RESULTS AND DISCUSSION

From our experience so far, we feel that it is feasible to bypass the pre-rooting stage and have the propagules go directly into an artificial soil mix. If a suitable environment is maintained there is a high percentage of root initiation within 3 to 4 weeks. To include the pre-rooting test tube stage would mean handling the plantlets an additional time in the laboratory, increasing production costs.

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TRANSPLANTATION AND POST-TRANSPLANTATION OF MICROPROPAGATED TREE-FRUIT ROOTSTOCKS

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Abstract. The principal factors that have affected success at transplantation of micropropagated tree fruit rootstocks are presented. The factors discussed include pre-transplantation conditions (laboratory culture) and the conditions at transplantation and field-planting; examples are taken from the Kelowna Nurseries production.

Abbreviations used in the text:

NAA	naphthalenacetic acid
IBA	indolebutyric acid
IAA	indoleacetic acid
BA	benzyladenine
MS	Murashige and Skoog nutrient formulation (8)

The use of micropropagation as a propagation tool is often viewed with skepticism. More specifically, it is the rooting and transplanting phases which need credibility. These stages present challenges at least equal to the initiation of cultures, and are more awesome because they mark the end of artificial control and a return to more traditional practices.

Recent publications concerning transplantation (2,3,6) and the factors influencing rooting in tree-fruit culture (5,9,10,11) give indications of diverse approaches.

Our approach is based upon survival and growth after transplation which relies on *in vitro* cultural history coupled with horticultural practices as follows.

The plant material that we have used includes 'M111', 'M7', 'M26' and 'M4' apple rootstocks (*Malus sylvestris* Mill.) and Mazzard 'F12/1' cherry rootstock (*Prunus avium* L.).

1. Laboratory Culture. Just as the condition of plants is important for more traditional propagation, so it is in micropropagation. The factors most likely to influence rooting and survival include the plant growth regulators, salt and sucrose availability, and time in the rooting medium.

(a.) *Growth regulators.* The cytokinin content of the last multiplication stage affects the subsequent rooting of excised shoots (Table 1). The table shows that for a given rooting response, shoots of 'Mazzard F12/1' required a lower auxin concentration in the rooting medium, when they came from cultures grown at the higher cytokinin concentration.

The auxin concentrations shown in Table 1 produced relatively broad peaks in the rooting response of shoots from either cytokinin source. Observations of rooting cultures showed that this occurred for all three major auxins when tested between 0 and 50 μM (0 to 9 mg/l *NAA*; 0 to 9 mg/l *IAA*; and 0 to 10 mg/l IBA).

Table 1. Rooting potential as affected by cytokinin content at the final multiplication stage. Results are the percentage of the propagules that were rooted at transplantation after 17 days on rooting medium. Mazzard 'F12/1'; 10 blocks with 15 shoots per block.

TO NAA (mg/l)	FROM BA (mg/l)	
	0.68	1.12
0.10	43%	69%
0.50	69	76
0.75	73	86
1.00	75	84
1.25	84	85
1.50	76	91

Taken in conjunction with the increasing quantity of roots per rooted shoot over the same range, our observations indicate that the higher auxin concentrations are superior. However, the quality of roots were altered to such an extent as to affect survival when transplanted. Increased auxin levels, particularly of NAA and IBA, caused the production of fused roots, as well as thickened roots associated with callus, which were prevalent on plantlets failing to survive. In consideration of survival, therefore, an auxin concentration that did not initially appear the best was finally selected (0.75 mg/l NAA), and the use of this concentration resulted in the need for 1.12 mg/l BA in the last cytokinin-containing medium for a good rooting response.

(b.) *Salt and sucrose availability*. Plant condition is further affected by nutrient and carbohydrate concentration during the *in vitro* rooting period. It is often assumed that optimum levels prepare plantlets for an autotrophic mode of life when transplanted. Several publications report the use of a diluted nutrient solution for rooting (1,4,7,9). Our observations (Table 2) agreed in principal with the above reports and revealed:

a. the requirement for sucrose in the rooting medium, irrespective of salt content.
b. the increase and then decrease of rooting potential with increasing salt levels, for each sucrose concentration.
c. the relative independence of sucrose concentration when using ¼ and ½ the concentration of MS nutrients (8).

However, we have noticed that survival was often better for cultures that were rooted on 21 and 28 grams of sucrose within the 92% range as shown in Table 2*.

Table 2. Rooting potential as affected by salt and sucrose content. Results show the number of propagules that were rooted out of a possible 25 (5 blocks of 5), recorded at transplantation. Mazzard 'F12/1'; 2.5 mg/l NAA.

Sucrose grams/l.	(MS) salts. Concentration Multiples						
	0	0.25	0.5	0 75	1.0	1.5	2.0
0	0	1	1	0	0	0	0
7	16	24*	20	11	7	2	0
14	20	25*	23*	18	17	6	1
21	21	23*	21*	22	22	14	4
28	12	23*	24*	21	18	11	4
35	16	23*	24*	24*	23*	11	5
42	17	24*	24*	21	21	16	2

(*represents over 92% rooting efficiency.)

(c.) *Time on rooting medium*. The time taken to produce desirable rooted shoots varies from 4 days on the rooting medium for 'F12/1' cherry rootstock to 10 days for 'M26' and 11 to 17 days for 'M111', 'M7' and 'M4' apple rootstocks. At these times roots are still quite short, 0.6 to 1.2 cm (¼-½ inch), and plantlets are easily transferred to soil for growing-on. Longer times than these have often reduced survival rates after transplantation.

The percentage rooting that we have achieved varies as follows:

'M7'	90 to 97%	'M4'	80 to 90%
'M26'	90 to 95%	'F12/1'	80 to 90%
'M111'	85 to 90%		

Although a figure over 90 percent is desirable, differences between one month's production and another do exist. We

have put over 156,000 shoots into *in vitro* rooting, a total for all the above cultivars, and we have achieved an average 85 percent rooting success.

2. Transplantation. Micropropagated plantlets for tree-fruits do not initially have the ability to withstand moisture loss from leaf surfaces, though this can be achieved by misting. Plantlets are routinely removed from their culture vessels by knocking out the agar and separating rooted plantlets, placing these in water until planting in two-inch-square (5 cm^2) containers — 36 in a flat of 11 × 21 inches (28 × 53 cm). Immediately after planting, flats are placed beneath frequent mist (Mistamatic leaf sensor with Flora Mist foggers) until they are ready to be moved to their final growing bed (usually 7 to 10 days).

The following factors influence success at this stage.

a. *Environment.* For the year-round production at Kelowna Nurseries Ltd., we have considered the use of a greenhouse and growth room (12,13,14). Although we have not found an answer to all our requirements, our greatest experience is in the use of the growth room. The environment of the 7000 square feet (650 m^2) growing area is controlled as follows:

i. lighting:

— high pressure sodium lamps (1000 W. Canadian G.E.)

— metal halide (1000 W. Canadian G.E.)

(1300 to 1500 foot candles, 16 hour photoperiod.)

ii. temperature:

— heating — gas-fired infra-red and heat output from lamps.

— cooling — greenhouse-type fan and cooling pads.
(25°C = 77°F)

Plantlets are placed in this environment from the moment they enter the mist bed until one month later. At that time they are about ⅛ inch (2 to 4 mm) in caliper. Mazzard 'F12/1' cherry rootstock usually grows to 5 to 6 inches (12 to 15 cm) high; 'M26', 5 to 7 inches (12 to 17 cm); 'M7', 'M4' and 'M111' 4 to 6 inches (10 to 15 cm).

b. *Potting mix.* We have had success using several of the more common mixes, though we have principally used a mix of peat, perlite, sand, at 3:4:1; v:v:v. This mix provides the support and drainage necessary for plant growth whilst reducing the conditions that sustain fungal growth.

c. *Irrigation.* Water supply can vary in salt content and should be considered when choosing the original location. When flats are moved from the mist beds they are suitably

moist, but close attention must be given to subsequent irrigation. Whilst plants are small, watering with a hand-wand is adequate, though taller plants are often unable to stand up in the water flow. This can lead to crooked growth if the plants are not righted. Automation of our system with Toro heads has reduced this problem.

d. *Fertilization*. Although plants have grown in mixes not adjusted for pH, we now adjust to near pH 6.0 with dolomite lime. Several mixes of granular fertilizer have been tested but their speed of release has caused burning. We now use a 14:14:14 Osmocote with a three to four month release formulation, plus added micronutrients. Superphosphate, iron chelate, magnesium, and calcium salts may be necessary to correct nutritional problems.

Using the growth room environment described, we have achieved varying degrees of transplantation successes:

'M7'	70 to 90%	'F12/1'	70 to 90%
'M26'	80 to 95%	'M4'	60 to 85%
'M111'	70 to 90%		

Perhaps the single greatest factor affecting these survival figures is disease, commonly damping-off fungi which become evident at removal from mist. Pasteurization with steam, or fumigation of the soilpile with Basamid (B.A.S.F., Ontario), followed by proper hygiene at transplantation and in the growing area should significantly reduce the problem. Dilute sprays or drenches of fungicide may control localized problems.

3. Post-Transplantation. From a controlled environment to an uncontrolled environment in one step is possible, though in our experience this has led to delay in top growth. We favour an intervening partially exposed environment into which plants are placed before full exposure. During the spring, the growth room can gradually be cooled off before transferring plants to a partially exposed environment which has minimal bottom heat. During the summer, plants can be moved to 50 percent shade. Following acclimatization, plants should be able to be transferred to full exposure, if conditions permit.

We have transplanted to field conditions without pre-conditioning in late April and May. Freshly planted material was subsequently hit by frosts and chilling winds and underwent a fall colouration and defoliation within a few days. Following a two month quiescent period most plants have shown good shoot growth and should be able to grow a further 30 to 60 cm (1½-2 feet) in the remaining two months of the growing season. Our total field planting is almost 30,000 from the cultivars mentioned above.

The losses that we have made would be similar for seed-

ling material as for micropropagated material, once the preliminary misting has passed. The condition of plantlets when they are transplanted can be greatly affected by alterations in kind or concentration of grown regulators, by the availability of salts and sucrose, and by the length of time on the rooting medium. We have found that this area may require month to month attention, and that newly isolated sources of a cultivar may require different conditions from existing stock. However, there is now little skepticism within our nursery that we can suitably adapt micropropagated material to the external environment on a commercially feasible scale.

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SMALL FRUIT CULTURE AFTER THE TEST TUBE

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There is an important transition period which tissue cultured plantlets must experience between the protected environment of the laboratory and the harsh world of the greenhouse. In fact, the ability to survive this transition is limiting the commercial use of tissue culture for some species. However, plants of some cultivars survive this crucial stage more easily than others.

Growers know that field-grown plants require good growing conditions: fertile, well-drained soil, proper watering, and nutrients. Tissue-cultured plants also require good growing conditions: controlled environment of heat, light, and chemical nutrients. Between these two very different growing conditions is a transition facility for preparing *in vitro*-propagated plants for growing on. The requirements for this facility differ according to the cultivar being grown.

This year we propagated 40,000 strawberry plants by tissue culture for growers who sell certified strawberry plants. We grew 'Hood,' 'Benton,' 'Olympus,' 'Totem,' 'Shuksan,' and 'Quinault' cultivars (2). We took the plants out of culture jars and put them directly into bedding plant containers in the greenhouse; our mortality was essentially zero. Caneberries, on the other hand, take considerably more care.

We programmed for field-ready strawberry plants on April 1. Strawberry meristems were started in culture in July. They multiplied in test tubes, then in mason pint jars, for four or five months, multiplying, in some cases, as much as six to one in ten days. Following the multiplication stage they were placed in rooting agar in pint jars for four to six weeks. We transferred the rooted plantlets from the jars into the greenhouse mostly between January 15 and February 15. The days were short, mostly cloudy and rainy, and cool to cold. Because of woody hillsides, we have less than five hours of direct sunlight on our greenhouses on February 1. The greenhouses are quonset style pipe houses (14′ by 90′) with inflated double-