

# Stimulation of flowering in *Picea abies* by gibberellins

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## Summary

Female cone production in grafted plus tree clones of Norway spruce, *Picea abies* (L.) KARST., was significantly stimulated by a mixture of gibberellins A<sub>4</sub> and A<sub>7</sub>. The effect was further enhanced by joint application of gibberellin A<sub>9</sub>. The synthetic auxin naphthylacetic acid had no effect. There were great differences between clones and also between years in spontaneous cone abundance and in response to the gibberellin treatments. At present gibberellins cannot be used for large-scale stimulation of flowering in seed orchards of Norway spruce, but for purposes of speeding up a breeding program the gibberellin technique can be used immediately. Provisional recommendations are given.

**Key words:** flowering, seed orchard, gibberellin, Norway spruce, clonal variance.

## Zusammenfassung

Die Produktion von weiblichen Blüten an Pfropflingen der Fichte wurde signifikant durch eine Mischung von Gibberellin A<sub>4</sub> und A<sub>7</sub> stimuliert. Die Wirkung wurde weiter verstärkt durch zusätzliche Applikation von Gibberellin A<sub>9</sub>. Synthetisches Auxin (Naphthyllessigsäure) hatte keine Wirkung. Es gab große Unterschiede sowohl zwischen den Klonen als auch zwischen den Blühjahren bezüglich der spontanen Zapfenblütenbildung ebenso wie als Reaktion auf die Gibberellinbehandlung. Gibberelline können z. Zt. noch nicht in großem Maßstab für die Blühstimulierung in Samenplantagen mit Fichte benutzt werden; zur Beschleunigung eines Züchtungsprogrammes kann die Gibberellintechnik jedoch sofort eingesetzt werden. Es werden vorläufige Empfehlungen gegeben.

## Introduction

Flowering in perennials like coniferous trees has long interested scientists, forest tree breeders and practical foresters. From a physiological point of view flowering is a very complex process, proceeding through many consecutive phases, and depending on external conditions, internal regulatory mechanisms and the general ontogenetic development. The idea that plant hormones play an important role in regulating flowering is very old but has so far received little direct experimental support. "Flowering hormones" have been discussed but never identified. One of the most conspicuous examples of hormonal control of flowering has been the gibberellin-induced cone production in species belonging to Taxodiaceae and Cupressaceae first demonstrated by Japanese workers and later studied extensively by PHARIS and co-workers (for a review see DUNBERG, 1974). The recent extension of these results to include a few species of Pinaceae (*Pseudotsuga menziesii* [MIRB.] FRANCO, ROSS and PHARIS 1973, 1976; *Pinus contorta* DOUGL. ex LOUD., PHARIS et al., 1975) has prompted formulation of a number of questions of scientific and practical importance. Can the results obtained be extended to Pinaceae species in general? What particular gibberellins are active, and for what reason? Can this new technique be used practically by forest tree breeders and for large-scale seed production?

The purposes of this investigation were:

- to investigate whether and to what extent male and female cone production can be promoted by application of gibberellins to mature but poorly flowering grafts of *Picea abies* (L.) KARST., a major forest species in Europe and representative of a genus so far not tested,
- to establish quantitative relations between hormone dosage and flowering response, and evaluate differences in efficiency between different gibberellins,
- to investigate possible additive or synergistic effects of auxin,
- to investigate the influence of year-to-year climatic fluctuations and differences in spontaneous cone abundance on response to gibberellins.

Table 1. — Female flowering in the year 1976

Treatment number	GA <sub>3</sub> µg	GA <sub>4/7</sub> µg	GA <sub>9</sub> µg	NAA <sup>1)</sup> µg	Female cones per graft	Mean for subgroups of five grafts
1 a	Untreated control <sup>2)</sup>				0.2	
1 b	Ethanol-treated control				0.2	
1 c	"-				0.2	0.12 ± 0.05 a <sup>3)</sup>
2 a	"-				5	
2 b	"-				25	n = 5
3 a	200				0.2	
3 b	300				0.6	
3 c	600				1.6	
4 a	200			5	0.4	
4 b	300			5	0.2	1.31 ± 0.60 a
4 c	600			5	0.8	
5 a	200			25	2.0	n = 9
5 b	300			25	0.2	
5 c	600			25	5.8	
6 a	200				6.4	
6 b	300				4.4	
6 c	600				1.0	
7 a	200			5	3.6	
7 b	300			5	2.2	3.67 ± 0.70 b
7 c	600			5	4.0	
8 a	200			25	1.8	n = 9
8 b	300			25	2.4	
8 c	600			25	7.2	
9 a		200			1.2	
9 b		300			0.8	
9 c		600			0.4	
10 a		200	5		1.8	
10 b		300	5		0.0	0.49 ± 0.22 a
10 c		600	5		0.2	
11 a		200	25		0.0	n = 9
11 b		300	25		0.0	
11 c		600	25		0.0	
12 a	300	300			6.2	
12 b	300	300	5		1.6	3.13 ± 1.53 ab
12 c	300	300	25		1.6	n = 3
13 a	300		300		3.0	
13 b	300		300	5	1.8	1.60 ± 0.87 ab
13 c	300		300	25	0.0	n = 3
14 a		300	300		10.0	
14 b		300	300	5	6.4	8.27 ± 1.04 c
14 c		300	300	25	8.4	n = 3
15 a	200	200	200		6.0	
15 b	200	200	200	5	2.6	4.47 ± 1.00 bc
15 c	200	200	200	25	4.8	n = 3

1) naphthylacetic acid

2) mean for the entire seed orchard (2,500 grafts) was also 0.2

3) figures not followed by the same letter differ significantly (p = 0.05, Mann-Whitney U-test)

## Materials and Methods

The experiments were performed in the seed orchard Björkebo near Umeå in northern Sweden (lat 64° N). This orchard contains grafted plus tree clones derived from mature *Picea abies* (L.) KARST. trees from northern Sweden. The scions were grafted on seedling rootstocks of "local provenance" and were about nine years old from grafting in 1975, when the first hormone applications were made. A total of 220 grafts with flowering records for the two previous years were selected from among the 2,500 grafts in the seed orchard. Grafts were matched for clone, size, vigour, growth form, flowering record and position in groups of 15 and allocated at random to the major treatment groups (see table 1). Each treatment group was further subdivided into three groups of five grafts; matching was attempted also at this stage but had to be less rigorous.

Gibberellins originated from Imperial Chemical Industries Ltd. and were provided by R. P. PHARIS, University of Calgary. The gibberellin A<sub>4</sub>/A<sub>7</sub> mixture contained approximately equal amounts of the two components. Different batches of gibberellin A<sub>9</sub> were used in 1975 and in 1977. None of them was free from unknown contaminants.

In 1975 the gibberellins and the auxin naphthylacetic acid were applied alone and in various combinations (see table 1) as 5 µl ethanol injections into the central pith of the elongating terminal shoot of each of four branches in the second whorl from the stem apex. Treatments were repeated three times, on June 4, June 24 and July 8. The treated shoots had just begun to elongate on the first date and had just reached their final length on the third. The method of application was time-consuming, frequently caused treated shoots to bend, and resulted in the loss of some few shoots.

Concentrations of gibberellins (200, 300, and 600 µg per injection) and of naphthylacetic acid (5 and 25 µg per injection) were selected within the range known to be reasonably efficient in other species. Gibberellin concentrations were intended to facilitate quantitative comparisons between different gibberellins and gibberellin combinations (see table 1).

In 1976 and again in 1977 female conelets were counted, and male flowering was quantified by means of a five-grade scale (DUNBERG, 1974).

In 1977 the same 220 grafts plus 15 additional grafts were allocated at random to the six treatment groups (see table 2), which were again subdivided into groups of five grafts. It was hoped that the high number of grafts per treatment in combination with group sampling would guard against chance errors caused by heterogeneity of the experimental population and thus make matching unnecessary.

The gibberellins were applied in 10 µl of ethanol and spread along the surface of the elongating terminal shoot of each of four branches in the second whorl from the stem apex. Treatments consisted of one single concentration of hormone(s) and were applied only once, on June 23.

In 1978 female conelets were counted.

Female cone production varied greatly between clones and was seldom normally distributed even within one clone. The experimental population of grafts consisted of a number of subpopulations. These difficulties were known when the work was planned, and measures were taken to minimize experimental errors caused by heterogeneity of the experimental population. These measures were matching in the first experiment and group sampling in the second. Differences between treatments were analyzed by the non-parametric Mann-Whitney U-test. The significance level was set at  $p = 0.05$ .

## Results

No consistent effects on male flowering were recorded. Male flowering was generally more abundant than female

Table 2. — Female flowering in the year 1978

Treatment	Cones per graft, mean of 5 grafts	Mean of means
Gibberellin A <sub>4</sub>	4.4	
	12.2	
	0.8	
	0.4	
300 µg	1.4	3.60 ± 1.54 a <sup>1)</sup>
	2.0	n = 7
	4.0	
Gibberellin A <sub>4</sub> /A <sub>7</sub>	0.2	
	1.0	
	7.2	
	0.4	
300 µg	0.0	1.60 ± 0.96 a
	0.8	n = 7
	1.6	
Gibberellin A <sub>9</sub>	10.8	
	13.4	
	0.4	
	1.0	
450 µg	1.4	6.43 ± 2.79 a
	17.8	n = 7
	0.2	
Gibberellin A <sub>4</sub>	0.0	
150 µg	2.6	
	4.2	
+	9.6	
Gibberellin A <sub>9</sub>	0.6	5.00 ± 2.48 a
225 µg	0.2	n = 7
	17.8	
Gibberellin A <sub>4</sub> /A <sub>7</sub>	0.2	
150 µg	27.4	
+	0.6	
	6.2	
Gibberellin A <sub>9</sub>	1.6	5.40 ± 3.75
225 µg	1.8	n = 7
	0.0	
Control	0.6	
	4.6	
	0.0	
	0.0	
ethanol only	1.0	
	9.2	2.30 ± 0.78 a
	0.8	
	0.8	n = 12
	3.4	
	4.4	
	1.8	
	1.0	

1) figures not followed by the same letter differ significantly ( $p = 0.05$ , Mann-Whitney U-test)

and normally occurred in the grafts far from the points of application of hormones.

Female flowering in 1976 is shown in table 1. The original intention was to consider each individual treated branch as one experimental unit. However, on many occasions conelets developed in positions closer to the shoot apex, and since this seemed to be the result of translocation of the applied compound, the entire graft had to be considered as the experimental unit. The observation also indicates that exogenously supplied gibberellins are translocated within the graft, and suggests that translocation effects may be important for the efficiency of different gibberellins.

No effects on female flowering of naphthylacetic acid alone or in combination with the various gibberellins or of differences in concentration of the latter could be detected. Treatments differing only in these respects were therefore pooled before differences between the various gibberellins were analyzed.

The heterogeneity of the experimental material severely impaired the reliability of the results obtained. Thus the 26-fold increase in conelet production caused by a combination of gibberellins A<sub>3</sub> and A<sub>4</sub>/A<sub>7</sub> was not statistically significant. Nevertheless the results clearly show that the gibberellin A<sub>4</sub>/A<sub>7</sub> mixture was the only active compound among those tested, and that the effect was further enhanced by joint application of gibberellin A<sub>9</sub>, although this compound by itself was without effect.

In 1977 only a few female cones were produced in the seed orchard. The generally low level of flowering thus made it impossible to evaluate possible persisting effects of the 1975 hormone treatments.

The great year-to-year fluctuations in flowering were further demonstrated by the unusually abundant flowering in 1978, with a mean of 2.30 female cones per graft in the control group. The variation between clones and even between grafts within a clone was considerable. As a consequence, quantitative comparisons suffered so severely that there were no statistically significant differences between any two treatments, although the mean number of cones ranged from 1.60 to 6.43. Results obtained in 1976 could thus not be verified by this second series of experiments.

#### Discussion and recommendations

The outcome of this investigation clearly demonstrates the difficulties connected with the experimental material. Great differences occurred between clones and between years in spontaneous cone setting. Results obtained in the 1975/1976 experiment could not be verified in the 1977/78 experiment. One obvious conclusion is that the response to gibberellins is strongly influenced by year-to-year fluctuations, and by clonal differences in spontaneous cone production. A certain number of the clones have never produced female cones spontaneously in the seed orchard, and some of these could not be forced into cone production by any treatment or combination of treatments.

In spite of the failure of the 1977/78 experiment it seems safe to conclude that gibberellin application promotes female flowering in mature *Picea abies* grafts. Practical work not presented here has demonstrated the usefulness of gibberellin applications in breeding programs. Gibberellin-induced stimulation of flowering has recently been reported also for *Picea sitchensis* (BONG.) CARR. (TOMPSETT, 1977) and may thus be a general possibility within the genus *Picea*.

No quantitative relations between gibberellin dose and flowering response could be established. This may have been caused by a certain quantitative unreliability of the application technique. It is also possible that such quantitative relations were obscured by variation within the plant material. Comparisons between the different gibberellins were based on a larger number of grafts, and some quantitative conclusions could be drawn. A mixture of gibberellins A<sub>4</sub> and A<sub>7</sub> was active, gibberellins A<sub>3</sub> and A<sub>9</sub> were not, although the latter further enhanced the effect of gibberellin A<sub>4</sub>/A<sub>7</sub> when applied jointly. A further question therefore is whether both compounds in the gibberellin A<sub>4</sub>/A<sub>7</sub> mixture are active or only one of them. The investigation failed to answer this question.

The time of application of gibberellins may be very important. Obviously, the application must take place some time between the formation of bud primordia and the differentiation of these into vegetative or floral. Unfortunately, no anatomical observations have been made on the material used in this investigation. Thus, the lack of success with the 1977/1978 experiment may have been in part caused by unsuitable timing of the single application.

In spite of recent achievements it seems improbable at present that gibberellins can be used to increase seed production in seed orchards of *Picea abies*. Some other means of applying the compound must be developed, preferably a spraying technique that could perhaps be combined with insecticide or fertilizer distribution. This will still be very uneconomical unless the efficiency of the applied gibberellins can be improved considerably. At present, an efficiency around one cone produced per milligram gibberellin applied appears valid for many *Pinaceae* species. From a scientific point of view, one might question whether the effects brought about by such high amounts of gibberellin have any relation whatsoever to the endogenous processes that regulate flowering. Gibberellin concentrations found in shoot tissue of *Picea abies* reached a maximum of around 100 ng per g fresh weight (DUNBERG, 1976) or around one microgram per shoot. These data were however based on bioassay of unidentified active compounds, and may be very misleading. Further critical work is obviously needed.

For the purpose of producing cones for controlled crosses in breeding and testing programs the gibberellin technique can be used immediately. Provisional recommendations to breeders of *Picea abies* are:

- use a mixture of the gibberellins A<sub>4</sub>, A<sub>7</sub> and A<sub>9</sub>, or if that is not possible the gibberellin A<sub>4</sub>/A<sub>7</sub> mixture that can be obtained commercially,
- use a concentration of around 500 µg applied in 5 µl of ethanol injected into the terminal shoots of the second and/or third branch whorl from the top of the tree,
- repeat the injections with weekly or bi-weekly intervals during the entire period of shoot elongation growth,
- do not expect the technique to succeed with all clones.

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