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A way to utilise the advantages of clonal forestry for Norway spruce?

Dag Lindgren

Swedish University of Agricultural Sciences, Department of Forest Genetics and Plant Physiology, Umeå, Sweden; dag.lindgren@genfys.slu.se

It is suggested that vegetative propagation of offspring from the genotypes with the highest breeding values is an efficient way of harvesting genetic gain. This may be at least 20% superior to stand seeds (only half of Swedish spruce plantings are with seed orchard seeds). Controlled crosses may have a similar superiority even to a recently established elite seed orchard over the life time of the seed orchard, as the latest breeding material can be used for controlled crosses. The additional gain for forestry with tested clones seems to be less than 10% compared to controlled crosses, but this option is not technical available today, as rooted cuttings require juvenile stock plants and the SE technique is not yet mature.

Keywords: rooted cuttings, somatic embryogenesis, full sib crosses, controlled crosses, family forestry

Repeated efforts in Sweden and elsewhere to use tested clones of Norway spruce in commercial clonal forestry have been going on for forty years, but have failed, and new efforts are commercially unsafe. Mono-clonal forestry of e.g. Norway spruce is not well-tested enough to head for a major fraction of the plant market in the near future; it seems expensive; it has been frustratingly difficult to maintain clones juvenile during tests and propagation, much technical and biological development seem needed and there are legal; administrative and social problems to get monoclonal forestry accepted at a major scale, thus commercialisation should head only for a minor segment of the market. It is a much more cost-efficient and safer way to get a better forests over the whole cultivated area to see to that the seed orchard program is optimised and seed orchards are efficient than to head for vegetative propagation. But once this is done or on the way, some foresters want still more. The seed orchard situation in Sweden is rather good, it could be better, but it is developing well enough to consider methods to get still higher gain a limited part of the attention.

Vegetative propagation of seeds from controlled crosses with selected tested parents is a way to get most of the potential advantages of clonal forestry which has been advocated for long (e.g. Lindgren 1977). It is possible to get it implemented with fine-tuning of available techniques, without discounting major new break-throws. Implementation may be justified for a limited fraction of the forest if there is a will to make a large but justified investment and take some extra trouble and risk for a substantial gain. By choosing the very best parents with the top breeding-values for the desirable characters, a considerable higher gain than with seed orchards is possible, and by using recent measurement results for the parent selection a long delay in a seed orchard to utilize the gain is avoided. By controlled crosses pollen contamination and inbreeding, which reduces the seed orchard gain, can be eliminated. Controlled crosses can be custom tailored and head for more specific targets nearer in time than seed orchards. Most of the gain is in the controlled cross. Clones originating from controlled crosses are tested and a gain can be done above that of the original cross, but that is reflected as better parents to cross and not a real advantage with tested clones. The use of rooted cuttings is facilitated if the first cycle of stock plants can be done by Somatic Embryogenesis. Cryo-storage of the seed genotypes as cell-cultures will assure safer access of material to propagate. If those techniques are applied for those purposes, it means a considerable step and a need experience for the use SE plants of tested clones directly in the forest. The controlled cross can be seen as the first step towards forestry with tested clones for those who believe in that. “True” commercial clonal forestry using copies of field tested clones at an acceptable price is still not an option without discounting considerable future progress in technology.

Fig. 1.: The gain is in the seeds! The major gain of vegetative propagation seems to be to multiply seeds with superior parents, not tested clones. (Photo Anders Fries).

Gain estimates

Detailed gain calculations are long, tedious and based on many specific assumptions and therefore very specific and difficult to translate to general cases. Here rough estimates are made instead, more details about factors considered and how they are considered and methods and values used is mainly in Rosvall et al. (2002) and something in Weng et al. (2008), and Danusevicius & Lindgren (2002).

Plus tree seed orchards and amplified crosses of the best tested plustrees

The gain comparison is summarized in Table 1. Possible provenance effects are not considered or rather, the comparison is with stand seeds of comparable provenance. The Swedish seed orchards composed or untested plus trees give a gain of approximately 10% composed of selection gain at plus tree selection 8 percent, which is higher than Swedish estimates but epigenic effects are neglected. A gain by heterosis or release of inbreeding as plus trees are not related by 2%. The realised gain is reduced as a share of forest production in cultures originates from naturals, this is not considered. The plus trees have been progeny tested and the new seed orchards are established with selecting progeny tested clones with high breeding values. For the seed orchards it is assumed they are constructed by selecting the best 20 clones out of a number of tested trees and the corresponding crosses are made among the best 6 clones of the same number of tested trees. It is somewhat debatable how many trees where are (actually it is usually clone-testing nowadays for Norway spruce) and how well they are tested but the estimated gain is rather well documented (Rosvall et al. 2002). The selection intensity will be higher when selecting families, it the staring number was 200 it would be 30% higher which is assumed. The larger flexibility of controlled crosses is not given a value. There is a considerable time advantage of the controlled cross. A Norway spruce seed orchard has an economical life time of 40 years and the average plant appear on then market more than 30 years after the selection of the clones for seed orchard (Moriguchi et al. 2007). The average time from initiation of a crossing program till the cuttings are sold may be 6 years. That means that crosses are much closer to the breeding population. The figures here would indicate 24 years difference. It takes time to reach sexual maturity but that time can be used for longer genetic testing and is not lost. The gain per year in Norway spruce breeding is estimated to 0.5 percent per year of improvement for 20 years cycles, but here a more moderate estimate of 0.4% per year (somewhat higher than the figures by Danusevicius & Lindgren (2002) suggest, but lower than Skogforsk estimates) is used meaning about 24 years of breeding which is expected to give an extra about 10% gain.

Table 1. Gain comparison between seed orchards with selected clones and crosses among selected clones. The reference level is unselected clones from a stand.

Factor	Elite plus tree seed orchards	Crosses among elite plus tree clones
Selfing	0	+1
No mating among relatives, Gain by selecting plus trees.	+10	+10
Gain by selecting parents or clones among tested plus trees	+15	+19
Pollen contamination	-5	0
Crosses are closer the breeding population that seed orchards	0	+10
Gain compared to stand seeds considering selfing, initial plus tree selection, no relatives, gain by selection among tested clones and pollen contamination	20	30
Gain compared to stand seeds over the expected life time of the seed orchard	20	40

The values from Table 1 indicates that the gain in using crosses is 30% compared to stand seeds, and 20 percent compared to elite seed orchards. However, estimates are usually unsafe and have uncertainties and differ among situations, so I reduce them a little in the summary.

Added gain from using selecting tested clones instead multiplying their offspring

Once plants from somatic embryogenesis become cheaper than plants from rooted cuttings, it is little doubt SE plants will gradually take over the market from rooted cuttings, and the share on the market of tested clones will increase, as tested clones will be superior to mass-multiplicated families. But the additional gain maybe lower than many believe or trust decisions on. If clones are tested and preserved in juvenile form by somatic embryogenesis, they can be multiplied, but this is not done today. Tested clones of Norway spruce are kept in a form so they can be crossed at least during some time window as an integrated part of long term breeding in Sweden.

- Dominance variation and epistasis variation adds to gain if selected clones are planted instead of used as parents. The dominance variation in reasonable old experiments seems often to be around a quarter of the additive, implying that the dominance gives a contribution to selection gain, maybe 2% additional genetic gain to clonal forestry over CP forestry (gain is proportional to root of genetic variance, if variance increases by a factor 0.2 by non-genetic variation, gain increases by a factor 0.1). As dominance and epistasis variation are low, clone testing is sufficient to estimate breeding values and the gain in the additive variation, it is probably no good idea to reproduce tested families (Weng et al 2008) compared to crossing the best recently tested clones, because of the time and effort it takes to test a family compared and make use of the rather small extra gain added above the breeding value of its parents which is available without testing the family.
- There is a considerable genetic variation within in a full sib family, if the best tested clone is selected a considerable extra gain can be made compared by the corresponding CP (controlled pollination) cross. That variation is used in Swedish long-term breeding and creates parents which can be used for crosses to maintain the superiority of family forestry. If SE becomes operational, this within family clones can be multiplied as tested clones, but it is a time delay compared to family forestry crossing parents of the previous generation.
- If only the best clones were planted, the selection intensity would have been slightly higher for clonal forestry than for parents, but for Norway spruce in the foreseeable future mixtures of several clones and the similar requirements of non-relatedness as for family forestry are envisaged. Further on, it seems that many tested clones will not be found suitable for vegetative propagation. Thus the selection intensity for use as tested clones will not be higher than for use as parents.
- For long-term breeding of Norway spruce in south Sweden, the progress (made by within family selection) seems to be 8 percent production in a breeding cycle which takes 20 years, of which field testing is 15.
- It takes some years to get seeds and carry out the vegetative propagation with CP-seeds, for clonal forestry this might be started faster after testing, Thus clonal forestry is closer to the breeding population than the same clones used as parents, and that may give an advantage of say 3% (=7-8 years genetic progress in the breeding population).
- Thus clonal forestry may raise forest production about 5 percent above CP-forestry.
- CP-forestry requires sexual maturity; above I assumed 15 year old tests. If younger tests are used the time advantage is larger, but on the other hand the gain is lower and more doubtful. But considering this and that clonal forestry can be a bit opportunistic in entering material where prospects are best, it may be more fair to estimate the possible superiority to 7%.

More arguments against deploying single well-tested clones ("varietal forestry"):

- Superior clones may get trade identification and brands and owner rights following legal procedures. They are then likely to stay in the market even after they have been genetically outdated and passed their "best before" date. The market forces may encourage dominance of a few clones used over long time, even if it is not the best option for forestry.
- Testing and maintaining clones cost. If that is charged on a small market, it may mean high additional costs per plant sold.
- It is argued that clones are able to combine different characters; I suggest different factors can usually be combined into a single index and mainly dealt with as a single character. Thus this does not look as a major advantage for well-tested clones.
- Different clones are differently difficult to multiply vegetatively, thus the best tested clone may not be worth the added plant production cost.
- If SE-clones are tested, the cost of starting up a clone is considerable and thus the selection intensity may be considerable lower than with rooted cuttings. Maybe only 5 clones among 100 cell-lines started develop into clones easy to multiply.
- Somaclonal variation is likely to exist and increase during the life time of clones. Clones are likely to change performance during testing and use. That includes mutations. The risk of such changes seems smaller if clones live for as short time as possible.
- Clones quite likely accumulate diseases and genetic scrap. The argument why cloning is less common in animals is health considerations rather than diversity.
- The life time - and thus life-time associated problems - of clones become longer the better tested they are.
- A monoclonal stand is unsuitable for seed collection and natural regeneration (also in the neighbourhood)
- Worries about monoclonal forestry will limit the legally permitted and publically acceptable areas of vegetative propagation in forestry with Norway spruce and easily cause a legal limbo. Family forestry sounds more attractive to public and authorities.
- In terms of production value of long rotation major species I would recommend withdrawing around 2% of production for monoclonal culture for lack of diversity when making comparisons.
- Conclusion: Family forestry mixing two families seem unproblematic compared to a single tested clone from the forestry point of view.

SE for the first cycles?

- The logistics of artificial crosses become a major problem to get beyond some million cuttings annually.
- If SE makes the first cycle(s) in multiplication and cuttings just a last amplification by a factor 40-100, a high cost of each SE-plant is acceptable, the quantitative seed need would not be a problem, seeds can be blown up to any quantity and stock plants need not be shared many seasons. This is used for Sitka spruce on Ireland, where SE plants are multiplied by a factor 150.

- SE does not succeed in always and some are difficult to propagate. This matters less, when mass propagation of seeds rather than clones is important. Different number and success of different lines does not cause a major problem.
- SE can be stored, that means that suitable material is always available for mass multiplication and the dependence on that crosses can be made regularly is much reduced.

Genetic diversity

Absence of genetic variation may offer advantages:

- Homogeneity is an advantage for the market, for the forest manager and for the plant producer;
- If the rotation time is short, and the land controlled by the company owning one large mill, the characteristics can be fine-tuned to the end use;
- If rotation time is short clones can be changed when found sensitive or susceptible.

The most pessimistic risk scenarios expressed some decades ago seem not supported by the accumulated experience today.

Genetic variation got advantages:

- Different genotypes will utilize the different micro-niches on a site better together
- Diseases and pests are likely to make less harm in a diverse stand
- Diversity makes it possible to expand on what becomes the best share of the trees when they become older.
- The environments are variable and unpredictable, genetic variation contributes to that at least a share of the trees will be adapted to future conditions and thus give a higher stability.
- A genetically uniform stand may offer fewer niches for other living beings.
- Plants which propagate by cloning in nature typically have 3-15 varieties, indicating that there often is an advantage with clone mixtures.
- A mix of two full sib families is likely to be as variable as a natural stand from most relevant aspects.
- A genetically variable stand is a better seed source.
- More likely to get public acceptance and green certification;

The advantages of absence of genetic variation are probably small for a species like Norway spruce;

- The rotation time is long.
- The end use of the harvest is unpredictable.
- A felled stand is currently a small unit for the end user, thus homogeneity of individual stands is unimportant.
- Much of the harvest comes at thinning.

- A clone forest in boreal conditions may not become very uniform even when its genetics is uniform.
- The environments of plantations are variable and unpredictable;
- The sites are not much homogenized by management.
- The crop stays for a century, a mistake remains almost forever.

For Norway spruce in Sweden, the main line in the foreseeable future should be diversity, even when vegetative propagation is utilized. This may change but not until vegetative propagation is actually used for planting in some million copies over some years.

Concluding suggestion about clone number and relatedness

I suggest heading for mixtures of clones (mass-multiplied crosses) from full-sibs, which have near as much gene diversity as two unrelated full sibs. The gene diversity could more pedagogically be expressed as status number, which is half the inverted value of gene diversity. A large full sib family has status number 2 (all genes from both parents are where). But 6 clones from a full sib family catches most genes from the parents and has status number 2*6/(6+1)=1.7. Two full sib families with 6 equally represented clones get status number 3.4, which is "near" and which I suggest as the current tolerance limit. However, asymmetries will always appear. It is expensive and virtually impossible to avoid them, neither is it needed, as they can be compensated for by higher number so the gene diversity is still the same. It is inconvenient to do actual calculations. I suggest at least 25 clones from the progeny of at least 6 parents and hope that this compensates for the asymmetries and still results in a status number near 4 in field plantations.

Experience elsewhere

Systems of mass-multiplication of cross families by rooted cuttings have been successfully used for decades in P radiata in NZ and hoop pine in Queensland and replaced open pollinated seed orchards since a decade. Controlled crosses for seedling forestry is expanding in southern United States and comprises several percents of the seedlings, and this practice is seen so favourable as it is used in some commercial operations even without vegetative propagation.

Variants of "family-forestry", thus multiplying assumed good families mainly by rooted cuttings, is practised in Ireland and UK for Sitka spruce. The recent magnitude is about 10 million plants annually and it has been in use since more than a decade. Even with Swedish Norway spruce where exists forestry based on controlled crosses, but not at a large scale (100 000 plants/annually), which in itself is somewhat discouraging (if it was profitable "the market forces" would expand the use).

Web (functioned early 2009):
http://www-genfys.slu.se/staff/dagl/Meetings/Finland08/VegPropFinland08.htm

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