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## *Picea abies* breeding in Sweden is based on clone testing

**Abstract:** In the last decades, clone testing has become an important component of the long-term breeding and seed orchards for Norway spruce in Sweden. For more than three decades, considerable resources have been spent on testing clones intended for clonal forestry, but the Swedish forestry never saw it worth to pay the added cost involved in the added gain. The efforts, however, resulted in many clone trials and developments in the technique for clone production and propagation. Theoretically, clone testing is faster and cheaper than progeny testing and more reliable than selecting individuals forwards. Nowadays, the main line in long-term breeding is to make crosses between the best trees and test-cloned full-sibs as a recruitment population for long-term breeding and seed orchards. Since controlled crosses are a bottleneck for long-term breeding, a possibility is to rely on wind pollination (Breeding Without Breeding; BWB) in trials for testing clones. The seed parent is known, and that the pollen parent is a desirable genotype can be checked by molecular markers. BWB has the potential to eliminate the waiting time between selection and recombination, which is particularly important in a late and irregularly flowering species such as Norway spruce. Clone testing ensures that the breeding values are known from the same tests as those used for BWB. Another option for BWB is to place in seed orchards a few ramets of clones belonging to the breeding population, but normally not deserving such a use, with the hope that their presence will make it possible to rely on wind pollination to recombine the whole breeding population.

**Additional key words:** Norway spruce, rooted cuttings, seed orchards, long-term breeding, Breeding Without Breeding

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### Introduction

Norway spruce is the economically most important species in Sweden. Nowadays, around half of the planted Norway spruce plants originate from seed orchards. Considerable resources have been spent on unsuccessful efforts to get clonal forestry implemented; currently, only one per mille of the spruce plants produced in Sweden are clones (rooted cuttings). The company Hilleshög AB initiated a clone-test programme in 1975 based on selections in nurseries from suitable provenances, and heading for plantation of rooted cuttings of tested clones in forestry. The gain was initially not spectacularly high

and did not justify the high additional production cost, so the programme was closed before superior tested clones were marketed to any extent. The Norway spruce clonal forestry in Sweden was last reviewed by Sonesson et al. (2001). However, the large programmes performed with clone-testing and the development of technology for rooted cuttings have been a real blessing for long-term breeding and the seed orchard programme. There are many tested clones in trials available, and the best clones are used in long-term breeding and to some extent also in seed orchards. The technology to establish trials and cutting propagation for trials and juvenility archives is well developed.

## Cloning full-sibs can be a superior tree-improvement technique

Clonal testing often appears as a superior testing method for selection in tree breeding and deployment to seed orchards. This idea was first mentioned and developed by Libby (1964), and the currently last review and development was presented by Shelbourne et al. (2007). The idea that it might be attractive to use cloned full-sib families as a base for deployment to Norway spruce seed orchards was first formulated by Lindgren and Werner (1986, 1989), and was subsequently translated into operational long-term breeding (Karlsson and Rosvall 1995). Since Norway spruce has a late and irregular flowering, the situation 15 years ago was that there were plenty of clone tests available to the breeders, while the progenies were delayed, which stimulated interest in clone tests. Some of the seed orchards established in Sweden today use rooted cuttings of tested clones, but even if clones are selected on the basis of rooted cuttings, grafts of these are often chosen for seed orchard deployment. The superiority of testing cloned full-sibs has been confirmed and optimised in calculations in later studies (Rosvall et al. 1998; Danusevičius and Lindgren 2002). Broad-sense heritability matters; high heritability motivated many individuals and few ramets. In the practically unimportant case when heritability is close to 1, it does not pay to make copies of the offspring. Clonal testing was found equally superior if the budget was half as high or double as high as the current Swedish breeding budget.

The major advantage of selecting the best tested clones over selecting the best individuals is that a clone can be tested with replications and in different localities, thus the error caused by environmental variation can be estimated and much reduced. In addition, since shifting the environment is the major cause of variation, it is very advantageous to get that source of variation reduced. While propagating the clones in a nursery, early observations can be made and the least promising clones can be culled without field testing. Destructive tests or tests that harm or disturb the plants can be made. Vegetative propagation may in itself cause additional variation, but even genetically identical seedlings may vary. Another potential advantage is that while testing a clone in the field, copies can be kept in archive for crosses. Compared to progeny testing and going back to the good parents to generate new candidates for selection, clone testing saves much time and is much cheaper per tested genotype, which more than compensates for some lower precision. One can say that the view on clone testing has reversed. Some decades ago, the aim was to deploy tested clones in the forest, thus clones should constitute the deployment popu-

lation. Nowadays, clone testing is a tool to get better sexual mass propagation in seed orchards.

## Clone test is the main track in the current Swedish breeding of Norway spruce

The current praxis is that the Swedish breeding population of Norway spruce is structured in 22 subpopulations, each with 50 or more tested founders so mated that each parent gets offspring in two families (Karlsson and Rosvall 1995). Between 20 and 40 individuals in each family are vegetatively propagated in 10–25 copies and field tested. Although the cloned full-sib programme is implemented, from a quantitative point of view it has not advanced that far. Less than a tenth of the planned clones from controlled crosses among tested plus-trees or plus-tree clones were in field trials 2005, but thanks to good spruce flowering in the last years the programme is now advancing on a wide front. A design which seems reasonably near to a supposed optimal based on the methods developed by Danusevičius and Lindgren (2002; Table 2) seems to be to test 20 clones from a family, each with 20 ramets, and to make selections 20 years after the establishment of field trials (a “20-20-20” strategy). Current ideas in Swedish spruce breeding seem to be to use more clones, shorter selection age, and maybe fewer ramets, but actual decisions have not been made for the main part of the breeding programme. Selection may mean that scions are taken and top-grafted in a clone archive for serving as females or to harvest pollen for use as males. An alternative is to preserve cuttings in archives at breeding stations while testing, and to make crosses at the station, but this requires much space and effort.

The experiences with cloned sibs of Norway spruce in Sweden have been so successful and the simulation so encouraging that development has been initiated for other species, too. Cloning full-sibs for Scots pine was suggested by Lindgren (1985) as a means of identifying genotypes with a high survival capacity since the survival of genotypes can be tested with clones, but not individuals, as an individual either survives or not. Andersson (2002) has developed a share of the Scots pine breeding stock with cloned full-sibs, and even for lodgepole pine and silver birch the development is or has been going on.

To perform controlled crossing to get families for a new breeding generation is a bottleneck in Norway spruce breeding. Flowering happens seldom and irregularly and takes typically more than a decade, and it may take two decades from grafting to crosses (the scenarios by Danusevičius and Lindgren (2002), mentioned above, are probably optimistic). It may thus take a long time to cycle generations after the se-

lections are done. Controlled crosses are administratively and logistically demanding. The crosses must be produced within a short time frame, the exact timing is difficult to predict and tends to happen at holidays. Insect damage is a major threat to Norway spruce seeds. The success depends on weather, luck, functioning equipment, staff availability at short notice, and the occurrence of different types of physical and biological injuries. In the following paragraph, a method is suggested which may be the remedy for most of these problems.

### Breeding Without Breeding

Nowadays, the new molecular tools of ancestry identification open an option which avoids controlled crosses and clone archives. These are based on widening the concept “Breeding Without Breeding” (BWB) as coined by Yousry El-Kassaby (El-Kassaby et al. 2007). Nature makes the crossings (we call it open pollination or wind pollination) with no cost or trouble, but the pollen parents are unknown. Phenotypic selection without pedigree control is in itself a very useful tree-improvement method in many situations (Lindgren and Wei 2007), but its combination with co-ancestry control is a powerful combination (El-Kassaby and Lindgren 2007). The applications suggested here are suitable for Norway spruce. Flowering is highly irregular so the advantage from making measurements first when a good seed crop is actually obtained is substantial. Norway spruce has so late flowering that the trees are too tall to make controlled crosses in field trials an option, but collecting a few cones is possible. Testing for Norway spruce is done by clones, thus much fewer genotypes are handled than with seedlings, which more than ten-fold reduces the need for marker analyses and the number of desirable pollen parents to search for and see difference among. When fathers can be identified, they are tested clones. BWB can be done in tests with the design headed for in the Swedish Norway spruce breeding today, thus if BWB does not work satisfactorily, that does not change the possibility to use the trials for the currently intended purposes.

The starting point for applying the suggested variant of BWB is a clone test. The test is continued to see if it has reached the stage when it can produce pollen (the first cone crop may occur before the first pollen crop, and when the idea with BWB is abandoned). If it is foreseen that the relevant field trial will produce good seeds and that a reasonable part of this is pollinated from the trial, the field trial (and replications in other locations) is measured in the late summer – early autumn. The waiting time between the measurements, on which selections will be based, and the start of the new generation is completely eliminated. It is actually better than that: because pollination takes place in the early summer but measurements

are made in the early autumn, the matings actually occur before the measurements! Cones are collected from the top-ranking clones when measurements have been compiled to identify such clones. The elite clones can also be harvested for scions to be used in seed orchards, thus BWB is compatible with the selection of superior clones to seed orchards. Seeds should be collected from the top-ranking mothers only. A percentage of good clones in the trial (say, the top-ranking 30%) are genotyped. Seedlings are planted and DNA from the seedlings is extracted and used for identifying the father. The genotypes where the pollen parents belong to the reasonably high ranking clones are subject to group merit selection (Lindgren and Mullin 1997). New clones are produced by rooted cuttings from the selected genotypes, constituting the recruitment population for the next cycle of testing and selections. Thus, the breeding cycle is completed without controlled crosses or archives of selected clones. Disadvantages to this BWB application, compared to controlled crosses, are that the pollen parents are likely to be selected with a rather low intensity and that the identification of pollen parents may be expensive and technically demanding.

### A design with wind pollination in a seed orchard

Another BWB design is to delay collecting open-pollinated seeds till they can be collected in a grafted seed orchard with selections based on the clone tests. Besides the top 20–25 clones which go into a normal seed orchard with tested clones (Lindgren and Prescher 2005), additional 50 clones from among the high-ranking ones and after some consideration about pedigrees are planted in the seed orchard, but only with two ramets each. This is because a long-term breeding population should be larger than a seed orchard population, but these selections to the breeding population can also be placed in seed orchards to get an adequate pollen cloud for BWB. As the extra clones account for only a few percent of the seed orchard ramets, and are good even if not the best, the influence on gain is minimal, and the compensation is that they have a small increasing effect on the gene diversity of the seed orchard crop. One has to wait till the seed orchard produces sufficient male flowering. In a year with good seed set, open-pollinated progenies are collected from the extra clones (the fathers will be among the ordinary seed orchard clones). Seedlings are produced, the father is identified by markers, and a group merit selection is performed for the next recruitment population. The selected genotypes are cloned, and a new cycle of clone trials is established. The advantage of this design is that the fathers are considerably more stringently selected, but the disadvantage is that there is

a long unproductive wait for sufficient male flowering in a seed orchard.

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