far-red enrichment was not observed until June 8, when leaves on the hardwood trees became fully expanded. On May 8, zeta was 1.20 in direct sunlight and 1.28 in the shade. On May 13, zeta in sunlight was 1.20 and 1.15 in the shade and on June 8, zeta in sunlight was 1.20 but only 0.77 in the shade. Whereas some evidence from the literature indicates strobili production in the *Picea* genus may be influenced by a phytochrome system

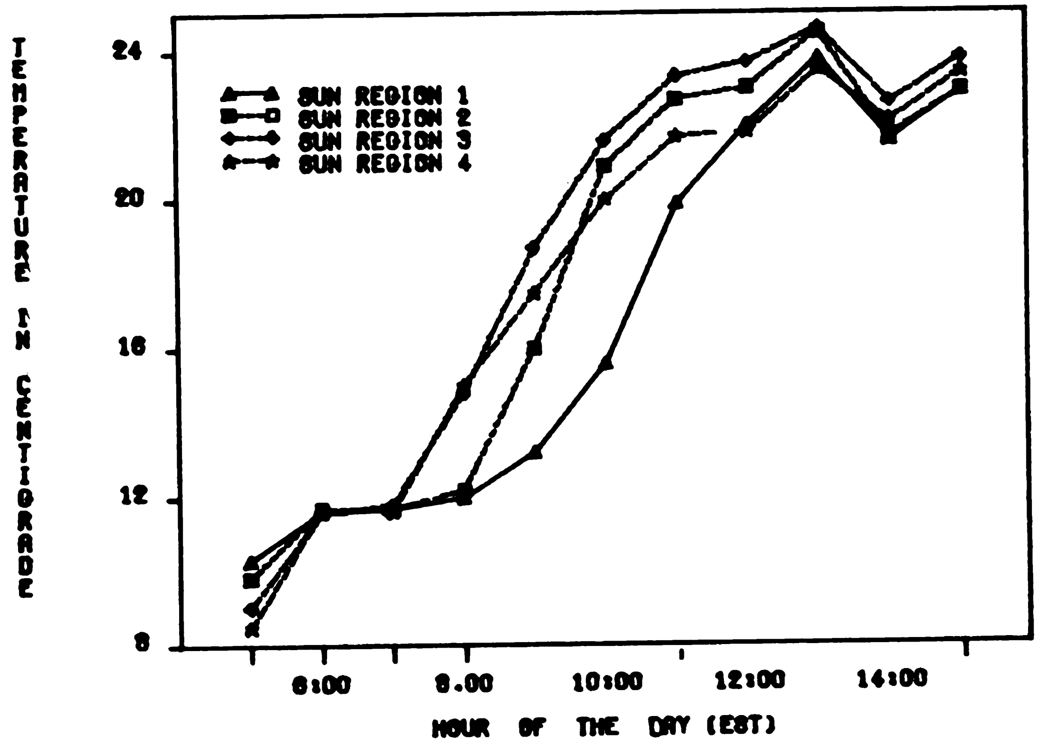


Figure 18. Mean hourly temperatures of 4 sun regions established in a *Picea* study plantation.

Table 8. Environmental parameters measured within 4 sun regions established in a spruce plantation.

SUN REGION	TIME(EST) REGION 1ST RECEIVED DIRECT SOLAR RADIATION	PAR[1] ACCUMULATION ($mol m^{-2} day^{-1}$)	HEAT SUM[2] (DEGREE-HOUR)	DAILY MAXIMUM TEMPERATURE (CENTIGRADE)	DAILY MINIMUM TEMPERATURE (CENTIGRADE)
1	10:15	41.1	369.7	24.1	9.2
2	8:30	46.9	375.8	24.5	9.0
3	7:45	48.5	386.4	24.6	8.2
4	7:00	50.1	373.6	23.5	7.6

[1] Photosynthetically active radiation (400 - 700nm).

[2] The sum of hourly mean temperatures (°C) for a 24 hour period.

(Durzan *et al*, 1979; Kosinski and Giertych, 1982), it seemed improbable that differences in fecundity observed in this study were caused by spectral differences. Gradients in spectral quality were observed only during morning hours and during a short period of the growing season that preceded meristematic differentiation.

Strobili Production After Elevated Crown Temperatures

The uppermost crown region of selected trees which were enclosed in polyethylene bags were subjected to significantly higher air temperature. On each of the 4 sampling dates, air temperature in the bags surrounding portions of tree crowns was significantly higher than ambient temperatures by 0.8°C to 3.6°C (Table 9). The effect of elevated crown temperature on shoot water potential was less definitive. Differences in mean shoot water potential between the two treatment groups ranged from 0.2 to 1.5 bars, and bagging significantly decreased shoot water potential on 1 of the 3 sampling dates (Table 9).

Strobili production on trees bagged from late-June to mid-July was not expected to be enhanced since meristematic differentiation was thought to occur during late-June. However, neither bagged group produced significantly more strobili than the control group (Table 9). A large variation within treatment groups may have masked treatment effects.

Several explanations can be offered for the insignificant effect of increasing air temperature

Table 9. Effects of enclosing the uppermost whorl and internode of white spruce in polyethelene bags.

TREATMENT	MEAN FEMALE STROBILI / TREATED REGION	ST. DEV.	MEAN TEMP(C)				WATER POTENT(-bars)		
			JUNE 11	JULY 18	JULY 7	JULY 15	JUNE 11	JULY 7	JULY 15
Crowns enclosed June 3 to June 23	18.3	29.7	28.5**	30.6**	-	-	13.8**	-	-
Crowns enclosed June 24 to July 18	2.3	7.2	-	-	35.7**	26.0**	-	13.7	10.7
Controls	8.9	12.7	25.5	26.0	33.0	25.0	12.3	12.6	10.5

** Significant different from the control at the 5% level.

surrounding tree crowns. Strobili enhancement of 'poor' flowering individuals may be more difficult to achieve than strobili enhancement of more precocious individuals. Secondly, the bagging treatment may not have elevated air temperature nor decreased shoot water potential sufficiently to change endogenous hormone levels to favor generative production. Lastly, the duration of bagging may not have been adequate to cause a positive flowering response.

In other studies, warm and sunny days during the period of meristematic differentiation period have been related to flower promotion in some species (Fraser, 1958; Daubenmire, 1960; vanVrendenbunch and LaBastide, 1969). Likewise, trees of a given species growing in more southerly regions of their range generally produce more strobili than trees growing in more northerly latitudes (Wright *et al*, 1966; Ganzel, 1973). To maximize seed production, a suitable site for seed orchard establishment is critical. In addition to moderately fertile, well drained soils, seed orchards should be established in open areas with trees planted at a wide spacing to reduce shade and competition.

Shade strongly influenced photon flux density, spectral quantity, and ambient temperature received by trees in the study plantation, but photon flux density most adequately explained differences in tree size and fecundity. Additional experimentation under environmentally controlled conditions would be helpful to verify the observed relationship between shade and fecundity of white spruce.

CHAPTER VI

THE RESPONSE OF JUVENILE *PICEA PUNGENS* TO ROOT-PRUNING

Abstract

Two groups of juvenile 11-year-old blue spruce (*Picea pungens* Engelm.) were mechanically root-pruned. One group of trees had been grown under accelerated-optimum-conditions for 8 months as seedlings, whereas trees in the second group were grown under nursery conditions for 3 years. Root-pruning did not enhance strobili production of either group but reduced vegetative bud break, shoot water potential, and terminal shoot elongation of accelerated grown trees. A significant reduction in shoot water potential and spring bud break was measured for nursery grown trees. Root-pruning apparently was not severe enough to induce strobili initiation.

Introduction

Throughout the United States, blue spruce (*Picea pungens* Engelm.) is highly prized as an ornamental and Christmas trees species. In Michigan alone, more than 16 million blue spruce seedlings were grown commercially in 1981 (Levenson, personal communic.). However, seedlings grown from wild seed show a large variation in foliage color, branching pattern, and needle sharpness.

Interspecific and intraspecific hybridization is a technique used to produce progeny from parents with superior phenotypic characteristics. The hybridization technique has produced some progeny from an interspecific cross between *P. glauca* (Moench) Voss and *P. pungens* that possess the desirable characteristics of bluish foliage, rapid growth rate, cold hardiness, and moderately soft needles. Whereas plantable numbers of a superior phenotype may eventually be obtained by vegetative propagation, sexual reproduction presently remains the only way to achieve further genetic improvement and mass production of either blue or hybrid spruce.

To determine the genetic variability and basis for selection in blue spruce, Michigan State University established a range-wide provenance/progeny test in 1970 that included 236 seed sources and 47 populations. While the plantation contains significant potential for genetic improvement, gains cannot be realized until trees are mature. Through 1981, all of the trees in the plantation (more than 7,000) remained juvenile and sexually unproductive.

This study reports on an attempt to induce strobili production of trees within the blue spruce plantation by root-pruning. Mechanical root-pruning was chosen as the cultural treatment due to the large plantation size and reports of root-pruning enhancing strobili production in other studies (Hanover, personal communic.; Stephens, 1961, 1964; Silen, 1973b; Quirk, 1973). Vegetative phenology, terminal shoot elongation, and shoot water potential were also evaluated following the root-pruning treatment.

Materials and Methods

Seed collections from 236 sources and 47 populations of blue spruce were made during 1968 throughout the natural range of blue spruce. Seed was sown during 1970 and seedlings were grown under two environmental regimes. One group of seedlings was grown in a greenhouse under high levels of light, temperature, moisture, and nutrition. These trees will be referred to as accelerated (AC) trees since a seedling of plantable size was obtained in 8 months. AC seedlings were machine planted in southwestern Michigan before the 1971 growing season at a 1.8m X 2.4m spacing. Sources were planted in southwestern Michigan as 4 tree plots in 5 replications. More than 4,500 AC seedlings were planted. The second group of seedlings were grown under nursery conditions. These non-accelerated (NA) trees were lifted as 3-0 seedlings and planted adjacent to AC seedlings in April, 1973. Spacing between trees was 1.8m X 2.4m and sources were planted as 4 tree plots in 3 replications. More than 2,500 NA trees were planted.

Trees in two AC and 1 NA replications were randomly chosen to be root-pruned. Trees in one replication in each of the NA and AC portion of the plantation served as the control. To sever tree roots to a vertical distance of 36cm, a tractor pulled 2 hardened cultivator tines down rows of width 2.4m. Root-pruning was conducted on April 8, 1981, well in advance of spring bud break. The distance between tines was 1.55m. Therefore, trees planted with a row width of exactly 2.4m would have been root-pruned at a distance of 42cm from the root collar. However, due to irregularities in row width, the actual pruning distance from the root collar ranged from 23cm to 55cm.

Trees were assigned a numeric value from 1 to 5 based on vegetative phenology on May 21,1981 (Table 10). Shoot water potential of a randomly chosen pair of root-pruned and control trees was measured with a pressure bomb (PMS Instrument Co., Corvallis, Oregon) during the afternoon of July 29, 1981. Twenty pairs of trees from the AC and NA group were evaluated for shoot water potential. Each pair consisted of one root-pruned and one control tree growing within 4.9m of each other. Water potential was measured twice for each tree and means were used in a paired t-test.

On August 25, 1981, total tree height and terminal shoot elongation during 1981 were measured with an accuracy of

Table 10. Phenological index and biological characteristics used to quantify bud break of blue spruce.

NUMERIC VALUE	CHARACTERISTICS
1	All tree buds remain dormant
2	Buds on uppermost whorl are dormant Buds on lower whorls have swollen
3	Buds on uppermost whorl have swollen
4	Uppermost whorl has broken bud and shoots are less that 3 centimeters
5	Uppermost whorl has broken bud and shoots are more than 3 centimeters.

± 2.5cm. To remove inherent differences in shoot elongation potential as a result of tree size, terminal shoot elongation was expressed as a percentage of total tree height. Strobili production was assessed in the spring, 1982, on all trees within the plantation. A chi-squared analysis tested the effect of root-pruning on vegetative phenology and an analysis of variance tested the effect of root-pruning on terminal shoot elongation. Statistical analysis of strobili production was not necessary in that only two trees flowered in the plantation.

Results and Discussion

Root-pruning did not induce strobili production. Throughout the entire plantation, only 2 trees bore strobili (8 female strobili were produced on AC and root-pruned trees). This response provided no evidence that root-pruning can hasten flowering of blue spruce. Root-pruning sigificantly reduced terminal shoot elongation of AC trees during the growing season after root-pruning. Root-pruned trees elongated an average of 13.5% of their total height and control trees elongated an average of 15.4%. Root-pruning did not suppress terminal shoot elongation of NA trees but reduced shoot water potential of both NA and AC trees (Table 11).

NA trees were field planted 2 years after AC trees and were not as strongly affected by root-pruning. Two additional growing seasons in the field apparently resulted in the establishment of a more extensive root system by AC

Table 11. Effects of root-pruning on shoot elongation and shoot water potential of 11-year-old blue spruce grown as seedlings under accelerated and nursery conditions.

PARAMETER	ACCELERATED[1]		NURSERY GROWN	
	Root-pruned	Control	Root-pruned	Control
Shoot Elongation (% total height)[2]	12.9**	15.4	18.1	18.3
Shoot Water Potential (bars)	-13.8***	-11.8	-14.4**	-11.5

[1] Trees were grown under accelerated optimum conditions for 8-months.

[2] Percent total height = terminal shoot elongation / total tree height.

** Significantly different from the control at the 5% level.

*** Significantly different from the control at the 1% level.

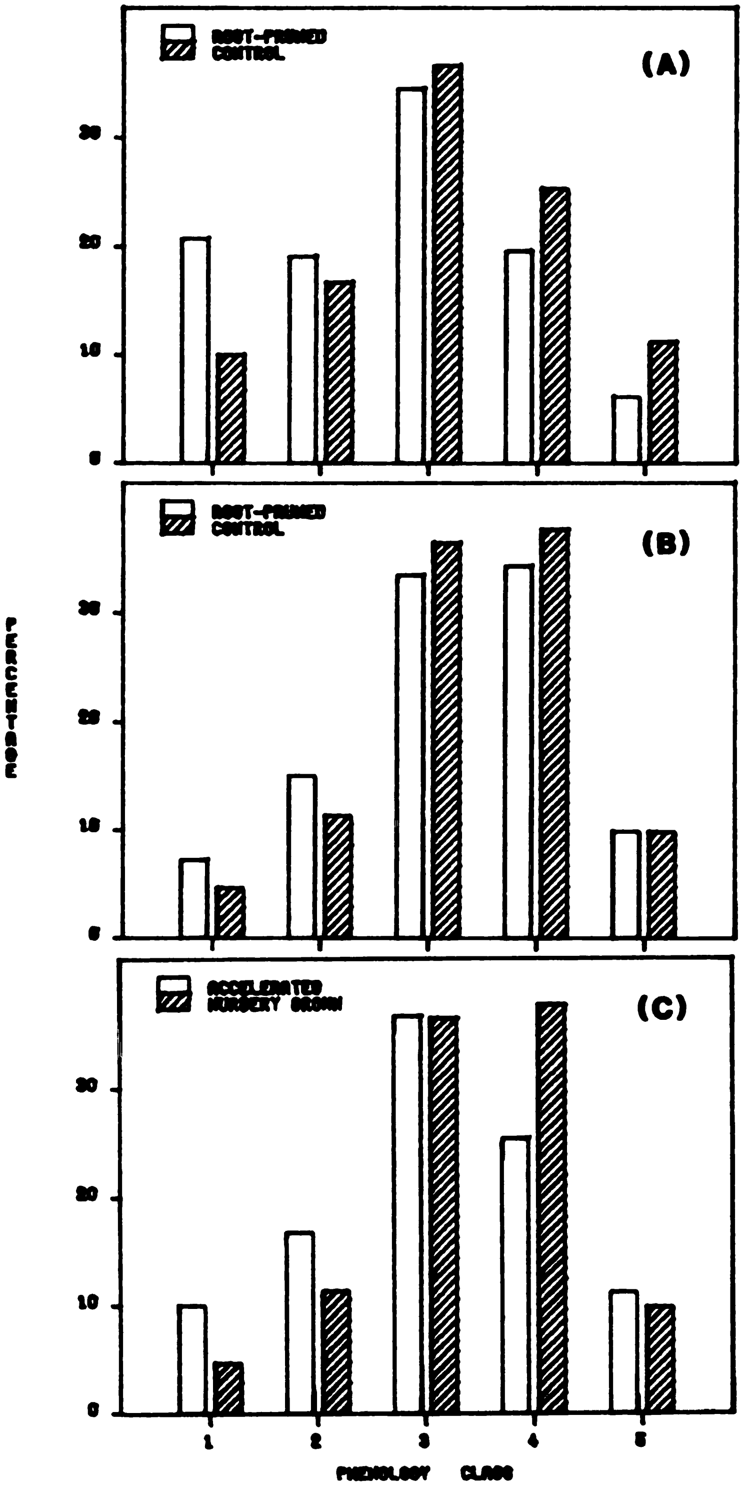
trees. With root-pruning conducted at a relatively uniform distance, AC trees would have had a larger proportion of their roots severed when compared to NA trees.

Root-pruning of both AC and NA trees significantly delayed bud break of vegetative shoots. A greater portion of root-pruned trees were assigned to a phenological class that represented a slower stage of vegetative phenology (Figure 19a,b). Again, this response by AC trees was probably a result of a greater reduction in root volume.

Trees of the *Picea* genus have needle primordia preformed in the apical bud and the rate of spring growth is related to water uptake. Shoot elongation and bud break was probably slowed, in part, by the reduced capacity of root-pruned trees to absorb water in the spring. Lavender *et al*, (1973) presented evidence that gibberellin (GA) may be exported from the roots of Douglas-fir (*Pseudotsuga mensiezii* (Mirb.) Franco) in the spring. A reduction in GA production or other growth regulators necessary for spring growth may also have contributed to the slowing of spring bud break and the reduced growth.

One objective of accelerating early seedling growth is shortening of the juvenile phase. The juvenile phase of white spruce can be shortened to 4 years by accelerating early seedling growth (Hanover *et al*, 1976), and apples can flower 20 months from seed if grown correctly in a greenhouse (Aldwinckle, 1975). However, shortening of the juvenile phase of blue spruce by accelerating early growth

Figure 19. Distribution of root-pruned and control 11-year-old blue spruce by phenology class. Trees assigned to phenology class '1' were slowest to break bud in the spring A) accelerated trees B) non-accelerated trees C) unpruned accelerated and non-accelerated trees.


ROOT-PRUNED
CONTROL
(A)
ROOT-PRUNED
CONTROL
(B)
ACCELERATED
NURSERY GROWN
(C)
PERCENTAGE
0
10
20
30
1
2
3
4
5
PHENOLOGY CLASS

has not been demonstrated.

One indication of ensuing maturation in spruce may be a shortening of the growth phase which has been shown to accompany an increase in age (Nienstaedt, 1972; Hanover, 1981). In addition, work reported in Chapter 5 indicated that mature white spruce tended to be more conservative (late) in breaking bud in the spring than juvenile trees of the same age. In this study, vegetative development of the control AC trees was significantly slower ($p < .05$) than NA trees (Figure 19c). The observed difference in phenology between AC and NA trees has significant biological implications in that maturation of AC blue spruce may be hastened by the accelerated growth treatment.

The utility of root-pruning to promote strobili production of juvenile blue spruce remains in question. Tree age would not explain the inability of root-pruning to promote flowering since many blue spruce of a similiar age flower profusely after transplanting (personal observation). The most plausible explanation for the lack of a flowering response in this study was that trees were not sufficiently stressed. Unpublished data (Ebell's work reported in Ross and Pharis, 1982) indicated that root-pruning is effective when accompanied with a 40-60% reduction in vegetative growth. In this study, vegetative growth of AC trees was suppressed 17%. In addition, tree roots remained intact on 2 of 4 sides and were severed only in a vertical direction (in contrast to a Vermeer transplanter).

CONCLUSIONS AND RECOMMENDATIONS

White spruce can be added to the growing list of species in the *Pinaceae* family which flower in response to exogenous $GA_{4/7}$ treatments. However, many factors influenced overall fecundity of white spruce: genotype, plant age, individual bud and crown position, GA concentration, time of treatment, adjunct treatments, and environmental conditions. With many factors governing strobili production, a better understanding of flowering can be achieved with careful partitioning of the physiological process. In Chapters 2 and 4, variation within and between trees was removed by considering branch positions as experimental units. This consideration may be necessary to detect treatment effects. In contrast, when entire trees were used as experimental units (Chapter 3), a 93-fold increase in female strobili production was statistically insignificant.

Two-year-old white spruce were unaffected by hormonal and cultural treatments that readily induced female strobili production on juvenile 6-year-old trees. In addition, female strobili production of mature white spruce was reduced at a relatively higher concentration of $GA_{4/7}$ compared to lower GA levels. The interaction af age with GA concentration makes a general treatment prescription difficult. Further study would be useful to verify this interaction.

Crown position influenced reproductive capacity, responsiveness to GA treatment, and sexuality. Differences between crown regions may reflect a chemical gradient, meristematic vigor, an environmental gradient, or a subtle difference in ontogeny. The influence of crown position is extremely important to seed orchard managers who may use exogenous chemical sprays to increase flower production.

Individual bud location influenced differentiation on selected crown positions. How differentiation is regulated remains unknown, but it is likely controlled by a hormonal balance. It has been proposed that distal branch positions may preferentially produce female strobili in response to GA treatments because of a suboptimal level of an endogenous factor. In contrast, proximal branch positions may be inhibited by exogenous GA treatments because of a supraoptimal concentration of an endogenous gradient. Quantification of endogenous plant hormones of buds from specific branch regions would be extremely beneficial.

Strobili enhancement of white spruce was most successful when exogenous hormonal treatments preceded meristematic differentiation and treatments that closely bracket the period of differentiation are recommended. In Chapter 2, female strobili production was enhanced by 3 weekly GA applications. GA treatments of varied frequency and duration should be tested to determine the most efficient and cost effective treatment.

Male strobili production was enhanced in 1 of 3 studies. Experimentation is needed to delineate the physiological requirements of each sex type. Differences are expected to be subtle based on the observation of hermaphroditic strobili and the promotion of male and female strobili following identical GA treatments (Chapter 4). Generally, female strobili are enhanced by exogenous GA treatments, whereas male strobili are less frequently enhanced. If endogenous GA levels are involved in differentiation, perhaps the requirement for male strobili production is more exacting than the requirement for female strobili production, or perhaps elevated GA levels must preceed differentiation for a longer period of time.

Naphthalene acetic acid and root-pruning can enhance the $GA_{4/7}$ effects on white spruce. Experimentation conducted in Chapter 3 should be repeated with more replicates to determine yearly interactions with hormonal and adjunct treatments and to reduce treatment variation. Root-pruning in combination with exogenously applied GA treatments can be recommended as an experimental treatment to enhance strobili production of white spruce. Neither treatment was prohibitive in cost or in the expenditure of time. However, root-pruning must adequately reduce vegetative growth and hormonal treatments must precede differentiation.

Additional flower enhancement work with blue spruce should be conducted to utilize the genetic resource of this species in Michigan. A root-pruning treatment could be repeated or combined with a hormonal treatment on a smaller scale. However, root-pruning must be severe enough to reduce vegetative growth a minimum of 40%.

Finally, Puritch and McMullan (1981) have described an injection technique to introduce plant hormones directly into the vascular system of tree species and Ross and Pharis (1982) believe that low volume sprayers eventually may be used to apply chemical treatments on a larger scale. The feasibility of applying exogenous hormones to larger trees and on a commercial scale should be tested.

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