THE USE OF VEGETATIVE PROPAGULES IN FOREST GENETICS AND TREE IMPROVEMENT

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ABSTRACT

Vegetative propagation, in theory and in practice, offers advantages for research in several areas of forest biology. It also has a direct practical application in the use of rooted cuttings or other forms of vegetative propagules in afforestation. The most severe difficulties which presently prevent these theoretical advantages from being realised are associated with phase change and maturation of apical meristems. Many of these difficulties can be avoided by propagating from very young trees, or by manipulating clones to keep some members in the juvenile phase of growth. These latter can continue to serve as donors of vegetative propagules which will have a consistent stage of maturation at the time of planting.

SOME USES OF VEGETATIVE PROPAGULES IN FOREST GENETICS AND TREE BREEDING

Gene Storage and Packaging

The most common and probably most important use made of vegetative propagation by forest geneticists and tree breeders has been to move genes from selected trees to some convenient location, usually designated a gene bank, clone bank, clone-holding orchard, or seed orchard. Here the genes may be recombined in pedigreed offspring via controlled pollinations, or multiplied in seedlings or additional vegetative propagules for production plantations, or just safely maintained for some possible future use. As long as the primary purpose is storage, multiplication, or recombination of the genes contained in the vegetative-propagule package, the form of this plant can depart greatly from that of an ideal timber tree. In some cases, unusual forms are created purposely to better serve the functions of multiplication, controlled breeding, or gene storage. In other cases, aberrant forms reflect the present inadequate state of the science and art of vegetative propagation. As far as the genes are concerned, their integrity appears to be unaffected by such departures from normal forest tree development.

Population Architect Studies

Burdon and Shelbourne (1974) have developed such studies from their theoretical basis. I agree with their conclusions, that vegetative propagules used as clones in well-designed experiments offer considerable theoretical advantages for estimating such things as the genetic variance, the broad-sense heritability, genotype-environment interactions, and genetic and environmental covariances between characteristics. In the absence of important biological problems (discussed below), these theoretical advantages of clones have been and will increasingly be realised. Burdon and Shelbourne also showed how clones may be valuable even in the estimation of the additive genetic component of variation and of narrow-sense heritability.

Such studies allow both practical and theoretical people to understand better the amount and distribution of genetic variability which is available in a population or a species. Both direct and correlated responses to natural or artificial selection can thereby be anticipated. A less-discussed component of variability is the environmental component, well estimated in valid clonal studies. The ratio of this to total variability (and thus analogous to a heritability ratio) describes the plasticity of a characteristic within the operational environment of the experiment. It is a useful estimator of the likely success environmental manipulations such as spacing control and fertilising may have.

Selection

Burdon and Shelbourne also covered the statistical details of using clones in selection. An important class of artificial selection falls under the general heading of "family selection", and clonal selection is one form of family selection. It has been shown that, theoretically, clonal selection is the most efficient of all types of family selection (Libby, 1964). However (given a fair comparison, such as the same number of trees grown in the contrasted schemes), other types of selection schemes which combine information from individuals and their relatives in a weighted index, with the individual being the unit of selection, are more efficient than clonal selection (Libby, 1969a). The use of clones in selection thus must be reserved for special cases. I will suggest five such cases.

First, as Burdon and Shelbourne suggested, there may be all-or-none characteristics like survival, forking, etc., of sufficient importance that it is useful or even necessary to present each genotype to the fortunes of the environment several or many times in order to adequately evaluate it for such characteristics.

Second, when the experiment is defined such that some percentage of trees in a limited area *must* be selected (in contrast to selecting some given number of genotypes out of the entire experimental population), this constraint produces a situation in which clonal selection is the most efficient option of all (Libby, 1969b). A "seedling seed orchard", where a round of selection and rogueing is based on the performance of the plants on the orchard site, includes such a constraint. Thus, paradoxically, a "seedling" seed orchard should consist of clones which will generally be the original seedlings plus many rooted cuttings of those seedlings taken when the seedlings are very young.

Third, if genotype-environment interaction is important, it may be desirable to test each genotype over the sets of major environments serviced by the selection programme, and restrict selected trees to those environments where they perform well. The fourth case, which is perhaps a part of case three, involves selection for phenotypic stability. It may be that some genotypes are labile (performing very well under some conditions, but performing badly or even failing in others), while other genotypes are more buffered, and that among the latter some perform relatively well over most or all relevant environments. One might eliminate the labile genotypes and select the better performers among the more buffered genotypes. Vegetatively replicating the genotypes over several or many environments is useful for this type of selection.

Finally, if production plantations are to be made with vegetative propagules, it is sensible to select genotypes based on their performance *as* vegetative propagules, not as seedlings.

Environmental Investigations

In many studies of tree physiology, nutrition, silviculture, etc., genetic variability is viewed mostly as contributing to greater variability in the experiment and a larger error term. By using vegetative propagules, and giving each treatment to identically the same set of genotypes, the precision of such experiments can be increased. It is clear, though, that one must not sacrifice generality for precision by using only one or a few clones.

As an example of such an experiment, we are adapting the Lederbergs' (1952) replica plating technique for investigating the potential range of radiata pine (*P. radiata* D. Don) in California. A replication consists of 36 clones, six chosen over the variety of ecological conditions in each of the four native populations at Ano Nuevo, Monterey, Cambria, and Guadalupe Island, and six each from among New Zealand's and Australia's best progeny-tested families. Differences in performance between replications will be wholly due to environmental differences, i.e., free of genetic sampling error. There should thus be no surprises in consequent plantations arising from misinterpretation due to an unusual group of test genotypes used for the evaluation of a particular site. Genetic variability from the entire native range is included in the test, as well as twelve clones derived from outstanding seed parents. Additionally, we should learn a great deal about these 36 clones, including the types and magnitude of genotype-site interactions expressed by them.

Competition

The various types of competitive interaction between adjacent trees can be investigated by using vegetative propagules. The effects of a genotype on its neighbours, and their effects on it, can be described with precision by sufficient replication of the particular permutations of genotypes of interest.

SOME USES OF VEGETATIVE PROPAGULES IN PRODUCTION FORESTRY, AS AN ADJUNCT TO BREEDING

Planting Partly Mature Propagules

In some cases, as perhaps with radiata pine, there may be advantages in starting a forest with vegetative propagules at some stage of maturity beyond juvenile (see, for instance, Tufuor, 1973; Tufuor and Libby, 1973). Thus, manipulation of the developmental phase of tested clones or even untested trees may, in a practical sense, make the phenotypic expressions of genotypes better than they would have been had these same genotypes been planted as seedlings.

Phase Change

The phenomena included in this term can significantly influence the form and growth characteristics of a vegetatively propagated tree. In this discussion, phase change should not be viewed as a single event, but as a number of generally irreversible changes affecting the form, sexual maturity, and vigour of a tree. These phase changes occur concurrently or sequentially as apical meristems develop from juvenile through adolescence to maturity (Sweet, 1964; Zimmerman, 1972).

Vegetative propagules, taken at different times as a tree matures, or (in some species) from different parts of a full-crowned tree, will then grow exhibiting consistent within-clone differences in form and vigour. If these different maturation states can be stabilised within some members of a clone, then such clones can be propagated at the particular maturation states which most nearly maximise their value in production forestry. Within a clone, these differences are probably associated with the developmental states of the genes, and are clearly not associated with differences in the gene complement of the clone.

SOME PROBLEMS OF USE AND INTERPRETATION ASSOCIATED WITH VEGETATIVE PROPAGATION

Improper Technique

If some cuttings in a clone develop weak or one-sided root systems, this will introduce an erratic growth response within clones making for a greater error variance in experiments utilising clones. Inadequate graft unions caused by poor grafting technique, or graft incompatibilities resulting in partial or complete failure of the graft union, will have a similar effect on the reliability and general usefulness of clones.

Monoculture

A monoclonal stand is the most extreme form of monoculture, and is particularly susceptible to epidemic attack by insects, diseases, dendrophagous animals, or widespread injury by some environmental event.

Maturation

If the purpose of an experiment is to evaluate a clone for some future use, the continued maturation of the clone while the experiment is in progress is a serious problem. For instance, in 1971 I asked Charles Pawsey how many of the hundred-plus clones of radiata pine grown to useful size in South Australia he would now recommend for widespread use in production plantations. He answered that several had proven themselves good enough to receive such a recommendation, but he could not make such a recommendation. The problem was that all had been cloned while relatively juvenile, but that all ramets and the ortet were now fully mature, and cuttings would be difficult to root. Furthermore, those cuttings which were successfully rooted, while of a proven genotype, would be unlikely to grow with either the form or vigour of the earlier juvenile ramets used to evaluate the clone.

Abnormal Growth

In many species, rooted branch cuttings tend to continue to grow with the horizontal orientation and bilateral symmetry of branches for varying periods of time, until the terminal meristem converts to radial symmetry and a vertical growth orientation, or a sprout with such symmetry and orientation initiates and assumes dominance. Such return to normal growth orientation is frequently erratic within and between clones (Tufuor, 1973), and would generally disqualify the vegetative propagation technique for either reducing the error variance in experiments or for evaluation of genotypes in a selection experiment.

Reduction of Vigour

As a tree matures, vegetative propagules of it commonly grow more slowly. At advanced stages of ortet maturation, this may result in slower height growth of the ramets. More commonly, it results in a reduced rate of ramet diameter growth, even if height growth is comparable to that of seedlings. Sweet (1973) has estimated a loss of about 40% in the lower stem volume growth of ramets from mature radiata pine compared to juvenile ramets or seedlings after 3 years in the field. This is very close to the 38 % and 44% reductions observed in independent sets of experiments with ramets from 12- to 14-year-old radiata pine trees compared to their seedling offspring after 5½ years in the field (Tufuor, 1973). Clearly, the alternative advantages of using such vegetative propagules in production plantations would have to be large to offset such a considerable loss in early volume growth.

It seems likely, by the way, that this slower diameter growth of adolescent and mature cuttings is due to the fewer and smaller branches (and therefore smaller leaf area) produced, rather than an intrinsically slower growth rate of the cambial cells.

SOME STRATEGIES TO DEAL WITH THESE PROBLEMS

Monoculture

As Schreiner (1971) and others have repeatedly suggested, a prescribed mixture of clones should be as well buffered against epidemic attack or environmental crisis as a diverse seedling population. Adaptation of insects or diseases to the trees in a plantation may in fact be more difficult with the discontinuous array of tree biotypes in a mixed clonal planting than with the virtually continuous array of tree biotypes in a seedling planting.

Technique of Vegetative Propagation

Important advances have already been made in both rooting and grafting technique. The work of Thulin and Faulds (1968, and more recent unpublished results) on rooting media quality and rooting aftercare (such as a root-pruning schedule) has already made possible the routine large-scale production of radiata pine cuttings with fibrous, symmetrical root systems of a quality comparable to that of nursery-grown seedlings. The work of Copes (1973) on graft union interactions and incompatibilities gives promise of more reliable grafted clones by identifying compatible stock-scion combinations. The uncertain effect of the stock root system on the clone's performance, however, still dictates a preference for rooted cuttings over grafts for many purposes.

Young Seedlings

If the purpose of the experiment is to investigate various components of a species' genetic architecture, to select genotypes for later sexual reproduction, or to increase the sensitivity of any experiment by control of genetic variation, most of the problems associated with vegetative propagation can be eliminated by rooting from very young seedlings. Such very juvenile cuttings generally root easily and quickly, compared to cuttings from older ortets. With a few minor exceptions, such vegetative propagules are soon indistinguishable from their seedling ortets in both growth and morphology. They, of course, do not have cotyledons, and we have found in such experiments with redwood that they generally do not develop the basal burl characteristic of redwood seedlings.

As an example, we grew radiata pine seedlings in the greenhouse until they were about 50 cm tall, and had many secondary needles. A terminal cutting about 10 cm long was taken from each seedling and placed in a rooting medium. With the top thus removed, the hormonal inhibition on the short-shoot meristems was also removed, and about 8-12 of the upper fascicles began long-shoot growth. After several weeks, most of these had attained a length of about 10 cm, and they were in turn removed and placed in the rooting medium. Following their removal, additional fascicle meristems further down the stem began long-shoot growth. This procedure was repeated several more times, and 16 months from germination most clones had 15 or more well-rooted and plantable ramets.

Hedging

It appears that the maturation of clones can be arrested by repeatedly pruning back all new growth above a specified height. This remains at the level of descriptive phenomenology, but work with several kinds of fruit trees (Garner and Hatcher, 1957), three species of oak (pers. comm. J. M. Tucker and W. P. Hackett, Univ. California, Davis, 1973), and radiata pine (Libby, Brown, and Fielding, 1972), indicates that such phenomena may be general for woody perennials. Furthermore, it appears that the maturation state of a clone can generally be set and maintained with fair accuracy by the height at which the hedging is done (Garner and Hatcher, 1962).

Thus, it would be possible to keep some ramets of candidate clones hedged while others are grown as trees for evaluation. When the clones are selected on the performance of the tree-form ramets, the production-scale propagation of cuttings of those clones from hedged ramets can commence with the expectation that they will duplicate the performance of the test ramets, since cuttings from the hedged ramets will still be at the approximate stage of maturation of the ramets used for the evaluation at the time they were rooted.

The hedging technique also provides a convenient way to produce and harvest many cuttings efficiently and from a small area (Libby, Brown and Fielding, 1972). It is possible that in some species, where bilateral horizontal growth of cuttings is a problem, cuttings from the top of a hedged plant will grow vertically with radial symmetry from the time of planting. There is, however, little available evidence on this speculation.

Serial Propagation

Serial propagation involves the vegetative propagation of a primary ramet from the original ortet (preferably at a young age), followed by propagation of a secondary ramet from the primary ramet a few years later, a third-stage ramet from the second-stage ramets, etc. It seems clear that this technique at least slows maturation of the clone compared to continued propagation from the crown of the ageing ortet. Whether it in fact arrests maturation is not presently clear.

Herrnmun (1957) claimed that serial propagation of Norway spruce and other species would maintain juvenility, and Kleinschmit (1972) reported no decline in rooting with successive propagations of Norway spruce. Hatcher (1960) similarly suggested that cycles of newly-established plants, established for limited duration, could substitute for permanent hedges in certain fruit tree species.

Pawsey (1971) reported serial propagations commencing in 1940 with five 5-year-old radiata pine seedlings. As of 1966, serially-propagated rooted cuttings of these five clones were 26, 21, 15, and 10 years old, and grafts taken from the fourth-stage rooted ramets were 4 years old. Scions from all of these were grafted in 1966. Grafts from the most recent of these grew in a more juvenile manner than grafts from the earlier ramets. This experiment clearly indicated a slowing of maturation via serial propagation, but did not critically show whether maturation was thus fully arrested, as implied by Herrnmun and Hatcher.

Delisle (1954) reported five serial propagations of eastern white pine at four-year intervals over the period 1938-54. Rooting showed a marked decrease with each attempt to root the increasingly older clones. Fielding (1970) included data on female strobilus production of: 5-year-old radiata pine seedlings; primary ramets which had grown two years after being rooted from 3-year-old seedlings; and secondary ramets which had grown two years after being rooted from primary ramets which had grown four years after rooting from 3-year-old seedlings. None of the seedlings or primary ramets had produced female strobili, while all five clones of secondary ramets had produced female strobili. These observations by Fielding and Delisle do not support the belief that serial propagation fully arrests maturation.

Rejuvenation

In general, attempts to change a mature apical meristem to a more juvenile one have been unsuccessful or at least inconclusive. Some apparent cases of rejuvenation may be nothing more than improving the nutrient status of a cutting, making it appear to regain vigour or increase rooting potential (Cameron, 1968). One enticing case of reversion to a juvenile state is reported following treatment of mature English ivy with gibberellin (Robbins, 1960).

SOME OPEN QUESTIONS

Can a true rejuvenation be accomplished by any certain means besides allowing chromosomes to experience meiosis. If so, then the foresight to arrest maturation by techniques such as hedging while clones are being tested will become unnecessary.

What happens to lower-stem wood production after the upper crowns of seedlings become mature, and the lower crowns are pruned off or shaded? If the tops of rooted cuttings from mature ortets and of seedlings which have completed phase change are very similar (as they appear to be) in number and distribution of leaves, then perhaps later stemwood production of the two types of tree will be similar too. If so, then the main difference between mature rooted cuttings and seedlings may be that the lower logs of seedlings have larger, knottier, and more tapered corewood centres. This difference may actually be an advantage of adolescent or mature cuttings compared to seedlings.

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