PRESENT LIMITATIONS AND FUTURE PROSPECTS FOR COMMERCIAL MICROPROPAGATION OF SMALL FRUITS

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Micropropagation has had a significant impact on the commercial propagation of several small fruit crops, especially bramble fruits. Tissue culture is commercially viable under certain conditions, primarily because these crops are relatively slow and labor intensive to multiply by conventional techniques, and because of the demand for virus indexed registered stock. Red raspberry, for example, is difficult to root from cuttings, and hence is conventionally propagated mainly by division of canes arising from root suckers. Typically, a multiplication rate ranging from about 4 to 1 per year is achieved with stock infected with common viruses. At Congdon and Weller Nurseries, however, multiplication rates of about 10 to 1 per year are achieved from division-propagated stock largely because of a virus index registration program which has been in place since 1964 (11). These relatively low rates of multiplication combined with stringent cultural requirements required for participation in the New York State Virus Tested Plant Material Program made conventional propagation particularly daunting. Virus indexed nuclear stock was kept free of tobacco streak virus and mosaic viruses by growing it in screen houses to exclude aphids and leaf hoppers which are the vectors of these viruses. Foundation I stock was propagated from nuclear stock by division and replanted. Although no longer screened, Foundation stock was still protected from reinfection by eliminating native brambles within a 1000 foot radius and a rigorous pesticide spray program. Control of the digger nematode (*Xiphinema americanum*) which transmits tomato ringspot virus (the cause of crumble berry in brambles) required the additional considerable expense of soil fumigation before planting. In the past several years, micropropagation has completely eliminated the use of screen houses for red raspberries and reduced the amount of time required to produce 30,000 plants of a newly released cultivar from 5 or 6 years to about 2 years. Under these circumstances the advantages of micropropagation become readily apparent. Furthermore, field performance of brambles propagated through tissue culture may surpass that of convention-

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ally propagated material. Tissue culture propagated thornless blackberries, for example, exhibited greater vegetative vigor and uniformity than tip layer propagated material (9).

Considering these obvious advantages of small fruit micropropagation and the fact that relatively straightforward techniques are available for each of the major crops, the conclusions of a recent survey conducted by Swartz and Lindstrom (8) are somewhat surprising. They reported that only approximately 13% of bramble fruit nursery stock in the U.S. is propagated via tissue culture. Micropropagation accounts for about 5% of blueberry nursery stock and less than 1% in the case of grape and strawberry. The remainder of this review will consider some of the economic and cultural factors which limit the commercialization of small fruit micropropagation and discuss some research currently underway directed towards overcoming some of these limitations.

Simple economics is the most important impediment to increased commercial micropropagation of both strawberries and bramble fruits. The selling price is about 30 to 50% higher for micropropagated brambles and 4 to 7 times higher for micropropagated strawberries than their conventionally propagated counterparts (8). The existing demand for micropropagated red raspberries (13% of the market) despite their relatively high cost stems largely from the fact that they are produced and marketed as virus-indexed, registered and, as such, they can be counted on to perform better than nonregistered stock.

Despite whatever horticultural (noneconomic) advantages which might accrue from the planting of tissue culture propagated small fruits, there is little doubt that demand would grow significantly if the costs of micropropagation were to decrease. One of the most significant contributors to the relatively high cost of micropropagated small fruits is the considerable amount of skilled hand labor involved. Several cost analyses of commercial micropropagation have consistently identified labor as the major expenditure. Labor costs as a fraction of the total cost of propagation range from low of 39% for chrysanthemum (2), 67% for foliage plants (6), and as much as 76% for broccoli (1). Recently, Borgman (3), working in the principal author's laboratory, has completed a detailed microcomputer-based analysis of the costs and potential profitability for commercial scale micropropagation of red raspberry. Net present value analysis was used to correct for the effect of inflation on fixed and variable costs incurred over a 10 year period. Despite the use of a variety of labor and time saving devices available to the modern tissue culture lab including an autoclave, multiple transfer hoods, automatic dishwasher, and media dispenser, labor was still the major cost of production, accounting for about 50% of the total expense. Axillary shoot culture, the technique used in the micropropagation of small fruits and practically all other

commercially micropropagated crops, is inherently labor intensive. The technique involves the cytokinin-stimulated proliferation of basal axillary and/or adventitious shoots from a single shoot tip explant (stage II). Typically, during this most labor intensive stage, a technician, working under aseptic conditions, with scalpel and forceps, must meticulously subdivide a rosette consisting of several shoots, and transfer each individual shoot to fresh medium. For red raspberry, multiplication rates are on the order of 4 to 1 over a 4 to 6 week period. Thus, many successive subcultures (5 or more), (and hence much time and labor) are commonly required to generate commercial numbers of plantlets (thousands to hundreds of thousands) from several initial (stage I) explants. The transfer of axillary shoots does not lend itself easily to automation because the delicate microshoots are easily damaged and each must be precisely oriented in the new medium. When sufficient numbers of shoots are available they are rooted, either in vitro (Stage III) by transfer to an auxin-containing medium, or ex vitro (Stage IV) by removal from culture to a high humidity greenhouse where they are treated as microcuttings. With small fruits, rooting is mostly ex vitro, because high rooting percentages are achieved, and because it is less labor intensive than in vitro rooting.

One potentially labor saving approach to micropropagation being investigated in the principal author's laboratory is root organ culture. This would involve culturing root rather than shoot tissue during stage II. After the desired level of root multiplication is achieved, plantlet regeneration would be accomplished by inducing adventitious bud formation on the root cultures (Stage III). The savings in labor would arise from the fact that roots can be subdivided (chopped up) without regard for the need to keep buds and leaves intact or the need to accurately place the explant on the new medium as is so time consuming in subculturing of shoots. If bud initiation and shoot growth could be delayed until the last passage in culture it would be unnecessary to subdivide shoots at all.

Red raspberry is particularly well suited for investigating the potential usefulness of this approach to micropropagation, because of it's natural tendency to sucker. Suckering begins with the formation of adventitious shoot buds on the roots of a plant growing in soil. This is physiologically quite similar to the induction of adventitious buds on root organ cultures in vitro, and suggests that root organ culture followed by plantlet regeneration (in vitro suckering) might be successfully exploited for the sake of micropropagation. In our laboratory we have succeeded in culturing roots of several cultivars of red raspberry including 'Titan', 'Heritage', 'Sentry', and 'Latham' (4). Root explants were severed from in vitro rooted stage III shoot cultures and placed in liquid Anderson's medium. In the presence of the auxin IBA they grew mainly by initiation and elongation of latèral roots. Figure 1 shows the effect of IBA concentra-

tion on lateral root formation. For newly initiated root cultures there was a sharp optimum at 0.5 mg/L, but root cultures which had been subdivided and transferred through 5 passages had a significantly lower optimum IBA concentration (0.1 mg/L), suggesting that auxin habituation may have occurred. Root cultures also grew readily on agar or gelrite solidified media. Bud regeneration followed by shoot development has been achieved on both liquid and solidified culture medium, but the frequency of bud initiation has been low. Bud regeneration frequency was higher from 'Heritage' than from 'Titan' root cultures. This was as expected because the former also has the greater tendency to sucker naturally. The principal focus of the research at the present time is to determine the optimal hormonal and/or other conditions necessary for shoot bud initiation, and eventually to adapt the technique to commercial scale micropropagation.

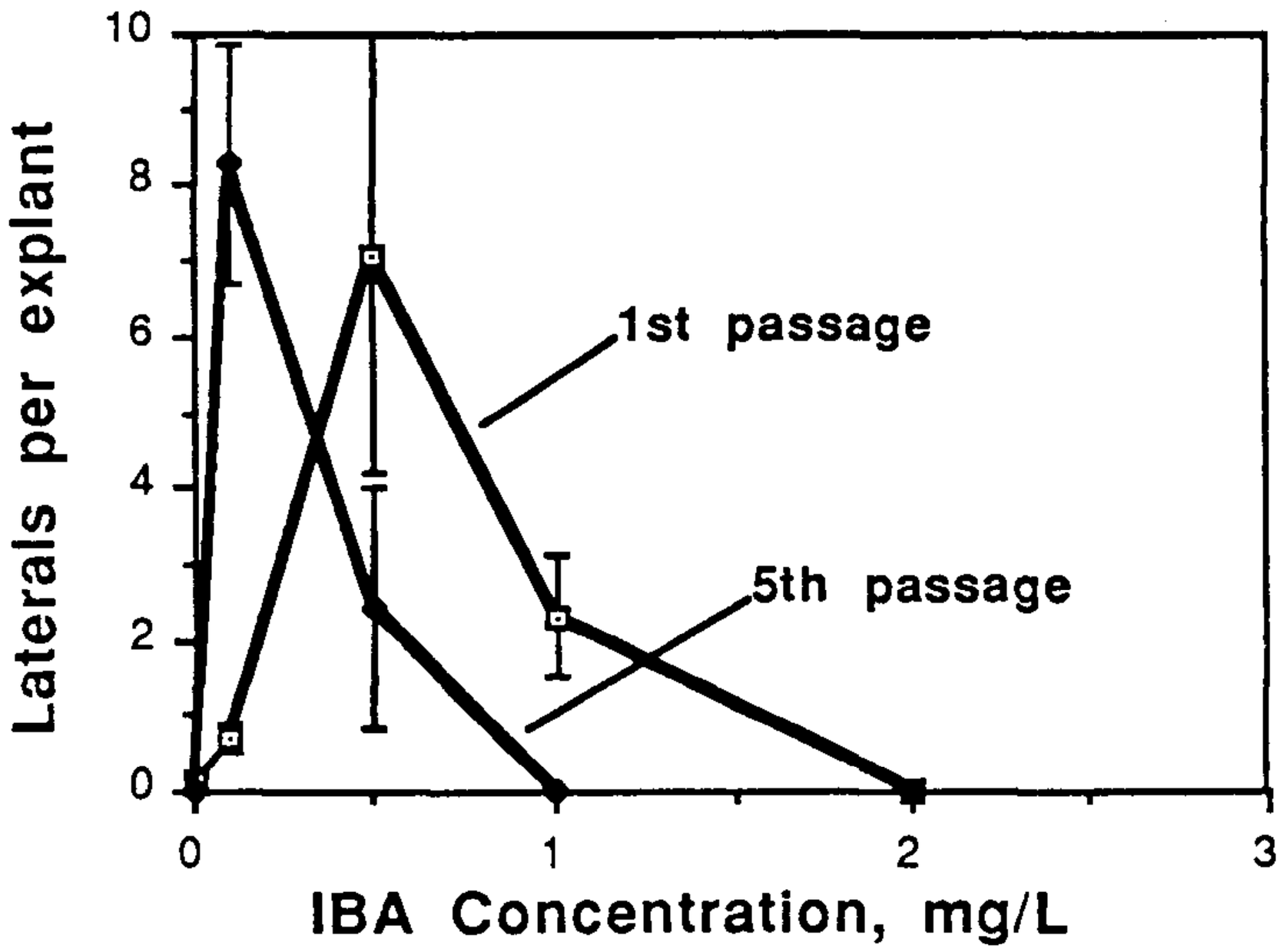

Figure 1. Effect of IBA concentration on lateral root formation on root organ culture of 'Titan' red raspberry.

In addition to economic factors which limit the commercialization of small fruit micropropagation, there are biological limitations as well. Included in this category are concerns about the occurrence of off-type plants resulting either from genetic mutations or from more or less transient phenotypic (epigenetic) changes. For example, increased thorniness has been observed in micropropagated gooseberry (10). Another related concern is increased susceptibility to certain diseases and biocides in plants which have been micropropagated. After out-planting, tissue culture propagated strawberries have been shown to have increase susceptibility

to the pathogens which cause red stele and Verticillium wilt (7). It has been observed at Congdon and Weller nurseries that field plantings of micropropagated brambles were injured by lower-than-recommended rates of the herbicide Princep (Simizine), during the first growing season, but unaffected by the maximum recommended rate during the second growing season. Recently, one of us (JCN in collaboration with M. Pritts) has conducted greenhouse and field experiments at Cornell University which confirm these observations. Micropropagated and conventionally propagated (tip layered) 'Royalty' purple raspberry plants were treated after outplanting with recommended rates of several herbicides registered for this crop. Table 1 shows that growth (dry weight) of micropropagated, but not of conventionally propagated (tip layered), plants was significantly reduced by both Princep and Surflan, compared to untreated controls. These and similar observations concerning enhanced disease (7) and biocide (5) susceptibility in micropropagated plants suggests that more research in need to develop alternative pest and weed control practices.

Table 1. Effect of three herbicides on growth of micropropagated and conventionally propagated 'Royalty' purple raspberry.

		Growth as percent of control	
Herbicide	Rate, # a.i./A	Micropropagated	Tip-layered
Control	0	100	100
Simizine (Princep)	1	43*	74
Oryzalin (Surflan)	2	57*	69
Diphenamide (Enide)	6	65	70

Commercial micropropagation of small fruits is an excellent example of the impact of biotechnology on horticultural production. Further research directed toward developing more efficient and cost effective methods should result in increased use of this already important form of propagation.

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Tuesday Afternoon, December 9, 1986

The afternoon session was convened at 2:00 p.m. with Carla Patore serving as moderator.

MODIFIED SIDE GRAFT FOR DECIDUOUS TREES

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Our basic approach to outdoor grafting of deciduous trees had always been coupling or triangling. When the scarcity of good, capable grafters to make intricate triangle grafts became a problem, we decided to try modified side grafting. We felt that this procedure would solve two of our problems; the need for experienced and skilled grafters, and the poor "takes" that we encountered with various species. This resulted in the loss of the full standard. When only a portion of the stem is lost, the tree is made less uniform and not as readily saleable.

To overcome both of these problems we decided to see if side grafting, as we were using in evergreen grafting, might work. We were grafting around 30,000 evergreens at that time, had plenty of experienced help, and time to train more, as grafting is done during our slacktime—winter.

Let me describe to you the procedure using *Morus alba* 'Pendula', which is grafted onto *M. alba* or *M. alba* var. *tatarica* as