

## **Practical use of GA<sub>4/7</sub> to stimulate flower production in *Picea abies* seed orchards in Sweden**

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### **Abstract**

In 2005 we conducted the first practical attempt to stimulate flower production in *Picea abies* seed orchards in Sweden by applying GA<sub>4/7</sub>. Four seed orchards were treated: two old orchards at the end of their production phase, and two young orchards at the start of their production phase. Only one treatment of GA<sub>4/7</sub> was applied to each orchard, and treatment was done at one single occasion in each orchard. Every second row of trees was left untreated and used as a control. Male and female flowering was scored during the flowering season in 2006 from a random sample of trees in all four orchards. In autumn 2006, the number of cones produced per tree was counted in three of the four orchards, and the seed quality was assessed in two of them. Our results show that the GA<sub>4/7</sub> treatment had a significant positive effect on male flowering in the two young orchards, but not in the two old orchards. GA<sub>4/7</sub> treatment also had a significant positive effect on female flowering in three of the orchards, but in one of the old orchards it had no significant effect on female flowering. A positive significant GA<sub>4/7</sub> effect was found in the number of cones produced in only one of the young orchards. Seed quality was not affected by the GA<sub>4/7</sub> treatment. Although the GA<sub>4/7</sub> treatment did not always have a clear positive effect, economic calculations based on cone count data showed that GA<sub>4/7</sub> treatment is still a cheap and highly profitable way of increasing the seed production in *P. abies* seed orchards.

### **Introduction**

Flower stimulation using GA<sub>4/7</sub> has shown positive effects in a large number of conifer species (see e.g. Owens & Blake 1985). Also in *Picea abies* positive results has been reported (Dunberg 1980, Högberg & Eriksson 1994, Fogal et al. 1996). Consequently, flower stimulation, using GA<sub>4/7</sub> treatment, has been used routinely for a long time in order to facilitate crossings in the Swedish breeding programmes. However, seed orchard managers

have hesitated to use GA<sub>4/7</sub> in seed orchards, especially in *P. abies*, due to its irregular and unpredictable flowering patterns.

In 2005 we conducted the first practical, commercial-scale attempt to stimulate flower production by GA<sub>4/7</sub> treatment in *P. abies* seed orchards. The objectives of the study presented here were to measure the effects of the GA<sub>4/7</sub> treatment on flower, pollen and cone production.

## Material and methods

### *Location of seed orchards*

Four *P. abies* seed orchards were treated with GA<sub>4/7</sub>: two old orchards near the end of their production phase and two young orchards at the beginning of their production phase. The locations of the orchards are shown in figure 1, and descriptions of the orchards are given in table 1.

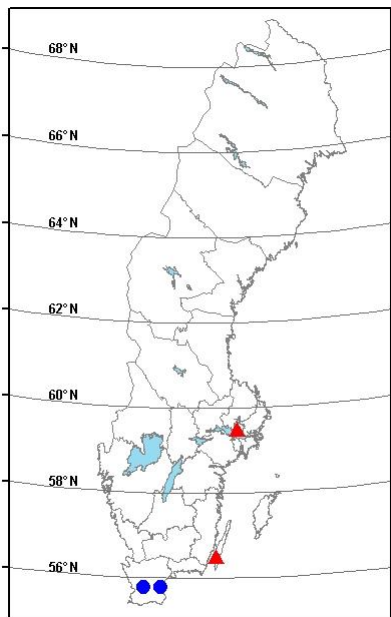


Figure 1. Locations of the seed orchards. Blue circles show the locations of the two old orchards: 52 Maglehem and 68 Slogstorp. Red triangles show the locations of the two young orchards: 501 Bredinge on the island of Öland and 504 Ålbrunna, outside Stockholm.

### *GA<sub>4/7</sub> treatment*

A single GA<sub>4/7</sub> treatment was applied to each orchard, and the dates of application are shown in table 1. Every second row was left as untreated control to enable statistical assessment of the treatment. Each graft was given a GA<sub>4/7</sub> dose corresponding to its diameter at breast height, table 2. In all orchards, a standard method of GA<sub>4/7</sub> application was used, i.e. a hole was drilled into the xylem in the trunk and a GA<sub>4/7</sub> solution was applied using a micropipette.

However, in 504 Ålbrunna we also evaluated a different injection method, using equipment from ArborSystems that was developed for injecting insecticides into trees, figure 2.

Table 1. Description of the seed orchards studied, GA<sub>4/7</sub> treatment dates in 2005, and the numbers of grafts and samples included in this study.

	504 Ålbrunna	501 Bredinge	52 Maglehem	68 Slogstorp
Established	1984	1983	1958	1965
Seed orchard managers	Bergvik Skog AB	Södra Odlarna AB	Svenska skogsplantor AB	Svenska skogsplantor AB
Size (ha)	25	26	5	19
Number of clones	137	100	36	45
Spacing (m)	7 × 4	7 × 3.5	5 × 5	7 × 3.5
GA <sub>4/7</sub> treatment dates in 2005	July 6–8	June 30 – July 2	July 7	July 4–5
No. grafts, flower score	1600	243	363	400
No. grafts, cone count	210	99	100	-
No. samples, seed quality	9	4	2	-
DBH (mm) of grafts in cone count. Min – mean – max	73 – 171 – 278	126 – 185 – 298	111 – 290 – 445	-



Figure 2. The Wedgle™ Direct-Inject™ tree injection unit from ArborSystems (Omaha, USA) tested in 504 Ålbrunna.

Table 2. Doses of GA<sub>4/7</sub> and the number of application points on each graft used in the GA<sub>4/7</sub> treatment of the seed orchards.

Graft diameter at breast height (cm)	GA <sub>4/7</sub> dose (mg/graft)	Number of application points (drilled holes using the standard method or injection points using the injection method)
< 7	15	1
7–15	30	2
15–20	45	3
20–25	60	4
> 25	100	5

### *Flower scoring*

Female and male flowering were scored during flowering in 2006 by examining a random sample of trees in all four orchards, as listed in table 1. Flowering was scored in one of ten classes; from 0 (no flowers), to 9 (corresponding to the most abundant flowering observed in each orchard).

### *Cone count and seed quality assessment*

In the autumn of 2006, the number of cones produced per tree was counted in three of the orchards, table 1. From each graft all cones, including vital cones and those affected by insects and fungi, were collected to estimate the production potential of the trees. Seed quality was assessed for the different treatments in the two young orchards 504 Ålbrunna and 501 Brelinge. Each sample consisted of 30 cones. In Ålbrunna three samples of each treatment were analysed, and in Brelinge two samples of each treatment were analysed. At 52 Maglehem, the samples for the control and the GA<sub>4/7</sub> treatment were unintentionally mixed up, so for this orchard only data from two samples consisting of cones from both control grafts and GA<sub>4/7</sub> treated grafts could be analysed. Seed quality traits analysed were 1000-grain weight and the number of filled seeds per cone.

### *Statistical analyses*

The Proc GLM module of the SAS program (SAS 1999) was used for statistical analysis. Data were analysed separately for each seed orchard, and the traits included in the analysis were the numbers of female and male flowers per graft, and the number of cones per graft. We analysed the data using the following linear model:

$$y_{ijk} = \mu + b_i + c_j + g \cdot x_{ijk} + e_{ijk}$$

where:

$y_{ijk}$	=	dependent variable, <i>i.e.</i> the number of female flowers per graft
$\mu$	=	overall mean
$b_i$	=	fixed effect of the block
$c_j$	=	fixed effect of the treatment
$g$	=	regression coefficient
$x_{ijk}$	=	diameter at breast height (only used in the analyses of cone count data)
$e_{ijk}$	=	residual (N(0, $\sigma_e^2$ ))

The significance of any differences between the control and treatment least square means was tested within the GLM procedure.

### *Economic calculations*

Based on the cone count and seed quality results from 504 Ålbrunna, along with costings for the GA<sub>4/7</sub> solution and GA<sub>4/7</sub> application, we calculated the cost of production for one additional kilogram of filled seeds. The GA<sub>4/7</sub> cost was €14 per gram, the labour cost was

€28 per day and it was possible to treat 600 grafts per day. We assumed that 80 percent of the originally planted grafts were alive and that 75 percent of the cones contained filled seeds. Rate of exchange used: 1 €= 9.20 SEK.

## Results and discussion

### *Flower and pollen scoring*

We found a significant positive effect of the GA<sub>4/7</sub> treatment on male flowering in the young orchards, but not in the old orchards (table 3), and scores for female flowering showed a significant positive effect of the GA<sub>4/7</sub> treatment in three of the orchards. In one of the old orchards, the treatment had no significant effect on female flowering, table 4. The standard GA<sub>4/7</sub> treatment method yielded higher, but not significantly higher, flowering scores than the injection method for both male and female flowering at 504 Ålbrunna. A possible reason for this difference between the treatment methods is that the GA<sub>4/7</sub> is introduced into the xylem of the tree with the standard method, and into the phloem with the injection method. Further comparative studies between these two methods are needed to confirm whether or not there is a real difference in the results obtained using these application methods.

The higher average flower scores in the GA<sub>4/7</sub>-treated trees, compared to the controls, were due to larger proportions of control grafts in the lowest scoring classes, and larger proportions of GA<sub>4/7</sub>-treated grafts in the highest scoring classes, figure 3. The finding that more GA<sub>4/7</sub>-treated grafts flowered than the controls implies that more GA<sub>4/7</sub>-treated clones participated in the flowering, suggesting in turn that the treatment enhanced the genetic diversity in the seed crop. The finding that more grafts flowered abundantly after GA<sub>4/7</sub> treatment also implies that the treatment enhanced the genetic diversity of the seed crop.

Table 3. Results of male flower scoring.

Seed orchard	Flower score, controls	Flower score, GA <sub>4/7</sub> -treated grafts	GA <sub>4/7</sub> treatment effect (percent)	Pr >  t	
504 Ålbrunna, standard method	4.08	4.55	11.6	0.003	**
504 Ålbrunna, Injection method	4.08	4.50	10.4	0.008	**
501Bredinge	3.55	3.95	11.2	0.041	*
52 Maglehem	4.78	4.87	1.9	0.596	n.s
68 Slogstorp	4.32	4.34	0.6	0.909	n.s.

Table 4. Results of female flower scoring.

Seed orchard	Flower score, controls	Flower score, GA <sub>4/7</sub> -treated grafts	GA <sub>4/7</sub> treatment effect (percent)	Pr >  t	
504 Ålbrunna, standard method	3.18	4.52	42.1	<.0001	***
504 Ålbrunna, Injection method	3.18	4.30	35.4	<.0001	***
501 Bredinge	3.46	4.20	21.3	<.0001	***
52 Maglehem	4.54	4.59	1.2	0.740	n.s.
68 Slogstorp	2.84	3.31	16.7	0.048	*

The absence of any significant effect of GA<sub>4/7</sub> treatment in the old orchards may be explained by the doses given, since they may have been too small in relation to the graft size. The highest doses of GA<sub>4/7</sub> (100 mg/graft) were used for grafts with diameters larger than 25 cm, and at 52 Maglehem and 68 Slogstorp the grafts had an average diameter of 29 cm and a maximum diameter of 44 cm.

#### *Cone count*

In the young orchard 504 Ålbrunna the standard GA<sub>4/7</sub> treatment resulted in a significant ( $P < 0.049$ ) 69 percent increase in the number of cones compared to the controls, while the injection method resulted in a non-statistically significant 33 percent increase, table 5. In the other young orchard, 501 Bredinge, there was no positive effect of the GA<sub>4/7</sub> treatment on cone production. However, in this orchard both the treated and control grafts' production levels were extremely high; on average 877 cones were produced per graft, with a maximum cone production for one graft of 2828. In years when high natural flower production occurs, the grafts probably cannot bear any further cones, so the increased flowering indicated by the flowering scores may not result in a larger cone crop.

Table 5. Results of cone counts.

Seed orchard	Cones per control graft	Cones per GA <sub>4/7</sub> -treated graft	GA <sub>4/7</sub> treatment effect (percent)	Pr >  t	
504 Ålbrunna, standard method	169.7	286.9	69.0	0.049	*
504 Ålbrunna, Injection method	169.7	225.5	32.9	0.352	n.s.
501 Bredinge	876.8	830.0	-5.3	0.749	n.s.
52 Maglehem	479.4	494.7	3.2	0.850	n.s.
68 Slogstorp	-	-	-	-	

### Seed quality

The seed quality data show, as expected from other investigations (e.g. Eriksson et al. 1998), that the GA<sub>4/7</sub> treatment resulted in slightly lighter seeds. The differences between the treated and non-treated grafts were not statistically significant in this respect (table 6), but this may have been due to the small sizes of our small samples.

Table 6. Seed quality results.

Seed orchard	1000-grain weight, controls (g)	1000-grain weight, GA <sub>4/7</sub> -treated grafts (g)	No. of filled seeds per cone, controls	No. of filled seeds per cone, GA <sub>4/7</sub> -treated grafts
504 Ålbrunna, standard method	8.1	7.8	105	106
504 Ålbrunna, Injection	8.1	6.9	105	135
501 Bredinge	9.5	8.9	147	140
52 Maglehem	9.0 <sup>1)</sup>	-	150 <sup>1)</sup>	-
68 Slogstorp	-	-	-	-

<sup>1)</sup> Average for both control and GA<sub>4/7</sub> treated grafts.

### Economic calculations

Results from the economic calculations based on data from 504 Ålbrunna showed that the cost of producing an additional kilogram of seeds was €14 (table 7), much lower than the market price of high quality orchard seed in Sweden (ca. €100). Even if only one in every seven treatments with GA<sub>4/7</sub> was successful, the cost of producing an extra kilogram of seed would be less than €100; less than 10 percent of the market price of the seed. So, GA<sub>4/7</sub> treatment appears to be a cheap and highly profitable way to increase seed production in *P. abies* seed orchards.

Table 7. Economic calculation results.

Cost of GA <sub>4/7</sub> treatment (€ha)	311
Extra production of filled seed (Kg/ha)	22
Cost of the extra production of filled seed (€Kg)	14
Success rate	Cost of the extra production of filled seed (€Kg)
Every time	14
1 out of 3	42
1 out of 5	70
1 out of 7	99



## Conclusions

The treatment of *P. abies* with GA<sub>4/7</sub> increases both the genetic diversity and size of the seed crop in seed orchards. Although a positive effect of GA<sub>4/7</sub> treatment is not always obtained, economic calculations based on our cone count data indicate that GA<sub>4/7</sub> treatment is a cheap and highly profitable way to increase the seed production in *P. abies* seed orchards, even if only one treatment in seven is successful.

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