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Improvement of Flowering Competence and Capacity with Reference to Swedish Conifer Breeding

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SWEDISH UNIVERSITY OF AGRICULTURAL SCIENCES

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Abstract

The objective of this thesis was to gain more knowledge related to flowering, and to assess its implications for the Swedish *Pinus sylvestris* L. and *Picea abies* (L.) Karst. breeding programmes.

Scions of young trees can be induced to flower by grafting them into the crown of reproductively older trees (topgrafting). The effects of the interstock clone and gibberellin ($GA_{4/7}$) application on topgrafting were studied in *P. sylvestris*. The interstock clone significantly influenced topgraft flowering and survival, and interacted significantly with the topgraft for female flowering. $GA_{4/7}$ treatment significantly increased female flowering. The results show that topgrafting can be used to induce early flowering and accelerate generation turnover in *P. sylvestris* breeding programmes.

The effects of the timing of $GA_{4/7}$ applications on flower production were studied in *P. sylvestris* using stem injections. Maximal female flowering was obtained from the latest applications in both of the two years examined. The treatment had no effect on male flowering. The characters shoot and needle elongation were poor indicators of optimal application time. However, heat sums (degree-day summation), can be used to determine the optimal time for treatment, which occurs at about 700 degree-days (threshold +5°C) for female flowering.

In breeding, trees are often selected for height. Strong genotypic correlations between height growth and reproductive traits could, if present, adversely affect genetic gain and diversity. So, genotypic correlations between early cone-set and height growth traits were studied in *P. abies* clonal trials. Only weak, non-significant genotypic correlations were found, implying that selection for growth traits should not affect fecundity in the species. Strong genotypic correlations (0.7 – 1.0) for cone-set were found between trials, indicating low G×E interactions for early flower initiation.

The rooting ability of cuttings decreases and flowering competence increases with age of the tree. Rooting success and cutting performance of flowering and non-flowering clones of *P. abies* were studied. No significant differences in rooting or cutting performance was found between clones with and without cone-set, indicating that genetic selection for rooting success or cone-set will not cause unwanted correlated responses in the other trait.

Key words: cuttings, early flowering, flower stimulation, genotypic correlations, gibberellin, Norway spruce, precocious flowering, Scots pine, topworking.

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To Lena, Gunnar and Malin

Contents

Appendix

Papers I – IV

This thesis is based on the following papers, which are referred to in the text by their corresponding Roman numerals.

I. Almqvist, C. & Ekberg, I. Interstock and $GA_{4/7}$ effects on flowering after topgrafting in *Pinus sylvestris*. Accepted for publication in *Forest Genetics*.

II. Almqvist, C. Effect of timing of $GA_{4/7}$ application on flowering in potted grafts of *Pinus sylvestris* in the greenhouse. *Manuscript*.

III. Almqvist, C., Jansson, G. & Sonesson, J. 2001. Genotypic correlations between early cone-set and height growth in *Picea abies* clonal trials. *Forest Genetics* 8(3): 197–204.

IV. Hannerz, M., Almqvist, C. & Ekberg, I. 1999. Rooting success of cuttings from young *Picea abies* in transition to flowering competent phase. *Scandinavian Journal of Forest Research* 14: 498–504.

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Introduction

Geographical range and population structure of *Pinus sylvestris* and *Picea abies*

Pinus sylvestris L. has the largest geographical distribution of any pine species (Richardson & Rundel 1998). In the northwest of its range it covers the Scandinavian countries, extending eastwards to the east coast of Asia and southwards to the Mediterranean Sea (Boratyn´ski 1991). *Pinus sylvestris* is a pioneer species with low demands for water and nutrients. It therefore naturally tends to occupy dry to mesic sites that are also frequently subjected to forest fires.

Picea abies (L.) Karst. also has a wide geographic distribution, which covers the area from the European West Coast to the Asian East Coast (Schmidt-Vogt 1977). *Picea abies* is a shade tolerant species with higher demands for water and nutrients than *Pinus sylvestris*. It is also sensitive to forest fires. Naturally it tends to grow in forest fire refuges and as a secondary species emerging from under canopies of broadleaved trees or *Pinus sylvestris* on wet and mesic sites.

Pinus sylvestris and *Picea abies* are both wind-pollinated, predominantly outcrossing species with a capacity for long distance dispersal of viable pollen (Lindgren & Lindgren 1996). Together with their almost continuous distributions, this gives the species characteristics such as clinal variations in adaptive traits along latitudinal and altitudinal gradients. Large within-population variation in adaptive traits is also characteristic of both species (see *e.g.* Eriksson 1982). In Sweden *Pinus sylvestris* and *Picea abies* are the two main conifer species: *Pinus sylvestris* constitutes 39% and *Picea abies* 44% of the total growing stock (Anon 2001).

Reproductive characteristics of *Pinus sylvestris* and *Picea abies*

In this thesis the term "flower" is used to mean all kind of reproductive structures in forest trees (Romberger 1967).

Reproductive cycle

The reproductive cycle covers the period from the initiation of reproductive structures through to the release of mature seed. In *Pinus sylvestris*, the reproductive cycle extends over three years. Reproductive structures are initiated in the first year, pollination occurs in the spring of the second year when the pollen tube and ovule development is initiated, but development is then paused. It resumes in the third year when fertilization and seed maturation take place. The reproductive cycle in *Picea abies* is completed in two years. In the first year the reproductive structures are initiated followed by flowering, pollination, fertilization, and seed maturation in the second year (Figure 1).

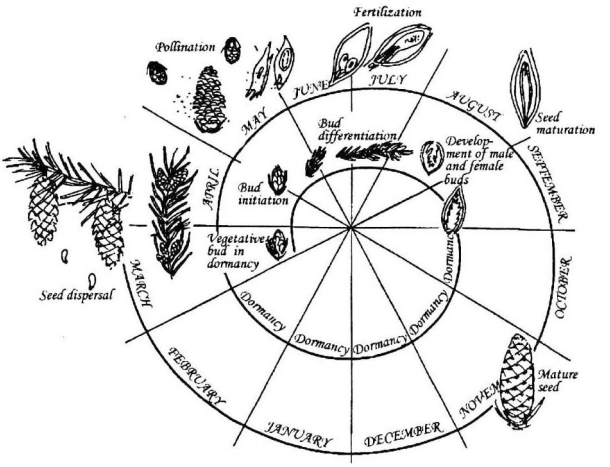

Figure 1. The reproductive cycle of *Picea abies* (from Eriksson 1996).

Initiation of reproductive buds

In *Pinus* species, male and female buds differentiate at different times during the development of the long-shoot bud (see *e.g.* Owens & Blake 1985). The long-shoot buds are composed of a series of cataphylls, which are initiated throughout the growing season. The time of initiation determines whether the axillary apices of the cataphylls will differentiate into dwarf shoots, lateral buds or reproductive buds, as described for *Pinus contorta* by Owens & Molder (1984a). At the base of the long-shoot bud where the axillary buds are initiated early in summer they will differentiate into dwarf shoot or male buds. At the top of the long-shoot bud the most distal axillary buds, initiated later in summer, will differentiate into either lateral branch shoot buds or female buds. The time of long-shoot bud development is influenced by the origin of the material and the environmental conditions it is subjected to.

In *Picea* species bud development starts with bud scale initiation in spring simultaneously with shoot elongation (Owens & Blake 1985, Hejnowicz & Obarska 1995). Differentiation into specific bud types (vegetative or reproductive) occurs during a two-week period after the termination of bud-scale initiation and at the end of shoot elongation (Dunberg 1979, Owens & Molder 1984b). In central Sweden reproductive bud differentiation in *Picea abies* normally occurs sometime between late June and mid July. The time of differentiation is dependent on weather conditions during spring and early summer and on the origin of the material.

Picea abies does not normally flower abundantly for two consecutive years (see *e.g.* Tirén 1935, Lindgren *et al.* 1977). In *Picea abies* female flowers differentiate from the terminal buds of branches. Abundant female flowering will therefore reduce both the current year's foliage and the number of meristems that could

differentiate into flowers the next year. For this reason *Picea abies* needs several years to build up its crown before it can flower abundantly again. This is in contrast to *Pinus sylvestris*, where female flower buds are formed in distal axillary buds and therefore their production does not stop the continuation of branch development.

Swedish long-term breeding programmes for *Pinus sylvestris* and *Picea abies*

Swedish long-term tree breeding strategy (Danell 1993) is based on the Multiple Population Breeding System (MPBS) concept (Namkoong *et al.* 1980, Burdon & Namkoong 1983). This means that high-intensity breeding for adaptation, biomass production and wood quality are integrated with dynamic gene conservation to safeguard the potential for adaptation to possible future environmental changes. For *Pinus sylvestris* and *Picea abies* the MPBS consists of meta-populations of about 1000 individuals, each sampled in each generation (Danell 1993). The meta-populations are divided into 20-24 sub-populations with about 50 trees in each, and the sub-populations are each allocated to adaptation targets with specific photoperiod and temperature parameters (Figure 2).

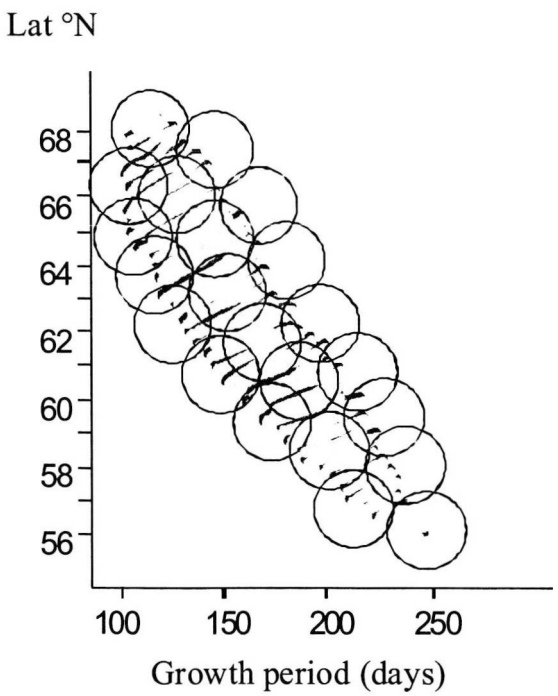

Figure 2. Principal distribution of the breeding populations used in the Swedish breeding programmes for *Pinus sylvestris* and *Picea abies*. Sub-populations are represented by circles and have target climates described in terms of photoperiod (Lat °N) and temperature (length of growth period). The shaded area approximates the present climatic range in Sweden (from Danell 1993).

In *Pinus sylvestris* (Figure 3A), the breeding cycle of a sub-population is based on double-pair matings of the 50 trees to create a new generation (Wilhelmsson & Andersson 1993). Following polycross matings, individual trees in this progeny are tested in field trials. Trees to be used in the next breeding cycle are selected, mainly on a within-family basis, taking one tree from each family, according to the results of the progeny testing. Note that the earlier the polycross and double-pair matings can be done, the shorter the breeding cycle will be. Topgrafting, accelerated growth conditions and hormonal treatment to stimulate flowering can all help speed up the generation turnover.

In contrast, for *Picea abies* (Figure 3B), the progeny obtained from the double-pair matings are tested in clonal trials (Karlsson & Rosvall 1993). Since *Picea abies* has a prolonged juvenile phase (20–25 years) there is a risk that only a limited proportion of the clones will have reached flowering competence when the clones to be used in the next breeding cycle can be selected, based on the results of the clonal trials. This may force the breeder to select only from among the clones with early flowering competence in order to avoid excessive delays. If early flowering competence is correlated with inferior height growth, this will cause a problem. One way to avoid this problem and to promote naturally late-flowering clones to flower earlier may be topgrafting.

In clonal forestry programmes of *Picea abies* a major problem has been the ageing of clones, which reduces rooting ability and increases plagiotropic growth (Roulund 1981). To keep the costs and quality of the rooted cuttings at reasonable levels, clones with poor rooting characteristics are removed from the programme. Since rooting ability decreases and flowering competence increases with age one may suspect that selection for high rooting capacity will negatively affect the flowering competence of the selected population.

Therefore, better knowledge of flowering competence and capacity is important for the future development of the breeding programmes. Increased genetic gain per year from each breeding cycle can be achieved if the time to flowering can be reduced. Genetic gain can also be increased if correlations between early flowering and such traits as height growth and rooting capacity of cuttings are clarified and, if necessary, compensatory measures are taken.

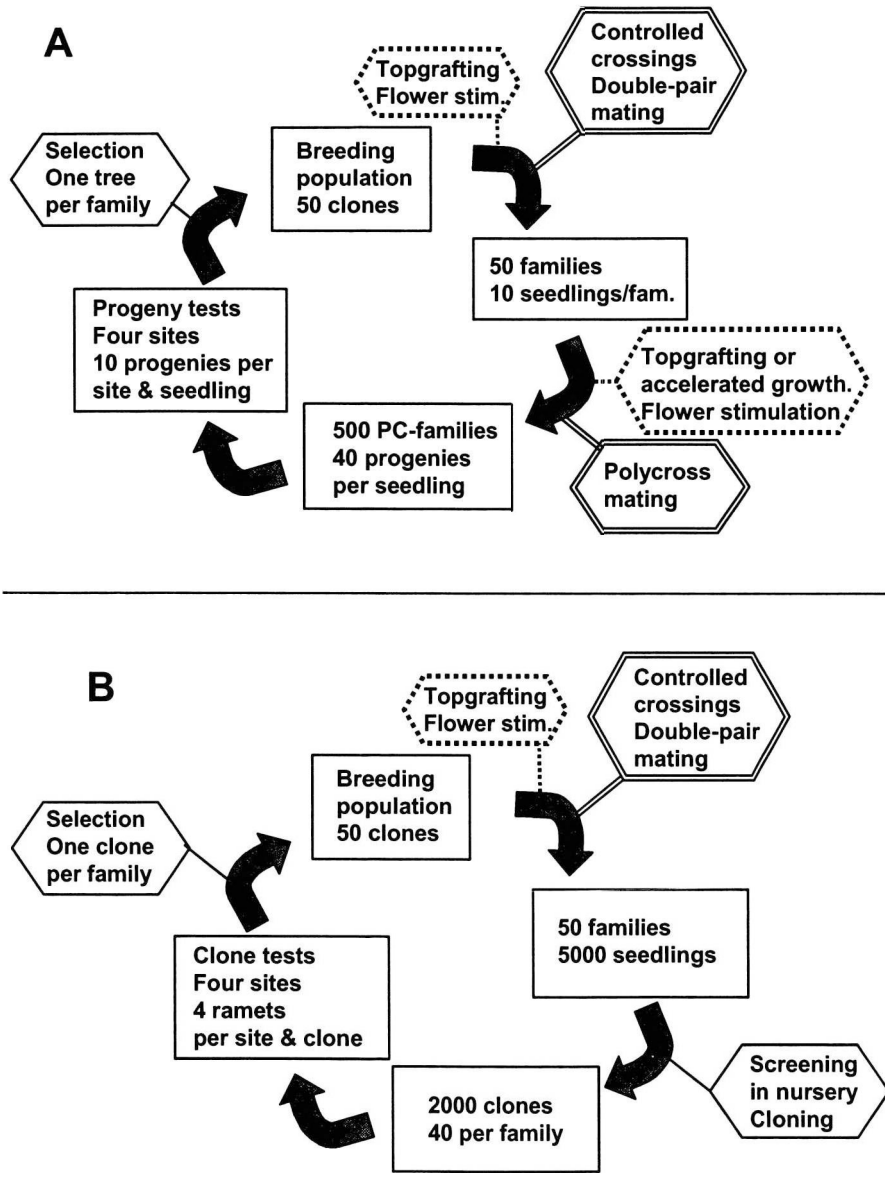

Figure 3. Schematic view of one sub-population of the *Pinus sylvestris* breeding programme (A) and *Picea abies* breeding programme (B). Rectangles symbolize material and hexagons activities. Matings are symbolized by double-bordered hexagons. Activities promoting early flowering, and discussed in this thesis, are symbolized by dashed hexagons.

Length of juvenile phase – phase change

The genetically programmed passage through phases, such as the juvenile and reproductive phases, is defined as ontogenetic ageing (Fortainer & Jonker 1976). In any plant a complex web of simultaneous processes, each taking place at different rates in different parts of the plant, leads to ontogenetical ageing. Studies of the ontogenetical ageing processes have stressed the need to characterise the developmental stages. Most studies have defined four stages: embryonic, juvenile, transition, and mature reproductive (Greenwood 1987 & 1995, Poethig 1990, Meier-Dinkel & Kleinschmit 1990). The term phase change is often used as synonymous to maturation or ontogenetic ageing.

The juvenile phase, in its most general definition, is the period after seed germination during which seedlings cannot be induced to flower (Zimmerman *et al.* 1985). There are large differences in the duration of the juvenile phase, with a trend from short durations for pioneer species towards longer durations for climax species (Table 1).

Table 1. Duration of the juvenile phase and age of first flowering in some forest trees (*cf.* Chalupka & Cecich 1997).

Species	Duration (years)	Age of first flowering		References
		Natural	Induced	
Pinus sylvestris	8–20 [a]	2 years		Wright et al. (1966)
			4 years	Almqvist (unpubl. data)
Pinus banksiana	3–5 [b]		12 months	Rudolph (1979)
Pinus contorta	4–8 [b]		30 months	Wheeler (1979)
Pinus taeda	5–10 [b]	4 years	2 years	Greene (1969)
Larix decidua	10–15	4 years	2 years	Wareing (1959)
				Holst (1962)
Picea abies	20–25 [b]	9 years	12 years	Chalupka (1972)
				Bonnet-Massimbert (1987)
Picea glauca	30	8 years	6 years	Young & Hanover (1976)
Pseudotsuga menziesii	12–15 [b]		4 years	Ross (1976)
Picea sitchensis	20–35 [c]			
Fagus silvatica	30–40 [c]			

[a] Sarvas (1964) [b] Anon. (1974) [c] Clark (1983)

In forest trees the juvenile phase is often expressed not only by the absence of flowering but also in traits such as specific leaf shape, high rooting capacity, and low degree of plagiotropic growth behaviour in grafts and cuttings (Hackett 1985, Greenwood 1987, Poethig 1990, Greenwood & Hutchison 1993). Also, within trees phase change gradients occur (Figure 4). In *Picea abies*, for instance, there is an increase in the rooting success of cuttings of 2.5% per branch whorl as cuttings are taken from the top towards the bottom of the crown (Roulund 1973).

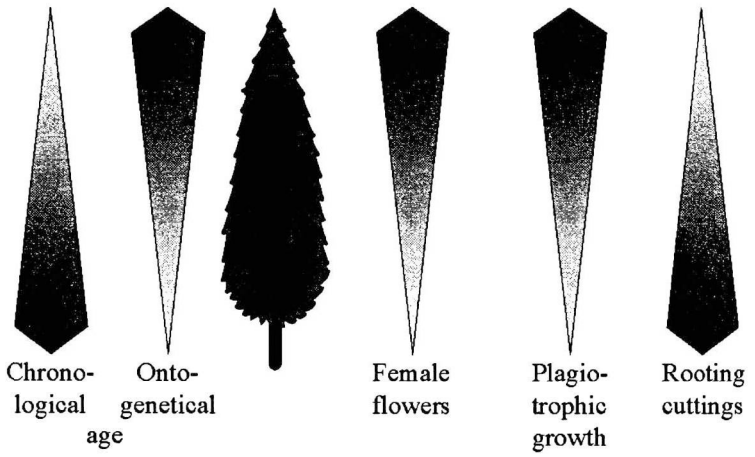

Figure 4. Ageing within a tree and its effect on female flower production, plagiotrophic growth and rooting ability of cuttings (from Almqvist & Ekberg 2000).

Flowering that occurs exceptionally earlier than normal is called precocity or precocious flowering (Chalupka & Cecich 1997). Precocious flowering can be induced naturally or artificially. A tree that flowers precociously should still be regarded as juvenile, since the most consistent criterion of a tree having changed to a mature phase is that it has not only attained but also maintained flowering competence (Hackett 1985).

The physiological and molecular basis of the ontogenetic ageing of woody plants still remains largely unknown (Meier-Dinkel & Kleinschmit 1990, Greenwood & Hutchison 1993, Greenwood 1995). Poethig (1990) hypothesized that the controls of the age-related processes are largely independent of each other. It has also been proposed, first by Robinson & Wareing (1969), that the number of cell divisions in the apical meristems controls the ontogenetical ageing of a plant. Greenwood & Hutchison (1993) concluded, based on grafting experiments, that the continuing cell divisions of apical meristems may be what drives the maturation process, although they pointed out that the physiological effects of increased size and complexity of trees are not well understood. Greenwood (1995) contended that the switch mechanism could be located inside the cells of the apical meristems, and that the expression of mature traits could be a function of the ratio of juvenile to mature cells in the apex.

Factors influencing phase change

Genetic control

Early or precocious flowering is a complex trait that is probably polygenic, even if major genes may be present. The inheritance appears to be under strong additive genetic control (Visser 1976, Schmidtling 1981, Chambers *et al.* 1997), so it should be possible to select for the trait. However, the number of studies on the genetic control of precocious flowering is limited.

Few reports have given heritability estimates of precocious flowering. In *Pinus taeda* Schmidtling (1981) reported a broad-sense heritability of 0.13 for precocious flowering compared with 0.61 for mature flowering. In the same species Byram *et al.* (1986) studied one young and three mature seed orchards and found broad-sense heritability for cone production to be lower in the young orchard (0.35) than in the old orchards (0.46 – 0.56). In Paper **III**, the broad-sense heritability for precocious flowering in *Picea abies* was found to be half of the corresponding value for more mature flowering, at around 0.2 and 0.4, respectively. However, precocious flowering in *Eucalyptus* species has been found to be highly heritable, with narrow-sense heritability in the range 0.31 – 0.59 (Chambers *et al.* 1997, Wiltshire *et al.* 1998).

Even though precocious flowering most likely is polygenic and therefore additively inherited there are reports indicating that single genes with either dominant or recessive inheritance control flowering in conifers. In *Pinus banksiana* Rudolph (1981) found indications of dominant inheritance of precocious flowering in inbred lines. Precocious ovulate production in *Pinus sylvestris* also seems to have a recessive inheritance (Heimburger & Fowler 1969). In the same species Teich & Holst (1969) found that a single major gene, either being dominant or recessive, depending on which clone was used as second parent, might control precocious flowering.

Tree size

Hackett (1985) stated that "The best strategy for obtaining rapid sexual maturation in many tree species is to grow the seedlings as rapidly as possible to a certain species- or genotype- dependent minimum size and then apply the flower-inducing treatment that is appropriate for the species".

Why the size seems to be a prerequisite for the ability to flower is unclear. One hypothesis is that the distance from the root is important due to unknown factors produced in the roots that act as inhibitors of flower initiation. In support of this hypothesis, re-rooting of shoots before the plants reach a critical size inhibits flowering and delays the decrease in rooting capacity to some extent. The use of

serial propagation (St Clair & Kleinschmit 1985, Dekker-Robertson & Kleinschmit 1991) and hedging (Bentzer 1981) in *Picea abies* cutting production are examples of the practical application of this effect.

In *Pinus banksiana* Bolstad *et al.* (1992) and Cecich *et al.* (1994) found that there was a strong positive correlation between seedling size and flowering. The time at which flowering first occurred was a function of the relative size within the family, rather than the absolute size. They contend that there is probably a threshold size for flowering within families, but the threshold size depends on the environment to which the seedling is exposed.

An adequate size before flowering is also important for the ability to produce viable seeds in trees (Chalupka & Cecich 1997).

Hormones

Conifers of the *Pinaceae* family were first successfully induced to flower by gibberellin applications in the mid-1970s, when use of the relatively non-polar gibberellins GA_4 and GA_7 (and, to some extent, GA_5 and GA_9) started (Owens & Blake 1985). Generally, $GA_{4/7}$ is more effective in promoting female flower production than male flower production (Greenwood & Hutchison 1993). The effect of GA stimulation is not persistent and must be repeated to keep the flower production at an enhanced level. Flower stimulation treatments today usually consist of a combination of hormone application (primarily $GA_{4/7}$) and other cultural treatments such as water stress and high temperature.

Growth regulators other than gibberellins (*e.g.* cytokinins, auxins, and abscisic acid) have not been shown to have a direct effect on floral initiation in conifers if applied alone (Owens & Blake 1985). However, in some cases cytokinins and auxins have an effect if applied in conjunction with $GA_{4/7}$ (Ross *et al.* 1983, cit. in Owens & Blake 1985).

The mechanisms by which the endogenously produced or exogenously applied gibberellins control flowering still remain largely unknown. Pharis *et al.* (1987) suggested in a review that gibberellins, either endogenously produced or exogenously applied, are used preferentially for vegetative growth processes, with an increase in flowering occurring only if a threshold concentration of active gibberellins is reached.

Treatments with $GA_{4/7}$ to promote flowering have not always been successful, however. Some failures to stimulate flowering in conifers by treatment with $GA_{4/7}$ could have been due to them being applied at the wrong time. The optimal timing of application depends on both the species and the growth environment, reflecting differences in ontogenetical and anatomical development. Anatomical studies of the development of vegetative apices in relation to shoot elongation, which may be highly relevant for gauging optimal times of gibberellin

application, have been done for several species (*e.g.* Owens & Molder 1984a, Hejnowicz 1987), and examples of timing studies in different conifers are listed in Table 2 (page 18–19).

If flowering is to be stimulated it is clearly important to affect the biochemical processes that precede the anatomical differentiation into reproductive or vegetative structures. This means that the $GA_{4/7}$ application should be timed so its effects are present during the period preceding differentiation.

For *Pinus sylvestris* it has been shown that applying $GA_{4/7}$ early during shoot development promotes male flowering and application at later stages of shoot development promotes female flowering (Chalupka 1984). It may be speculated that to stimulate flowering in ontogenetically relatively young material, *e.g.* seedlings that have been subjected to accelerated growth treatment, the timing of application probably needs to be close to optimal.

Even though the mechanisms of GA action still remain unknown in detail, it is quite clear that the less polar gibberellins like $GA_{4/7}$ play a direct morphogenetic role, and do not act by inducing non-specific physiological stress as suggested earlier by McMullan (1980). The activity of different GA metabolic pathways in conifers and angiosperms is affected by the growth environment and the cultural treatments applied to the plants. Moritz *et al.* (1990) found markedly higher concentrations of GA_9 and GA_9-conjugates during and after shoot elongation in *Picea sitchensis* grafts grown under conditions favourable to flower induction (hot and dry) than in grafts grown in conditions unfavourable to flower induction (cool and wet). Odén *et al.* (1995) also presented results from a *Picea abies* study showing that the relative activity of different GA pathways depends on the growth environment. In *Pinus sylvestris* Wang *et al.* (1996) found indications that exogenously applied GA is absorbed, translocated, and readily metabolised through pathways closely related to those found for endogenous GAs. They also found that GA_9 metabolism follows two different pathways: In one, GA_9 is converted to GA_4 and then to GA_1, while in the other GA_9 is converted to GA_{20} and then to GA_{29}. However, they did not investigate whether the growth environment changes the relationship between the activities of the two pathways.

Transfer of flowering-initiating genes

Three major classes of genes regulating flower initiation and morphogenesis have been identified in *Arabidopsis thaliana*, the model species mainly used in studies of these processes (*e.g.* Haughn *et al.* 1995, Weigel 1995, Levy & Dean 1998). The first class includes flowering-time genes that promote (early flowering) or repress (late flowering) phase change. The second class includes the meristem-identity genes which are early acting genes regulating flower initiation. The third class includes the homeotic genes which act later, specifying the identity of the separate organs of the flower, the so-called organ-identity genes (Parcy *et al.* 1998). Examples of the second and third classes are the intensively studied genes *LEAFY*, *APETALA1* and *APETALA2*. Genes such as these are candidates for insertion into tree species to shorten the juvenile phase and thus promote rapid generation turnover. Comparative studies of these genes should also significantly contribute to our understanding of the evolution of flower initiation and development in angiosperms and gymnosperms. There are also ongoing studies that should reveal the role of GAs in regulating the flower-initiating genes (Blázquez *et al.* 1998).

In conifer species, a number of genes homologous to the *Arabidopsis* flowering genes have been identified. In *Picea abies*, for instance, three different homeotic genes have been identified, two of which are expressed in both vegetative and reproductive shoots (Tandre *et al.* 1995). The third gene is expressed only in a specific part of the female strobilus (Tandre *et al.* 1998). Three additional organ-identity genes, homologous to homeotic genes in angiosperms, have been identified in *Picea abies* (Sundström *et al.* 1999). Observations have also shown two *APETALA2*-like genes to be differentially expressed in vegetative and reproductive organs in *Picea abies* (Vahala *et al.* 2001). In *Pinus radiata* a homologue of the *Arabidopsis* flower meristem-identity genes *FLORICAULA* and *LEAFY* has been identified and shown to be involved in the determination of male cone primordia (Mellerowicz *et al.* 1998). Similarly, Mouradov *et al.* (1999) found genes homologous to *LEAFY/FLORICAULA* in *Pinus radiata*, and homeotic genes which were expressed both in the early stages of differentiation of cone buds (female and male) and in vegetative buds.

The gene LEAFY from *Arabidopsis thaliana* has been inserted into hybrid aspen (*Populus tremula* x *tremuloides*) and transgenic plants were generated that constitutively expressed this gene (Weigel & Nilsson 1995). Precocious flowering in these plants was observed after a few months growth in tissue culture. To further promote an early phase change, reports indicate that overexpression of both appropriate flowering-time genes and meristem-identity genes should be considered, as discussed by Araki (2001). Recently, Peña *et al.* (2001) showed that constitutive expression of the *Arabidopsis LEAFY* or *APETALA1* genes in transgenic citrus plants induced flowering within a year of germination and thus could accelerate the generation turnover in this species.

Table 2. Effect of timing of $GA_{4/7}$ application on flowering in some conifer species.

Species	Application	Location	Application time	Effects on flowering	Reference
Pinus sylvestris	Spray	Poland	May 29 -Aug 17, (5 times/graft)	Reduced male, Increased female	Chalupka (1980)
Pinus sylvestris	Spray	Poland	May 21 – Jun 19, (5 times/graft) July 9 – Aug 6 (5 times/graft)	Increased male Increased female	Chalupka (1984)
Pinus sylvestris	Spray	Finland	May 24 – Jul 24, (3-6 times/graft)	Increased male & female	Luukkanen & Johnsson (1980)
Pinus sylvestris	Stem injection	Sweden	Start at 50% shoot elongation (May 26), 3 appl. at biweekly intervals. Start 6 weeks later (Jul 6), 3 appl. with one week intervals.	Early appl. increased male Late appl. increased female	Eriksson et al. (1998)
Pinus radiata	Bud appl.	New Zealand	Feb 1 – Mar 30, 1 appl./graft	Sig. increase in female Feb 17 – Mar 5.	Siregar & Sweet (1996)
	Stem inj.		Feb 1 – Mar 30, 1 appl./graft	Sig. increase in female Feb 1 – Mar 9.	
Pinus taeda	Bud appl.	USA	Biweekly appl. from Jun to Oct, and parts thereof	Best effect on female, Jun-Oct treatment. No effect on male	Greenwood (1982)
Pinus taeda	Spray	USA	Biweekly appl. 2-6 times during parts of period May to Aug	2-3 appl. during Jul to Aug gave best effect on male. No female result reported	Hare (1984)
Pinus strobus	Spray	Canada	May-Jun, 6 appl./graft Aug-Sep, 6 appl./graft	Increased male No effect	Ho & Eng (1995)

Pinus banksiana	Stem Injection & implants	Canada	Jul 5, Aug 15 Jul 5 + Aug 15	Increased male, not female No effect Increased male, not female	Fogal et al. (1996)
Picea engelmannii	Spray	Canada	During early, rapid or late shoot development and combinations thereof	No clear indications of optimal treatment time	Ross (1985)
Picea mariana	Spray	Canada	1-6 weekly applications starting 1-6 weeks after veg. bud burst	Best effect on female if starting 2 weeks after veg. bud burst & continuing for 5 weeks	Ho (1991)
Pseudotsuga menziesii	Stem injection	Canada	During early, rapid or late shoot development	Best effect on male & female during rapid shoot elongation	Ross (1983)
Tsuga heterophylla	Spray	Canada	Starting at swollen bud, veg. bud burst or early shoot development. 1–3 weekly sprayings.	Best effect on male & female from 3 applications starting at swollen bud.	Owens & Colangeli (1989)

Methods for generating transgenic conifer plants are available for *Picea abies* (Wenck *et al.* 1999, Clapham *et al.* 2000a), *Picea* spp. (Clapham *et al.* 2000b), *Pinus taeda* (Wenck *et al.* 1999), *Pinus radiata* (Walter and Grace 2000) and *Larix* spp. (Lelu and Pilate 2000). Whether these techniques can be exploited in practical tree breeding relies heavily on early testing using marker-aided selection. However, much work remains to be done for validation of marker-aided selection.

In conclusion, the main significance of studies like these for developing early flowering methods in conifers is that they will increase our basic understanding of the regulation of flowering and phase change. They may also offer additional scope for measuring the effect of different cultural treatments on maturation and phase change.

Methods to stimulate early flowering

The hypotheses on which early-flowering induction can be based are summarised in Figure 5, and can be stated as follows (Almqvist & Ekberg 2000): juvenile trees are not flowering incompetent, just very reluctant to flower; the juvenile phase can be regarded as a suppressed mature phase; the closer the plant is to a naturally occurring phase change, the weaker the suppression will be; and ontogenetic ageing takes place in the apical meristems.

Two approaches are being used to stimulate early flowering by cultural treatments: topgrafting alone or in combination with artificial flower stimulation, and accelerated growth followed by artificial flower stimulation. It is also possible to combine the two approaches, *i.e.* to subject the seedlings first to an accelerated growth treatment and then topgraft scions from the seedlings.

What is needed from a breeder's point of view is methods promoting precocious flowering and that the seed produced are of high quality. However, to meet these goals, the materials must probably be treated in a way that makes them ontogenetically older. Otherwise it will probably not be possible to induce precocious flowering in all genotypes.

Topgrafting

In topgrafting, or topworking as it's also called, new genetic material is grafted into the crown of ramets of older, reproductively mature clones. The resulting ramet consists of three parts with different genotypes, the rootstock, the interstock, and the topgraft. The hypothesis is that the reproductive competence of the interstock will be transferred to the topgraft scion. The method originates from horticulture, where it has been used in breeding fruit trees for many years (Hartman & Kestler 1968). In conifers, marked effects of rootstocks on processes such as growth and reproduction have been reported (see review by Jayawickrama *et al.* 1991). However, a specific rootstock will only cause the desired effect within a limited range of scions.

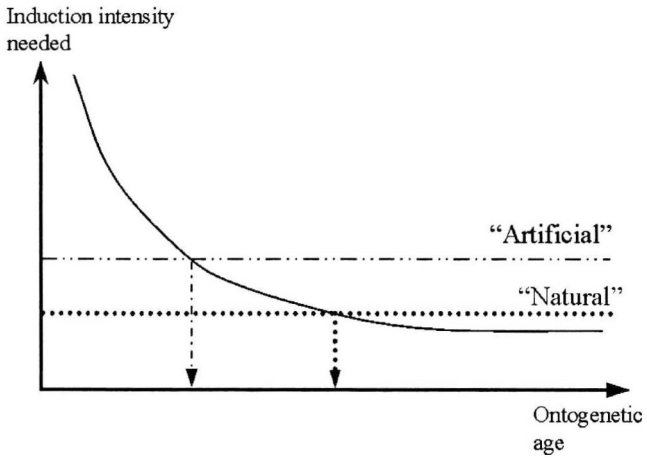

Figure 5. Schematic presentation of the relation between ontogenetic age and the flowering induction intensity needed to initiate flowering. At the point where the intensity of the induction treatment needed decreases below the "natural" induction intensity, the phase change is complete and the tree has become mature. With "artificial" flower stimulation the induction intensity can be increased, and thus the ontogenetical age at which flowers can be initiated can be decreased (from Almqvist & Ekberg 2000).

Most attempts to develop topgrafting methods for conifers have focused on *Pinus taeda*. Greenwood & Gladstone (1978) were the first to report successful topgrafting in this species, with pollen production being observed in 59% of the living grafts 3–4 years after grafting. Bramlett *et al.* (1995) also presented a topgrafting method for *Pinus taeda*, which they called surrogate pollen induction, in which pollen cones were initiated in the year of grafting. Furthermore, female flowering occurred in 21–80% of topgrafts surviving a year after grafting in the same species, using topgraft scions taken from 1–5 year old seedlings (Bramlett and Burris 1995).

In *Pinus taeda* McKeand & Raley (2000) found strong interstock effects on both topgraft survival and flowering, but no significant interaction between interstock and topgraft in these traits. Gooding *et al.* (2000) found no differences between interstocks of *Pinus elliottii* and *Pinus taeda* as regards survival and flowering of *Pinus taeda* topgrafts.

In *Pinus sylvestris* Simak (1978) tried to induce flowering in scions from 3-year old seedlings by topgrafting them into the crown of 16–25 year old trees. In the second year after grafting 1 of 73 grafts tested produced flowers.

There are no reports in the literature on the combined effect of topgrafting and $GA_{4/7}$ treatment in conifers.

Accelerated growth and flower stimulation

The aim of accelerated growth is to speed up the ontogenetic ageing of the seedlings. This is done by subjecting the seedlings to optimum growth conditions for vegetative growth, which allows the apical meristems to undergo a large number of cell divisions per unit time. The optimal conditions are achieved by extending the growth periods with long-day treatments, providing favourable supplies of nutrients and water, and optimising other parameters where possible (see *e.g.* Young & Hanover 1976, Cecich *et al.* 1994). In the following flower stimulation step, optimal treatments for inducing the initiation of flower buds are applied. These treatments are species-specific, but normally include a combination of gibberellin application, enhanced temperature and water stress.

In accelerated-optimal-growth tests for *Picea pungens,* Young & Hanover (1976) treated seedlings with 14-hour photoperiods with different light intensities. They found no effect of light intensity on free growth, which indicates that accelerated growth treatments can be performed in greenhouses with ordinary supplemental light equipment. They also tested the free growth capacity of seedlings 0–9 years old, and found that seedlings retained free growth capacity only in their first two years. This highlights the importance of utilizing the first years for accelerated growth, even though the duration of free growth capacity varies between species. Furthermore, under accelerated-optimal-growth conditions, flowering was initiated in 4-year-old seedlings of six *Picea* species and hybrids of some species (Young & Hanover 1976). Accelerated growth protocols have also been tested in *Pinus banksiana* and found to be effective in decreasing time to first flowering (Rudolph 1979, Bolstad *et al.* 1992, Cecich *et al.* 1994).

In *Pinus contorta* the effects of accelerated growth on flowering were studied by Wheeler *et al.* (1982). In the 5th year from seed both the proportion of female flowering and the number of female flowers per flowering tree were about six times higher for seedlings grown under accelerated growth conditions than for controls (81% and 18 flowers per tree versus 12% and four flowers per tree, respectively).

In *Picea abies* Kang at al. (1994) cultivated full-sib families under different photoperiodic, light-intensity and temperature treatments during the first growth period. Responses in juvenile growth traits were observed during the following two growth periods. The only significant factor found to affect growth performance in such a way as to promote more mature behaviour was the length of time the seedlings were subjected to continuous light. These results are in accordance with the hypothesis that the activity of the apical meristems controls the maturation process.

Relation between flowering and other traits

Reproductive organs are known to be strong competitors for nutrient resources within the plant (Linder & Troeng 1981). Seed cones in *Picea abies* develop at the same time that the following year's buds are being formed. One may therefore assume that abundant flowering and heavy cone-set in any year will have an effect on the height growth the following year, or even for several years.

In breeding programmes with species like *Picea abies* that have a very long juvenile phase, the breeder may be forced to select parents for the next generation only from among clones that start to flower relatively early, ignoring others that will start flowering later, in order to avoid excessive delays. However, if there is a negative genetic correlation between early flowering and height growth, the genetic gain in height growth will be reduced due to the selection of early flowering trees.

In the literature, both positive and negative genetic relationships between flowering and growth traits have been reported (*e.g.* Schmidtling 1981, Nienstaedt 1985, Byram *et al.* 1986 & El-Kassaby and Barclay 1992).

The rooting ability of cuttings of *Picea abies* has been found to decrease gradually as the mother-tree becomes older and to drop sharply after 10 years of age (Roulund 1975). The decline in rooting ability is accompanied by an increase in plagiotropic growth of the cuttings (Dormling *et al.* 1976, Roulund 1979, Dekker-Robertson & Kleinschmit 1991).

Picea abies has a long juvenile phase and generally starts to flower after 15–20 years under good conditions (Wareing 1959), but it can take as long as 30–40 years before cone-set reaches appreciable levels (Wright 1964). This is in contrast to other conifer species, in which abundant cone-set appears faster, *e.g.* *Picea mariana* (Tousignant *et al.* 1995), *Picea glauca* (Wright 1964) and *Pinus radiata* (Sweet 1973).

Both the decrease in the rooting ability of cuttings and the increase in flowering are results of the complex web of processes that occur within the plant during maturation. From a breeder's point of view, it is important to clarify whether these two "maturation traits" are genetically linked to each other. If so, there is a risk that selection for either one of the traits will lead to an undesired response in the other trait.

Objectives

The purpose of the work described in this thesis was to gain more knowledge concerning the following questions related to flowering competence and capacity, and to assess their implications for Swedish *Pinus sylvestris* and *Picea abies* breeding programmes:

- How can early flowering be stimulated to shorten the length of breeding cycles? (Papers **I** and **II)**
- Does selection for early flowering influence height growth? (Paper **III**)
- In clonal forestry, does selection for high rooting capacity reduce flowering competence? (Paper **IV**)

Paper **I** presents a study on interstock and $GA_{4/7}$ effects on flowering after topgrafting in *Pinus sylvestris*. The study forms part of our ongoing work to develop improved topgrafting methods for initiating early flowering in *Pinus sylvestris*.

Paper **II** describes a study in which potted grafts of *Pinus sylvestris* were transferred into the greenhouse and given applications of $GA_{4/7}$ at different points during shoot and needle development. The purpose was to access the impact of application time on flowering and to find useful indicators for determining the most effective time of treatment. The results are being used in our ongoing work to develop methods for early flowering in *Pinus sylvestris* via accelerated growth and flower stimulation.

Paper **III** reports the genotypic correlations found between cone-set and height growth characters in young clonal field tests of *Picea abies* in several localities, and to evaluate the impact these correlations may have on the breeding programme.

In Paper **IV**, the rooting success and cutting performance of *Picea abies* cuttings taken from clones during their transition to the flowering competent phase were studied. The aim was to determine how cutting and flowering characteristics are correlated with each other.

Materials and methods

The experiments described in Papers **I** and **II** were performed at Brunsberg field station, SkogForsk (lat. 59° 37' N, long. 12°58' E, alt. 80 m.a.s.l.), (Figure 6).

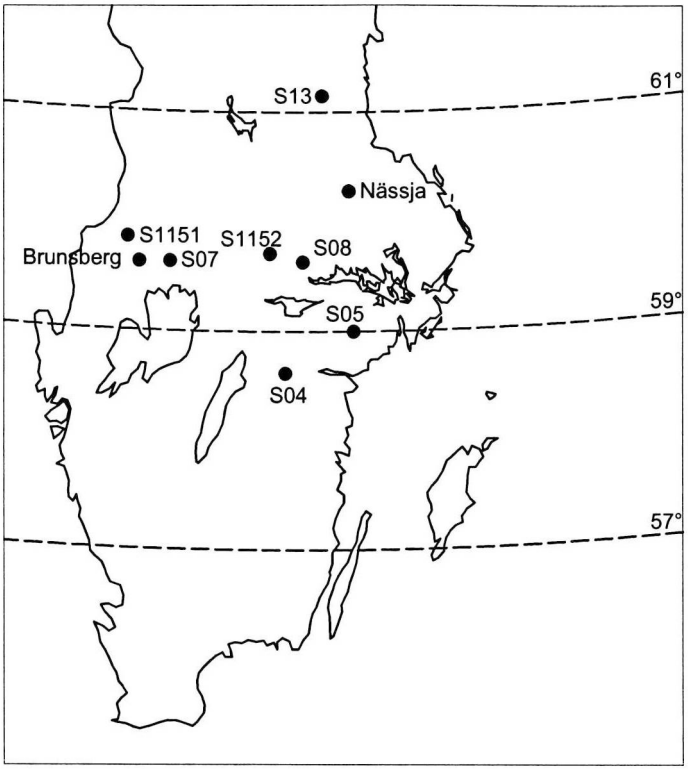

Figure 6. Map showing the location of Brunsberg field station (Papers **I**, **II** & **IV**), Nässja demonstration trial (Paper **IV**), and the seven clonal trials (Paper **III**) (S04-S13, S1151 & S1152).

In the study described in Paper **I**, field grown grafts of six clones from old plus-trees were used as interstocks, and seven clones selected from a 19-year old progeny test were used as topgrafts. Two grafts of each topgraft clone were topgrafted into the crown of each of two different ramets of the six interstock clones. As a comparison, each topgraft clone was also grafted onto four 2-year old rootstocks. One year after grafting, half of the interstocks were treated with $GA_{4/7}$ to stimulate female flowering. This treatment was repeated the second year after grafting. Survival of the topgrafts was recorded throughout the experiment. Male and female flowers were counted in spring one to three years after grafting. The growth of the topgrafts was measured in spring three years after grafting.

In Paper **II**, experiments on potted grafts of ten clones from old plus-trees are described. During attempts to stimulate flowering they were placed in a greenhouse with a 25°C/20°C (day/night) temperature regime. Four different times of $GA_{4/7}$ application during shoot and needle elongation were tested, and there was an untreated control. Each graft was given one 50 mg $GA_{4/7}$ application as a stem injection. The final lengths of the shoots and needles in the year before treatment, their lengths at the time of treatment, and their final lengths in the year of treatment were recorded. The number of female flowers was counted and male flowering was scored on each graft in spring the year after treatment. Temperatures both outdoors and indoors were monitored, and three different heat sum accumulation models were used to calculate accumulated heat sums up to the dates of treatment. The experiment was performed in two consecutive years.

Variables that did not meet the requirements for normal distribution were transformed into normal scores (Gianola & Norton 1981) in both Papers **I** and **II**. Analysis of variance was performed using the Mixed SAS procedure in Paper **I** and the GLM procedure in Paper **II** (SAS 1999).

Data presented in Paper **III** were collected from two series of clonally replicated tests of *Picea abies*. Series 1 consists of five field trials and Series 2 of two field trials (Figure 6).

In Series 1 cone-set occurred for the first time in 1995, 14 years after planting, and measurements were taken in autumn, after 14 and 16 growing seasons in the field. In Series 2 the first cone-set also occurred in 1995, six years after planting, and measurements were taken in autumn six and nine years after planting. The total height and height increment variables were measured, growth disturbances were assessed, and cone-set was scored in three classes.

The cone-set variables were transformed into normal scores to meet the requirements for normal distributions (Gianola & Norton 1981). Single-trait mixed linear models were used to estimate genetic parameters and genetic correlations within trials. The analysis was performed using the LSMLMW and MIXMDL program package (Harvey 1990). Two-trait mixed linear models were used to estimate genetic correlations between height, height increment and cone-set between trials. These analyses were performed using ASREML software (Gilmore *et al.* 1999).

In Paper **IV**, materials from a demonstration trial with *Picea abies* cuttings (established with 3-year old cuttings taken from 4-year old seedlings) located on former farmland in Nässja were evaluated (Figure 6). In 1995, eight years after planting in the field, the first flowering in the trial was recorded, and 58% of the clones bore cones. From 15 clones without flowers and 15 heavily flowering clones, dormant twigs from upper and lower parts of the crown were collected. The 30 clones were all in intermediate classes with respect to time of bud break.

The twigs were inserted in rooting media in a greenhouse at Brunsberg field station shortly after twig collection. Rooting success was recorded when the rooting process was complete, and after one growing season outdoors in the nursery leader length was measured, and the degree of plagiotropic growth behaviour was assessed. Analysis of variance was performed using Proc mixed (SAS 1999).

Results and discussion

In the topgrafting experiments discussed in Paper **I** the interstock had a substantial effect on the survival of the topgrafts, which ranged between 54% and 89% in the spring of year 3. These results are in accordance with findings in *Pinus taeda* (McKeand & Raley 2000).

Even though all topgraft clones produced some female flowers as early as year 2, there was on average a 5-fold increase in female flower abundance between years 2 and 3, from 0.8 to 4.2 female flowers per topgraft.

There was a statistically significant effect of interstock for both female and male flowering. Unlike findings in *Pinus taeda* (McKeand & Raley 2000) there was a significant interaction for female flowering between interstock and topgraft (see Table 3 in Paper **I**). There were also significant 3-way interactions between interstock, topgraft and $GA_{4/7}$ treatment for both female and male flowering.

The $GA_{4/7}$ treatment of the interstocks resulted in a 55% increase in female flowering in year 3, but had no significant effect on female flowering in year 2 and no effect at all on male flowering. However, the treatment was timed to stimulate female flowering, so no effect on male flowering was expected. The positive effect of injecting $GA_{4/7}$ into the trunk of the interstock on topgraft flowering supports the idea that the hormonal balance of the interstock influences the topgraft.

As in *Pinus taeda* (McKeand & Raley 2000), there was no relationship between the flowering of the interstock itself and its capacity to induce flowering on the topgrafts. Therefore screening, using a set of topgrafts, is needed to identify good interstock clones.

In Paper **II**, the effectiveness of monitoring the phenological traits shoot and needle elongation was evaluated as an indirect way of gauging the morphological development of next year's buds, and thus deciding when to apply $GA_{4/7}$. The results of the timing experiments showed that there were such large differences in absolute values of final shoot and needle length between the two consecutive years that the lengths attained in the previous year cannot serve as indicators of the current year's final shoot or needle length. The correlation between the shoot

and needle lengths in the two consecutive years was also quite low. Thus, there is no reliable way to predict the final length of the shoots or needles, and no way to estimate how large the shoot or needle length will be at the optimal time of treatment.

Accumulated heat sums could potentially be used to decide when $GA_{4/7}$ should be applied. Since all three tested heat sum variables gave equally accurate predictions, the simplest and most robust should be recommended for practical use, *i.e.* summed degree-day values (Sarvas 1967).

The lower limit of the timing window (Siregar & Sweet 1996), defined as the period in which treatment results in significantly ($p<0.001$) higher flowering than in the control, was found to be about 500 degree-days (threshold +5°C). However, the duration of the timing window remains unknown since the upper limit was not detected (the last $GA_{4/7}$ application time used in the study resulted in the largest effect on female flowering, see Figures 1 and 2 in Paper **II**). Based on the study in Paper **II** the best time for $GA_{4/7}$ application in the greenhouse is at about 700 degree-days.

In an ongoing experiment on the accelerated growth of *Pinus sylvestris*, seedlings first subjected to three growing seasons of accelerated growth conditions were stimulated to flower according to the findings in Paper **II**. Flower stimulating treatment, designed to induce female flowering, was performed in the greenhouse during the 4^{th} growing season. In the beginning of the 5^{th} growing season, four years after sowing, 4.2% of the seedlings treated with $GA_{4/7}$ had female flowers. In the control seedlings, subjected to normal nursery growth conditions, $GA_{4/7}$ treatment in the greenhouse did not induce any female flowering.

In a pilot study of *Pinus sylvestris* combining accelerated growth treatments and flower stimulation by $GA_{4/7}$, 41.5% of the plants that were subjected to accelerated growth conditions for three growing seasons and then planted out on former farmland had female flowers in spring 2001, seven years after sowing. None of the control plants had female flowers, although half of them were treated with $GA_{4/7}$.

Both Papers **I** and **II** focus on ways to promote flowering. In attempts to develop methods for shortening the juvenile phase and thus induce flowers, this is the first but most difficult step. Next, the quality and characteristics of the seed produced by plant material subjected to accelerated growth and flower stimulating treatments, and by topgrafts, must be evaluated. The quality of seeds produced in field-grown seed orchards with and without $GA_{4/7}$ treatment has been investigated by Eriksson *et al.* (1998). The only significant difference observed was that the $GA_{4/7}$ treatment was associated with a decrease in seed weight. In *Picea glauca* Beaulieu *et al.* (1998) found no effect on seed traits or any increase in segregation distortion in allozyme loci following flower induction treatment.

The risk that specific factors in the maternal environment may have significant after-effects on seedling behaviour requires thorough evaluation. In *Picea abies*, for instance, effects of the reproductive environment on the phenology and frost tolerance of the next generation have been reported (see review by Skrøppa & Johnsen 1999). After-effects caused by latitudinal transfer of clones have also been observed in *Pinus sylvestris* (Andersson 1994).

In most trials of both clonal series described in Paper **III** the genotypic correlations between cone-set and height increment variables were weak and mostly non-significant, both before and after the studied cone-set year (see Figures 1 and 2 in Paper **III**). The genotypic correlations between cone-set and height were also weak and non-significant in five out of seven trials in both series. These results are consistent with reports concerning genetic correlations between fecundity and height traits in other conifers (Zhou *et al.* 1999, Hannerz *et al.* 2001).

Estimated broad-sense heritability of the cone-set trait was on average about 0.4 in Series 1, which is in accordance with heritability estimates for flowering and cone-set traits in other conifer species (Schmidtling 1981, Nienstedt 1985, Byram *et al.* 1986, Matziris 1997, Han *et al.* 1999, Hannerz *et al.* 2001). This implies that the fecundity of *Picea abies* is under strong genetic control. The broad-sense heritability for height in Series 1 was only half to a third of the cone-set heritability, although within the normal range of heritability for height obtained in clonal field tests.

In the young material in Series 2, the proportion of clones showing cone-set was much lower than in Series 1. Thus, the lower estimate of broad-sense heritability (about 0.2) for what must be considered precocious cone-set was consistent with expectations, due to the low cone-set. It is also in accordance with the few reports on the heritability of precocious flowering in conifers that have been published previously (Schmidtling 1981, Byram *et al.* 1986).

The genotypic correlations between cone-set in different trials were all strong and significant, and within the range of 0.7 – 1.0 in both series. This implies that the G×E interactions in flower initiation were weak, at least within the geographic range of our trials in the particular year studied.

In Paper **IV,** there were no statistically significant differences between clones with and without cone-set in rooting success, frequency of leaders from the apical bud, leader length, or degree of plagiotropic growth. The lack of correlation between the treatments and these phenological characters is in accordance with data from studies of *Picea mariana* (Tousignant *et al.* 1995). The results suggest that the ability to flower and rooting capacity are independent, age-related processes. Our results are also consistent with the hypothesis by Borchert (1976) that the onset of flowering is not correlated with vegetative maturation traits such

as rooting ability. In a review Meier-Dinkel & Kleinschmit (1990) concluded that maturation might proceed more rapidly for vegetative traits than for flowering competence.

Of the variables evaluated in Paper **IV**, the crown position of the twigs had the greatest impact on the rooting results. Twigs from the lower crown had 4–5 times higher rooting success than twigs from the upper part (Table 1 in Paper **IV**). This indicates that the lower branches are ontogenetically younger than branches higher up in the crown (Hackett 1985), and it also supports earlier results in *Picea abies* by Dormling *et al.* (1976) and Roulund (1979).

Conclusions and implications for tree breeding

How can early flowering be stimulated?

According to our hypothesis, both topgrafting and accelerated growth have the potential to effectively decrease the time to flowering and thus the generation time in Swedish conifer breeding programmes.

The topgrafting method (Paper **I**) can reduce the time from grafting to flowering compared to grafting on young rootstocks. For clones selected from plantations or progeny tests it is often necessary to bring them into and maintain them in a breeding archive before they can be included in crossing programmes. Topgrafting of these selections could reduce the time to flowering. In both *Pinus sylvestris* and the *Picea abies* breeding programmes (Figure 3) there is a risk that at least some of the clones selected for the next generation of the breeding population have not attained flowering competence at the time of selection. Topgrafting of these clones could be an effective way to force them to flower, and thus enable their inclusion in double-pair matings. For *Picea abies*, however, the topgrafting method has yet not been tested.

Distributing the scions from one topgraft clone into the crown of two to three interstock clones is one way to avoid possible adverse effects from the interaction observed between topgraft and interstock (Paper **I**). This increases the probability of getting at least one good combination of topgraft×interstock.

The findings in Paper **II** show that one properly timed $GA_{4/7}$ application is enough to stimulate female flower production in *Pinus sylvestris*. Normally the $GA_{4/7}$ application is divided between two to three occasions to ensure that at least some is applied during the sensitive period for female flower induction. A reduction to one occasion would significantly reduce the cost of flower stimulation. Better timing of the application would also probably result in increased flowering, and thus reduce the time it takes to complete a crossing programme.

Precisely defining the timing window for $GA_{4/7}$ treatment should improve the results of flower stimulation in ontogenetically young materials and thus reduce the time required for producing the polycross families needed to establish progeny tests in the breeding programme (see Figure 3).

The financial benefits of stimulating flower production in operational seed orchards of *Pinus sylvestris* are very sensitive to labour costs. With a reliable method to decide when the timing window for $GA_{4/7}$ application is "open", it should be possible to reduce the number of applications to one. Two-year results are available from an ongoing experiment studying the effects and costs of one (150 mg/graft) versus two (2×75 mg/graft) applications of $GA_{4/7}$ for stimulating female flower production in a field-grown operational seed orchard. The data indicate that with proper timing as many cones are produced with one application as with two applications, but at only half the labour costs.

Does selection for early flowering influence height growth?

In Paper **III**, only weak and negligible genotypic correlations between cone-set and both height and height increment were found, both before and after the cone-set year. In this respect there were no differences between the very young, precociously flowering material and the older material, which could be considered close to phase change. The breeding implications of this are that selection for height or height increment will not influence the cone-set capacity of the selected population either positively or negatively. Furthermore, the ranking of clones for height growth will not differ in a clonal test whether the clones have started to flower or not at the time of assessment. Thus, if the breeder has to restrict selections based on height to clones that have started to flower, in order to avoid excessive delays, the genetic gain in height will of course decrease, but only because of the reduction in selection intensity.

In cases where clones are being selected for inclusion in a seed orchard, the selection of early flowering clones will not have any effect on the height growth traits of the seed orchard crop.

Does selection for high rooting capacity reduce flowering competence?

Rooting performance is a trait that has great economic and practical impact on breeding and mass propagation in *Picea abies*. In clonal forestry programmes high rooting capacity is an important trait to select for (Högberg *et al.* 1995). In the breeding programme for *Picea abies* clonal tests are utilized for testing the performance of the new breeding generation (Karlsson & Rosvall 1993). The findings in Paper **IV** imply that rooting capacity and flowering ability are

independent processes within the tree, although both are age-related. This has important implications for mass propagation and breeding, since selection of clones with high rooting capacity in clonal forestry programmes will not affect the flowering competence of the selected clones.

Clonal seed orchards have normally been established with grafts. When the clones to be included originate from old plus-trees no real alternative is available. As the *Picea abies* breeding programme progresses, the clones from more advanced generations will be young enough at the time of selection for inclusion in seed orchards to retain some rooting capacity as cuttings. Since cuttings are much cheaper to produce than grafts, they could be used in seed orchard establishment. Among the candidate clones to be included in the orchard, those with the highest rooting capacity could be selected without risk of prolonging the time until flowering in the orchard or reducing flowering capacity. Clones for a seed orchard could also be selected for early flowering to decrease the time to seed production. In that case the expected rooting capacity of the selected clones would be equal to the average figure for the whole material.

In the breeding programme, rooting capacity could be included as one of the traits being selected for in the nursery screening stage (see Figure 3), without the risk of undesired correlated responses in flowering ability.

Further research

In the *Pinus sylvestris* breeding programme, topgrafting is one option for inducing early flowering and thus reducing the time it takes to complete the polycross matings needed for the progeny tests (see Figure 3A). Therefore, the effect of topgrafting ontogenetically young materials needs to be studied, considering both the grafting technique and the age of the topgrafting material. Effects of seedling treatment prior to topgrafting on topgraft survival and flowering are also of great interest.

The observed interaction between interstock and topgraft clone needs to be confirmed. Similarly, the variation in flower inducing capacity within interstock clones (*i.e.* between ramets of the same clone) also needs to be investigated.

The topgrafting method has been suggested as a way to replace existing tree crowns with new clonal selections in seed orchards (Bramlett 1997). Whether this is feasible from both practical and economic standpoints needs to be verified.

The topgrafting method needs to be tested on *Picea abies*. In the *Picea abies* breeding programme topgrafting could be used to induce flowering in clones that start flowering late, so they can be included in the double-pair matings (see Figure 3B).

The timing window for $GA_{4/7}$ application in *Pinus sylvestris* must be better defined for female flowering. To increase its effectiveness in stimulating ontogenetically young materials to flower we need to know whether there is any age-dependency in the limits and duration of the timing window for $GA_{4/7}$ application. Stimulation of male flowering with respect to both time of application and number of $GA_{4/7}$ applications must also be further studied.

There is a need for studies to confirm that the low genotypic correlations observed between fecundity and height growth traits are also the general pattern in *Pinus sylvestris*. These studies should examine both precociously flowering materials and materials that mainly flower when mature.

A sub-set of the clones included in the clonal tests of Series 1 (Paper **III**) have been selected and included in a potted indoor seed orchard. When cone production data for the clones in this seed orchard become available it will be possible to study the relationship between flowering events in clonal field trials at a relatively young age and flowering characteristics in the seed orchard.

The lack of correlation between rooting capacity and flowering ability noted in Paper **IV** is encouraging. Since both rooting capacity and flowering competence are fundamental traits with large impacts on the utilization of the breeding material, the lack of correlation between them needs to be verified by new studies in *Picea abies*.

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