VOICE: Do you have an algae problem in the porous concrete?

BRIAN HUMPHREY: No.

VOICE: How do you root apple rootstock cuttings?

BRIAN HUMPHREY: We only root apple rootstock from hardwood cuttings. We can root MM.111 and MM.106 reasonably well but M.7 is very difficult.

VOICE: Can *Acer griseum* hardwood cuttings be rooted?

BRIAN HUMPHREY: *A. griseum* is extremely difficult, if not impossible, to root from any type of cutting.

CARMINE RAGONESE: Have you run into any problems with an excessive amount of callus on rhododendrons.

BRIAN HUMPHREY: The sure sign of too much callus on any cutting is either that you have an extremely difficult plant to root or that you have used too weak an auxin.

TISSUE CULTURE OF FRUIT TREES AND OTHER FRUIT PLANTS

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The uses of tissue culture in plant propagation have been amply reviewed in the IPPS Proceedings of the past several years. In the Fruit Laboratory, we are interested in (a) rapid propagation of new selections from our breeding programs, (b) rapid increase of plants that have been indexed for freedom from known viruses, (c) preservation of germplasm, and, in the future, (d) production of haploids for plant breeding. The crops with which we are working are apple (*Malus sylvestris* Mill.), thornless blackberry (*Rubus* sp.), strawberry (*Fragaria* × *ananassa* Duch.) and blueberry (*Vaccinium* sp.). We also have four peach (*Prunus* sp.) understocks in culture but in the future Dr. Hammerschlag of the Cell Culture and Nitrogen Fixation Laboratory will be doing most of the work on peaches at Beltsville.

Tissue culture of fruit crops is underway at numerous locations around the world. In the United States, most such work is in state or federal research stations although several nurseries are now beginning to join in. In Europe, and possibly elsewhere, both commercial laboratories and nurseries are using

tissue culture for production of strawberries and of apple, plum (*Prunus domestica* L.) and cherry [*Prunus avium* (L.) L.], rootstocks.

In this paper, I will discuss tissue-culture propagation of apple, thornless blackberry, strawberry, and blueberry, as done both in our laboratory and elsewhere.

Apple. Research on growing apples through tissue culture is underway at numerous laboratories in more than 10 countries. The chief goal is development of a rapid propagation technique, particularly for certain of the clonal rootstocks, but development of virus-indexed plants is also a goal in many of these laboratories. Our goal is a rapid propagation technique for apple cultivars because we want to investigate the potential of own-rooted trees in high-density plantings.

Cultures are established using either actively growing shoot tips 5 to 20 mm long or apical meristems 0.2 to 0.5 mm high dissected from buds. Establishing cultures from meristems is more difficult but the technique can rid clones of viruses and is suitable for mass multiplication.

Apples have generally been cultured on variants of the Murashige and Skoog (MS) medium (10); the addition of phloridzin or phloroglucinol, as suggested by Jones (7), is the best-known modification. A somewhat different chloride-free salt mixture has been developed and used in P. Boxus's laboratory at Gembloux, Belgium, and W.C. Anderson has used his rhododendron medium (1) successfully with apples (personal communication). We have used only the MS medium, originally with phloroglucinol. Since we found no particular benefit to using phloroglucinol, we have eliminated it from our medium. The medium we use for culture establishment and shoot proliferation has been detailed elsewhere (4). Often the inclusion of 1 mg/liter indolebutyric acid (IBA) produces too much callus on apple shoot cultures so we now reduce IBA content to 0.5 or 0.1 mg/liter. As a general rule, we establish the shoot tips in a liquid medium and after 2 to 3 days transfer them to a solid medium for shoot proliferation.

For rooting, we have tried both an agar medium and a liquid medium saturating a sterile 1:1 mixture of perlite and vermiculite. Rooting has been better with the latter support medium. The nutrient medium mentioned above is modified by halving the salt concentration and eliminating the benzyladenine (BA) and gibberellic acid (GA_3). We have tested IBA at concentrations ranging from 0.01 to 5 mg/liter; the optimum concentration depends on the cultivar and the support medium. Results to date indicate that IBA concentrations higher than 2 to 3 mg/liter inhibit rooting. In contrast, Huth (5) reported that

rooting of 'Jonathan' shoots was best with 10 mg/liter of either IBA or napthaleneacetic acid (NAA).

Cultures on agar media are grown at 25°C. Light during the 16-hr photoperiod is provided by deluxe warm white fluorescent tubes (40 watt) giving 2.2-4.3 klux (200-400 ft-c) at the culture jar. Light for liquid cultures is provided by high-output cool-white fluorescent tubes (Power Groove[1]) giving about the same intensity at the culture flasks.

Rooted plantlets are removed from the culture tubes, the agar (when present) is washed from the roots, and the plantlets are planted under mist. We have used 1:1 peat-perlite as a planting medium but other media could serve as well. After 7 to 10 days, the plants are removed from mist and transferred to a greenhouse bench. If the plantlets are well rooted in the culture tube, they are easily acclimated under mist. Losses after that stage have been very infrequent.

Using this technique, we have produced plants of 'Spartan', 'Ozark Gold' and 'MM 106'. Shoot proliferation of 'Golden Delicious', 'Summer Rambo', 'York Imperial', 'Stayman', and 'Northern Spy' is progressing well, and we will start rooting experiments with these cultivars soon. Plants of some of these and other cultivars and rootstocks, e.g., 'Golden Delicious', 'McIntosh', 'Cox's Orange Pippin', 'M 26', 'M 27', 'MM 106', and 'MM 111', have been produced in other laboratories. Large-scale production of 'M 26' and 'M 27' is beginning in some commercial laboratories and nurseries.

The main problem now is to refine the conditions necessary for obtaining consistently high rooting percentages of apple shoots. Improving the shoot proliferation rate is necessary for some cultivars, but this seems to be less of a problem than rooting. Acclimating plants from tissue culture to greenhouse conditions may require additional study but it has not been a major problem in our research.

Thornless blackberry. Rapid propagation of genetically thornless cultivars of blackberry has been the goal of our program with this crop. Similar work is underway at the University of Illinois, whereas the USDA program at Oregon State University has the goals of obtaining plants free of viruses and developing completely thornless plants from cultivars that are chimeral for this condition. Such cultivars have thornless shoots but produce thorny adventive suckers from the roots.

The details of our technique have been published (4). Ac-

[1] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

tively growing shoot tips 10 to 20 mm long are established in a modified MS liquid medium. After 2 or 3 weeks, the explants are transferred to the same medium with added agar for shoot proliferation. Cultures are grown under the light and temperature conditions described above for apple. Cuttings have been rooted in an agar medium but we prefer to take the small (7 to 15 mm long) cuttings and root them directly under mist in the greenhouse. This is quicker, requires less labor and is more effective. We have used the technique successfully on 'Smoothstem', 'Thornfree', 'Black Satin', 'Dirksen Thornless' and several advanced selections that may be introduced soon.

We have also successfully stored rooted plants of 'Smoothstem' at 4°C. These plants were produced by rooting cuttings directly in peat pellets. The well-rooted plants in pellets were sealed in plastic bags and placed in a refrigerator. They receive low intensity light for 16 hours per day to prevent etiolation. The plants have been stored for 14 months now with losses of less than 5 percent; the experiment is continuing.

Strawberry. Production of strawberry plants by tissue culture is rapidly becoming an established procedure. Freeing plants of viruses was the first goal of strawberry tissue-culture research. Once this was accomplished, development of rapid propagation techniques followed (2,3,8). In addition, long-term storage of tissue-cultured strawberries is feasible (9), and some attempts have been made to develop haploid plants for breeding purposes (12). In the Fruit Laboratory, the research objectives are those just outlined. Since 1962, Dr. John McGrew of the Fruit Laboratory has demonstrated freedom from known viruses by indexing of more than 100 cultivars and selections following tissue culture; he has also been testing the long-term storage of strawberry cultures at low temperature for several years. We began our work on rapid propagation of strawberry earlier this year to determine the usefulness of the technique for the strawberry breeding program in the Fruit Laboratory. Research on this crop has been carried out at a number of laboratories around the world. The techniques have been developed enough that large-scale production of strawberries is underway in commercial laboratories and nurseries in several European countries.

Cultures are established using apical meristems 0.1 to 1.5 mm high dissected from actively growing runner tips. The plants from which the runners are obtained are usually grown at 38°C for some weeks before the meristems are excised to increase the probability that the meristems will be free of virus.

A number of different media have been used for growing strawberries in culture (3) but most workers now use either a

modified Knop's solution (2) or the MS salts (3,6). Meristems are established on an agar medium containing no or a very low level of BA. For shoot proliferation, the cytokinin level is increased, the concentration varying according to the laboratory. Although Boxus (2) uses 1 mg/liter BA, other researchers have reported that lower levels are better (6). It appears that the cytokinin level interacts with the mixture and concentration of salts, the type and amount of sugar used, or both. We have been using the technique described by Boxus (2,3) and it works quite well with the cultivars we have tried. We achieved proliferation rates of 3:1 to 4:1 within 3 weeks in our first attempts, and somewhat better proliferation rates have been obtained more recently. The proliferation rates vary among cultivars, so the composition of the medium may need modification for maximum proliferation of each cultivar. We observed that proliferation rates are better if we transfer clumps containing several crowns rather than transferring individual crowns. Separating the clumps into individual crowns may damage the crowns slightly, thus reducing the proliferation rate or delaying the growth of axillary buds.

Rooting is achieved by transferring clumps containing several to many crowns to a medium containing no BA or GA_3. After 4 to 6 weeks, rooting is sufficient to permit transfer of the plantlets to the greenhouse. This is done by washing the agar from the roots, separating the clumps by hand into individual rooted crowns, and planting these in pots. The young plants are placed under mist or a plastic tent for a few days until they have become acclimated to the greenhouse environment. Boxus (personal communication) takes unrooted and small crowns and scatters them on sphagnum peat under mist. These crowns root readily providing additional plants to those rooted in the culture jars. We have done this also, with good success. We have also taken unrooted crowns directly from the proliferation medium and rooted these in peat pots under mist. Rooting percentages of 60 to 80% in 4 weeks were obtained in our first trial but these were reduced to 50 to 70% when lightly rooted crowns were excluded. I am confident that a better mist control and a longer rooting period would improve these results appreciably.

Since March of this year, we have proliferated and rooted several thousand plants of four cultivars and now have proliferated cultures of an additional 10 cultivars ready for rooting. Boxus has used the technique for more than 150 cultivars and selections and has found none for which the method failed to work (personal communication).

Long-term storage of strawberry plantlets *in vitro* has been

accomplished by Mullin and Schlegel (9) and McGrew (personal communication). Boxus (3, personal communication) stores proliferating or rooting cultures in jars for 4 to 6 months at 4°C when necessary; rooted plants in plastic containers can be stored for 3 to 4 months. This storage capability is useful for adjusting production schedules to meet needs for shipping or planting.

Blueberry. Propagation of blueberries by tissue culture is being studied at several laboratories in the United States and Canada in addition to our own. Our goal is to develop a rapid propagation technique to speed the testing and introduction of new cultivars.

Cultures have been established from rapidly growing shoot tips 5 to 20 mm long or from parts of germinating seedlings (11). An agar medium is used for establishing the cultures.

The culture media used have been those developed by Anderson (1) for rhododendron and similar media developed by us which contain neither the sodium nor the chloride ions found in Anderson's. We have tested the combination of indoleacetic acid (IAA) and 6-ς-dimethylallylaminopurine (2iP) used by Anderson as well as IBA plus BA. The IAA plus 2iP have been superior in tests to date.

Both axillary shoots arising from buds and adventitious shoots arising directly from leaves have been produced on cultures of lowbush blueberry (11) and of a number of highbush, rabbiteye and other hybrid blueberries (unpublished results from our laboratory). We are now working on techniques to maximize shoot proliferation rates and are just beginning rooting studies. One problem has been the very small size of the proliferating shoots, which makes separating and transferring them tedious. We anticipate good progress with this crop in the coming year.

This has been a brief overview of work in progress and the present status of research on tissue culture of four fruit crops. Work has been done or is underway on several other fruit crops. I think that tissue culture has a definite role in production of fruit plants in the future and the foundation for this role is now being laid. Tissue culture is not the answer to all production problems but it is an extremely useful tool when applied in the proper situations.

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JOERG LEISS: Do you have an open house at Beltsville?

RICHARD ZIMMERMAN: Yes, in January but I am not sure when exactly it is. You are welcome to come by and we will show you what we are doing.

LARRY CARVILLE: How susceptible to pathogens are the transplants when you take them out of the sterile environment into an open environment?

RICHARD ZIMMERMAN: We have had no problem with pathogens.

LARRY CARVILLE: Do you use any sterilants?

RICHARD ZIMMERMAN: No. The only problem is mildew and we have that anyway.

RICHARD ZIMMERMAN: I missed the question box last night and understand that there was a question on what woody plants were being tissue cultured. There is a lot of woody plant culture being done in Europe. At a research station in France I saw work on ornamental woody shrubs with something like 26 different genera.

BILL FLEMER: What type of roots do you get on your tissue cultured apples? Are they satisfactory?

RICHARD ZIMMERMAN: So far they have been satisfactory. The first had an enormous amount of callus and we thought that this was what we did not want. As it turned out those were the only cuttings that rooted. Those were the tallest apple plants that I showed in the talk.

VOICE: Could you review how long it took you to get the 1800 strawberry plants mentioned in your talk?

RICHARD ZIMMERMAN: It takes 8 to 9 weeks from the initial meristem isolation to have material ready to go into the multiplication stage. At that point you have a culture that can be divided into 2 pieces; you would then subculture at 3 week intervals. Rooting requires 4 weeks and this is followed with a growing on period of 4 weeks. Starting in April with well established cultures, 1800 strawberry plants were produced by September.

Thursday Afternoon, November 30, 1978

The Thursday afternoon session convened at 2:00 p.m. with Burke McNeil serving as moderator.

PROPAGATION OF UMBRELLA PINE — CLONAL DIFFERENCES IN ROOT INITIATION[1]

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The Japanese umbrella pine, *Sciadopitys verticillata*, has long been considered extremely difficult to propagate by cuttings (4). Lowry (2), reported rooting less than 14 cuttings out of a total of 1100 taken. DeFrance (1) was more successful and obtained 50% rooting in 1938. Waxman (4) reported a relationship between the stage of plant development and the ease of root initiation. Cuttings taken after the chilling requirements were partially or completely satisfied had the highest rooting percentage. The recommended period for taking cuttings was from January through March.

Subsequent attempts to root Sciadopitys cuttings have given highly variable results even though the cuttings were taken during the recommended period. A considerable number

[1] Scientific Contribution No. 748, Storrs Agricultural Experiment Station, University of Connecticut, Storrs, Connecticut 06268.