their rooting ability. An additional ten kinds of plants were then tested on the most promising formulations. Many of the easier rooting plants were almost equal in their response as would be expected. The most promising solvent in our tests proved to be dimethyl formamide. I will admit my evaluations were based on how quickly they rooted and with thirty years experience, which ones I would rather have to plant on. I would like to thank the Environmental Protection Agency for the way they worked with me in the approval of this formulation.

Now to address the title of this paper, research has commenced on several different projects. One a hydrophilic dip containing a fungistat and a rooting hormone for those bareroot trees that are hard to establish after storage. Another is a pressurized spray can containing a callusing agent, a fungistat, and with enough mechanical strength to hold a field bud in place without desiccation and allow the resultant growth to be unimpeded. Work is progressing on the rooting of plants in polyethylene in storage instead of in conventional media. Hopefully one or more will work out.

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GENETIC STABILITY OF HORTICULTURAL PLANTS PROPAGATED BY TISSUE CULTURE

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The term tissue culture, as popularly used, covers a multitude of cell, tissue, and organ culture techniques. The aspect of most interest to plant propagators is micropropagation, the rapid asexual multiplication *in vitro* of a desired plant. Most commonly, the explant used in micropropagation is a meristem-tip, shoot-tip, or bud that is induced to grow and then proliferate in culture. The basis of this procedure is the stimulation of new shoots *in vitro* by treatment with an appropriate plant growth hormone. A cytokinin in the culture medium stimulates growth of axillary and/or adventitious buds. The resulting shoots can then be rooted by transferring them to a medium free of cytokinin and containing an appropriate auxin

concentration, or by rooting them directly in the greenhouse using more or less standard procedures. Micropropagation can also include the production of somatic embryos in culture, a process which is also under hormonal control. Propagation by somatic embryogenesis is not yet typical of commercial applications.

Questions concerning the genetic stability of plants produced by tissue culture often arise. Will these plants look like and grow like the source plant from which the cultures were initially established? The answer to the question depends upon a number of factors including the type of plant in culture and its inherent stability, the culture techniques being used, the growth regulators and other chemicals employed in the media, and the cells, tissues, or organs being cultured.

Generally, plants regenerated from axillary shoots are considered most likely to be phenotypically identical to the parent plant (2,5), while plants derived from adventitious shoots or somatic embryos are considered to be more likely to differ phenotypically, particularly if they arise from callus.

In actual practice, however, genetically stable plants, phenotypically identical to the original, are produced by all of these methods. On the other hand, aberrant or mutant plants are also produced by all of these methods. The result is that one must know well the plants being tissue cultured and develop experience to know the best means of handling them. For example, Boston fern (*Nephrolepis exaltata bostoniensis*), which tends to be unstable, may yield up to 25% aberrant plants from tissue culture (A. Donnan, personal communication). This problem can be overcome by limiting the number of subcultures and starting new cultures often using clean stock plants. Some ornamental plants, e.g. *Alocasia* sp., are regularly micropropagated using shoots derived from callus without any loss of phenotypic identity (J. Rowe, personal communication).

Another example of genetically stable plants produced from callus is that of 'Seyval' grape (3,4). Callus cultures were established from various parts of the plants of 'Seyval' and somatic embryos were induced to form from the callus. Many of the embryos were successfully cultured until they grew into plants which were transferred to soil and then planted in a vineyard. These vines have now all fruited for several years. While they are phenotypically identical to one another, none resemble exactly the standard plants of 'Seyval', differing in such characteristics as anthocyanin content of the stems and shape of the fruit cluster (3,6). However, all match the original description of this cultivar more closely than do the current

standard plants of 'Seyval'. The reason for this difference is unknown but it is unlikely to be due to a difference in virus content. A reasonable possibility is that the differences are due to epigenetic changes that occurred during the years since the cultivar was first described. They would be comparable, for example, to the changes in leaf shape, growth habit, etc., occuring in *Hedera helix* as it develops from the juvenile to the mature phase.

Besides phenotypic stability, the field or greenhouse performance of micropropagated plants is a prime consideration. Evaluation for both factors is well illustrated with micropropagated strawberries in a large field experiment at Beltsville (7). Meristem-tips were cultured from virus-indexed plants of 'Earliglow', 'Guardian', and 'Redchief' and then were proliferated and rooted on a modified Boxus medium (1). Plants were set in the field at the end of May, 1979, and allowed to runner freely throughout the summer until the plants filled a square 60 cm × 60 cm. Additional runners were then cut off. The plants in the squares were evaluated in the fall for vegetative characteristics. Tissue-cultured plants produced more crowns, more runners, better filled the square, and were generally more vigorous than the standard runner-propagated plants. The following spring, the tissue-cultured plants had many more flowers in each square because each square had more crowns and each crown produced more trusses. However, the number of flowers per truss was not increased on the tissue-cultured plants. The flowering data indicated a potential for a 160% increase in yield but only a 25% increase (based on fruit weight) was realized. This difference resulted from a reduction in fruit set and a 25% reduction in average fruit weight on the squares of tissue-cultured plants. Fruit size reduction resulted mainly from a size decrease in the normally larger fruit harvested in the first 2 pickings. Much of the size reduction is thought to result from the greater competition between crowns in the squares of tissue-cultured plants.

A small portion of these tissue-cultured strawberries and all the runner-propagated control squares were grown a second season and yields were again taken. With 'Earliglow', the squares grown from tissue-cultured plants produced larger fruit and more crowns in the second fruiting season. For all cultivars combined, the fruit yield from the squares of tissue-cultured plants was about double that of the runner-propagated controls, whether measured as number of fruit or total fruit weight.

For the strawberry experiment described above, the cultural practices were those normally used for runner-propagated plants. Since the tissue-cultured plants are much more

vigorous, they could probably be planted much later in the season and still produce enough runners for an adequate crop of fruit. This has been done in a preliminary trial at Beltsville. Late planting would reduce the overcrowding that was observed in the squares produced from tissue-cultured plants. It would lead to other changes in the cultural system as well. Thus to take full advantage of the increased vigor and runnering ability of tissue-cultured strawberry plants, it will be necessary to modify the entire cultural system now being used. Studies on such modified systems are now under way at the University of Maryland.

The idea of modifying the complete cultural system to derive maximum benefits from tissue-cultured plants may be applicable to most, if not all, horticultural crops propagated in this way. Any change will depend on the use to which the plants are put, the length of time it takes them to mature, and the persistence of characteristics, such as increased vigor, which are associated with the tissue culture process.

The evidence is clear, I think, that genetic stability, as represented by phenotypic appearance and performance, will not be a serious obstacle to greater application of tissue culture propagation to horticultural crops. This does not mean that genetic abnormalities or changes will not occur, as they most certainly will, but that they can be dealt with by careful attention to detail during the tissue culture process and by careful inspection and roguing of the resulting plants.

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MODERATOR CARVILLE: We will now have questions for our panelists but first Don Dillon has something to say.

DON DILLON: One of the important products of the whole Society is the annual *Proceedings*. Now that we have grown from just the Eastern Region to the Western, the Southern, the Great Britain and Ireland, the Australian, and the New Zealand — the whole bit, there are so many papers now being produced that it is hard to keep up with everything. The International Board has been concerned that we need some way to know what each of the Regions are doing, what happened in the prior years, what progress has been made. So a plea went out to anyone with any idea on how to develop an Index that would cover the past 30 years of papers produced by this organization. The point I am making is that we are very privileged to have someone who responded to that plea; he is here today and he is up here at the table. He just presented the last paper — Dr. Richard Zimmerman. He has volunteered, free, to index the past 30 years of the *Proceedings*. This work is already underway; he has completed the author index thus far, and the subject index, and is now starting to work on the plant material index. We are most appreciative. So sometime in the future we will be getting an additional product from our Society — a 30 year Index. We are much indebted to Dr. Zimmerman for this.

MODERATOR CARVILLE: This is probably the best news that I have heard since I have been here. I have been on the International Board for a few years, and I might say that this is one of its concerns — first of all to update the current Index of the *Proceedings*, and then to find a way to do it within a budget, and along comes a sincere, dedicated member of the Eastern Region who is willing to put forth his time and effort into doing this tremendous task of identifying all speakers' topics, all generic plant names mentioned in all of these many papers. Dick, my sincere thanks to you.

Now to the subject at hand — any questions from the audience on our previous topic — plant growth regulators?

HUDSON HARTMANN: Dr. Zimmerman used the term "sub-clone" in his presentation. I would like to know how he defines "sub-clone".

RICHARD ZIMMERMAN: But, really, this is a very tricky

question. We had a symposium at the American Society for Horticultural Science meeting in Atlanta a few weeks ago on just the topic of the clone.

I really don't know what we should call these, but we had four different meristem tips that came from the same mother plant. We proliferated tissue from them so, basically, I suppose you can say they are the same clone — but what do you call them? We called them "sub-clones" in our slide. We are referring just to the plants derived from that particular meristem tip. They were all of the clone 'Earliglow,' if you will accept cultivar and clone being equivalent in this case. So this was the only way of keeping them separate. Of course, you can get into all sorts of things, for example when you start talking about Shepard's work with potato where he took the leaf of a potato and got protoplasts for regeneration, with a whole range of variation from the plants regenerated from that potato leaf. I don't know. It is a real problem. We really didn't resolve definitions or the proper usage at the Atlanta ASHS symposium.

RALPH SHUGERT: Ed Wood — would you explain your philosophy on rooting cuttings using a liquid hormone preparation: length of time that cutting should be in the hormone — 1 second, 5 seconds, 10 seconds, 24 hours; also the depth that that cutting should be inserted in the liquid.

ED WOOD: Well, the second part first. In my opinion, you dip the cutting to the depth at which you want roots to form. The hormone material tends to penetrate right through the tissue and forms roots to that depth. To approach the problem of how long an immersion — say a 5 second dip; if you dip it in and out, the liquid is going to be on for more than 5 seconds anyway, so I am not sure 5 seconds is going to penetrate that much. If you have very difficult-to-root material, you keep raising the concentration, but you may end up burning the cutting. We used to do this with *Photinia* × *fraseri*. I burned the devil out of the bottom of the cutting but the hormone seeks its own level so roots form above the burned part and you just cut the dead bottom off when you pull the cuttings out of the flat. Rather than do that, however, maybe you should try a little longer soak at a lower concentration. You are going to have to work that out because it is going to be different not only for every plant, but how that particular plant was grown — what kind of wood you are using. I think there is a good reason on hard-to-root material to use a longer soak where you get more penetration.

RALPH SHUGERT: From an economic standpoint, you can use the proper hormone but at a lesser concentration and a

longer dip. I don't think we have paid much attention to this. We are in a hurry and we just dip the cutting in and we bring it back up. Try cutting the concentration down and count to ten rather than five. You might be surprised at some of the results.

LARRY CARVILLE: I think one of the points that was made in that first question from Shugert to Wood, when he talked about a quick dip is — what is a "quick dip"? We read in the literature that it means 5 seconds to one person, or 10 seconds to another. Bob Ticknor mentioned something about his concentration as being 1 to 5; and we read in the literature 1 to 10, 1 to 15, etc. Now, is that 14 parts water and 1 part liquid, or is it the other way around. I always thought for 1 to 5 to take 1 of Jiffy Grow, for example, and 5 parts water. Bob uses it the other way — 1 part of his root stimulant, then bringing it to 5 total parts by adding 4 parts water. This can make a substantial difference in your results, and what your records show, obviously.

ED SCHULTZ: Question to Ingemars Karlsson. What hormone did you use on rooting yellow cedar and what medium was in the containers?

INGEMARS KARLSSON: When we obtained good rooting we used Rootone. We had started mixing our own but we burned the cuttings. The rooting medium was ⅓ coarse sand, ⅓ peat, and ⅓ perlite. We kept the surroundings around the cuttings very dry. We find that yellow cedar cuttings just rot if we root them under the same conditions as used for western hemlock cuttings. They need a dryer environment.

VOICE: Question about Dip-and-Grow. I noticed a large variation in the color of the liquid, while on the store shelf. I try to pick the clearest bottle but it changes color over time. Is that changing the effectiveness of the material? What would be the shelf life?

ED WOOD: Well, the chemists tell me that there may be a very slight lessening of the rooting effectiveness once it is discolored by sunlight. All organic materials of this type should be stored in darkness. To find out if there was a loss in effectiveness I tried it myself and I could not tell any difference in rooting. So I am not too worried about the discoloration.

LARRY CARVILLE: You should be aware in selecting this material from your supplier how it is stored and how it is displayed, because obviously it should be stored in a place away from direct light. If you are buying in large quantity, in gallons and then breaking it down into usable sizes, store it in a dark place.

JIM SAHLSTROM: Is there any health hazard in using the Dip-and-Grow rooting material?

ED WOOD: In the first place use good common sense. Don't take a bath in it. It has almost 80% alcohol and 20% DMSO in it. I don't really think that there are that many health hazards. Always follow the label and you are safe.

LARRY CARVILLE: Use of gloves in handling any of these compounds is good practical advice and you should have them available in your propagation department.

BRUCE BRIGGS: A general question for the panel. David Lane, you mentioned that the literature states that IAA breaks down fairly fast. You showed that when you used NAA at high concentrations in the tissue culture medium, you had better rooting. My question would be to you and the panel, first of all, why did you not use IAA, it would break down still faster? To Dave, and to the other panel members — why do we use combinations of hormones in our tissue culture? I would like to hear you comment on both of those two phases. On the one question — on the quick breakdown; the other question would be on — why the combination of the two, not just one in your formulation?

DAVID LANE: There are a number of auxins that you have to choose from for rooting in tissue culture. The ones that you think of immediately are IAA, IBA, NAA, and perhaps even 2,4-D. These auxins have different levels at which they become phytotoxic, first of all — and they also have different activities as auxins so that they initiate different numbers of roots, depending on their activity at a specific concentration. We found that NAA is a more active auxin than IBA; in other words, there are more roots produced at a certain concentration than with IBA, or with IAA. It is less phytotoxic. So when we started out we tended to use NAA rather than the others because our objective at that time was to produce the maximum number of root initials as quickly as possible. For the chronic treatment, with the Malling 9 apple rootstock, where we had just the dip and then took it out, and put it into a zero hormone mix, I guess we were just following through with the NAA that we used before. But I think it would be quite possible to use IAA, perhaps at a higher concentration than would be necessary with NAA, and it would break down more quickly and, perhaps, be just as effective.

I have no comments on using mixtures, but by using mixtures I suppose you could adjust the rate of breakdown and the concentration at different times during the incubation. With IAA, for example, there are some reports that in media

exposed to light, that it decreases in concentration, with a half-life of three days, four days, or something in that order.

RICHARD ZIMMERMAN: I am not positive of the source, but I think in the book, "Tissue Culture for Plant Propagators," by Defossard from Australia, there is a recommendation for using mixtures of both cytokinins and auxins. I don't know of any other place where this has been recommended, or has really been used. It is a problem that you get into. Trying to sort out the effects of the different ones; we intended to stay just with a single one rather than getting into combinations, so far. We may get into combinations for some materials difficult to root. I will have to see if this might be useful.

WILBUR ANDERSON: In the propagation of peas in aseptic culture, we have had to go to double cytokinins to get multiplication of shoots. If we only use one we just get green shoots; if we don't use it we lose the good, green healthy condition. The other cytokinin is necessary for multiplication. So I think each of the cytokinins and auxins have specific functions in the culture environment, or in a growing plant outside the culture. I think that we do have to look at some of these things and not forget that there are possibilities of combinations.

KEITH TURNER: I have a comment on what we have been speaking. When you are working with combinations of auxins, two different auxins, or combinations of two different cytokinins, the size of your experiment becomes extremely large, because you can't pick one concentration of one auxin, and then test a range of the other auxins. You must have at least five concentrations of each auxin. So you end up with 25 different treatments and it gets to be a huge experiment.

But my main question is to Ed Wood. What is the carrier for the active hormone in your material? You mentioned dimethyl sulfoxide, but another was said to be more effective.

ED WOOD: Dimethyl formamide.

MODERATOR PHIL PARVIN: Our next panel has speakers from distant parts of the world, Arie van Vliet from the famous nursery center at Boskoop, Holland, and Ed Bunker, one of the founding members of the Australian Region, from Queensland, Australia. Arie van Vliet will speak to us now: