Operational Use of Vegetative Propagation in Forestry: World Overview of Cloning and Bulking

Gary A. Ritchie[1]

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Abstract-Rooted cuttings (stecklings) are used world-wide for cloning forest trees and for bulking scarce or valuable forest tree seed. In cloning, numerous copies are made of very few genotypes, and each genotype (clone) is tracked and managed separately. In contrast, bulking involves making relatively few copies of many genotypes of a selected family or seed lot. Management and tracking are normally at the family level. Greater genetic gains can be achieved by cloning, but extensive and lengthy testing programs are required. With bulking, genetic gains are not as high but can be captured more rapidly.

The world's oldest rooted cutting programs are the ancient cloning programs developed in China and Japan. In China, Chinese fir (*Cunninghamia lanceolata* [Lamb.] Hook.) has been clonally propagated from stump sprouts for at least 800 years. Clones are deployed in small, monoclonal blocks and clear-cut harvested. About 60 million Chinese fir cuttings were produced in 1991. In Japan, cloning with sugi (*Cryptorneria japonica* D. Don) cuttings has been carried out on a large scale for at least 500 years. These programs are highly regionalized, with the various prefectures propagating clones which are well adapted to the local climate and soils.

Hardwood cloning is accomplished primarily with eucalyptus (*Eucalyptus* sp.), poplars (*Populus* sp.) and willows (*Salix* sp.). The large eucalyptus programs began in 1953 in the Peoples' Republic of Congo, where today about 1.2 million cuttings are set annually. Soon after this (1967), the well known Aracruz program was launched in Brazil. In both of these programs, use of clones has resulted in a doubling of volume yield and dramatic improvement in wood quality. Poplar and willow cloning have been practiced throughout Europe, Mid-Asia and the near-East for centuries. Over 1,500,000 ha of these clonal plantations exist today. In the United States, interest in poplar cloning has grown appreciably during the past decade. About 20,000 to 30,000 ha of clonal poplar plantations are established here annually.

Bulking is practiced on a much smaller scale than cloning and involves primarily conifers. In Australia and New Zealand, several companies currently rely on radiata pine (*Pinus radiata* D. Don) rooted cuttings to meet their primary planting stock requirements. While methods vary across companies, most employ hedges for cutting production, followed by in-nursery rooting. Production across these programs was 3.3 million in 1992. An added benefit of rooted cuttings in these applications is that the trees derived from them tend to have higher quality stems (fewer and smaller branches and less taper) than seed-derived trees.

Several smaller spruce-based bulking programs have been developed in Europe and Scandinavia with Norway spruce (*Picea abies* L.) and in Canada with black spruce (*P. mariana* [Mill.] B.S.P.). Great Britain and Ireland have also developed programs with Sitka spruce (*P. sitchensis* [Bong.] Carr.) and, to a lesser extent, hybrid larch (*Larix x eurolepis* Henry). Owing to high stock costs and loss of government subsidies, some of these programs have been reduced or abandoned.

The largest bulking program in the United States is Weyerhaeuser Company's Douglas-fir (*Pseudotsuga menziesii*[Mirb.] Franco) program, where rooted cuttings are produced from elite control-pollinated seed. This process has achieved a bulking factor of about 21 (21 packable trees per

individual seed sown). Annual production is in excess of 2 million.

BULKING AND CLONING

Nearly all vegetative propagation systems employed in commercial forestry, whether based on rooted cuttings, tissue culture, or somatic embryogenesis, have as their objective either to "bulk" valuable families or to "clone" valuable individuals (Ritchie 1994). So we begin here with definitions of both terms. Bulking involves making few copies of many individuals. This is normally done at the family level and clones among these family bulks of little or no interest. Cloning, in contrast, involves making many copies of few individuals. This is done at the genotype level, and individual genotypes (clones) are tracked through the process and into the field.

Advantages and disadvantages of bulking and cloning.

Bulking is normally conducted using genetically improved, tested families. It is assumed that the vegetative propagation process has not altered the genetic constitution of the family, therefore, the mean performance of the bulked family will equal that of the original seedling family. Further testing, then, is not needed and this material can be planted into the field immediately. This is a major advantage over cloning, because even when clones are derived from elite families, individual genotype performance cannot be predicted without field testing. In addition, during the testing period it is necessary to hold members of each clone in a juvenile condition, so that the selected clones can be easily propagated when the testing period is completed. This is often very difficult except in cases when mature trees produce juvenile tissues (discussed later). Therefore, the time between propagation and deployment can drag out for a decade or more.

In contrast, the genetic gains offered by cloning are much greater than those possible with simple bulking (Figure 1). Bulking captures the mean performance of an improved family as compared to its wild counterpart. Cloning, however, captures the gain associated with the best individual(s) in that family, which are significantly greater than the overall family mean.

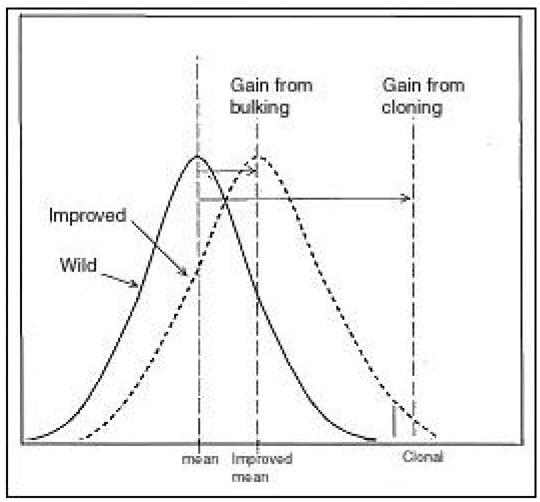

Figure 1. The genetic gains offered by cloning are much greater than those possible with simple bulking.

Whether cloning or bulking is employed depends largely on the species which is being propagated. In species which produce easily retrievable juvenile tissue from mature trees, cloning is often the preferred system. Some examples are poplars, willows, eucalyptus and Chinese fir. Bulking is normally used with species in which cloning is difficult or impossible. Many commercial conifer species would fall into this category.

BULKING SYSTEMS

As mentioned above, bulking systems are often used to amplify scarce supplies of valuable seed. This involves both species which are difficult to propagate from seed and elite families from breeding programs.

An example of the former is a system developed for bulking Alaskan yellow cedar (*Chamaecyparis nootkatensis* [D. Don] Spach.) in British Columbia. In this system, hedges are established from seedlings which are propagated from seed collected in the wild. These hedges are maintained at a very low height by periodic shearing, which holds them in a juvenile condition. In fact, the juvenile foliage is morphologically different from mature foliage and very easy to recognize. Cuttings are set in containers and rooted under mist in greenhouses. Details of this very successful program are outlined by Russell et al (1990).

Most bulking programs are aimed at amplifying the most valuable orchard seed which, by definition, is always in short supply. Most current programs are used to bulk open pollinated (OP) seed because few tree improvement programs are yet producing operational quantities of controlled pollinated (CP) seed. Ultimately, bulking CP seed will probably be the highest use of this technology.

OP Sitka spruce seed is now being bulked to commercial quantities in both Great Britain and Ireland (Mason 1991). The process used was pioneered at the British Forestry Commission's Northern Research Station in Scotland. Elite OP seed is sown into containers and grown as stock plants (cutting donors) in a greenhouse under accelerated conditions. After the second year, branches are harvested, rooted and lined out into a bareroot nursery. They are grown on for two additional years and a second crop of cuttings is removed from them. These are then rooted, grown on in the nursery and then sent to the field for planting. This program has suffered from the ebb and flow of reforestation subsidies from the British government. When subsidies are up, forest land owners can afford this high cost stock, when they are down, foresters go back to traditional lower cost seedling stock.

Several bulking programs have been developed in Canada using spruces, primarily black spruce (*Picea mariana* [Mill.] B.S.P.) (e.g. Vallée 1990). Most of these are similar to the Sitka program noted above, except that plants are rooted in containers and delivered to the field as container stock rather than bareroot stock. A program in Québec employs a novel tissue culture-like rooting system coupled with container production. As in Great Britain, however, many of these programs have fallen on hard times.

The only operational conifer-based rooted cutting program in the United States is Weyerhaeuser Company's Douglas-fir (*Pseudotsuga menziesii* [Mirb.] Franco) program in Washington State (Ritchie 1993). This program employs first generation CP seed which is selected for good volume production and excellent stem form. Stock plants are grown under accelerated greenhouse conditions for one year. Cuttings are harvested the following winter, set in early spring, rooted, and fall transplanted into a bareroot nursery. They are grown on for an additional growing season and lifted the following winter. Currently the program is producing about I million rooted cuttings annually. A CP orchard is currently being established, using second generation crosses, to more fully exploit this bulking program.

Other conifer programs have been developed in Europe (Kleinschmit 1992) and Scandinavia (Lepisto 1974; Roulund 1974; Johnsen 1985; Bentzer 1993). These are based primarily on Norway spruce (*P. abies* L.) owing to its slow, erratic seed production. The European (German) program involves several cycles of nursery selection, followed by cloning, hedging, and extensive field testing. A large industrial program in Sweden combines container production with high level of automation (Bentzer 1993). Many of these programs are on the decline because of lack of government subsidies and the high cost of the stock.

One area where bulking programs are expanding rapidly, rather than shrinking, is Australia and New Zealand (Menzies and Klomp 1988; Duryea and Boomsma 1992). Here, rooted cuttings of radiata pine (*Pinus radiata* D. Don.) are currently used by many companies and governmental agencies to bulk orchard seed. In fact, in several cases, the use of rooted cuttings has superseded the use of seedling-based planting stock. A particular advantage of radiata pine is that it can be readily rooted in open nursery beds rather than greenhouses. This enables the sticking, rooting, and growing-out phases of production to be accomplished in the same outdoor location in one year. This makes for a very efficient and low cost system. Another plus for vegetative propagation of radiata pine is that genetic gains in volume are attended by improvements in stem form - less taper, smaller and fewer branches on rooted cuttings. This effect is particularly marked when cuttings are taken from slightly mature (3-year old) cutting donors. This has been reported to improve both yields and value at rotation (Spencer 1987).

CLONING SYSTEMS

It was mentioned earlier that cloning with forest trees is commercially feasible only with those species which, when mature, maintain the ability to produce juvenile tissue. This tissue almost always emerges in the form of stump sprouts. With such species it is possible to make phenotypic selections on mature trees, fell the selected trees, then propagate those individuals (clones) from the juvenile sprouts which emerge from the stumps. These can then be tested as replicated individuals. It is not surprising that most of the world's large, successful cloning programs have emerged using trees with this capability, such as eucalyptus (*Eucalyptus* sp.), poplars (*Populus* sp.) willows (*Salix* sp.)., and Chinese fir (*Cunninghamia lanceolata* [Lamb.] Hook.)

The first large eucalyptus program was initiated in the Peoples' Republic of Congo in 1953 (Leakey 1987). The success of this effort lead to the establishment of even larger programs in Brazil, which are currently the largest operational clonal forestry programs in the world, according to Zobel (1993). Cloning, coupled with selection and breeding have produced remarkable gains. Early experience at the Aracruz operation in Brazil, for example, yielded first rotation gains in yield (112%), pulpwood density (25%), percent pulp (6%) pulp content (23%) and forest productivity (135%) (Branddo 1984). Cloning has also been very useful in combating herbivores such as leaf cutting ants, which have decimated large eucalyptus stands in Brazil. Identification of ant-resistant clones has made eucalyptus forestry economical in areas where it had been previously impossible.

Poplars have been clonally propagated throughout Europe, Asia and the mid-East for centuries, with over 1,500,000 ha of plantations in existence today. The introduction of the North American *P. deltoides* [March.] during the 1700s, and its subsequent crossing with the native *P. nigra* [L.] gave rise to a stream of highly successful poplar hybrids (*P. euroamericana*) clones which revolutionized poplar forestry (Zsuffa 1985). Today in the United States several industrial poplar programs have emerged in Washington and Oregon. Utilizing derivatives of fast growing hybrid clones (*P. deltoides x P. trichocarpa*) developed jointly by Washington State University and the University of Washington, these programs produce pulp in 7-8 year rotations. Owing to the short crop cycle, these plantations are considered agriculture and, hence, do not fall under the jurisdiction of forest practices boards.

The oldest and largest clonal forestry programs are in Asia. The sugi (*Cryptomeriajaponica* D. Don) program in Japan has been in existence for at least 500 years (Ohba 1993) while the much larger Chinese fir program has existed for perhaps one thousand years in south-eastern China (Li 1992; Li and Ritchie, in prep). This species is planted in 14 provinces In southern China and makes up about 25% of its national timber production. The sugi program has a long and successful history, with about 30 million rooted cuttings produced annually, as of 1989 (Ritchie 1991). The program is difficult to describe owing to its extreme diversity. Each prefecture in Japan has evolved, over the centuries, its own preferred techniques and clones. Many of the clones have been selected for the rooting ability as well as for their growth and yield characteristics. A detailed overview of this program is given by Ohba (1985;1993).

Chinese fir, as mentioned earlier, has the ability to produce juvenile stump sprouts if the site is burned following harvest. These "fire sprouts" can be collected and rooted to produce clones of the adult tree. Reforestation systems have been based on this approach for at least 800 years as indicated from these evocative lines from the 12th century Chinese poet Zhu Xi..."planting cuttings of the fir along the roads; enjoying the cool air in the moonlight of the future" (Li and Ritchie, in prep.). Li (1992) estimates that more than 20 selection cycles have been carried out using this system. Each is the equivalent of a 30-year clonal test. Yields of the most productive of these clones are dramatic. For example, one plantation yielded 1,170 m^3/ha at age 39, which is about six times the yield of a wild plantation on a similar site!

The Chinese clonal forestry program came under attack during the 1950's when Chinese agriculture came under strong influence of the Soviet Geneticist Lysenko. He convinced the government that cloning was leading to erosion of the productive base of the forests and cloning was largely replaced with seedling plantation. Recently, the folly of this action has been recognized and an effort is underway to find, and bring back into production, many of the priceless old clones.

CLONAL DEPLOYMENT

A key challenge of clonal forestry is how to capture the impressive gains associated with cloning without risking catastrophic plantation failures owing to narrowing of the genetic base - the often cited "monoculture" problem. Historically, there have been three approaches to clonal deployment in forestry: 1) monoclonal deployment, 2) mixed clone deployment, and 3) deployment in clonal mosaics. In monoclonal deployment, a large area of land is planted into only one clone. This method clearly gives the greatest clonal gains, but also carries the highest risk. By planting mixes of high yielding clones, high gains can be captured but with far less risk. However, when clones are planted in mixes, it is not possible to identify unique or abnormal clones within the mix, unless of course, every tree is tagged. For example, if a particular clone is maladapted so that many of its members die throughout the plantation, the maladapted clone will remain unidentified and there will be no way to remove it from the production base. Similarly, if a certain clone is particularly well adapted, its identification will also remain elusive. The third alternative, clonal mosaics, offers an attractive compromise. Here, clones are deployed in monoclonal blocks, but these are inter mixed with many other blocks containing different clones. This strategy captures the advantages of monoclonal plantations, but buffers risk by deploying many clones over small areas. In addition, the clonal boundaries may afford physical or biological barriers to destructive agents. With intelligent use of clones in forestry, it is also possible to create plantations, which carry much greater genetic diversity than natural stands. This is because artificial crosses can be made that could never occur in nature and these are then mixed in ways that nature could never accomplish.

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[1]*Senior Research Scientist, the G. R. Staebler Forest Resources Research Center, Weyerhaeuser Company, Centralia, WA 98531, USA.*

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