

INTERSTOCK AND GA_{4/7} EFFECTS ON FLOWERING AFTER TOPGRAFTING IN *PINUS SYLVESTRIS*

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ABSTRACT

In breeding programmes shortening the generation turnover should increase the genetic gain per unit time. Topgrafting (in which scions of young trees are grafted into the crown of older, reproductively more mature trees) may help meet this objective, since it can induce young material to flower. Therefore, we studied the effect of the interstock clone on the flowering of topgrafts in *Pinus sylvestris* (L.) over three years. We also tested the possibility of enhancing female flowering among the topgrafts by treating the interstocks with gibberellin GA_{4/7}. There were large differences in topgraft survival rates among the different interstock clones, varying between 54–89% in the third year. For both female and male flowering of the topgrafts, analysis of variance showed significant effects of both interstock and topgraft. The interaction between interstock and topgraft was significant for female flowering but not for male flowering. The length of the topgraft scion had a significant effect on the number of female flowers but not on male flowers. The effect of GA_{4/7} treatment was significant for female flowering, causing a 55% increase in the number of flowers per topgraft in the third year. No relationship was found between the female flowering of the interstocks and their capacity to induce female flowering in the topgrafts. The results indicate that topgrafting could effectively decrease time to flowering compared to conventional grafting using young rootstocks in *Pinus sylvestris*.

Key words: breeding, early flowering, flower stimulation, grafting, *Pinus sylvestris*, Scots pine, topworking

INTRODUCTION

In long-term tree breeding programmes involving several breeding cycles, shortening the generation turnover would be highly desirable since it would reduce the breeding cycle and thus increase genetic gain per unit time.

Topgrafting, or topworking as it's also called, is the method of grafting juvenile scions, topgrafts, into the crown of a graft of a reproductively mature clone, the interstock. The resulting ramet consists of three different genotypes, the rootstock, the interstock, and the topgraft. The rationale is that the reproductive competence of the interstock clone will be transferred to the topgraft clone. The method has been used for many years in fruit trees (HARTMAN & KESTLER 1968).

In *Pinus taeda* topgrafting was first reported by GREENWOOD & GLADSTONE (1978). In their study, 59% of the living topgrafts produced pollen 3–4 years after grafting. They conclude that the method was mainly useful for production of male strobili in young material. Similarly, BRAMLETT *et al.* (1995) described a topgrafting method for *Pinus taeda* they called surrogate

pollen induction, in which pollen cones were induced in the year of grafting.

Topgrafting of scions from 1–5 year old plants into the crown of old, reproductively mature *Pinus taeda* grafts resulted in female flowers in 21–80% of surviving topgrafts one year after grafting (BRAMLETT & BURRIS 1995). Topgrafts of all ages flowered, with the highest percentage of flowering grafts originating from the four year old seedlings. However, although some female flowers were produced in the first year after grafting, the number of flowers increased substantially between years one and two in *Pinus taeda*. BRAMLETT & BURRIS (1998) reported a 250 percent increase in female flowering, from 2.5 to 9 flowers per topgraft, between the first and second year after grafting.

The choice of interstock clone is important when topgrafting *Pinus taeda*, since there are strong interstock effects on the number of female flowers produced (MCKEAND & RALEY 2000). There are also large differences between interstock clones in the survival of the topgraft clones.

GOODING *et al.* (2000) found no differences between interstocks of *Pinus elliottii* and *Pinus taeda* in their

ability to promote topgraft survival or their capacity to initiate female flowers in topgrafts of *Pinus taeda*.

The topgrafting method has also been suggested as a way to replace existing tree crowns with new clonal selections in seed orchards (BRAMLETT 1997).

In contrast to the positive effects of topgrafting observed in *Pinus taeda*, studies concerning other species report variable results. In an investigation using western white pine (*Pinus monticola*) topgrafted scions had not flowered after five years, although the rootstock had flowered (BARNES & BINGHAM 1963). In *Larix decidua* and *Larix leptolepis*, ROBINSON & WAREING (1969) grafted scions of young seedlings and nine-year old plants onto flowering shoots of old trees. This resulted in very sparse female flower initiation in the young scions in both species. However, on some of the scions from the nine-year old plants both female and male flowers were produced.

In *Pinus sylvestris* MCDANIEL & EINERT (1976) mention that female flowers can be produced on young material by topgrafting, but they presented no data to illustrate their assertion. In an attempt to induce precocious flowering in *Pinus sylvestris*, SIMAK (1978) grafted scions from 3-year old seedlings into the crown of 16–25 year old trees. The only reported flowering from this experiment occurred two years after grafting, and in only one of 73 grafts. The flowering scion was grafted onto a 25-year old tree.

In conifers, the less polar gibberellins A₄ and A₇ have been successfully used to artificially induce flower formation (see e.g. OWENS & BLAKE 1985). The treatment effect is not persistent and must be repeated to keep the flowering at an enhanced level. PHARIS *et al.* (1987) suggest that the exogenously applied GAs seem to act in the same way as the endogenously produced GA in the tree.

To our knowledge, there are no earlier reports in the literature on the combined effect of topgrafting and GA_{4/7} treatment in conifers. Thus, the objective of this study was to assess the impact of the interstock clone on flowering of the topgraft clone in *Pinus sylvestris* L., and to assess the effect of treating the interstock with GA_{4/7} on the female flowering of the topgrafts.

MATERIALS AND METHODS

Materials and trial design

Six clones originating from old plus trees were used as interstocks, and the grafts used in this study were made in 1988. They were planted in a clonal row design with a spacing of 1 m within rows and 5 m between rows. The grafts have been pruned to a hedge orchard shape with a height of approximately 2.5 m. The interstocks

are located at Brunsbergs field station, SkogForsk, (lat. 59° 37' N, long. 12° 58' E, alt. 80 m.a.s.l.).

As topgrafts, seven clones from new selections made in a progeny test were used. The progeny test was established with one-year old seedlings and had grown for 19 growing seasons in the field when the scions were collected. Of these seven clones, four had started to flower in the field trials when the scions were collected and three had not. The scion material was collected from the upper part of the crown of the trees. In spring 1997 (year 0) two grafts of each topgraft clone were topgrafted into the crown of each of two different ramets of the six interstock clones. Altogether the experiment consisted of 2 topgrafts/ clone × 7 - topgraft clones × 6 interstock clones × 2 ramets/ interstock clone, i.e. 168 grafts. In addition, each topgraft clone was also grafted onto four 2-year old rootstocks. These grafts were used to compare the results of topgrafting and normal grafting.

In 1998 (year 1), the year after grafting, half of the interstocks were treated with gibberellin A_{4/7} (GA_{4/7}) to enhance initiation of female strobili. The timing of the injections was based on the findings of CHALUPKA (1980, 1984) and LUUKKANEN & JOHANSSON (1980), and was performed on July 8, 14 and 21. The GA_{4/7} was injected with a micropipette into the trunk of the interstock at the base of the graft into two drilled holes (2.5 mm diameter, 15 mm deep) on each occasion. After injection the holes were covered with grafting wax. To each interstock graft a total amount of 50 mg GA_{4/7} was injected, dissolved in ethanol (99%) to a concentration of 208.5 mg GA_{4/7}/ml. The GA_{4/7} treatment was repeated in 1999 (year 2). Application dates this year were July 6, 13 and 19.

Assessments and measurements

The survival of the topgrafts was recorded at four times: in the autumn of year 0 and the spring of years 1, 2, and 3. On each occasion the topgrafts were scored as dead or alive.

Both female and male flowers were counted on each topgraft after flowering in years 1, 2 and 3. In year 1 there was no flowering on the topgrafts, and in year 2 there were female flowering but only eight male flowers on all topgrafts, which was considered too low a number for meaningful evaluation.

On the interstocks, the number of female and male flowers was counted in year 3.

The grafts on 2-year old rootstocks were checked for flowering annually during the experimental period.

In the spring of year 3, before the start of the current year's shoot elongation, the total length of each topgraft was measured.

Statistical analysis

The number of flowers per topgraft was transformed into normal scores to meet the requirements for normal distributions (GIANOLA & NORTON, 1981).

The traits included in the analysis of variance were: number of female flowers in years 2 and 3, the number of male flowers in year 3, and topgraft survival in years 0–3. The Proc mixed module of the SAS program was used for these computations (SAS 1997), with the following model:

$$y_{ijkl} = \mu + b_i + c_j + d_k + bc_{ij} + bd_{ik} + cd_{jk} + bcd_{ijk} + f_l(cd_{jk}) + g x_{ijkl} + e_{ijkl}$$

where: y_{ijkl} = the dependent variable, e.g. number of female flowers in year 2; μ = the overall mean; b_i = fixed effect of GA_{4/7} treatment i of the interstock (GA_{4/7} or No GA_{4/7}, $i = 1, 2$); c_j = fixed effect of interstock clone j ($j = 1, \dots, 6$); d_k = fixed effect of topgraft clone k ($k = 1, \dots, 7$); $f_l(cd_{jk})$ = effect of scion l ($l = 1, 2$, $N(0, \sigma_l^2)$) nested within interstock j and topgraft k ; g = regression coefficient; x_{ijkl} = the scion length of the topgraft in spring year 3, used as covariable for flowering traits; e_{ijkl} = residual, ($N(0, \sigma_e^2)$).

RESULTS

There were substantial differences in the average survival rates of the topgraft clones between the interstock clones, although the overall survival was high (Table 1). On the interstock clones with the highest survival, 25 of 28 topgrafts were alive in the spring of year 3, a significantly higher proportion ($p = 0.003$) than on the interstock that gave the lowest survival, 15 of 28 topgrafts. For the different topgraft clones, the average survival ranged from 58 % to 92 % (data not

presented).

All topgraft clones produced female flowers in year 2 (Table 2), even those three that had not flowered in the field trial prior to collection of the scion material.

There was on average a 5-fold increase in the abundance of female flowers, from 0.8 to 4.2 female flowers per living topgraft, from year 2 to year 3 (Table 2). The difference between the topgraft female flowering on the most and least productive interstock was significant in both years 2 and 3 ($p = 0.01$ and $p = 0.03$ respectively).

The analysis of variance showed that there were statistically significant effects of interstock and topgraft for both female flowering in year 2 and 3 and male flowering in year 3 (Table 3). Also in the ANOVA the effect of GA_{4/7} treatment was significant for female flowering in year 3, but not year 2, and not for male flowering. There was a significant 3-way interaction between interstock clone, topgraft clone, and GA_{4/7} treatment for both female and male flowering. Interaction between interstock clone and topgraft was significant for female flowering but not for male flowering. The size of the topgraft scion had a significant influence on the number of female flowers but not on the number of male flowers.

The magnitude of the interaction between interstock clone and topgraft clone in female flowering in year 3 is shown in Figure 1. Of the seven topgraft clones, four flowered most prolifically on one of the two interstock clones that gave the highest average numbers of flowers. Figure 2 illustrates the lack of relationship between the female flowering of the interstock clones and their capacity to induce the topgraft clones to produce female flowers.

None of the topgraft clone grafts grafted onto juvenile rootstocks, as a comparison to the topgrafting method, produced any flowers up to and including year 3.

Table 1. Present surviving topgrafts on each interstock clone. Standard errors are given in the parentheses. In the spring of year 0, 28 grafts were grafted onto each interstock clone.

Interstock	Survival (%)			
	Autumn, year 0	Spring, year 1	Spring, year 2	Spring, year 3
A	85.9 (7.3)	78.9 (6.9)	75.8 (8.2)	73.1 (8.2)
B	57.1 (7.1)	53.6 (6.7)	53.6 (7.9)	53.6 (7.9)
C	96.4 (7.1)	96.4 (6.7)	89.3 (7.9)	89.3 (7.9)
D	75.0 (7.1)	75.0 (6.7)	71.4 (7.9)	71.4 (7.9)
E	89.3 (7.1)	89.3 (6.7)	89.3 (7.9)	89.3 (7.9)
F	96.4 (7.1)	96.4 (6.7)	96.4 (7.9)	89.3 (7.9)
Mean	83.4	81.8	79.3	77.7

Table 2. Mean values for the main effects Interstock and GA_{4/7} treatment for female flowering in years 2 and 3 and for male flowering in year 3. Mean values calculated as LsMeans on untransformed data are presented to indicate the level of flowering and the magnitude of the difference between Interstocks and GA_{4/7} treatments. For tests of significant differences LsMeans and standard errors calculated from the transformed data are presented.

	No. female flowers per topgraft				No. male flowers per topgraft	
	Year 2		Year 3		Year 3	
	Untrans- formed data	Transformed data	Untrans- formed data	Transformed data	Untrans- formed data	Transformed data
Interstock						
A	0.7	5.00 (0.21)	5.2	5.28 (0.22)	0.6	4.82 (0.24)
B	0.5	4.93 (0.26)	3.5	4.82 (0.27)	1.4	5.25 (0.29)
C	0.3	4.65 (0.18)	2.2	4.62 (0.19)	0.3	4.76 (0.21)
D	1.7	5.41 (0.21)	3.1	4.71 (0.22)	0.8	4.87 (0.24)
E	0.3	4.75 (0.21)	5.4	5.17 (0.19)	0.3	4.91 (0.21)
F	1.1	5.27 (0.19)	5.8	5.27 (0.20)	2.4	5.37 (0.22)
GA_{4/7} treatment						
No GA _{4/7}	1.0	5.06 (0.12)	3.3	4.77 (0.13)	0.9	5.01 (0.13)
GA _{4/7}	0.6	4.94 (0.12)	5.1	5.18 (0.12)	1.1	4.99 (0.12)

Table 3. Analysis of variance. Type 3 (SAS) tests of fixed effects for female flowering on the topgrafts in years 2 and 3, and for male flowering on the topgrafts in year 3.

Source of variation	Female flowering, year 2		Female flowering, year 3		Male flowering, year 3	
	F value	<i>p</i> -value	F value	<i>p</i> -value	F value	<i>p</i> -value
Interstock clone	6.38	<.001	4.87	0.002	2.80	0.032
Topgraft clone	15.85	<.001	15.13	<.001	6.50	<.001
GA _{4/7} treatment	1.90	0.184	17.34	<.001	0.45	0.512
Intrstock × topgraft clone	2.32	0.001	3.44	<.001	1.08	0.416
Interstock × GA _{4/7} treatment	1.96	0.130	5.38	0.003	4.27	0.009
Topgraft clone × GA _{4/7} treatment	2.11	0.098	2.28	0.079	1.01	0.448
Interstock × Topgraft × GA _{4/7}	3.11	0.007	3.40	0.005	3.61	0.003

DISCUSSION

The interstock had a large impact on the survival of the topgrafts. This is an important factor to consider when selecting clones to be used as interstocks in breeding programmes, and is in accordance with findings in *Pinus taeda* (MCKEAND & RALEY 2000).

There were large clonal differences between the interstocks in their capacity to induce flowering on the topgrafts, implying that the choice of interstock is important. Unfortunately, there was also a significant interaction between the interstock and topgraft as seen in Table 3 and Figure 1. This means that some topgraft clones produced few flowers on the interstock clone

that, on average, was the most productive. One way to overcome this is to distribute the grafts of each topgraft clone into the crown of two or three good interstocks. In *Pinus taeda*, MCKEAND & RALEY (2000) found no significant interaction between interstock and topgraft.

The GA_{4/7} treatment gave a positive response on female flowering in year 3 but not in year 2 and not on male flowering in year 3. The treatment was timed to stimulate induction of female flowers, so no effect on male flowering was expected. The absence of any effect on female flowering in year 2 may be related to the overall low response that year. That the injection of GA_{4/7} into the trunk of the interstock clone affects the formation of flowers in the topgrafts supports the idea that the hormonal balance of the interstock influences

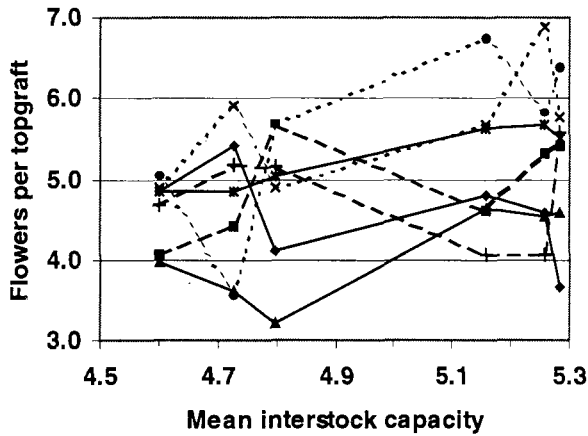


Figure 1. Mean number of female flowers produced by each of the seven topgraft clones on each of the six interstock clones, plotted against the mean capacity of the interstocks to induce flowering in the topgraft. Data from female flowering in year 3. Values in transformed scale.

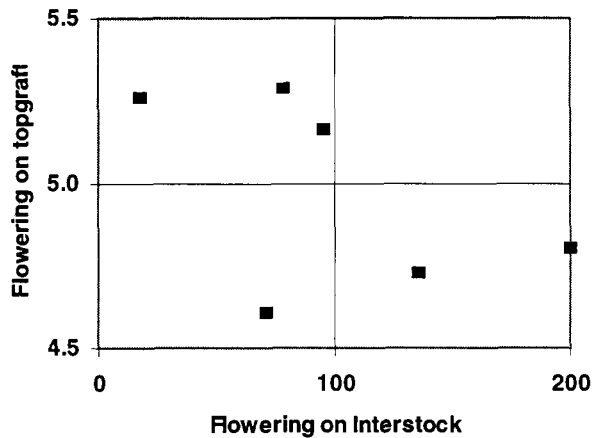


Figure 2. Mean number of female flowers produced by the seven topgrafts plotted against the relative number of flowers produced by the six interstocks themselves. Data refer to female flowering, year 3. Topgraft data in transformed scale. Interstock flowering in relative figures (average = 100).

the topgrafts. The increase of female flowering induced by the GA_{4/7} in year 3 was 55%. This implies that GA_{4/7} treatment of the interstocks could be an effective way for the breeder to ensure that enough flowers are produced to complete the crosses needed in the breeding programme.

The lack of relationship between flowering on the interstock clone itself and its capacity to induce flowering on the topgrafts is in accordance with findings in *Pinus taeda* (MCKEAND & RALEY 2000). A practical consequence of this is that candidate interstock clones need to be screened by topgrafting a number of scion clones into their crowns to evaluate their capacity as interstocks.

In this study, we have shown that topgrafting has the potential to decrease the time to flowering compared to grafting onto young rootstocks in *Pinus sylvestris*. For selections made in plantations or progeny tests that need to be put into a breeding archive for flowering, topgrafting is a method that should be considered. However, in our experiment we used scion clones that were close to, or already had shown, flowering competence. Before the method can be recommended for younger material more knowledge is needed about whether such material can be forced to flower using the topgrafting method. There is also a need to evaluate the seed quality produced with the method, especially when young scion material is used.

CONCLUSIONS

There is a large difference between interstock clones in their capacity to induce flowering in topgrafted scions. There is also a large variation in the survival of the topgrafts between the interstock clones, which should be considered when selecting interstock clones.

There is a significant interaction between the interstock and topgraft clones. Therefore the breeder should distribute the grafts of each topgraft clone onto two to three good interstock clones.

Treating the interstock with the flowering-stimulating hormone GA_{4/7} will enhance the flowering of the topgrafts.

There is no relationship between flowering of the interstock clones and their capacity as interstocks. Therefore, to identify good interstock clones their capacity as interstocks needs to be tested.

Topgrafting has the potential to decrease the time to flowering compared to conventional grafting using young rootstocks.

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