Influence of growth regulators and polythene covers on flowering of Scots pine and Norway spruce grafts*)

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Summary

Application of $GA_{4/7}$ at the turn of May and June significantly increased male flowering of Scots pine grafts. The effect of polythene covering from the beginning of July to mid August was similar. Spraying GA_3 in the second half of May and the beginning of June, as well as the polythene covers during July significantly promoted male flowering in Norway spruce grafts. Only very slight effects of NAA were observed on Scots pine and Norway spruce grafts. No synergism was observed between growth regulators used and polythene treatments, the effects being additive.

Key words: Flowering, GA_3, $GA_{4/7}$, NAA, *Pinus sylvestris* L.,*Picea abies* (L.) Karst., polythene covers.

Zusammenfassung

Nach Anwendung der Gibberellinsäuremischung $GA_{4/7}$ während der Monate Mai und Juni wurde an Kiefernpfropflingen die Bildung männlicher Blüten wesentlich erhöht. Ähnlich wirkte sich eine Umhüllung der Pfropflingszweige mit Polyäthylenfolie aus.

In der zweiten Maihälfte und Anfang Juni wirkten sich sowohl ein Besprühen der Fichtenpfropflinge mit Gibberellinsäure GA_3 als auch ihre Umhüllung im Juli auf die Bildung männlicher Blüten günstig aus. Ein Besprühen von Kiefern- und Fichtenpfopflingen mit Naphtylessigsäure NAA zeigte nur einen geringen Effekt.

Eine synergistische, blühfördernde Wirkung bei gleichzeitiger Anwendung von Wuchsstoff und Polyäthylenfolie konnte nicht festgestellt werden.

Introduction

The promotive effect of various gibberellins on the flowering of *Pinaceae* family is well demonstrated now. The application of less polar gibberellins, especially $GA_{4/7}$ mixture, influenced positively the flowering in 13 species of five genera: *Larix, Picea, Pinus, Pseudotsuga* and *Tsuga* (Pharis, Ross and McMullan 1980). It was found in many experiments that the stimulative effect of $GA_{4/7}$ mixture on flowering is more significant when gibberellins were applied in combination with other treatments, e.g. other growth regulators, girdling, mineral fertilization etc., however the lay-outs rarely allowed clear evaluation of the interactions.

In 1979 I combined three treatments affecting flowering, i.e. gibberellins, the auxin NAA and polythene covers. Results of these experiments are presented and discussed below.

Materials and Methods

Three experiments were conducted in 1979: one of them on Scots pine grafts and two on Norway spruce grafts.

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Pinus sylvestris: $GA_{4/7}$ + NAA + polythene covers

The experimental material consisted of the same five clones (12 grafts per clone) as in a previous experiment (Chalupka 1980). Three branches on each graft were designated as upper, middle and lower, and on these main branches two lateral ones were selected in the 1978 whorls. Thus six branches on each graft were treated with the same combination of growth regulators which were applied in a water solution with 0,05% of surfactant Aromox C12 added. Four combinations of hormones were used: $GA_{4/7}$(200 mg/l), NAA (30 mg/l), $GA_{4/7}$ + NAA(200 mg/l + 30 mg/l), and a water control. The sprays were done on the 24th and 29th of May, and on the 7th and 13th of June. Then half of the lateral branches, i.e. three on each graft, were wrapped in polythene from the 2nd of July to the 13th of August. The experiment was replicated trice.

In spring 1980 the number of shoots with male flowers, number of shoots with female flowers and the total number of female flowers were counted.

Picea abies: GA_3 + polythene covers

The experiment was set up on sixty 12-year-old grafts representing five clones. Four variants of treatments were used: 1. GA_3(100 mg/l in water solution with 0,1% Tween 20 added), 2. polythene covers, 3. GA_3 + polythene covers, 4. untreated control. The sprays of GA_3 in the 1st and 3rd variants were applied on the 15th, 22nd and 30th of May, and then polythene covers were placed in variants 2 and 3 for nearly one month, i. e. from the 18th of June to 24th of July. There were three replications.

Picea abies: GA_3 + NAA

For the experiment 4 clones were selected each represented by 12 grafts. The following combinations of growth regulators were used: GA_3(100 mg/l), NAA (30 mg/l), GA_3 + NAA (100 mg/l + 30 mg/l), and a water control. Tween 20 as a 0,1% water solutions was added. The grafts were sprayed on the 19th and 26th of May, and 4th and 9th of June. There were three replicates.

In both Norway spruce experiments a scoring was made of the number of female flowers. The intensity of male flowering was visually estimated on a 5-degree scale: 0 - no flowers, 1 - sparse flowers on a small part of crown, 2 - flowers on half of the crown, 3 - sparse flowers on the whole crown, 4 - numerous flowers on the whole crown.

All 1979 experiments were designed orthogonally and their results were evaluated by an analysis of variance.

Results

Pinus sylvestris: $GA_{4/7}$ + NAA + polythene covers

Table 1 shows the results of the analysis of variance for the Scots pine experiment. Application of the $GA_{4/7}$ mixture from the 24th of May to the 13th of June caused nearly a twofold increase in the percentage of shoots with male flowers, from 19,9% to 37,7%. This effect was manifested

Table 1. — Effects of $GA_{4/7}$, NAA and polythene covers on the flowering of Scots pine grafts. Calculated values of F.

Source of variance	DF	% of shoots flowering male	% of shoots flowering female	No. of female flowers per 100 shoots
Growth regulators /G/	3	8,12 ++	0,73	0,75
$GA_{4/7}$	1	23,11 ++	1,27	1,68
NAA	1	0,01	0,85	0,04
$GA_{4/7}$ x NAA	1	1,23	0,07	0,53
Polythene /P/	1	8,26 ++	24,32 ++	17,65 ++
Branches /B/	2	13,79 ++	3,40 +	1,13
Clones /C/	4	90,95 ++	3,21 +	2,27
G x P	3	0,03	0,48	0,27
G x B	6	3,45 ++	0,87	0,71
$GA_{4/7}$ x B	2	6,35 ++	-	-
NAA x B	2	3,55 +	-	-
$GA_{4/7}$ x NAA x B	2	0,45	-	-
G x C	12	3,37 ++	0,55	0,81
P x B	2	0,77	1,89	0,47
P x C	4	1,20	2,48	1,83
B x C	8	3,66 ++	1,35	1,44
G x P x B	6	0,52	1,42	1,30
G x P x C	12	0,66	1,27	0,73
G x B x C	24	1,22	1,47	1,07
P x B x C	8	0,55	0,89	1,18
G x P x B x C	24	0,48	0,58	0,70
Replicates	2	3,79 +	0,79	0,11

* significant at 0,05 level
** significant at 0,01 level

primarily on the upper and lower branches (*Fig. 1*). A significant interaction between NAA and branches was also observed. The effect of NAA was promotive on the upper branches, and negative on the middle and lower ones (*Fig. 2*). The proportion of shoots with male flowers was significantly increased by polythene covers from 23,2% to 33,9%. There was no interaction between growth regulators used and polythene covers.

The natural differences were confirmed in the distribution of male flowers within the crown (mostly on lower branches) and the interclonal differences were also statistically significant.

Female flowering of Scots pine grafts was only influenced by the polythene cover treatment which decreased percentage of shoots with female flowers from 0,3% to 0,0%, and the number of female flowers per 100 shoots from 2,2 to 0,2. These results concern a small number of female flowers which were observed only on 28 shoots out of 180 covered, and on 3 shoots out of 180 uncovered ones. The natural differences in the distribution of female flowers within the crown (mostly on upper branches), and between clones were statistically confirmed.

Picea abies: GA_3 + polythene covers

Both above mentioned treatments positively affected male flowering (*Table 2A* and *B*). The proportion of shoots with male flowers was increased by GA_3 supply from 46,7% to 83,3% and by polythene covers from 56,7% to 73,3%.

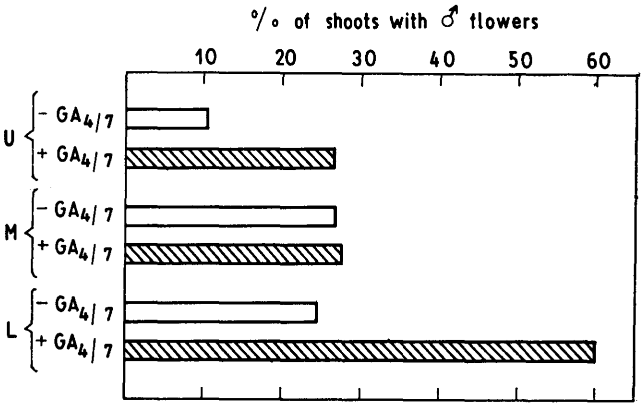

Figure 1. — Effect of $GA_{4/7}$ on male flowering in upper, middle and lower branches of Scots pine grafts.

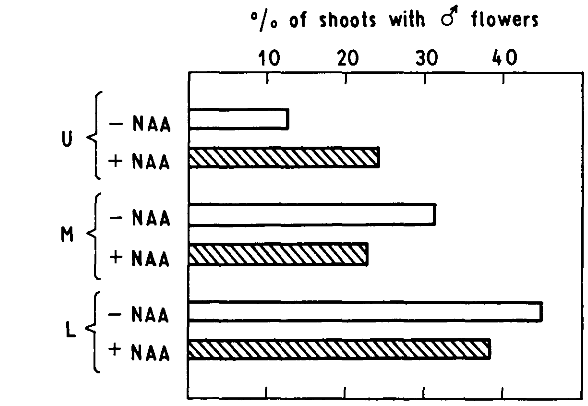

Figure 2. — Effect of NAA on male flowering in upper, middle and lower branches of Scots pine grafts.

Table 2. — **Effects of GA_3 and polythene covers on the flowering of Norway spruce grafts. Calculated values of F.**

A. Proportion of flowering grafts.

Source of variance	DF	% of grafts flowering male	% of grafts flowering female
GA_3	1	38,24 ++	4,08
Polythene /P/	1	7,02 +	0,08
GA_3 x P	1	0,78	0,08
Clones	4	5,29 +	0,33

B. Flowering intensity

Source of variance	DF	Intensity of male flowering	Number of female flowers
GA_3	1	5,08 +	5,13 +
Polythene /P/	1	2,86	0,10
Clones /C/	4	4,14 ++	0,42
GA_3 x P	1	1,26	0,11
GA_3 X C	4	0,71	0,42
P x C	4	0,38	1,68
GA_3 x P x C	4	0,48	1,67
Replicates	2	10,06 ++	1,99

* significant at 0,05 level
** significant at 0,01 level

Application of GA_3 enhanced also the intensity of male flowering from 0,93 to 1,47 according to the scale of estimation used. No interaction between these treatments was found.

Only seven female flowers were observed and all of these were initiated on the grafts sprayed with GA_3 *(Table 2B)*. A non significant positive effect of GA_3 sprays on the proportion of shoots flowering female was also found *(Table 2A)*.

Picea abies: GA_3 + NAA

Application of GA_3 significantly increased the intensity of male flowering from 1,1 to 2,0 of the adopted scale and the percentage of grafts with male flowers from 58,3% to 83,3%. Supplying NAA caused a significant decrease in the proportion of shoots flowering male from 78,2% to 62,5% *(Table 3)*. No effect was observed of growth regulators used on female flowering, and no interaction between them was found.

Table 3. — **Effects of GA_3 and NAA on the flowering of Norway spruce grafts. Calculated values of F.**

Source of variance	DF	Intensity of male flowering	% of grafts flowering male
Growth regulators /G/	3	4,12 +	7,50 +
GA_3	1	10,85 ++	16,00 ++
NAA	1	1,44	6,25 +
GA_3 x NAA	1	0,08	0,25
Clones /C/	3	3,41 +	2,83
G x C	9	0,56	-
Replicates	2	19,45 ++	-

* significant at 0,05 level
** significant at 0,01 level

Discussion

A positive effect of $GA_{4/7}$ application on male flowering in Scots pine grafts, also found in earlier work (CHALUPKA 1978), confirmed my suggestion on the relationship between the time of $GA_{4/7}$ application and the sex of flowers initiated (CHALUPKA 1980). Promotive effect of $GA_{4/7}$ mixture on male flowering in pines was also recently found by LUUKKANEN and JOHANSSON (1980 a und b), and WHEELER, WAMPLE and PHARIS (1980).

The strongest promotive effect of $GA_{4/7}$ on male flowering was observed on the upper and lower branches of Scots pine grafts. In the 1978 experiment the same $GA_{4/7}$ mixture was also most effective on lower branches where it stimulated female flowers (CHALUPKA 1980). It seems therefore that gibberellins do not affect directly the sexuality of flower primordia. They presumably induce the apical domes to various types of mitotic activity which is followed by the later differentiation of primordia as suggested by ROMBERGER and GREGORY (1974).

A great variability was revealed in the reaction of individual clones to $GA_{4/7}$ supply as was found for male flowers in 1980 *(Fig. 3)*, and female flowers in 1979 *(Fig. 4)*. This is also a confirmation of the suggestion about a non specific effect of gibberellins on flower induction. The highest efficiency of $GA_{4/7}$ in poor flowering clones and crown parts indicates a possibility for improvement in the gene pool of Scots pine seed orchard progeny.

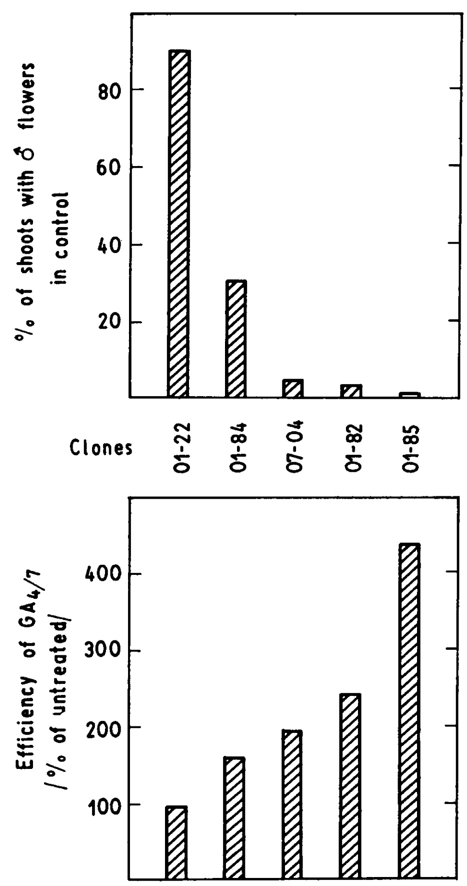

Figure 3. — **Effect of $GA_{4/7}$ on the initiation of male flowers in 1980 in different Scots pine clones.**

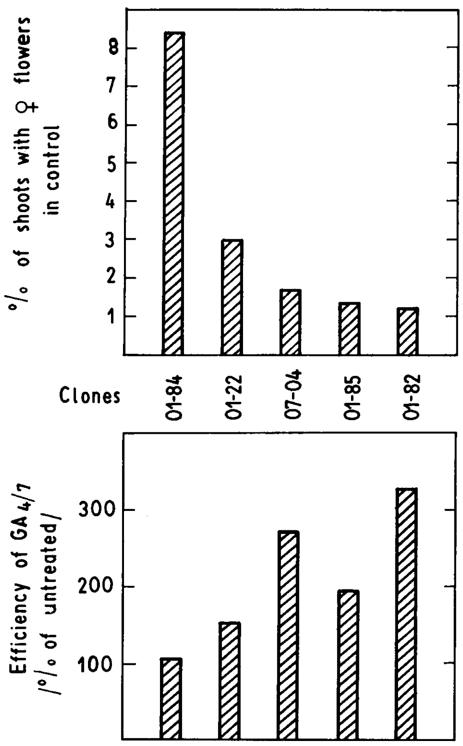

Figure 4. — Effect of $GA_{4/7}$ on the initiation of female flowers in 1979 in different Scots pine clones.

The results of both Norway spruce experiments confirmed the promotive effect of GA_3 on the male flowering as was already noticed (Chalupka 1979). A slight positive effect of GA_3 on female flowering was observed in one of the experiments, but it concerned only 7 cones initiated on 5 grafts.

Data from literature confirm the positive role of GA_3 in the regulation of flowering in different species of the *Pinaceae* family (Bleymüller 1978, Ross and Greenwood 1979, Greenwood 1979). In many works GA_3 was effectively used when combined with other growth regulators (Tompsett 1977, Tompsett and Fletcher 1979, Luukkanen 1980, Luukkanen and Johansson 1980 a and b). The hormonal experiments in Kórnik indicated clearly that GA_3 was a good stimulator of male flowering in Norway spruce in our conditions.

The application of NAA was in fact ineffective in Scots pine and Norway spruce grafts. There are controversial opinions about the role of NAA in floral stimulation in the *Pinaceae* family. The $GA_{4/7}$ mixture combined with NAA significantly increased female flowering in *Pinus radiata* (Sweet 1979) and *P. contorta* (Wheeler, Wample and Pharis 1980), however these effects could not be statistically compared with the effect of $GA_{4/7}$ and NAA acting independently. Hare, Snyder and Schmidtling (1979) noticed the positive influence of $GA_{4/7}$ and NAA (combined with girdling) on the female flowering in *P. palustris*, but the real effect of NAA alone was not deducible from their data. Luukkanen and Johansson (1980 a and b) found in *P. sylvestris* that the joint effect of $GA_{4/7}$ and NAA was the same as $GA_{4/7}$ alone. In *Picea abies* Dunberg (1980) found no effect of NAA. It stimulated flowering more effectively in *P. sitchensis* (Tompsett 1977) and *Pseudotsuga menziesii* (Pharis, Ross and McMullan 1980, Ross, Pharis and Heaman 1980, McMullan 1980), although in the last mentioned species no effect of NAA on flowering was also observed (Puritch *et al.* 1979).

To summarize the above data one must conclude that the role of NAA in the regulation of flowering of the *Piaceae* species seems to be uncertain. Among other things the reason for that is the evaluation of data not always using a proper statistical procedure.

Results obtained following the covering of individual grafts of Norway spruce with polythene confirmed the promotive effect of this treatment on male flowering of *Picea abies* (Brøndbo 1969, Remröd 1972, Chalupka and Giertych 1977) and in *P. sitchensis* (Tompsett and Fletcher 1977). The same male flowering effect was also obtained for the first time on Scots pine grafts after covering branches with polythene. This treatment influenced negatively female flowering on covered branches of Scots pine grafts while in *Picea abies* and *P. sitchensis* a positive effect was sometimes observed (Remröd 1972, Tompsett and Fletcher 1977). Absence of interaction between growth regulators and polythene covers indicates that their effects are only additive in Scots pine and Norway spruce grafts.

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Genetic Base Populations, Gene Pools and Breeding Populations for Eucalyptus in Brazil

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Abstract

Eucalyptus is a major exotic species in Brazil. To be successful in use of this exotic, care must be taken to make maximum gains in both the short term and long term. To do this successfully requires a dual tree improvement program; an operational — (or use) phase for immediate gains and a developmental (research) phase for long-term, advanced-generation gains.

To make the desired type gains for either the short or long term, a proper gene base is needed. This is generally lacking for the eucalypts in Brazil, and some very inferior sources like the "Brazil source" originating at Rio Claro have been and still are being extensively used. This paper outlines some general approaches necessary to obtain, improve and maintain genetic base populations of *Eucalyptus* in Brazil. Several options are available but it is urgent to obtain action for all.

Methods are described in the paper; they consist of developing land races from current plantations, use improved sources from other areas and developing sources from the original populations in their native habitat. The few very best genotypes are used for the current operational phases for maximum genetic gain while a much larger number are held in clone banks and used in ongoing breeding programs for advanced generations.

The huge advantage of the eucalypts with quick and large gains was mentioned. Especially fortunate is the potential for narrowing the genetic base for desired economically important characters while at the same time broadening the base for adaptability and pest resistance. This is possible because the two groups of characteristics are usually genetically independent.

Key words: adaptation, base population, Brazil source, *Eucalyptus*, exotic species, gene base, land race, seed source, vegetative propagation.

Zusammenfassung

Eucalyptus ist die am meisten angebaute exotische Baumart in Brasilien. Um bei *Eucalyptus* durch züchterische Maßnahmen einen maximalen genetischen Gewinn zu erreichen, sind Lang- und Kurzzeitprogramme notwendig. Für beide Programme ist geeignetes züchterisches Ausgangsmaterial erforderlich. Bisher wurden in Brasillien in der Hauptsache weniger wertvolle Bestände, wie der bei Rio Claro, zur Vermehrung benutzt. In dieser Arbeit werden Methoden beschrieben, die auf die Entwicklung von für Brasilien am besten geeigneten Populationen abzielen. Die besten Bäume aus bereits bestehenden Beständen sollen selektiert, vegetativ vermehrt und ein Teil davon in Samenplantagen genutzt werden. Der Rest soll in Klonbanken erhalten werden, um für Züchtungsprogramme in späteren Generationen verfügbar zu sein. Zusätzlich soll Saatgut aus wertvollen natürlichen und Sekundär-Beständen importiert werden. So kann eine Ausgangsbasis mit erwünschten, ökonomisch wichtigen Merkmalen,wie z. B. Anpassungsfähigkeit und Krankheitsresistenz, geschaffen werden, die genetische Gewinne in späteren Generationen ermöglicht und zu einer gesunden Forstwirtschaft in Brasilien beiträgt.

Introduction

The introduction of eucalypts when grown as an exotic generally follows the pattern of (1) introduction and testing of several species and provenances, (2) selection followed by improvement of the best provenance, (3) establishment of seed production areas for early seed availability, (4) production of seed orchards from the very best trees of the best provenances. Such programs are primarily geared for early and maximum seed production for large-scale operational programs. Too often there has been little or no concern about the long-range aspects and the best methods to produce continually improved planting material over a number of generations. This has been made worse in *Eucalyptus* because the initial gains have been so large and many persons have become quite satisfied with the initially improved stock. For example, gains as large as 20% have been obtained from a simple seed production area; with more intensive programs, especially those including vegetative propagation, gains of 100% over the stock originally planted are not uncommon.

In Brazil a common pattern in the past has consisted of getting the original seed from Rio Claro and development of seed production areas and even seed orchards from this source material. Derivative stands from the original Rio Claro source have many problems stemming from hybridization and resultant "hybrid breakdown" and from related matings, since the original stands contained a limited number of parents. Although many organizations initially used this "degenerate" source of eucalypts for operational planting, they have generally switched to newer introductions from Australia or in the case of *E. grandis* from the Republic of South Africa or from Zimbabwe.

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