

## A SUMMARY STATEMENT ON THE 1973 VEGETATIVE PROPAGATION MEETING IN ROTORUA, NEW ZEALAND\*

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This conference, I think, has worked very well. Over the past several days I have watched what appears to be a general consensus develop on a number of topics. I have been pleased to note that there has not been a division into armed disciplinary camps. Rather, I have perceived a mutual respect and a mutual understanding of one another's problems and contributions. In this summary, I am going to try to present some of the major topics of consensus as I see them.

### GRAFTING

Cross-species grafting, or grafting onto particular selected root stocks, have been widely attempted as techniques to dwarf trees and/or to increase their flowering. Generally these techniques have not worked out for forest trees, although there remain in horticulture and in fruit tree work a number of interesting and provocative examples.

Graft incompatibility now appears to be a general fact of life. It is a rare species among forest trees where graft incompatibility has not sooner or later appeared in one form or another, and been a serious problem where grafts are being used. There is evidence that in many forest tree species it is under strong genetic control, or at least is the expression of an interaction (between the two tissues) which has a genetic component. There are various environmental manipulations or treatments that can be applied to grafted plants that will postpone or perhaps in some cases even avoid the incompatibility. But by and large, graft incompatibility is serious. I am disturbed that in many countries around the world, when we begin tree breeding with a new species, or even begin in a new region with a species that is known to have graft incompatibility, one of the first things we do is establish grafted seed orchards. One of the things this conference has put on record is that this is neither wise nor prudent.

The above does not mean that grafts are not useful. I think they are useful for bringing genes from selected trees to some place where further manipulations can be made. These manipulations may include the use of grafted scion material to produce cuttings for seed orchards; or to make a limited number of crosses on the grafted scions to get their genes into *seedlings* for a progeny test, or a genetic architecture test, or for use in seed orchards. But increasingly, I think the nasty fact of graft incompatibility is forcing a choice of alternatives to grafts for such things as production seed orchards.

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\* This statement was the closing paper of the meeting.

## ROOTED CUTTINGS

One of the important alternatives to grafts is rooted cuttings, and the production of these has properly been a major topic of discussion at this conference. Techniques for rooting cuttings include such interesting manipulations as airlayering; grafting and deep planting; patch grafting of rooting tissue onto the scion we want to root; using root material which sprouts shoots; and prestimulating pine fascicles. (Fascicles should be considered under two categories: older material where fascicles apparently root better than conventional cuttings; and very young material where a large number of individuals can be produced very quickly with fascicles).

I will address the rest of these comments to our more conventional cutting, which is a 10-cm or so twig. As with fascicles, it is worthwhile to distinguish between older material (largely for use in seed orchards, breeding orchards, or temporary gene storage) and younger material (for genetic, physiological, and other research uses, and for production plantations).

There is a lot of conventional wisdom about rooting cuttings, such as the time of year to take the cuttings, and the type of cuttings to take. I have noticed some of this conventional wisdom has been subject to change in the last several years, and some of these changes have been discussed at this conference. Some of these changes are associated with shifts brought about by new technology. As an example, the availability of more effective fungicides and better moisture control allows us to use more succulent cuttings than before, and to take cuttings earlier in the growing season than before. But there have been warnings that one can get an over-dependence on this type of technology: fancy moisture controls can fail, or fungicides can be adapted to by the fungi. So maybe the conventional wisdom should still be respected, and perhaps we should try to stay away from too great a dependence on technological alternatives, and try to root cuttings in as natural a way as possible.

I have just a few comments on auxins, on moisture, on temperature, and on media, all of which have been covered in some detail at this conference. It seems that auxins and a few other chemical additives generally stimulate rooting, although we had a few reports where they were harmful to successful rooting. But in general there was an uncertainty on whether people wanted to use auxins, or needed to use auxins. I think that uncertainty is associated with the fact that we do not yet understand the sequence of rooting events well enough to allow auxins (and other chemicals) to be used effectively.

Clearly, moisture is a critical component of the rooting formula. One of the most interesting things that has come out of this conference is the data indicating that one of the times when moisture status of the cutting is most important is during those few hours or few days between severing the cutting from the donor plant and getting it established in the rooting medium. We still want to know more about this, and having learned more about it, I think we are going to be able to deal with the moisture requirement of the cutting during that particular critical period much more effectively than we do now.

The catchphrase, "warm feet, cold head" is nothing new, but it has been re-emphasized here. I think it is clear that too low a temperature retards rooting, and too high stresses the cutting. Combined with abundant moisture, high temperature invites pathogen problems. So there is somewhere near an optimum range for temperature,

and within that optimum range, we may want to keep the top of the cutting cooler than the bottom.

The rooting medium is something that has been relegated almost to the wives' tale shelf. Yet, I am personally and increasingly convinced that media really do make a difference. Different textures and different compositions of the rooting medium appear important both to the success of the rooting event, and to the subsequent form of the root system, and therefore rooting media deserve our continuing attention.

I have some thoughts developed during coffee-break discussions on the evolution of rooting, which may be useful. For some species (willow is a good example), the rooting of shoots or even branches is clearly of continuing selective advantage, and thus it is actively maintained by the selective process of evolution as an intact functional system. It is worth noting that many of the species that root twigs or branches as a normal reproductive process in nature have preformed root primordia in their stems.

For other species (such as some of the pines), rooting twigs or branches is not a normal reproductive process (and probably never was). It is perhaps surprising that a biochemical basis for rooting of stems exists in such plants at all. Most such species do not have preformed root primordia in their stems, but roots must be induced from tissues which normally would not root. New root initiation is a process that appears to be coded into the resident DNA of these cells, and we may ask how it comes to be there. I suspect that new root initiation may be, to a degree, tissue specific. It may operate well in root tissue, for that is where new roots are normally needed. The fundamental process we want to study may be how roots initiate in root tissue of a species that does not normally root from its stems, because this is probably the resident process which is maintained by evolution. It will then be appropriate to determine how this rooting process is failing in stem tissue. It may then be possible to modify stem tissue so that the genetically coded sequence of events leading to root initiation can be effective. Or perhaps we will discover which of the genes in the rooting sequence are turned off in stem tissue, and either figure out how to turn them on, or, by learning what is missing, to insert the missing products or some acceptable substitutes at the proper times so that root initiation and growth can proceed within stem tissue. We have also discovered the presence of rooting inhibitors. If a plant does not normally reproduce by rooting stems, its resident genetically-coded rooting process may best be over-ridden, because it is wasteful to initiate roots on stems. So it produces some kind of an inhibitor. Once recognizing that that is a reasonable thing for a plant to do, maybe we can figure out a reasonable way to overcome that inhibitor when we want that resident rooting sequence to work.

For still other species (black spruce may be an example), the layering of lower branches is a normal reproductive process, but the layering of upper branches is not. Here natural selection may make the rooting process more effective in lower crowns than in upper crowns. We have been talking during this conference as if rooting were a juvenile phenomenon. It may simply be a phenomenon of lower crowns, because that is where evolutionary selection found it to be useful. That may not be a very important idea, but I thought I would throw it in. It does bring me to the problem of maturation.

#### MATURATION

At least one of our papers said that an integrating feature throughout their programme (and I think an integrating feature of our conference) has been the association

of rooting with the maturation state of the cutting. This is important, and I will go back on the coffee-break idea I just presented, and suggest now that good rooting really is a juvenile characteristic, and rooting is highly tied to the maturation state of the cutting or tissue. Furthermore, clones will mature unless steps are taken to prevent such maturation. During maturation, the clones lose their high rooting capacity, generally have reduced growth vigour (particularly diameter growth), and show a changed tree form.

It was suggested that we need to distinguish clearly the reversible and non-reversible effects associated with the maturation, the chronological age, and the tree size, of the material we are working with. It was pointed out that ageing in the "Wareing" sense is probably a poor term. We never really came up with an alternative term (and I do not intend to propose one now), but it seems a clear terminology would distinguish reversible effects of maturation from the less reversible or non-reversible effects of maturation.

Important questions were asked about the maturation of root systems. Do they age? Are newly initiated roots from mature cuttings old, or are they juvenile? Is there a gradient of maturation states within a large root system? These questions, at least in terms of the knowledge available at this conference, have not yet been addressed.

It was suggested that there is a correlation between treatments which inactivate viruses in plant tissue, and treatments which lead to an apparent or real return to a juvenile condition of that treated tissue. This is an interesting lead, because intracellular viruses are a type of nucleic acid, and these treatments seem to lead them to change states, and perhaps go to a more dormant state. Heat treatment of tissue containing viruses, which tends to kill them, apparently does not result in a change in the maturation state of the host tissue. This leads me to suggest that maturation is correlated with genetic state changes in the DNA, and it is reversed at or about meiosis. I would like to use a computer analogy thus: the development of a plant may be viewed as the increasing storage of information via state changes in the DNA programme. This storage of information is relatively irreversible. What happens when juvenility is restored at meiosis is analagous to clearing the previous data from the computer memory. You bring it back to ground state and then you start putting information in again. In the computer and perhaps the organism, information is added by a change in the physical state (but not the chemical composition) of the memory, and "juvenility" is restored by returning the memory to its original physical state.

#### SCIENCE ORGANIZATION

As in most such meetings, there is a dichotomy between the fundamentalists and the empiricists that are here. The more fundamental research seems to be going after specific events, or specific chemicals, or specific processes, which may contribute to rooting or other propagation. The more empirical research, by contrast, is dealing (generally in a rather fuzzy way) with a series of integrated events and conditions which lead to successful vegetative propagation. What is needed is a synthesis on the fundamental level, with precise understanding and with the power that will come with such understanding to manipulate the total series of events and conditions which is now being empirically handled. We probably cannot expect this fundamental synthesis to come for many years, and we must in the interim continue to operate a two-front

war, one of isolated precision, and the other of integrated empiricism. I hope that during this period we maintain this mutual respect and feedback between these two theatres of action.

#### "C" EFFECTS

I re-emphasize that there are two kinds of "c" effects. One is the kind that occurs within clones or within families, frequently due to inequalities of the starting material, and (certainly in the recent history of our art and science) due to faulty techniques, or inadequately understood techniques. These will be reduced as our techniques get better. The second occurs between clones. It similarly may result from inadequate understanding, and it results in a nasty experimental bias that several of us have suggested modifies our enthusiasm for clonal techniques for such things as selection and other types of genetic experimentation. The within-clones "c" effects were viewed as a type of noise which confounds primary information interpretation, or reduces the desired uniformity within clones. The technique of analyzing relative growth rates will perhaps help us in a reasonable interpretation of experiments with starting propagules of unequal size, which can lead to "c" effects of both types.

#### TISSUE AND ORGAN CULTURE

Tissue and organ cultures were discussed as good ways to investigate cell maturation. Protoplast cultures, in which cell walls are removed, may provide ways to do quick genetics via somatic cell hybridization. It was agreed that there is no serious problem in getting callus or organs to grow in culture. A major priority for forest research is to develop methods of reliably recovering entire plants from such cultures. Thus, one strong recommendation of this conference is that we need to do more research of both a fundamental and empirical nature, to learn how to recover plants once we have genotypes reduced to cell culture of some sort. We were also warned that there are frequent genetic or chromosomal aberrations in cultures, and we are not always going to get back what we thought we had put in. Thus, good practice will frequently include the quick recovery of plants from the culture before such aberrations accumulate.

#### OPERATIONAL PROGRAMMES

We now have several examples of programmes around the world which are setting in excess of a million cuttings per year for operational production reforestation. We have clearly left the pilot-plant stage in these programmes, and I think many more such programmes with these and other species can confidently be expected in the near future.

#### DIVERSITY

Finally, I re-emphasize the warnings that have come from several members of this conference, that there are dangers in the vegetative propagation of forest trees with respect to the maintained diversity of forest stands. The unwise application of these powerful techniques of vegetative propagation, which appear to be at hand, could create the great danger of extensive monoclonal stands; or the more subtle but perhaps more serious loss of genetic diversity via the extensive replacement of native populations. Because we may be responsible for the effective development of these techniques of vegetative propagation, we have a particular obligation to guard against their abuse and to promote their wise use.